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MESSAGE FROM EDITORS' DESK

It gives us an immense pleasure in bringing out the sixth volume of International Journal of Science and Humanities with your incessant support. International Journal of Science and Humanities being published by Islamiah College has been successfully marching towards its sixth year by providing a platform for authors in exhibiting their talents in the form of their research articles on various disciplines such as English, Chemistry, Bio-Chemistry, Commerce, Management, History, Sociology, Public Administration, Political Science, Physics, Economics and Mathematics.

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Therefore, we humbly request you to provide your valuable suggestions in further strengthening this Journal and always extend your support by publishing your quality articles in our reputed International Journal of Science and Humanities.

Disclaimer

APPEAL

I am delighted to introduce this issue of International Journal of Science and Humanities (IJSH) to the students and research community on behalf of Islamiah College (Autonomous), Vaniyambadi, a century old institution serving for the cause of education to socially, economically and educationally weaker sections of the society. The IJSH, is a peer reviewed research journal of interdisciplinary nature that cater the needs of the teaching and research society. The aim of the journal is not only to provide a space for leading research work but also provide a platform for the budding researchers to publish their maiden attempt in the field of science and humanities. The objective of IJSH is to publish up-to-date, high-quality and original research papers alongside relevant and insightful reviews.

The initiative to start this journal was taken by Janab L.M Muneer Ahmed, the Secretary & Correspondent of this College with an aspiration to keep the research vibrant in this campus. Now, the torch is handed over to me from June 2016 onwards to run this journal on non-profitable basis without compromising its aims and objectives. At this juncture, I appeal to all teaching and research communities to concentrate on both teaching and research relevant to society, which are symbolically related as the two faces of the same coin. I also appeal to all reviewers and editors not to compromise with the quality of the input and promote this journal to the next level with excellent output. Finally, I pray Almighty to provide guidance for development and success of this journal. Best wishes and thanks for your contribution to the IJSH.

Mr. L.M. MUNEER AHMED
Secretary & Correspondent
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DIELECTRIC RELAXATION STUDIES OF ACRYLAMIDE WITH GLYCOLS IN NON-POLAR SOLVENTS USING FREQUENCY DOMAIN TECHNIQUE

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Abstract

The dielectric relaxation of Glycols (ethylene glycol, diethylene glycol and triethylene glycol) with Acrylamide in dilute solution of Benzene and 1,4-diaxone is measured at 9.37 GHz using Frequency domain (X-band) technique. Different dielectric parameters like dielectric constant (ε'), dielectric loss factor (ε'') at Microwave frequency, static dielectric constant (ε_0) and dielectric constant at infinite dilution (ε_∞) at optical frequency have been determined. From the measured dielectric data, the relaxation time (τ) calculated using Higasi method and activation energies ($\triangle F_\tau$ and $\triangle F_\eta$) have been determined. The relaxation time and molar free energy activation of 1:1 molar ratio is greater than other higher molar ratios (i.e. 3:1, 2:1, 1:2, 1:3) confirm that the existence of most likely 1:1 complex formation between the studied systems and also complex formation formed between free hydroxyl group of glycols and carbonyl group of amide.

Keywords: H-bonding, Acrylamide, Ethylene Glycol (EG), Di-Ethylene Glycol (DEG), Tri-Ethylene Glycol (TEG), Permittivity, Dielectric Constant, Dielectric Loss, Relaxation Time and Activation Free Energy.

1. Introduction

Amides are the simplest molecules containing a peptide linkage and a study of their hydrogen bonding yields into the nature of protein structure [1]. Amides are used as synthetic reagents. Mono alkyl ethers of ethylene glycol, propylene glycol and butylene glycol are commercially known as cellosolves. These are used as industrial solvents, surfactants, detergents and wetting agents. The molecules of these compounds have hydrogen bonding sites and can enter into intra and inter molecular hydrogen

bonding giving rise to several different conformations. In pure liquid state, the molecules of mono alkyl ethers of ethylene glycol, propylene glycol and butylene glycol exist in hydrogen bonded linear structure in dynamical equilibrium [2]. Hydrogen bonds constitute a very interesting class of intermolecular interactions, which are of extreme importance in many fields of chemistry and molecular biology. The dielectric investigation of hydrogen-bonded compounds in non-polar solvent provides valuable information regarding molecular complex formation in solution. The study of the H-bonds of the type O=H——O=C occupies a position of considerable importance as it relates to the study of biopolymers. Thus, the study and knowledge of dielectric properties of the mixtures of amide with glycols in non-polar solvents is expected to provide useful and vital process parameters for efficient design of transesterification processes of industrial interest.

Keeping both the industrial and scientific interests in mind, an attempt has been made in the present work to study the hydrogen bonding between free hydroxyl group of glycols and the carbonyl group of amides using dielectric method. This study is expected to provide better understanding of the nature of molecular orientation processes. The dielectric relaxation behaviours of amides are expected to be similar to that of n-alcohols [3,4]. The study of dielectric relaxation of polar liquids in non-polar, solvents from the microwave absorption studies gives valuable information about various types of the molecular associations present in the solutions as microwave can detect weak molecular interactions. Dielectric relaxation study can give precise information on the formation of H-bonded complex when a proton donor is mixed with a proton acceptor in nonpolar solvent. In our present investigation, the mixed solvents have been used a wide range of chemical, biological pharmaceutical, industrial, biophysics, condensed matter physics, and laboratory applications [5]. The dielectric investigation of hydrogen-bonded compounds in non-polar solvent provides valuable information regarding molecular complex formation in solution. The study of the H-bonds of the types (O-HC=O) occupies of position of importance as it relates to the study of biopolymers. Recently, dielectric relaxation behaviour of mixtures of polar molecules under varying conditions of complexation temperature and environment factors has evoked considerable interest [6]. The present work reports the dielectric relaxation behaviour of mixtures of acrylamide with glycols like Ethylene glycol, Di-ethylene glycol and Tri-ethylene glycol in Benzene and 1,4-diaxone to obtain knowledge of the dynamic dielectric behaviour of amides in glycol solutions.

2. Material and Method

The static dielectric constants were measured by heterodyne beat method at 308K using a commercial instrument, Dipole meter DM-01 supplied by Wissenschaijftlich Technische Werksatter, Germany operated by 220V [7]. The refractive indices (n_D), which was measured by Abbe's refractometer [8]. All measurements were made at 35°c

and the temperature was controlled within ±0.5°c by a thermostat. The uncertainties in the measurements of dielectric constants and refractive indices were ±0.0005 and ±0.0002 respectively. The measurements of dielectric constant at an angular frequency (ε') and dielectric loss (ε''), where carried put X-band microwave frequency at 9.37GHz. The viscosities were measured with the help of Ostwald's viscometer. A variable attenuator and a slotted line wave-guide with a slit in the broad face to accommodate a probe were connected with a liquid cell. The signal from the Klystron was fed to the attenuator through a ferrite isolator. This isolator allows free passage of power only in the forward direction and it attenuates the reverse wave strongly. The sample length of liquids is adjustable by the micrometer plunger assembly. The microwave power was transmitted from the slotted line into the liquid through a Teflon window, which has negligible dielectric loss. The probe is a crystal detector, which measures the microwave power. The microwave current is fed to a sensitive spot galvanometer. The temperature of the liquid inside the cell was kept constant by circulating water around it from a thermostat [9]. The physical parameters of all the chemicals used here have been checked against their literature values.

3. Dielectric Parameters

According to Higasi's method [13], the average relaxation time $\tau_{(1)}$ is described by

$$\tau_{(1)} = \frac{a''}{\omega(a' - a_\infty)}.$$

While the overall dielectric relaxation $\tau_{(2)}$ is given by

$$\tau_{(2)} = \frac{a_0 - a'}{\omega a''}$$
$$\tau_{(0)} = \sqrt{\tau_{(1)}\tau_{(2)}}$$

$\tau_{(0)}$ may be called the mean relaxation time, where ω is the angular frequency ε_0, ε', ε'' and ε_∞ are defined by equation (3)

$$\varepsilon_0 = \varepsilon_{01} + a_0 w_2$$
$$\varepsilon' = \varepsilon'_1 + a' w_2$$
$$\varepsilon_\infty = \varepsilon_{\infty\,1} + a_\infty w_2.$$

In which subscript refer to the solvent and 2 refers to the solute, 0 refers to the static frequency and ∞ refers to the infinite or optical frequency measurements and w_2 is the weight fraction of the solute.

The molar free energies have been calculated using the Eyring's equation.

$$\tau = \frac{h}{kT} \exp\left(\frac{\triangle F_\tau}{RT}\right)$$
$$\eta = \frac{Nh}{V} \exp\left(\frac{\triangle F_\eta}{RT}\right),$$

where $h-$ Planck's constant, $k-$Boltzmann's constant, $N-$Avagadro number and $V-$ the molar volume.

4. Result and Discussion

The ternary systems selected were acrylamide with proton donors (ethylene glycol, di-ethylene glycol and tri-ethylene glycol) using Benzene and 1, 4-dioxane as solvents. The Value of relaxation times $\tau_{(1)}$, $\tau_{(2)}$ and $\tau_{(0)}$ for all the systems were calculated by Higasi's method. The relaxation time τ, of amide with proton donors (ethylene glycol, di-ethylene glycol and tri-ethylene glycol) in Benzene and 1,4-dioxane as solvents at 35°c has been provided in tables 1 & 2. A perusal of table shows that the value of relaxation time τ increases with increasing chain length of amide and acidic nature of glycols.

The increase in relaxation time due to increase in the effective radius of the rotating unit. The observed high value of acrylamide can be attributed to larger size of acrylamide molecule in comparison molecules. Benzene and 1, 4-dioxane as solvents are symmetrical and non-polar molecule. But each carbon atom in this molecule in this polarized due to its three pair of electrons and therefore it can function as an electron donor. Therefore, there is a possibility of interaction between the positive hydrogen of alcohol group and carbon atom in Benzene and 1,4-Dioxane. The potential hydrogen bonding nature of benzene molecule may contribute to increasing the relaxation time [7]. The relative size of both amide and glycols molecule determines the predominance of a particular type of interactions over the other. Our results show that the relaxation time larger at 1:1 mole of amide with glycols as shown in fig. The relaxation time decreases conspicuously for the other mole ratios but are higher than either of the components. Parthiban [10] studied the H-bonding in some carbonyl + glycols system in different compositions. They also observed that the relaxation times of ternary mixtures is always much greater than either of the polar solutes in the inert solvents.

The relaxation time for dilute solution ethylene glycol, di-ethylene glycol and tri-ethylene glycol observed in the present studies range between 11 and 11.50 ps, with excess of glycols, the relaxation time in the benzene is slightly greater than 1, 4-Dioxane. This result is in agreement with the earlier investigation of Parthiban [10].

The result also shows that the molecular association between amide and glycols is maximum at 50:50 mol% ratio and then decreases at other mol%. From this we conclude that the 1:1 complex is dominant in amide in glycols as shown in fig1. The

Table 1: Value of dielectric constants and relaxation times for various weight fractions, Acrylamide with Glycols in Benzene

Ratio	Weight fraction W_2	ε_0	ε'	ε''	ε_∞	Relaxation Time (ps) using Higasi's Method			Activation energy (KJ mol^{-1})	
						τ_1	τ_2	τ_0	$\triangle f_\tau$	$\triangle f_\eta$
Acrylamide+EG+Benzene										
1:3	0.02202	2.3299	2.2956	0.1882	2.2751	6.27	8.81	7.43	8.46	11.37
1:2	0.02228	2.3586	2.3123	0.1921	2.2809	6.37	9.57	7.81	8.51	11.40
1:1	0.02278	2.3764	2.3345	0.1991	2.2865	6.47	10.40	8.20	8.58	11.42
2:1	0.02331	2.3612	2.3612	0.2023	2.2898	6.39	9.46	7.77	8.49	11.39
3:1	0.02357	2.4128	2.3712	0.2069	2.9323	6.30	8.89	7.48	8.43	11.34
Acrylamide+DEG+Benzene										
1:3	0.03334	2.8590	2.7405	0.1972	2.0150	6.06	9.94	7.78	8.54	11.28
1:2	0.03218	2.8823	2.7538	0.2049	2.0141	6.14	10.37	7.97	8.61	11.34
1:1	0.03032	2.9056	2.7649	0.2125	2.0133	6.23	10.94	8.25	8.69	11.47
2:1	0.02834	2.8704	2.7627	0.2098	2.0382	6.17	10.06	8.08	8.52	11.42
3:1	0.02734	2.8612	2.7390	0.1980	2.0451	5.96	9.67	7.59	8.64	11.37
Acrylamide+TEG+Benzene										
1:3	0.04883	2.9020	2.8458	0.2010	2.1127	6.20	10.70	8.14	8.77	11.46
1:2	0.04239	2.9926	2.8916	0.2098	2.1043	6.26	10.79	8.22	8.83	11.53
1:1	0.03786	3.0126	2.9934	0.2172	2.0954	6.34	10.92	8.32	8.89	11.60
2:1	0.03337	2.9896	2.8876	0.2105	2.1098	6.21	10.21	8.15	8.79	11.41
3:1	0.03111	2.9054	2.8678	0.1998	2.1099	6.16	10.58	8.18	8.80	11.45

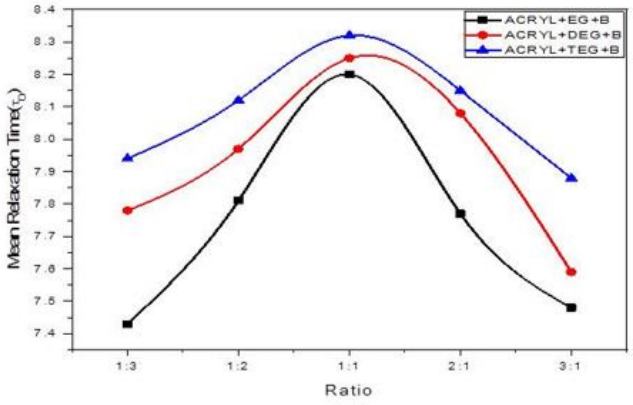

Figure 1: Variation of mean relaxation time with ratio of Glycols and Acrylamide in Benzene

Table 2: Value of dielectric constants and relaxation times for various weight fractions, Acrylamide with Glycols in 1,4 Dioxane

Ratio	Weight fraction W_2	ε_0	ε'	ε''	ε_∞	Relaxation Time (ps) using Higasi's Method			Activation energy (KJ mol^{-1})	
						τ_1	τ_2	τ_0	$\triangle f_\tau$	$\triangle f_\eta$
Acrylamide+EG+1,4 Dioxane										
1:3	0.01867	2.5591	2.4636	0.1813	2.2674	4.55	9.84	6.69	8.09	11.02
1:2	0.01889	2.5878	2.4959	0.1954	2.2695	4.77	9.93	6.88	8.17	11.11
1:1	0.01933	2.6287	2.5481	0.2029	2.2776	4.98	10.11	7.01	8.23	11.19
2:1	0.01977	2.5108	2.4780	0.1952	2.2648	4.59	9.79	6.83	8.14	11.13
3:1	0.01998	2.5806	2.4759	0.1840	2.2615	4.87	10.01	6.72	8.08	11.06
Acrylamide+DEG+1,4 Dioxane										
1:3	0.02827	2.5768	2.4228	0.1798	2.2502	4.87	9.96	6.96	8.19	11.17
1:2	0.02452	2.5988	2.4612	0.1834	2.2599	4.96	10.07	7.06	8.24	11.21
1:1	0.02572	2.6097	2.5034	0.1991	2.2681	5.05	10.19	7.17	8.32	11.25
2:1	0.02403	2.5843	2.4753	0.1878	2.2547	4.93	9.98	7.01	8.25	11.16
3:1	0.02318	2.5587	2.4631	0.1787	2.2511	4.89	10.02	6.99	8.22	11.19
Acrylamide+TEG+1,4 Dioxane										
1:3	0.03786	2.5119	2.4002	0.1687	2.2398	4.97	10.07	7.07	8.27	11.21
1:2	0.03595	2.5689	2.4362	0.1793	2.2405	5.04	10.16	7.15	8.30	11.28
1:1	0.03212	2.5990	2.4896	0.1895	2.2571	5.13	10.28	7.26	8.39	11.36
2:1	0.02829	2.5438	2.4297	0.1770	2.2478	5.02	10.03	7.19	8.36	11.31
3:1	0.02638	2.5099	2.4008	0.1693	2.2325	4.95	9.98	7.02	8.29	11.24

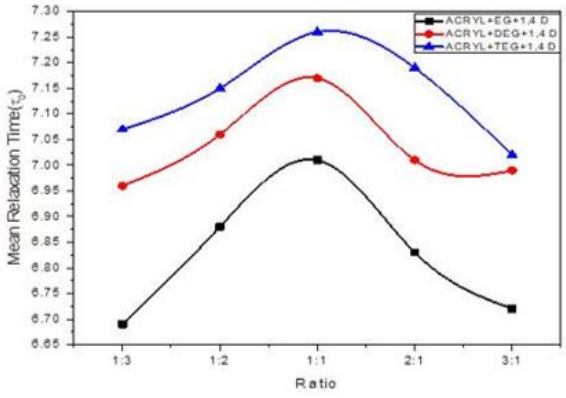

Figure 2: Variation of mean relaxation time with ratio of Glycols and Acrylamide in 1,4 dioxane

increasing relaxation time is due to increasing chain length of glycols and amide and offers hindrance to the rotation of the molecule. The increase in relaxation time may be due to the increase in effective radius of the rotating unit. At high concentration glycols in the mixtures, there is large number of glycols molecule surrounding the amide molecules. Thus dipole-dipole interaction occurs in such a way that the effective dipole moment gets increased and linear $\alpha-$multimers are formed. The dipole-dipole interaction is the interaction of the OH group of the glycols with C=O of amide. At low concentrations of glycols in the mixtures, other is only a small number of glycols molecule to enable dipole-dipole interaction through hydrogen bonding with non-associative amide molecules [11].

The relaxation times of amide with tri-ethylene glycol are slightly greater than the other glycols. The relaxation increases with increasing alkyl chain length of glycols and basic nature of amide donors, indicating that the degree of co-operations for reorientation of the molecules with increasing the chain length with the fact that the relaxation time is directly related to the size of the molecules. The molar free energy activation for viscous flow $\triangle f_\eta$ and the free energy $\triangle f_\tau$ are calculated and given table 1 & 2. It is evident from our data $\triangle f_\eta$ is $\triangle f_\tau$. This is in agreement with the fact that the process viscous flow, which involves both the rotational translational forms of motion, faced greater interference from neighbour than dielectric relaxation, which take place by rotation only. Subramanian [8], pointed out that the relaxation time of amide donor increases as the acceptor ability of the solvent environment increases. Similarly, for a given glycols acceptors, the relaxation must increase with the amide donor ability of the donor solute. Our results are in accordance with this conclusion.

5. Conclusion

The hydrogen bonded complexes of amide and glycols (ethylene glycol, di-ethylene glycol and tri-ethylene glycol) have been suited dilute solutions of benzene and 1,4-dioxane using dielectric method. The dielectric properties of the above systems studied are depending on the alkyl chain length of glycols and amide. The most likely association between glycols and amide is 1:1 complex through the free hydroxyl group of the glycols and the carbonyl group of amide. From the above result it may be conclude that, the acceptance ability of acceptor is increasing order EG > DEG > TEG.

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STUDIES ON PROTON DONOR-ACCEPTOR INTERACTION IN TERNARY MIXTURE OF PHENOL WITH METHYL ACETATE: ULTRASONIC AND FTIR STUDIES

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Abstract

The ultrasonic velocity, density and viscosity measurements were carried out for the mixtures of phenol with Methyl acetate in benzene at 303K. The derived acoustic and thermodynamic parameters namely adiabatic compressibility, free length, free volume, internal pressure, viscous relaxation time and Gibbs free energy were evaluated with a view to investigate the nature of molecular interaction. The obtained results support the occurrence of complex formation through intermolecular hydrogen bonding in these ternary liquid mixtures. FT-IR spectra confirm the expected interactions.

Keywords: Binary Liquid, Phenol, Methyl Acetate, Thermodynamic Parameters and FTIR.

1. Introduction

Ultrasonic velocity measurement in liquid mixture allows for accurate determination of some useful acoustic and thermodynamic parameters and their excess values which are highly sensitive to molecular interactions in their mixtures. Acoustic and thermodynamic parameters were used to consider numerous forms of interaction, molecular binding, molecular activity and specific styles of inputs and outputs [1-5]. The relationship between the molecules may be developed in theory by an analysis of the sudden departure characteristics from the ideal nature of other physical properties, such as volume, compressibility, viscosity etc. The ultrasonic method was used to analyze the properties of some material in order to consider the existence of molecular interactions in pure liquid and liquid mixtures [6-12].

The literature survey on the ultrasonic studies on Phenol and other liquids indicates that enormous work in this direction has been carried out binary and ternary liquid mixtures of weak and strong interacting systems and very few studies are reported on alkyl benzoates with Phenol and other solvent systems. Moreover, thermodynamic properties of these liquid mixtures are of interest for different branches of science and engineering and also play significant role in technological and biological process of living organisms and in nature. This fact has encouraged the author to carry out a series of systematic investigations on the solvent properties of these liquid mixtures.

In the Chemical industry, there exists a continuing need of reliable thermodynamic data of binary liquid systems. This is particularly true for systems involved in industrial process. With this objective in mind the author has chosen Phenol with Methyl acetate in benzene as ternary liquid mixture systems at the temperature 303.15K

The author has attempted to measure the ultrasonic velocity (U), density (ρ) and viscosity (η) of all the ternary liquid mixtures at 303.15 K. From these measured values, the following thermodynamic and transport parameters, which are useful for understanding the nature of the interaction in the ternary mixtures, are evaluated, adiabatic compressibility (β) Inter molecular free length (Lf) Free volume (Vf) Internal pressure (π), Gibb's free energy ($\triangle$G).

2. Materials and Method

The mixtures Phenol and Methyl acetate of various concentrations ranging from 0 M to 0.1M were prepared by taking analytical reagent grade and spectroscopic reagent grade chemicals with minimum assay of 99.9% and procured from E. Merck Ltd (India). The above experimental solutions are of equimolar concentrations of different solutions constituting the system which are under study.

3. Experimental Details

The ultrasonic velocity measurements in the Phenol with Acetaldehyde solutions are made in the ultrasonic Interferometer of fixed frequency 2 MHz (Model F-81 Mittal enterprises, New Delhi) at 303k temperature. The values of densities are measured at 303k temperature using specific gravity bottle by standard operating procedure and the viscosity using Ostwald's viscometer with an accuracy of ± 0.001% standardized with double distilled water. The FTIR spectra are collected for these samples using Fourier Transform Infra Red (FTIR) Spectrometer (Spectrum RX, PerkinElmer).

4. Thermodynamic Parameters

Adiabatic compressibility (β)

$$\beta = \frac{1}{U^2\rho},$$

where U is the velocity in m/s, ρ is the density in kg/m^3.

Free Volume (V_f)

$$V_f = \left(\frac{M_{\text{eff}}U}{K\eta}\right)^{\frac{2}{3}},$$

where V_f is free volume in m^3 mol^{-1} K is a constant independent of the nature of liquids and temperature. (K = 4.28×10^9) η is the viscosity in NSm^{-2} and U is the velocity in m/s.

M_{eff} is the effective molecular weight, which is expressed as

$$M_{eff} = X_1M_1 + X_2M_2 + X_3M_3.$$

Free Length (L_f)

$$L_f = K_T\sqrt{\beta},$$

where L_f is the free length in meter, β is the adiabatic compressibility, K is Jacobson's constant. This constant is a temperature dependent parameter whose value at any temperature (T) is given by $(93.875 + 0.345\ \text{T}) \times 10^{-8}$.

Internal Pressure (π_i)

$$\pi_i = bRT\left(\frac{K\eta}{U}\right)^{\frac{1}{2}}\left(\frac{\rho^{\frac{2}{3}}}{M_{\text{eff}}^{\frac{7}{6}}}\right),$$

where π_i is the internal pressure in P a, b is the packing fraction assumed as 1.78 for polymers R is the Universal gas constant 8.314, and T is the temperature in Kelvin.

Viscous Relaxation Time (τ)

$$\tau = \frac{4}{3}\eta\beta,$$

where η is the viscosity in NSm^{-2} and β is the adiabatic compressibility in N^{-1}m^2.

Gibb's Free Energy ($\triangle G$)

$$\triangle G = -KT \log\left(\frac{h}{KT\tau}\right),$$

where τ is the viscous relaxation time in meter, K is the Boltzmann's constants, and h is a Planck's constant.

5. Results and Discussion

5.1. Ultrasonic Studies

In the present study, the ultrasonic velocity, density and viscosity have been measured at the temperature of 303 K for phenol with Methyl acetate system. The experimental results of ultrasonic velocity, density and viscosity along with computed values of Adiabatic Compressibility (β), Free Volume(V_f), Free length (L_f), The values of relaxation time (τ), Internal pressure (π_i) and Gibbs free energy ($\triangle G$) for the ternary liquid mixtures at 303 K are given in Table 1-3.

Table 1: The value of density (ρ), viscosity (η), velocity (U) of phenol with methyl acetate at room temperature

Concentration		**Density(ρ)**	**Viscosity(η)**	**Velocity(U)**
X_1	X_2	**(Kg/m^3)**	**(Ns/m)**	**(m/s)**
0.0000	1.0000	839	0.6543	1214
0.0804	0.9196	841	0.6941	1252
0.2523	0.7477	843	0.7615	1270
0.4405	0.5595	846	0.9351	1312
0.6475	0.3525	849	1.1245	1389
0.8763	0.1237	851	2.1264	1402
1.0000	0.0000	854	2.6543	1422

It is evident from the table 1, that in the system the value of density, viscosity and ultrasonic velocity increases with increasing molar concentration of Phenol. This behavior is different from the ideal mixture and this attributed to the intermolecular in the system [20].

The adiabatic compressibility (β) values for various mole fractions of phenol with methyl acetate mixtures have been computed from the measured values of ultrasonic velocity and densities .The plots of adiabatic compressibility (β) vs mole fraction of the phenol are given in Table 1-2. The value of adiabatic compressibility decreases

Table 2: The value of adiabatic compressibility (β), free volume (V_f), free length (L_f) of phenol with methyl acetate at room temperature

Concentration		**Adiabatic Compressibility(β)** ($\mathbf{N^{-1}m^2}$) $\times 10^{-10}$	**Free Volume(V_f)** ($\mathbf{m^3mol^{-1}}$) $\times 10^{-7}$	**Free length(L_f)** (**m**)$\times 10^{-10}$
X_1	X_2			
0.0000	1.0000	8.0872	1.8142	5.6424
0.0804	0.9196	7.5857	1.7960	5.4646
0.2523	0.7477	7.3547	1.7069	5.3808
0.4405	0.5595	6.8669	1.4123	5.1993
0.6475	0.3525	6.1050	1.2552	4.9024
0.8763	0.1237	5.9783	0.5214	4.8512
1.0000	0.0000	5.7909	0.4035	4.7746

Table 3: The value of internal pressure (π_i), visco relaxation time (τ), Gibbs free energy ($\triangle G$) of phenol with methyl acetate at room temperature

Concentration		**Vis. Rel Time(τ)** $(s) \times 10^{-12}$	**Internal Pressure(π_i)** (**Pa**) $\times 10^{-4}$	**Gibbs free energy($\triangle G$)** ($\mathbf{KJmol^{-1}}$) $\times 10^{-20}$
X_1	X_2			
0.0000	1.0000	7.0553	394.57	1.9390
0.0804	0.9196	7.0203	390.88	1.9389
0.2523	0.7477	7.4675	386.57	1.9406
0.4405	0.5595	8.5617	400.13	1.9443
0.6475	0.3525	9.1535	403.80	1.9462
0.8763	0.1237	16.950	521.39	1.9630
1.0000	0.0000	20.494	562.01	1.9682

with increasing concentration of phenol. This indicates induced dipole induced dipole interactions exist.

The free length (L_f) of a system is a measure of intermolecular attraction between the components in phenol with methyl acetate mixtures. The increase in free length indicates weakening of intermolecular interaction. From the data in Table 2, it is seen that the free length values decrease with increase in concentration phenol. This shows that the intermolecular attraction Strong at higher concentration.

The free volume (V_f) value are calculated and presented in Table 2 for the phenol with methyl acetate system investigated. It is found that for all the systems at 303 K, free volume decreases with increase in concentration.

It is observed from Tables 3 that as the concentration of Phenol increases, the internal pressure increases. This suggests the close packing of the molecules inside the shield. The increase in nature pressure generally indicates association through hydrogen bonding [13-14] and hence it supports the present investigation.

The values of relaxation time (τ) are also given in the Table 3. The viscous relaxation time increase significantly with increase in concentration and the interaction between the molecules of components is stronger than the attractive forces between the molecules of each component. The relaxation time that is in the order of 10^{-12} s is due to the structural relaxation process showing the presence of molecular interaction.

Gibbs free energy confirms the same (relaxation time) from the measured values are given in the Table 3. This indicates that the need for smaller time for the cooperative process or the rearrangement of the molecules in the mixtures decreases the energy that leads to dissociation.

5.2. FT-IR Studies

The infrared spectrum, which gives significant information about the functional groups, can be substantially influenced by the surrounding condensed medium. Hence, FT-IR studies have been used to investigate solution structure and provide physical information about intermolecular interaction. In order to study the strength of molecular association at specific concentrations.

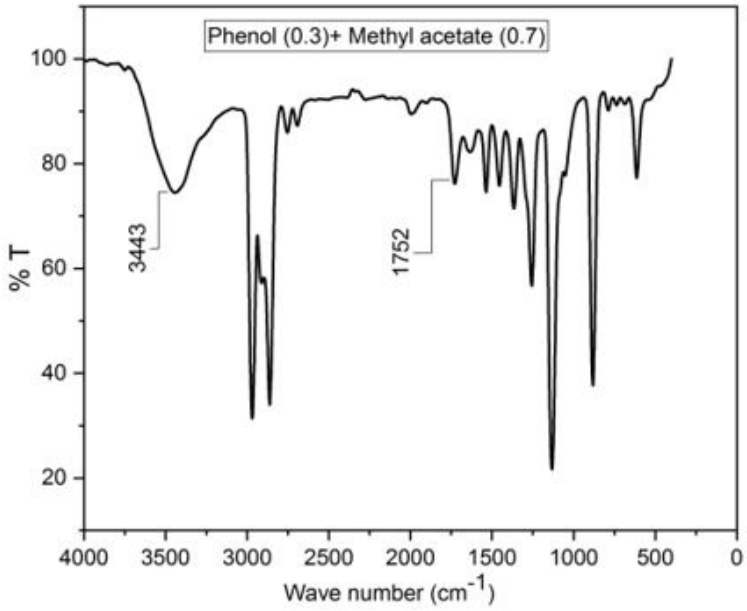

Figure 1: FT-IR spectrum of Phenol (0.3) with Methyl Acetate (0.7)

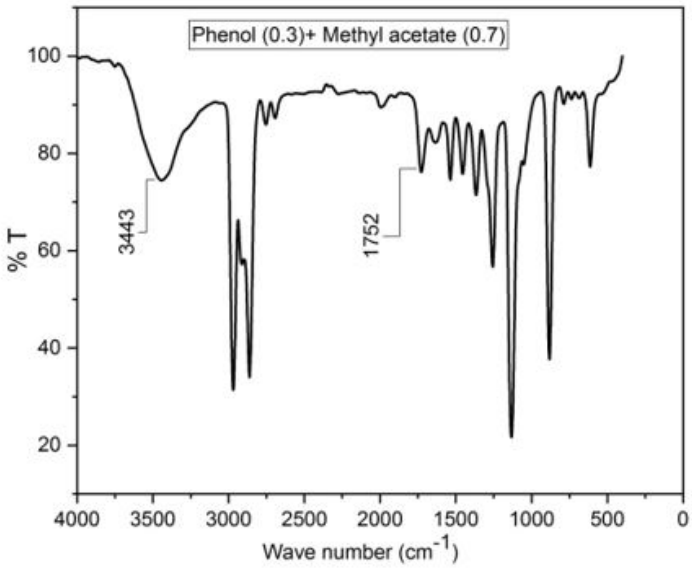

Figure 2: FT-IR spectrum of Phenol (0.5) with Methyl Acetate (0.5)

Figs. 1-3, show the FT-IR spectrum in the range of 350-4000 cm^{-1} of mixtures liquids such as phenol with methyl acetate and their liquid mixtures at equimolar composition. From these spectrums, it is necessary to analyze the -O-H, -C=O, and C-H stretching bands to predict the possible molecular interactions between the phenol with methyl acetate.

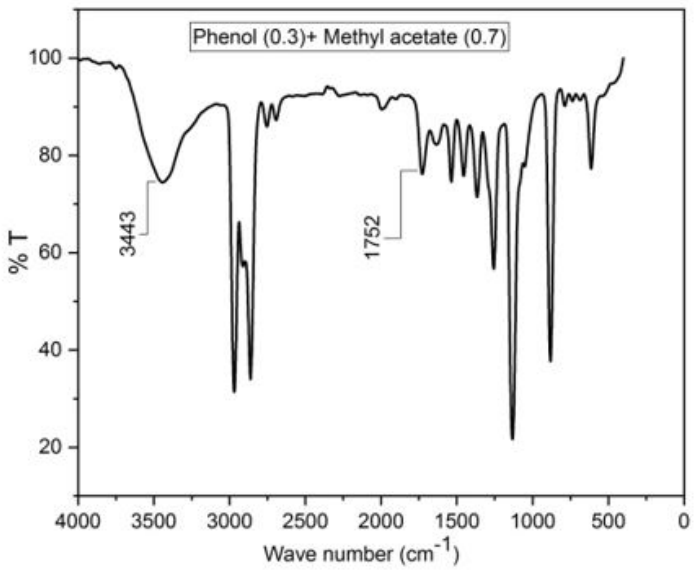

Figure 3: FT-IR spectrum of Phenol (0.7) with Methyl Acetate (0.3)

The FT-IR spectra of OH stretch, hydrogen bonded 3500-3200 cm^{-1}, in the present studies the O-H stretch frequency shifted to 3434 cm^{-1}, 3443 cm^{-1} and 3498 cm^{-1} for the binary mixture of phenol (0.3,0.5,0.7) with methyl acetate (0.7,0.5,0.3) respectively at equimolar composition, it is clearly states that disruption hydrogen bonded complexes in phenol with Methyl acetate.

Table 4: FT-IR Stretching frequencies for the binary mixtures of Phenol with Methyl acetate

Vibration Modes	Scheme 1 Phenol (0.3) + Methyl Acetate (0.7)	Scheme 2 Phenol (0.5) + Methyl Acetate (0.5)	Scheme 3 Phenol (0.7) + Methyl Acetate (0.3)
OH stretch	3443 cm^{-1}	3434 cm^{-1}	3498 cm^{-1}
C=O stretch	1752 cm^{-1}	1736 cm^{-1}	1786 cm^{-1}
C-C(O)-C Stretch	1256 cm^{-1}	1232 cm^{-1}	1289 cm^{-1}
C=C(ring) stretch	1543 cm^{-1}	1257 cm^{-1}	1533 cm^{-1}
stretch C-H	1455 cm^{-1}	1417 cm^{-1}	1465 cm^{-1}

From the data in Table 4, it is clear that the frequency of vibrational bands changes on mixing phenol with Methyl acetate, the shift is observed in the frequencies of υC=O

vibrational bands and the values of -OH stretching frequency for the equimolar mixtures of phenol with Methyl acetate, are observed to be shifted towards higher value in the order: Phenol(0.7)+MA (0.3) (1786, 3498 cm^{-1}); Phenol(0.3)+MA(0.7) (1756,3443 cm^{-1}); Phenol(0.5)+MA (0.5) (1736, 3434 cm^{-1}) Respectively; similar shift in the stretching frequency values of C=O band was reported [15].

From the frequency shift values observed in the studied the binary mixtures viz. phenol with methyl acetate it is clear that the maximum shift is observed in all the three mixtures at $\sim$ 0.5 mole fraction of Phenol, and the magnitude of shift is found to obey the order 0.7 - 0.3 < 0.5 - 0.5 > 0.3-0.7 because of dipole-dipole interactions. Hence, the IR spectra supports that the strength of molecular interaction between the studied binaries is in the order: Phenol (0.5)+ Methyl Acetate (0.5) > Phenol (0.3)+ Methyl Acetate (0.7) > Phenol (0.7)+ Methyl Acetate (0.3).

6. Conclusion

The various acoustical parameters such as adiabatic compressibility, relaxation time, free length, free volume, internal pressure, Viscous relaxation time, and Gibb' free energy have been evaluated from the measured ultrasonic velocity, density and viscosity values for the ternary mixtures. From the above studies it is concluded that, a hydrogen bond formation between phenol and Methyl acetate through C=O . . . . . H-O and specific interaction between the phenol and Methyl acetate molecules. The formation of intermolecular hydrogen bonding between the components of liquid mixtures in the present study has been confirmed by recording of IR spectra of liquid mixtures.

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MOLECULAR INTERACTION STUDIES OF PHENOL WITH ACETALDEHYDE USING ULTRASONIC AND FTIR TECHNIQUE

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Abstract

The densities (ρ), viscosities (η) and ultrasonic velocities (U) were measured for the binary mixtures of Phenol with Acetaldehyde at 303 K. Excess volumes (VmE) and isentropic compressibilities (ks^E) were determined from these experimental findings. To obtain their coefficients and standard deviations, the excess or deviation properties were based on RedlichKister polynomial equation. Depending on the molecular interactions between the components of hetero molecules, the excess or deviation properties were considered either negative, and the nature of liquid mixtures was studied in terms of molecular interactions by steric and electronic effects. The FTIR provides support to thermodynamic studies to clarify different interactions between molecules that are unlike them.

Keywords: Binary Liquid, Phenol, Acetaldehyde, Excess Values and FTIR.

1. Introduction

Thermal properties such as density, viscosity, sound speed (u) and their differences of binary liquid mix proportions have been used in the design of chemical and biological engineering processes [1] and in the creation of modern theoretical models for engineering applications [24]. Excess or variance parameters such as excess molar volume (VE), variation in istropic compressibility (in some cases) and excess intermolecular free duration (in other cases) can be derived from mass, viscosity and sound velocity. Such parameters are efficient in obtaining essential knowledge about molecular interactions between binary and ternary mixtures unlike molecules [5, 6]. The thermodynamic excess parameters are used for practical chemical engineering purposes such as chemical process, chemical separations, mass transfer operations (adsorption, evaporation and precipitation), heat transfer and fluid flow [7]. The Fourier-transform

infrared (FTIR) is a method used to identify the classification of the molecule by knowing its functional group. By using FTIR spectroscopy, we can observe [815] that hydrogen interaction between molecules takes place and it is a very interesting class of intermolecular interactions.

In the present work, we have revealed the nature of intra and inter molecular interactions between phenol and acetaldehyde molecules in the liquid mixtures with aid of excess thermodynamic properties and Fourier transform infrared spectroscopic (FT-IR) studies. By using the measured densities (ρ) and speeds of sound (u), excess volumes (Vm^E),isentropic compressibilities (k_s)and excess isentropic compressibilities (k_s^E) were also determined and details are displayed for these binary mixtures at temperature 303 K. The excess properties is associated with the polynomial equation Redlich-Kister. In comparison, the FT-IR spectrum reported for pure liquids and their mixtures confirms the molecular interactions between component molecules by changing the vibrational frequencies -O-H,-C-H and -C-O stretch.

2. Materials and Method

2.1. Materials

The mixtures of phenol and acetaldehyde of various concentrations ranging from 0 M to 0.1M were prepared by taking analytical reagent grade and spectroscopic reagent grade chemicals with minimum assay of 99.9% are procured from E-Merck Ltd (India). The above experimental solutions are of equimolar concentrations of different solutions constituting the system which are under study.

2.2. Experimental Details

The ultrasonic velocity measurements of phenol with acetaldehyde solutions was rendered in the 2 MHz (Model F-81 Mittal enterprises, New Delhi) fixed frequency ultrasonic interferometer at 303 k temperature. The density measurements are calculated at 303 k temperature using a specific gravity bottle by normal operating practice and the viscosity using Ostwald's viscometer calibrated with double distilled water sensitivity of ±0.001 percent. For such measurements the FTIR spectra are obtained utilizing the Fourier Transform Infra Red (FTIR) Spectrometer (Spectrum RX, PerkinElmer).

2.3. Thermodynamic Parameters

2.3.1 Molar Volume of the Binary Liquid Mixture (Vm)

The molar volume of the system at every mole fraction for the mixture is given by

$$V_m = \frac{M_{\text{eff}}}{\rho_{\text{mix}}}, \tag{2.1}$$

where $M_{\text{eff}} = M_1X_1 + M_2X_2/(X_1 + X_2)$.

2.3.2 Excess Molar Volume

The difference between the molar volume of the mixture and the sum of the individual molar volume times the mole fraction is defined as excess volume or excess molar volume

$$Vm^E = Vm - (Vm_1X_1 + Vm_2X_2). \tag{2.2}$$

2.3.3 Adiabatic Compressibility (Ks)

Adiabatic compressibility is a function of the interaction or dissociation or repulsion between molecules. Singh and Kalsh proved that the adiabatic compressibility for unassociated and weakly associated molecules would be independent of the temperature and pressure. This also defines how the solvent molecules are arranged around the liquid molecules. The structural arrangement of the molecule affects the adiabatic compressibility. It can be calculated using the equation.

$$ks = \frac{1}{U^2\rho}, \tag{2.3}$$

where U is the velocity in m/s and ρ is the density in kg/m^3.

2.3.4 Excess Isentropic Compressibilities (ks^E)

An ideal mixture of isentropic compressibility is considered to be additive in terms of volume fraction. The difference between the compressibility of a real solution and that of an ideal solution is called excess isentropic compressibility. The values of excess isentropic compressibilities (ksE) were calculated from the following relations

$$k_s^E = k_s - k_s^{id}. \tag{2.4}$$

3. Results and Discussion

It is seen that (Table 1) in the present systems of binary liquid mixtures, the viscosity () and the ultrasonic velocity (U) and the density () increases with increasing concentrations of phenol. The variance in ultrasonic velocity in a mixture depends on the increase (or) decrease in intermolecular free length after combining the components on the basis of a pattern, suggested by Eyring and Kincaid[16] for sound propagation.

The mole fraction (X_1), experimental densities (ρ) and calculated excess volume (Vm^E) data are presented in Tables from 1 - 2 at 303 K. The plots of excess volume (Vm^E) with mole fraction, X_1, for the binary mixtures of Acetaldehyde with phenol 303

Table 1: The value of density (ρ), viscosity (η), velocity (U) and Molar volumes (Vm), phenol with Acetaldehyde at 303 K

Concentration		Density (ρ) Kg/m^3)	Viscosity (η) ($\times 10^{-3}$ NSm^{-3})	Ultrasonic velocity U/(ms^{-1})	Molar volumes (V_m) (m^3/mol)
X_1	X_2				
0	1	877	1.8512	1011	5.02
0.0494	0.9506	884	1.9251	1025	5.26
0.1671	0.8329	910	2.0124	1075	5.76
0.3188	0.6812	925	2.2541	1150	6.49
0.522	0.478	931	2.4512	1200	7.54
0.8082	0.1918	944	2.5142	1230	8.95
1	0	955	2.6543	1311	9.85

Table 2: The value of Excess Molar volumes (Vm^E), Adiabatic Compressibility (ks) and Deviation in Adiabatic compressibility (Ks^E) of phenol with Acetaldehyde at 303 K

Concentration		Excess Molar volumes Vm^E (m^3/mol)	Adiabatic Compressibility(ks) (N^{-1}m^2) $\times 10^{-10}$	Deviation in Adiabatic Compressibility(ks^E) (N^{-1}m^2) $\times 10^{-10}$
X_1	X_2			
0	1	0	11.156	0
0.0494	0.9506	−9.61	10.767	−6.481
0.1671	0.8329	−9.12	9.5091	−7.739
0.3188	0.6812	−8.39	8.1745	−9.074
0.522	0.478	−7.34	7.4591	−9.789
0.8082	0.1918	−5.93	7.0019	−10.25
1	0	0	6.0924	0

K are shown. The values are negative in sign in the whole concentration region for phenol with acetaldehyde.

In the present study, phenol and acetaldehyde are self-associated through hydrogen bonding in the pure state due to the presence of electron donor and electron acceptor sites. When the phenol molecules are mixed with acetaldehyde, the disruption of

hydrogen-bonded structures present in pure phenol or acetaldehyde acid takes place with subsequent formation of the intermolecular hydrogen bond between component molecules in liquid mixtures. On the other hand, interstitial accommodation of phenol molecules in hydrogen-bonded acetaldehyde aggregates (packing effect) and makes a negative Vm^E.

The measured speed of sound (u), calculated isentropic compressibilities (ks) and excess isentropic compressibilities (ks^E) for the binary mixtures of Phenol with acetaldehyde are report in Table 2. The plots of excess isentropic compressibilities (ks) with mole fraction (X_1), for the binary mixtures of phenol with acetaldehyde at 303 K.

It is ascribed from table 2, that, ks^E property is negative for phenol with acetaldehyde in binary liquid mixtures. Figure also reveals that negative ks^E data in the binary mixture of phenol with acetaldehyde indicate that the liquid mixtures have low compressibilities and also show more effective binding than when compared to pure liquids. Furthermore, the negative values for the binary mixtures under study suggest that there are variations in molecular sizes and interstitial accommodation (H-bonding) between binary liquid mixture constituent molecules [17, 18]. It can be inferred that as the frequency of the interaction between the intermolecular forces decreases, the values of ks^E are more negative.

The excess property (Vm^E) was fitted by Redlich-Kister type polynomial equation and the calculated values of the polynomial coefficients a_0, a_1 and a_2 along with their standard deviations (σ) are presented in Table 3.

Table 3: Coefficients of the Redlich-Kister type polynomial and the corresponding standard deviations σ (Vm^E) – (ks^E) for the binary mixtures Phenol with Acetaldehyde at 303 K

Parameters	Temperature (K)	A_0	A_1	A_2	Standard deviations (σ)
Vm^E	303 K	−38.38	70.14	−28.74	0.2258
K_s^E	303 K	−26.33	5.34	22.76	0.6145

3.1. FT-IR Studies

3.1.1 Stretching Region (-OH)

Figures.1-3 confirms the FT-IR spectrum of pure phenol or acetaldehyde and their binary mixtures in the range of 350-4500cm^{-1} at equimolar composition. In Figs.1-3 we can find a wide band at 3558 cm^{-1} and 3522 cm^{-1} for pure phenol and phenol at equimolar composition with acetaldehyde, respectively. The -OH stretching level for the binary liquid mixtures of phenol with acetaldehyde at equimolar composition is changed from 3522 cm^{-1} respectively. It clearly states that, attributed to the presence

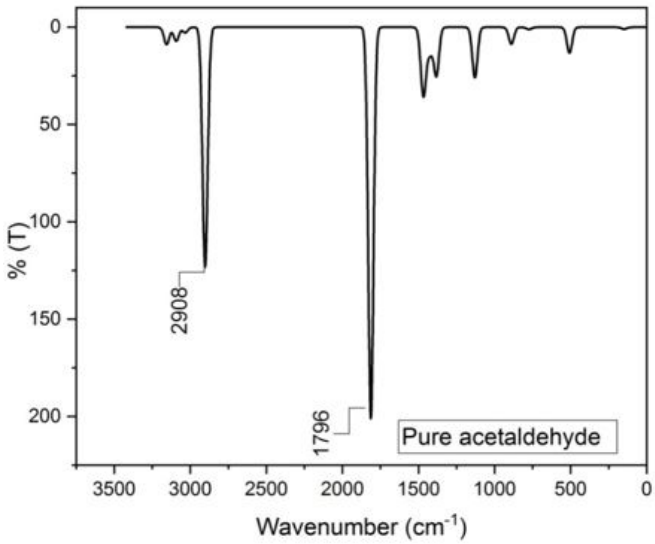

Figure 1: FT-IR spectrum of Pure Acetaldehyde

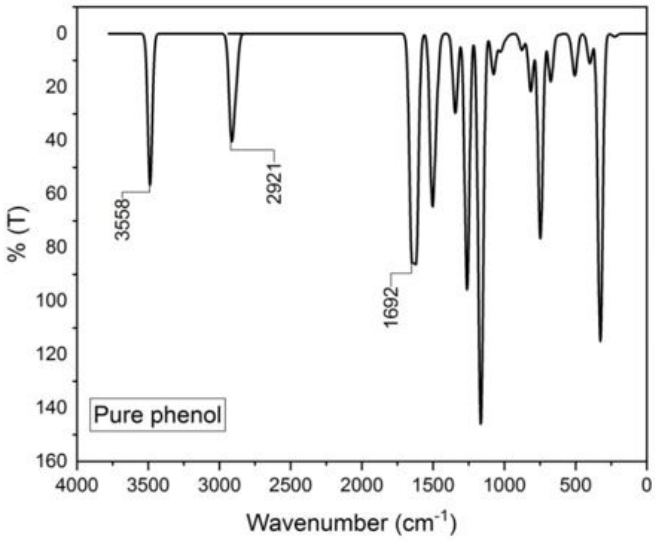

Figure 2: FT-IR spectrum of Pure Phenol

of acetaldehyde, degradation of the hydrogen bound complexes occurs in phenol molecules.

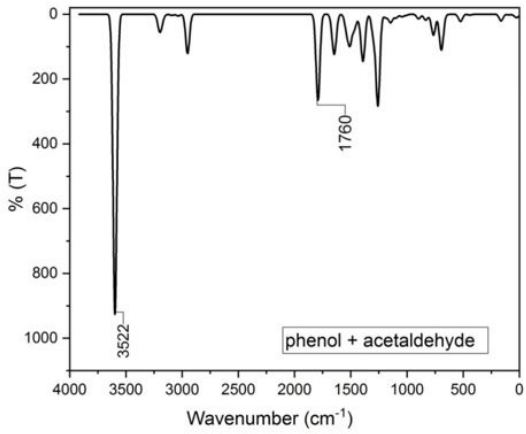

Figure 3: FT-IR spectrum of Phenol with Acetaldehyde at equimolar mixtures

3.1.2 C=O Stretching Region

The FT-IR spectrum of pure components and their liquid mixtures at a range of 1600-1800 cm^{-1} equimolar composition are shown in Fig. 13. Here in, at 1796 cm^{-1} a sharp band appears indicating the -C = O stretching acetaldehyde vibration frequency.The absorption rate of the strong C = O strclching band of get changed acetaldehyde was moved from 1796 cm^{-1} to 1760cm^{-1} for the binary liquid mixtures at equimolar composition respectively due to the introduction of acetaldehyde to phenol. This behavior is due to the formation of strong intermolecular hydrogen bonding between oxygen atom of carbonyl (C=O) group of acetaldehyde acid and hydrogen atom of -OH group of phenol molecules (-C=O——H-O-).

3.2. Discriminating Weak Interaction types by filling color to RDG Isosurfaces

The reduced density gradient (RDG) is calculated using Multiwfn [19]. Such RDG is then visualized which makes it easy to recognize the hydrogen bond. By integrating the RDG function with the leich $\Omega(r)$ function, we may differentiate between the form and strength of the weak interaction [20].

$$RDG(r) = \frac{1[\nabla(r)]}{2(3\pi^2)^{\frac{1}{3}}\rho(r)^{\frac{4}{3}}} \tag{3.1}$$

$$\Omega(r) = \sin n(\lambda_2(r))\rho(r), \tag{3.2}$$

where RDG is the diminished gradient of energy and where RDG is the overall mass of the electron. According to the principle of atom in molecule (AIM), [21-22] the relationship with the second-largest own value of the Hessian matrix of electron density as seen in Eq. (5). (6). $\rho(r)$ is an essential indicator of weak interaction strength, while the form of weak interaction is defined by the λ_2 function of sine. It is also possible to

evaluate the weak interactions of different forms by drawing the scatter graph between function λ_1 (RDG) and function λ_2 ($\Omega(r)$). Figure demonstrates the formula obtained by multiplying the equation of $\rho(r)$ and sine λ_2 and thereby producing the RDG isosurface. 4 (A) with a contour value of 0.5 and an isosurface of the RDG between -0.04 and 0.02. The hydrogen bond increase is situated at -0.036 a.u., which means that the hydrogen bond is particularly high in acetaldehyde-mixed phenol. Similarly, one can distinguish the weak interactions in Fig. 4 (2) (b). It can be ascertained directly from Fig. 4 (b) the hydrogen bond between OH ..... O = C is strong.

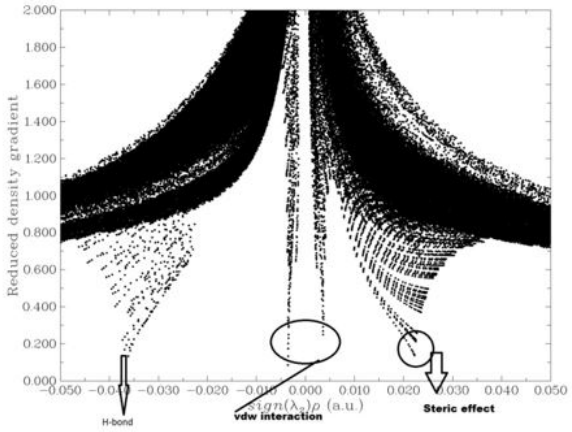

Figure 4: (Color online) Plot of reduced density gradient (RDG) versus $\Omega(r)$ for function value 1 and function value 2

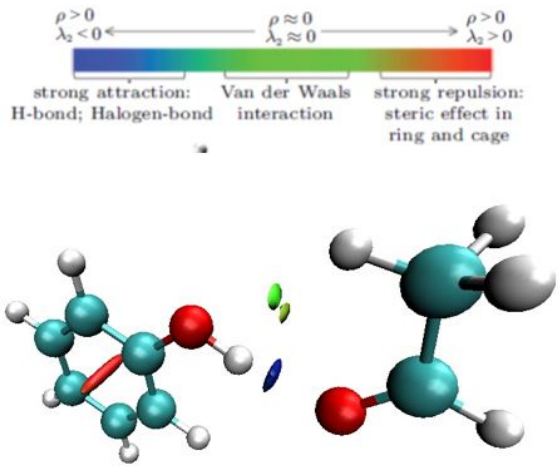

Figure 5: (Color online) (b) Different types of weak interactions represented by different color gradients as well as RDG isometric surfaces

4. Conclusion

In the present work, the excess properties (Vm^E and ks^E) were determined for the binary mixtures containing phenol with acetaldehyde. It was observed from the measured data that the excess volume data has inversion sign for the binary mixtures. On the other hand, the excess adiabatic compressibilities exhibited negative behaviour for the binary mixtures of phenol with acetaldehyde. However, these excess properties and FT-IR data indicates the formation of intermolecular hydrogen bonding (-C=O—H-O-) between carbonyl atom of the acetaldehyde and hydroxyl group of phenol by the strong hydrogen bonds in self-associated phenols. Furthermore, these excess properties ((Vm^E and Ks^E) were correlated with Redlich-Kister polynomial equation.

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SYNTHESIS AND ANTIFUNGAL ACTIVITY STUDIES OF NOVEL 2-(2-PYRIDYL)-2H-PYRAZOLE-3-CARBOXAMIDE DERIVATIVES

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Abstract

Novel 2-(2-pyridyl)-2H-pyrazole-3-carboxamide derivatives were prepared by multi step synthesis. We have prepared fourteen derivatives of 2-(2-pyridyl)-2H-pyrazole-3-carboxamide and all newly synthesized compounds are characterized by 1HNMR, 13C NMR, LC-MS and screened for their antifungal activity against Aspergillus flavus (NCIM No.524), Fusarium oxysporum (NCIM No.1072) and Candida albicans (NCIM No.3102).

Keywords: Pyrazole, 2-(2-Pyridyl)-2H-Pyrazole-3-Carboxamide, Synthesis and Antifungal Activity.

1. Introduction

Nitrogen containing heterocyclic compounds- especially pyrazole and its derivatives are broad spectrum of biologically active such as antimicrobial agents [1], anti-inflammatory [2], antifungal [3], herbicidal [4], antiviral [5], analgesic, antitumour, cytotoxic, antipyretic and obesity [6]. We report in the present work the synthesis and biological activity of novel 2-(2-pyridyl)-2H-pyrazole-3-carboxamide derivatives.

2. Chemistry

We have started with preparation of 2-hydrazino pyridine (**2**) from reaction of 2-chloropyridine (**1**) and hydrazine hydrate 120°C with yield of more than 90%. Further Ethyl 5,5-dimethyl-2,4-dioxohexanoate (**5**) was prepared by Claisen condensation of 3,3-dimethyl-2-oxo-butane (**4**) and diethyl oxalate (**3**) in presence of sodium ethoxide in ethanol.

Ethyl-3-(tert-butyl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxylate (**6**) was prepared by heating Ethyl 5,5-dimethyl-2,4-dioxohexanoate (**5**) with 2-hydrazino pyridine (**2**) in ethanol and Acetic acid mixture. Compound **6** was subjected to hydrolysis using lithium hydroxide to get 3-(tert-butyl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxylic acid (7) in very good yield.

In the final steps various amides were prepared by reacting 3-(tert-butyl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxylic acid (**7**) with different amines, using propane phosphonic acid cyclic anhydride (T3P) as coupling reagent. T3P found to better reagent for coupling since it is easy to handle, and yields are much higher when compared to other reagents like EDCI or HATU.

All the Compounds prepared are characterized by LCMS and NMR data.

3. Experimental

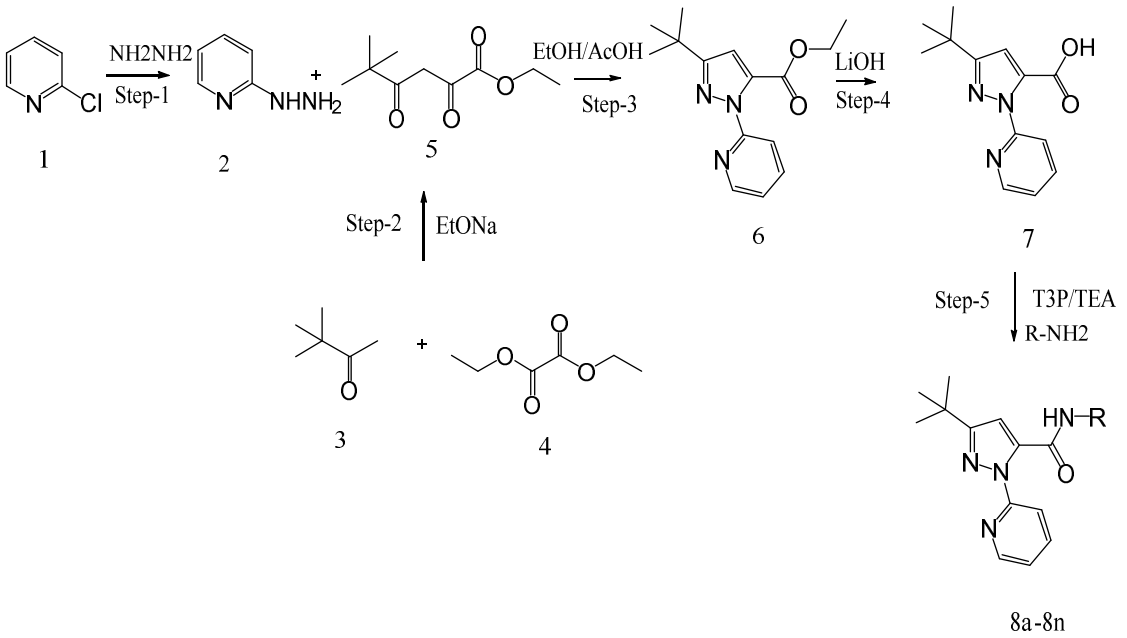
All reagents were purchased from Aldrich and used as received. Dry THF, Ethanol, Toluene were supplied by Spectrochem. All chemistry was performed under a nitrogen atmosphere using standard techniques. All the NMR spectra were measured using either Bruker AMX 400 instrument with 5mm PABBO BB-1H tubes. 1H and 13C NMR spectra were measured for approximately 0.03M solutions in d6-DMSO at 400MHz with TMS as internal reference. The IR spectra were measured as potassium bromide pellets using a Perkin-Elmer 1600 series FTIR spectrometer. LCMS were obtained using Agilent 1200 series LC and Micro mass zQ spectrometer. Column chromatography was performed using a silica gel (230- 400 mesh).

2-hydrazinylpyridone (2)

A mixture of 2-chloropyridine (**1**) (10.0 g, 0.088mol) and hydrazine hydrate (17.8 g, 0.355mol) was heated to 120°C for 12h. Reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue obtained was diluted with dichloromethane and washed with 10% NaOH solution, dried over anhydrous sodium sulfate and concentrated under reduced pressure to get 2-hydrazinylpyridine (**2**) as light-yellow oil. (Yield 5.75 g, 60%).

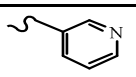
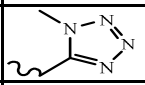
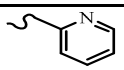
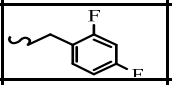
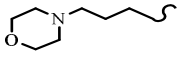
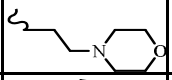
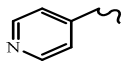
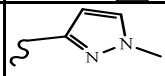
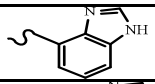
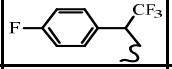
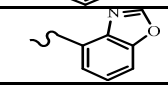
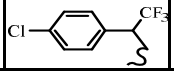
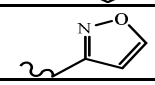
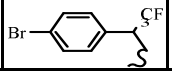
^{1}H NMR spectra: (CDCl3, 400MHz); $\delta-$value in ppm 8.14-8.15(d, 1H, N=CH), 7.47-7.51(t, 1H, Ar) 6.67-6.72(t, 2H, Ar), 5.94(bs, 1H, NH), 3.50(bs, 2H, NH2).

LC-MS: m/z 110.1(M+1).



Scheme 1: Synthesis of 2-(2-pyridyl)-2H-pyrazole-3-carboxamide derivatives

Table 1: Yields of various 2-(2-pyridyl)-2H-pyrazole-3-carboxamide derivatives prepared using Scheme 1

Compd no	R	%Yield	Compd no	R	%Yield
8a		70	8h		66
8b		65	8i		85
8c		85	8j		88
8d		71	8k		71
8e		65	8l		81
8f		60	8m		80
8g		64	8n		83

Ethyl-5,5-dimethyl-2,4-dioxohexanoate (5)

A solution of 2,2-dimethylbutan-2-one (**3**) (5.0 g, 0.05mol) and diethyl oxalate (**4**) (7.3 g, 0.05mol) in ethanol (50 ml) was added sodium ethoxide(3.4 g, 0.05mmol) at 0°C. The RM was then stirred at room temperature for 12h. Reaction mixture was concentrated under reduced pressure. The residue obtained was diluted with ethyl acetate and washed water, saturated brine solution, dried over anhydrous sodium sulfate and concentrated under reduced pressure to get Ethyl-5,5-dimethyl-2,4-dioxohexanoate (**5**) as light-yellow oil. (Yield 5.0 g, 50%).

^{1}**H NMR spectra:** (CDCl3, 400MHz); $\delta-$value in ppm

Enol form: 14.77(bs, 1H, OH), 6.55(s, 1H, C=CH) 4.33-4.40(q, 2H, OCH2), 1.37-1.4(t, 3H, CH3), 1.22(s, 9H, $C(CH3)_3$).

LC-MS: m/z 201.3(M+1).

Ethyl-3-(tert-butyl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxylate (6)

A solution of 2-hydrazinylpyridine (**2**) (5.0 g, 0.045mol) and Ethyl-5,5-dimethyl-2,4-dioxohexanoate (**5**) (9.17g, 0.045mol) in ethanol (100 ml) and acetic acid (10 ml) was heated to 100°C for 24h. The reaction mixture was then cooled to room temperature and was concentrated under reduced pressure. The residue obtained was diluted with ethyl acetate and washed water, saturated brine solution, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude residue was purified by column chromatography using ethyl acetate and petroleum ether to get Ethyl 3-(tert-butyl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxylate (**6**) as light-yellow oil. (Yield 5.7 g, 45%).

^{1}**H NMR spectra:** (CDCl3, 400MHz); $\delta-$value in ppm 8.5(d, 1H), 7.82-7.92(t, 1H) 7.56-7.58(d, 1H), 7.41-7.44(t, 1H), 6.8(s, 1H), 4.40-4.46(q, 2H), 1.39-1.42(t, 3H), 1.33(s, 9H).

LC-MS: m/z 274.3(M+1).

3-(tert-butyl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxylic acid (7)

To a solution of Ethyl 3-(tert-butyl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxylate (**6**) (2.5 g, 9.16mol) in tetrahydrofuran (10 ml) and methanol (5 ml) was added lithium hydroxide monohydrate (0.96g 22.8 mmol) in water (5 ml) drop wise at RT. Reaction mixture was then stirred at RT for 3h. Reaction mixture was then diluted with water(50 ml) and acidified with 1.5N HCl and extracted with ethyl acetate. The organic layer was washed water, saturated brine solution, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude residue was purified by column chromatography using ethyl acetate and petroleum ether to get 3-(tert-butyl)-1-(pyridin-

2-yl)-1H-pyrazole-5-carboxylic acid (**7**) as white solid. (Yield 1.8 g, 81%).

^{1}H NMR spectra: (CDCl3, 400MHz); $\delta-$value in ppm 8.58-8.59(d, 1H), 7.89-7.93(t, 1H) 7.54-7.56(d, 1H), 7.43-7.46(t, 1H), 6.84(s, 1H), 1.3(s, 9H).

LC-MS: m/z 246.2 (M+1).

4. General procedure for the preparation of 3-(tert-butyl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxamides (8a-8n)

To a solution of 3-(tert-butyl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxylic acid (**7**) (0.25g, 1.066mol) in ethyl acetate (5ml) was added corresponding amine (1.066mmol) and triethylamine (0.96g 2.03mmol). The reaction mixture was cooled to 0°C and T3P (50% solution in ethyl acetate, 1.52mmol) was added drop wise. Then reaction mass was stirred at RT for 16h. Reaction mixture was then diluted with ethyl acetate (50ml) and was washed water, saturated brine solution, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude residue was purified by column chromatography using ethyl acetate and petroleum ether to get 3-(tert-butyl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxamides (**8a-8n**).

3-(tert-butyl)-1-(pyridin-2-yl)-N-(pyridin-3-yl)-1H-pyrazole-5-carboxamide (8a)

Yield: 70% as white powder. M.pt: 152.2-154.2°C.

^{1}H NMR spectra: (DMSO-d_6, 400MHz); $\delta-$value in ppm 10.38(s, 1H), 8.96(s, 1H) 8.64-8.65(d, 1H), 8.28-8.29(d, 1H), 8.19-8.22(d, 1H), 8.10-8.14(t, 1H), 7.72-7.73(d, 1H), 7.63-7.65(t, 1H), 7.35-7.38(t, 1H), 6.83(s, 1H), 1.24(s, 9H).

LC-MS: m/z 322.3 (M+1).

3-(tert-butyl)-1-(pyridin-2-yl)-N-(pyridin-2-yl)-1H-pyrazole-5-carboxamide (8b)

Yield: 65% as white powder. M.pt: 134-135.8°C.

^{1}H NMR spectra: (DMSO-d6, 400MHz); -value in ppm 9.73(s, 1H), 8.64-8.65(d, 1H) 8.34-8.35(d, 1H), 8.17-8.19(d, 1H), 8.10-8.14(t, 1H), 7.84-7.88(t, 1H), 7.74-7.76(d, 1H), 7.63-7.64(t, 1H), 7.15-7.17(t, 1H), 6.92(s, 1H), 1.26(s, 9H).

LC-MS: m/z 322.3 (M+1).

3-(tert-butyl)-N-(3-morpholinopropyl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxamide (8c)

Yield: 85% as white powder. M.pt: 156.3-157.5°C.

^{1}**H NMR spectra:** (DMSO-d_6, 400MHz); $\delta-$value in ppm 8.61-8.62(d, 1H) 8.39-8.42(t, 2H), 8.05-8.10(t, 1H), 7.59-7.63(d, 1H), 7.61(s, 1H), 6.63(s, 1H), 3.33-3.52(t, 4H), 2.24-2.29(q, 2H), 2.3-2.33(m, 6H), 1.16-1.17(m, 2H), 1.21(s, 9H).

LC-MS: m/z 372.8 (M+1).

3-(tert-butyl)-1-(pyridin-2-yl)-N-(pyridin-4-yl)-1H-pyrazole-5-carboxamide (8d)

Yield: 71% as white powder. M.pt: 129-130.2°C.

^{1}**H NMR spectra:** (DMSO-d_6, 400MHz); $\delta-$value in ppm 10.49(s, 1H), 8.63-8.65(d, 1H) 8.43-8.45(d, 2H), 8.17-8.19(d, 1H), 8.09-8.11(t, 1H), 7.82-7.84(t, 1H), 7.69-7.72(d, 2H), 7.64-7.66(d, 1H), 7.62-7.64(t, 1H), 6.84(s, 1H), 1.23(s, 9H).

LC-MS: m/z 322.3 (M+1).

N-(1H-benzo[d]imidazol-4-yl)-3-(tert-butyl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxamide (8e)

Yield: 65% as white powder. M.pt: 134-135.8°C.

^{1}**H NMR spectra:** (DMSO-d_6, 400MHz); $\delta-$value in ppm 12.39(s, 1H), 10.05(s, 1H), 8.63-8.65(d, 1H) 8.07-8.17(m, 3H), 7.70-7.73(d, 1H), 7.64-7.65(t, 1H), 7.51-7.61(t, 2H), 6.81(s, 1H), 1.25(s, 9H).

LC-MS: m/z 361.4 (M+1).

N-(benzo[d]oxazol-4-yl)-3-(tert-butyl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxamide (8f)

Yield: 60% as white powder. M.pt: 182.2-184.2°C.

^{1}**H NMR spectra:** (DMSO-d_6, 400MHz); $\delta-$value in ppm 9.69(s, 1H), 8.75(s, 1H) 8.66-8.67(d, 1H), 8.1-8.2(m, 2H), 7.73-7.75(d, 1H), 7.68-7.73(t, 1H), 7.43-7.48(m, 2H), 6.81(s, 1H), 1.25(s, 9H).

LC-MS: m/z 362.4 (M+1).

3-(tert-butyl)-N-(isoxazol-3-yl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxamide (8g)

Yield: 64% as white powder. M.pt: 138-139.8°C.

^{1}**H NMR spectra:** (DMSO-d_6, 400MHz); $\delta-$value in ppm 11.05(s, 1H), 8.81-8.82(d, 1H) 8.62-8.63(d, 1H), 8.1-8.12(t, 1H), 7.71-7.73(d, 1H), 7.62-7.63(t, 1H), 6.96-6.97(d, 1H), 6.91(s, 1H), 1.25(s, 9H).

LC-MS: m/z 312.6 (M+1).

3-(tert-butyl)-N-(1-methyl-1H-tetrazol-5-yl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxamide (8h)

Yield: 66% as white powder. M.pt: 180-181.7°C.

^{1}**H NMR spectra:** (DMSO-d_6, 400MHz); $\delta-$value in ppm 8.59-8.60(d, 1H), 8.09-8.14(t, 1H) 7.61-7.63(t, 1H), 7.29-7.32(d, 1H), 6.91(s, 1H), 3.93(s, 3H), 1.21(s, 9H).

LC-MS: m/z 327.2 (M+1).

3-(tert-butyl)-N-(2,4-difluorobenzyl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxamide (8i)

Yield: 85% as white powder. M.pt: 148.5-149.9°C.

^{1}**H NMR spectra:** (DMSO-d_6, 400MHz); $\delta-$value in ppm 8.8(t, 1H), 8.61-8.63(d, 1H), 8.05-8.10(t, 1H), 7.63-7.67(d, 1H), 7.59-7.61(t, 1H), 7.34-7.39(t, 1H), 7.16-7.23(t, 1H), 7.01-7.07(t, 1H), 6.68(s, 1H), 4.41-4.43(d, 2H), 1.22(s, 9H).

LC-MS: m/z 372 (M+1).

3-(tert-butyl)-N-(2-morpholinoethyl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxamide (8j)

Yield: 88% as white powder. M.pt: 222.3-223.6°C.

^{1}**H NMR spectra:** (DMSO-d_6, 400MHz); $\delta-$value in ppm 8.61-8.62(d, 1H), 8.06-8.11(t, 2H), 7.61-7.61(d, 1H), 7.59-7.61(t, 1H), 6.64(s, 1H), 3.54-3.56(q, 4H), 3.33-3.34(t, 2H), 2.38-2.44(m, 6H), 1.21(s, 9H).

LC-MS: m/z 358.2 (M+1).

3-(tert-butyl)-N-(1-methyl-1H-pyrazol-3-yl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxamide (8k)

Yield: 71% as white powder. M.pt: 106.8-107.9°C.

^{1}H NMR spectra: ($CDCl_3$, 400MHz); $\delta-$value in ppm 9.2(bs, 1H), 8.58-8.6(d, 1H), 7.9-7.94(t, 1H), 7.54-7.56(t, 1H), 7.42-7.45(d, 1H), 7.28(s, 1H), 6.8(s, 1H), 6.87(s, 1H), 3.8(s, 3H), 1.31(s, 9H).

LC-MS: m/z 325.1 (M+1).

3-(tert-butyl)-1-(pyridin-2-yl)-N-(2,2,2-trifluoro-1-(4-fluorophenyl)ethyl)-1H-pyrazole-5-carboxamide (8l)

Yield: 81% as white powder. M.pt: 156.3-157.5°C.

^{1}H NMR spectra: ($CDCl_3$, 400MHz); $\delta-$value in ppm 8.62-8.63(d, 1H), 7.93-7.97(t, 1H), 7.43-7.55(m, 5H), 7.07-7.11(d, 2H), 7.28(s, 1H), 6.83(s, 1H), 5,87-5.92(m, 1H). 1.28(s, 9H).

LC-MS: m/z 421.1 (M+1).

3-(tert-butyl)-N-(1-(4-chlorophenyl)-2,2,2-trifluoroethyl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxamide (8m)

Yield: 80% as white powder. M.pt: 129-130.2°C.

^{1}H NMR spectra: ($CDCl_3$, 400MHz); $\delta-$value in ppm 8.62-8.63(d, 1H), 7.93-7.97(t, 1H), 7.47-7.54(m, 3H), 7.36-7.42(m, 4H), 6.83(s, 1H), 5,87-5.91(m, 1H). 1.28(s, 9H).

LC-MS: m/z 337 (M+1).

N-(1-(4-bromophenyl)-2,2,2-trifluoroethyl)-3-(tert-butyl)-1-(pyridin-2-yl)-1H-pyrazole-5-carboxamide (8n)

Yield: 81% as white powder. M.pt: 191.3-192.5°C.

^{1}H NMR spectra: ($CDCl_3$, 400MHz); $\delta-$value in ppm 8.62-8.63(d, 1H), 7.93-7.97(t, 1H), 7.47-7.54(m, 4H), 7.29-7.33(d, 2H), 6.83(s, 1H), 5,83-5.89(m, 1H). 1.26(s, 9H).

LC-MS: m/z 481 (M+1).

5. Antifungal Activity

The synthesized compounds (**8a**)-(**8m**) were evaluated for their antimicrobial activity against Aspergillus flavus, Fusarium oxysporus and Candida albicans are representing fungal organisms by diffusion disc method [12]. The results of antifungal effect of all tested compounds were reported as zone of inhibition in mm and are shown in Table 2. Amphotericin-B and Ketoconazole were used as the reference antifungal agent. The result revealed that most of the newly synthesized 2-(2-pyridyl)-2H-pyrazole-3-carboxamide derivatives exhibited moderate to good antifungal activities against *Fusarium oxysporus* and *Candida albicans*.

Table 2: Antifungal activities of the 2-(2-pyridyl)-2H-pyrazole-3-carboxamide derivatives (8a)-(8m)

Comp. No.	Aspergillus flavus	Fusarium oxysporuum	Candida albicans
8a	4	9	11
8b	NZ	11	12
8c	NZ	10	11
8d	5	13	13
8e	7	13	14
8f	8	12	13
8g	NZ	10	12
8h	NZ	8	10
8i	NZ	5	9
8j	6	10	12
8k	7	9	13
8l	6	11	10
8m	7	11	12
8n	7	11	12
Amphoterecin-B	10	15	Not used
Ketoconazole	Not Used	Not Used	17

Values are mean of three determinations, the ranges of which are less than 5% of the mean in all cases. Compounds 5μg compound in 500 μL DMSO, used for experiments, NZ= No Zone of activity.

6. Conclusion

We synthesized a series of Novel 2-(2-pyridyl)-2H-pyrazole-3-carboxamide derivatives in high yields. The advantages are the usage of low-cost starting chemicals and simple experimental procedure. These derivatives are having good antifungal activity.

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SYNTHESIS AND CHARACTERIZATION STUDIES OF 2-(2,6-BIS(3-CHLOROPHENYL)-3,5-DIPHENYLPIPERIDIN-4-YLIDENE) HYDRAZINECARBOXAMIDE

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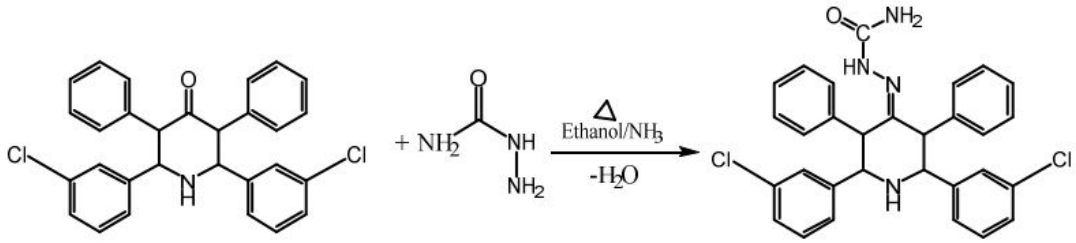
Abstract

The synthesis of substituted piperidin-4-one derivatives using Semicarbazide hydrochloride from1,3-diphenyl acetone, variously substituted aldehydes and ammonium formate (amine) in ethanol medium under reflux-free condition is described to get 2,6-bis(3-chlorophenyl)-3,5-diphenylpiperidin-4-one. Which after incorporated with Semicarbazide hydrochloride using reaction condition to get target compound 2-(2,6-bis(3-chlorophenyl)-3,5-diphenylpiperidin-4-ylidene) hydrazinecarboxamide, The characterized by FT-IR, 1HNMR, 13CNMR.

Keywords: FT-IR, 1H NMR and 13C NMR data.

1. Introduction

Heterocyclic compound with a piperidone skeleton are attractive target for organic synthesis and there is found to be significant in compound possessing aromatic substitution in 2 and 6th position in the piperidone rings [1]. From the survey of existing literature, it appears that heterocyclic compounds of mannich bases played a vital role in the development of piperidin chemistry. Literature reports show that a wide range of 2,6-as well as 3,5-disubstituted piperidin-4-one[2-11]. Amoung the piperidin derivatives, piperidones are important intermediates in several synthetic sections [12-13]. Due to the known therapeutic properities of piperidones and the presence of keto functional group that facilitates the introduction of other substituted derivatives of this class compounds have been found the posses biological activities such as herbicidal insecticidal, fungicidal, anti inflammatory, anesthetic, anticancer activity etc.



2-(2,6-bis(3-chlorophenyl)-3,5-diphenylpiperidin-4-ylidene) hydrazinecarboxamide

2. Materials and Methods

The compound (0.01 mol) was dissolved in 75 ml. of absolute ethanol. Semicarbazide hydrochloride (0.015 mol) was dissolved in ethanol by adding small amount of ammonia and taken in a pressure equalizing addition funnel. Then it was added drop by drop with constant stirring maintaining the temperature of the water bath at 70 80°C. After the addition, heating was continued until the product formed. The formation of the product was monitored by TLC. The solution was poured into ice-cubes. Then it was cooled in a refrigerator over night. A colourless solid formed. The solid thus obtained 88(a) was filtered at the pump washed several times with ammonia-water and acetone mixture and dried in a vacuum desiccator. It was recrystallised from ethanol. The precipitate formed was filtered and dried. The product was dried, m.p 220-222°C.

3. Spectral Characterization

2-(2,6-bis(3-chlorophenyl)-3,5-diphenylpiperidin-4-ylidene)hydrazinecarboxamide, Yield: 70-76%; mp: 220-222°C: FT-IR (KBr): 3137 (υN-H (2° amide))3062 (υN-H), 3066 (υC-H(aro)), 3036 (υC-H(ali)), 1721 (υC=N), 1698 (υC=O), 1491, 1404 (υC=C), 747 (υC-Cl)cm^{-1}, ^{1}H NMR (300MHz, DMSO-d_6, δ in ppm); 7.45-7.37(d, 8H, aromatic-**H**), 7.76-7.03(m, 10H, aromatic-**H**); 6.07 (2° amide s, 1H, NH proton), 5.45 (s, 3H, semicarbazone moiety), 4.57-4.54(dd, 2H, benzylic-**H** at C_2 and C_6, 4.30-4.27(d, 2H, benzylic-**H** at C_3 and C_5, 3.37(Hump, 1H, **NH**), ^{13}CNMR (100MHz, DMSO-d_6, δ in ppm): 206.5(¿C=O), 141.3, 136.8, 131.8,129.0, 138.7, 127.6,127.5, 126.1, 65.5, 63.7.

4. Conclusion

The synthesis of the compound described in this work. Nitrogen containing piperidine-4-ones are obtained, when more convenient ammonium formate is employed instead of

the deliquescent ammonium acetate. The synthesized compound was characterized by FT-IR, ^{1}H NMR and ^{13}C NMR data.

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ANALGESIC POTENTIAL OF SESAME SEED EXTRACTS BY THERMAL HEAT METHOD IN ANIMAL MODEL

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Abstract

To investigate the analgesic activity of the ethanolic,hexane and chloroform extracts of sesame seeds by thermal heat method(hot plate) in albino mice.

Keywords: NSAID, GIT Symptoms, Addiction Property and Respiratory Depression.

1. Introduction

Pain is a common symptom having huge public health importance. Conventional analgesics like NSAIDs, opioids all are effectively reduce pain but causes unwanted side effects like GIT symptoms, renal, addiction property and respiratory depression. So search for a novel analgesics with favourable risk benefit profile is essential.Natural products have a great role in the discovery of analgesics drugs along with determining complex mechanism involved in pain transmission and pain relief.Hence we selected natural product sesame seed for our study.

Sesame is a medicinal plant that belongs to the pedialiaceae family.Sesame seeds are rich in biologically active phytochemicals which contribute its pharmacological

properties.It contains two unique substances sesamin and sesamolin known to have a cholesterol lowering effects in humans and to prevent B.P.It also possess antiproliferative,neuroprotective,anti-inflammatory properties. These seeds are rich in polynutrients like omega-6-fatty acids ,flavonoids, phenolic antioxidants,vitamins and dietary fiber with potential anticancer as well as health promoting properties. Sesame seeds have been widely used traditional medicine for their nutritivepreventive and curative properties.

The present study evaluated the analgesic potential of three extract of sesame seeds(ethanol, hexane & chloroform) by hot plate method in albino mice.

2. Methods

2.1. Chemicals

The standard drug - aspirin was procured from Dabur pharma Ltd, Tarapur, Thane. Ethanol and other chemicals were purchased from Sigma Aldrich Pvt Ltd, Bengaluru, India. All chemicals were of analytical grade.

2.2. Preparation of Plant Extract

The sun dried Sesame seeds were powdered in a mortar. The dry powder was taken in soxhlet apparatus and extraction was done using different solvents like ethanol, n-hexane and chloroform. Rotary evaporator was used for concentrating the filtrate. After extraction excess solvent was distilled at 80°C. The extract so obtained was stored in sterile bottles.

2.3. Animals

Albino mice weighing 30-40 g were used. They were kept at 12hr :12hr light dark cycle. Standard pellets and water were provided ad libitum. 12 hours before

drug administration, food was withdrawn and reinstituted only after completion of the experiment. Institutional Animal Ethical Committee approved the study protocol.

2.4. Experiment

The animals were divided into 5 groups. Group 1 was given normal saline (0.1 ml/kg) and served as control. Group 2 was administered the standard drug - Aspirin (50 mg/kg). The test groups (Groups 3 to 5) were administered the various Sesame seed extracts (ethanolic, hexane and chloroform) at the doses of 100, 150 and 200 mg/kg respectively. All drugs were administered by oral route. Analgesic activity was evaluated by hot plate method.

2.5. Hot Plate Analgesometer

Eddy and Leimbach were first described this method in 1953. So this apparatus is calledas Eddys hot plate. Mice were placed on a hot plate maintained at 55°C within a restrainer. The cut-off time was 10 seconds to prevent any thermal injury to the paws. The time taken by the mice to react to the thermal stimuli either by licking their paw or jumping was recorded as the latency period. The latency period was recorded before and at various intervals after administration of the respective treatments (30, 60, 120 and 180 minutes). A compound with analgesic activity increases the latency period.

2.6. Statistical Analysis

The data was stated as mean ± standard error of mean (SEM). The data was analysed by one way ANOVA and Dunnets test as post hoc. P value < 0.05 was considered significant.

3. Results

The ethanolic extract of Sesame seed at the dose of 100 mg/kg and 150 mg/kg exhibited significant analgesic activity at 120 and 180 minutes whereas at a dose of 200 mg/kg it exhibited activity even at 60 minutes. The maximum analgesic effect was showed at 120 minutes. The activity of extract at 200 mg/kg was comparable to Aspirin. Both the hexane and chloroform extract of Sesame seed did not exhibit significant analgesic effect at a dose of 100 mg/kg and 150 mg/kg. But at a higher dose (200 mg/kg), both hexane

and chloroform extract showed significant analgesic activity which was maximum at 180 minute.

Table 1: Analgesic Effect of Ethanolic Extract of Sesame Seed

Treatment group	Treatment given	Latency period in seconds				
		0 min	30 min	60 min	120 min	180 min
Group 1	Normal saline (0.1 ml / kg)	1.4 ± 0.05	1.4 ± 0.07	1.5 ± 0.01	1.52 ± 0.08	1.6 ± 0.02
Group 2	Aspirin (50 mg / kg)	1.38 ± 0.07	1.9 ± 0.02	4.6 ± 0.08*	5.4 ± 0.07**	5.9 ± 0.03**
Group 3	Sesame seed extract (100 mg / kg)	1.39 ± 0.04	2.0 ± 1.02	2.2 ± 0.05	4.7 ± 0.02*	4.6 ± 0.06*
Group 4	Sesame seed extract (150 mg / kg)	1.4 ± 0.06	2.3 ± 0.09	2.6 ± 0.03	4.9 ± 1.02*	4.8 ± 0.07*
Group 5	Sesame seed extract (200 mg / kg)	1.4 ± 0.04	3.2 ± 0.06	4.2 ± 0.04*	5.1 ± 0.11**	5.0 ± 0.13**

Data is presented as mean ± SEM *=P<0.05, **=P<0.01 compared with control

Table 2: Analgesic Effect of Hexane Extract of Sesame Seed

Treatment group	Treatment given	Latency period in seconds				
		0 min	30 min	60 min	120 min	180 min
Group 1	Normal saline (0.1 ml / kg)	1.39 ± 0.07	1.39 ± 0.09	1.36 ± 0.08	1.33 ± 0.05	1.36 ± 0.01
Group 2	Aspirin (50 mg / kg)	1.4 ± 0.01	1.8 ± 0.02	4.8 ± 0.08**	5.5 ± 0.02**	5.8 ± 0.07**
Group 3	Sesame seed extract (100 mg / kg)	1.39 ± 0.03	1.7 ± 1.0	2.8 ± 0.09	2.9 ± 0.03	2.9 ± 0.05
Group 4	Sesame seed extract (150 mg / kg)	1.39 ± 0.02	1.8 ± 0.14	2.9 ± 0.47	3.3 ± 0.49	3.1 ± 0.47
Group 5	Sesame seed extract (200 mg / kg)	1.4 ± 0.04	2.4 ± 1.03	3.8 ± 0.06*	3.9 ± 0.05*	4.1 ± 0.12*

Data is presented as mean ± SEM *=P<0.05, **=P<0.01 compared with control

Table 3: Analgesic Effect of Chloroform Extract of Sesame Seed

Treatment group	Treatment given	Latency period in seconds				
		0 min	30 min	60 min	120 min	180 min
Group 1	Normal saline (0.1 ml / kg)	1.4 ± 0.01	1.41 ± 0.04	1.47 ± 0.05	1.51 ± 0.09	1.45 ± 0.04
Group 2	Aspirin (50 mg / kg)	1.4 ± 0.05	1.8 ± 0.08	4.7 ± 0.02**	5.3 ± 0.03**	5.8 ± 0.01**
Group 3	Sesame seed extract (100 mg / kg)	1.4 ± 0.03	1.9 ± 0.13	2.0 ± 0.05	2.19 ± 0.10	2.8 ± 0.06
Group 4	Sesame seed extract (150 mg / kg)	1.39 ± 0.02	2.0 ± 0.01	2.1 ± 0.11	2.2 ± 0.04	2.9 ± 0.17
Group 5	Sesame seed extract (200 mg / kg)	1.38 ± 0.06	2.3 ± 0.10	3.9 ± 0.02*	4.0 ± 0.03*	4.5 ± 0.06*

Data is presented as mean ± SEM *=P<0.05, **=P<0.01 compared with control

4. Conclusion

The ethanolic, hexane and chloroform extracts of Sesame seeds have analgesic potential. The analgesic activity of ethanolic extract is comparable to aspirin.Further studies are required to confirm the exact analgesic mechanism.Sesame plant gives a future scope in the medical field to improve the quality of life in a better way.

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KINETICS AND MECHANISM OF OXIDATION OF 1,2, DIHYDROXY PROPANE-1,2,3 TRICARBOXYLIC ACID BY PFC IN AQUEOUS ACETIC ACID MEDIUM, A COMPARITIVE STUDY WITH 2-HYDROXY PROPANE-1,2,3-TRICARBOXYLIC ACID AND 1-PROPENE-1,2,3 TRICARBOXYLIC ACID

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Abstract

The oxidation of 1,2, dihydroxy propane-1,2,3-tricarboxylic acid, 2-hydroxy propane-1,2,3-tricarboxylic acid and 1-propene-1,2,3-tricarboxylic acid by PFC in aqueous acetic acid medium leads to the formation of a mixture of compounds as the products. Earlier studies on oxidation reaction involved mainly PFC (pyridinium fluorochromate) as oxidant with many organic substrates and 1) 1,2-dihydroxy propane-1,2,3-tricarboxylic acid, 2) 2-hydroxy propane-1,2,3-tricarboxylic acid 3) 1-propene-1,2,3-tricarboxylic acid as a substrate with many other oxidants. In the present study the kinetics is investigated in acetic acid medium and the effect on solvent composition is also studied.

Keywords: Acetic Acid, 1,2-Dihydroxy Propane-1,2,3-Tricarboxylic Acid, 2-Hydroxy Propane-1,2,3-Tricarboxylic Acid and 1-Propene-1,2,3-Tricarboxylic Acid.

1. Introduction

The kinetics of oxidation reactions and the investigation of the reaction mechanisms from the kinetic data have been the most interesting subjects in chemistry always. In any kinetic investigation, one may be interested to arrive at the relationship between the rate and the various factors like concentrations of the reactants, temperature, reaction medium etc., and interpretation of the empirical rate laws in the light of the mechanism proposed.

2. Experimental Methods

Kinetics of oxidation of 2-hydroxy propane-1,2,3-tricarboxylic acid

The burette is washed with water, rinsed with distilled water and then with the thio solution. It is filled with same thio solution up to zero mark. The initial reading of the burette is noted. 5 ml pipette is washed with water, rinsed with distilled water and then with the 1:5 molar oxidant and the substrate reaction solution. The solution is then pipetted out in to a clean conical flask. Small pinch of KI is added into the flask. Suddenly titrated with burette solution, the light yellow colour was converted to dark yellow and then 2-3 drops of starch indicator was added. The end point of the titration is the appearance of dark blue colour to green colour. The same titration is repeated to every 5 min interval, the obtained volume of thio solution was tabulated and then the rate constant was calculated.

Similarly, the kinetics is followed by varying the oxidant and substrate concentration and also by varying the solvent concentration. The obtained values were tabulated and the rate constants were also calculated. Similar procedure is followed for all the substrates.

3. Result and Discussion

In most of the chemical reactions, it is only the disappearance of starting materials and the appearance of final products that can be detected. In general, the net reaction simply represents a summation of all the changes that occur. The net change may actually consist of several consecutive reactions each of which constitutes a step in the formation of final products.

Kinetics of oxidation of 1,2, dihydroxy propane-1,2,3 tricarboxylic acid, 2-hydroxy propane-1,2,3-tricarboxylic acid and 1-propene-1,2,3 tricarboxylic acid

The 5 ml of test solution was titrated against thio solution under pseudo first order reaction condition. The rate constant values were calculated using the decreased trends of volume of thio solution and thus obtained rate constants values were given in Table 2. Similarly, rate constant values were calculated for the variation of acetic acid (20%) and it was given in Table. The obtained rate constants (Figure 1) revealed that 10% acetic acid solution showed lesser rate constants value. On increasing the percentage of acetic acid, the rate constant values also increased due to the higher acidic nature of the solution.

3.1. Experimental Results

Kinetic study for the oxidation of 1,2, dihydroxy propane-1,2,3-tricarboxylic acid, 2-hydroxy propane-1,2,3-tricarboxylic acid and 1-propene-1,2,3 tricarboxylic acid by PFC

1,2, dihydroxy propane-1,2,3-tricarboxylic acid, 2-hydroxy propane-1,2,3-tricarboxylic acid and 1-propene-1,2,3 tricarboxylic acid = 0.05×10^{-2}mol/lit, PFC=0.01×10^{-2}mol/lit.

Table 1: (10% Acetic acid)

Time (sec)	1,2-dihydroxy propane -1,2,3-tricarboxylic acid		2-hydroxy propane -1,2,3-tricarboxylic acid		1-propene-1,2,3-tricarboxylic acid	
	$\log(a-x)$	Rate constant ($k \times 10^{-4}$ sec^{-1})	$\log(a-x)$	Rate constant ($k \times 10^{-4}$ sec^{-1})	$\log(a-x)$	Rate constant ($k \times 10^{-4}$ sec^{-1})
300	0.6798	1.5954	0.6722	1.4211	0.6707	1.3123
600	0.6601	1.5548	0.6534	1.4195	0.6459	1.3374
900	0.6442	1.5548	0.6331	1.4578	0.6212	1.3558
1200	0.6246	1.5694	0.6127	1.4827	0.6017	1.3612
1500	0.6071	1.5214	0.5911	1.4925	0.5893	1.3745
1800	0.5843	1.5284	0.5683	1.4721	0.5609	1.3851

Table 2: (20% Acetic acid)

Time (sec)	1,2-dihydroxy propane -1,2,3-tricarboxylic acid		2-hydroxy propane -1,2,3-tricarboxylic acid		1-propene-1,2,3-tricarboxylic acid	
	$\log(a-x)$	Rate constant ($k \times 10^{-4}$ sec^{-1})	$\log(a-x)$	Rate constant ($k \times 10^{-4}$ sec^{-1})	$\log(a-x)$	Rate constant ($k \times 10^{-4}$ sec^{-1})
300	0.6782	1.6494	0.6628	1.4145	0.6457	1.4787
600	0.6584	1.6354	0.6432	1.4512	0.6265	1.4967
900	0.6407	1.6215	0.6236	1.4878	0.6054	1.5121
1200	0.6258	1.5984	0.6022	1.5114	0.5805	1.5392
1500	0.6084	1.5945	0.5794	1.5545	0.5623	1.5858
1800	0.5893	1.5651	0.5568	1.5987	0.5406	1.5472

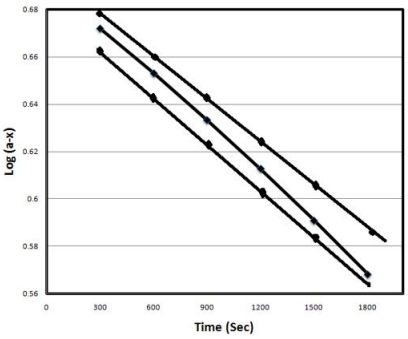


Figure 1: 10% Acetic acid

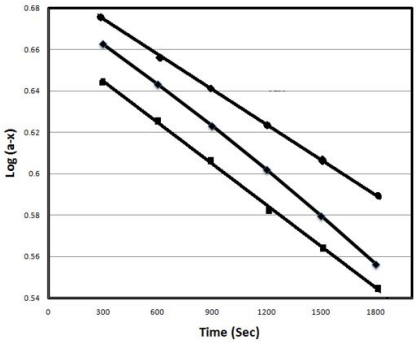
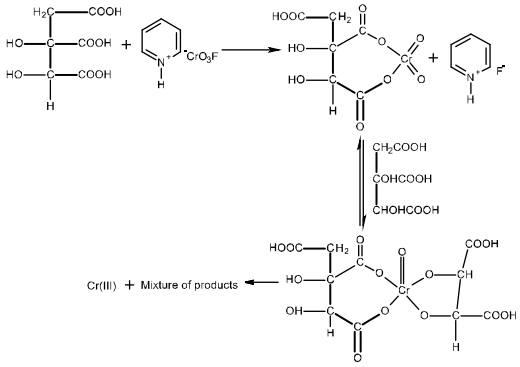
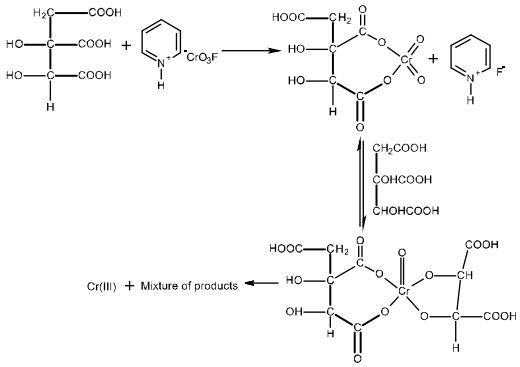


Figure 2: 20% Acetic acid

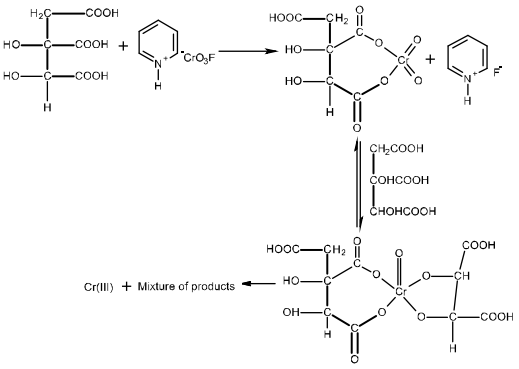
4. Mechanism



(Scheme-1) Oxidation of 1,2, dihydroxy propane -1,2,3-tricarboxylic acid



(Scheme-2) Oxidation of 2-hydroxy propane -1,2,3-tricarboxylic acid



(Scheme-3) Oxidation of 1-propene-1,2,3 tricarboxylic acid

5. Summary and Conclusion

In the present study the kinetics and mechanism of oxidation of 1,2, dihydroxy propane-1,2,3 tricarboxylic acid, 2-hydroxy propane-1,2,3-tricarboxylic acid and 1-propene-1,2,3 tricarboxylic acid by PFC in aqueous acetic acid medium is investigated and the effect on solvent composition is also studied. oxidation of 1,2, dihydroxy propane-1,2,3 tricarboxylic acid, 2-hydroxy propane-1,2,3-tricarboxylic acid and 1-propene-1,2,3 tricarboxylic acid has been achieved due to formation of the complex between the substrate and the oxidant. The reaction is found to be increasing with the increase in the concentration of acetic acid. Acetic acid being a weak organic acid, is able to catalyze the reaction and the reaction follows pseudo first order kinetics.

After thorough analysis from the obtained results, we arrived at the following conclusions:

1. Oxidation of 1,2-dihydroxy propane-1,2,3-tricarboxylic acid, 2-hydroxy propane-1,2,3-tricarboxylic acid and 1-propene-1,2,3-tricarboxylic acid by PFC is observed to follow pseudo first order Kinetics.

2. The rate constant values for the oxidation is found to be increasing with the increase in the % of acetic acid.

3. The rate constant for the oxidation of 1-propene-1,2,3-tricarboxylic acid is found to be slower than 1,2-dihydroxy propane-1,2,3-tricarboxylic acid and 2-hydroxy propane-1,2,3-tricarboxylic acid.

4. The rate constant of 1,2-dihydroxy propane-1,2,3-tricarboxylic acid is higher than 2-hydroxy propane-1,2,3-tricarboxylic acid and 1-propene-1,2,3-tricarboxylic acid.

5. The oxidation of 1,2-dihydroxy propane-1,2,3 tricarboxylic acid and 2-hydroxy propane-1,2,3-tricarboxylic acid by PFC in aqueous acetic acid medium leads to the formation of a mixture of compounds as the products.

6. The oxidation of 1-propene-1,2,3-tricarboxylic acid by PFC in aqueous acetic acid medium is very slow and leads to the decarboxylation of 1-propene-1,2,3-tricarboxylic acid with reduction of PFC. The formation of any organic product in the oxidation of 1-propene-1,2,3-tricarboxylic acid cannot be confirmed by chemical analysis.

7. The oxidation of 1,2-dihydroxy propane-1,2,3 tricarboxylic acid by PFC in aquous acetic acid medium is faster than the oxidation of 2-hydroxy propane-1,2,3-tricarboxylic acid. The substrate is found to be reactive and the isolation of any keto or hydroxyl compound is not possible due to their further oxidation with the reagent. The reduction of PFC containing Cr(VI) to Cr(III) confirm that there is oxidation of the substrate.

8. A negative test with acrylonitrile rules out the formation of any radical in the oxidation reaction.

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CYTOCHROME OXIDASE SUBUNIT 1 (CO1) PROTEIN SEQUENCE ANALYSIS OF TWO COLONIAL ASCIDIANS, PEROPHORA AND EUDISTOMA SPECIES

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Abstract

Two colonial ascidians collected were taxonomically identified and their COI genes were sequenced. The forward and reverse sequences were translated to amino acid sequences and structure modelling tool was employed to assess and understand the protein structure in silico. The results of normalized QMEAN4 score confirm that the colonial ascidians of the present study are not thermophiles.

Keywords: COI, Amino Acid, Colonial Ascidians.

1. Introduction

Ascidians (Phylum: Chordata, Class: Ascidiacea), or sea squirts, are the largest and most diverse class of the sub-phylum Tunicata (also known as Urochordata). Classification of the Ascidiacea has been based on the structure of the branchial sac, but molecular studies have shown that certain groups at family, genus or species level may need reorganization (Stach & Turbeville, 2002). However, at the present time few molecular studies are available and all existing identification keys use the classification based on morphology.Advancement in computational and sequencingtechnology has made DNA sequence the key source of new information for advancing our knowledge of evolutionary and genetic relationship. The animal mitochondrial genome is a better target for analysis than the nuclear genome because of its lack of introns, limited exposure to recombination and its haploid mode of inheritance (Saccone et al 1999). The choice of mitochondrial gene as a universal marker was mostly driven by the fact that mitochondria is maternally inherited, avoiding problems with recombination. Mitochondrial genome has a high mutation rate when compared with the nuclear

genome, which results in high degree of intra-specific polymorphism and divergence, important in evolutionary studies (Williams and Knowlton 2001, Wheat and Watt 2008, Hlaing et al 2009).The mitochondrial cytochrome c oxidase subunit is a protein coding gene, where indels are rare, since most may lead to a shift in the reading frame.

In this context,the present investigation was carried where a molecular marker was sequenced and the protein sequence analyzed using different tools. The mitochondrial DNA was isolated and COI genes were sequenced. The sequenced genes were translated into amino acid language and protein homology was done.

2. Materials and Methods

Sample collection

Two colonial ascidians Perophora sp and Eudistoma sp were collected from the Tuticorin Harbour. Both the species were collected from shallow water by hand picking method. The collected ascidians were narcotized using menthol crystals and were identified morphologically using taxonomic keys by Dr. H. Abdul Jaffar Ali, Ascidiologist, Islamiah College (Autonomous), Vaniyambadi.

Sample Preservation

The collected ascidians were preserved in 10% formalin for conventional taxonomy and for COI gene sequencing, it was preserved in 95% ethanol. The 95% ethanol preserved samples were brought to the laboratory in ice cold condition and stored at -20°C and the formalin preserved samples were preserved at room temperature.

Genomic DNA Isolation

Genomic DNA was isolated using DNeasy Blood and Tissue Kit (Qiagen) following manufacturer's animal tissue protocol.

PCR Amplification and Sequencing

COI gene was amplified using Universal primer (Folmer et al, 1994). PCR amplifications were carried out in 100.0 μ1 reaction volumes containing; 1 μ1 of AmpliTaq Gold DNA polymerase enzyme, 400 ng of both primers and 1 μ1 of template DNA. Thermocycling conditions consisted of: 94° C for 5 minutes, one cycle; 94° C for 30 seconds, 55° C for 30 seconds, 72° C for 30 seconds; 35 cycles; 72° C for 3 minutes, one cycle. Amplified products were purified and sequenced in both directions using BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) by a commercial lab. PCR products were sequenced in both the reverse and forward direction using the appropriate PCR primer to prime the sequencing reaction. Sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequence was carried out using BioEdit(Hall 1999).

GC % Calculation

The GC % was calculated using online software.

Sequence Alignment

The forward and reverse sequences were aligned using online alignment tools.

Translation of the COI gene sequences

Translation of COI gene sequences were done by Expasy Translate using ascidian mitochondrial as the genetic code.

Protein Homology modeling

The protein homology modeling for the Perophorasp and Eudistomasp were done using SWISS-MODEL workspace.

Template Search

Template search with Blast and HHBlits has been performed against the SWISS-MODEL template library. The target sequence was searched with BLAST (Altschul et al., 1997) against the primary amino acid sequence contained in the SMTL. A total of 13 templates were found. An initial HHblits profile has been built using the procedure outlined in (Remmert, et al., 2011), followed by 1 iteration of HHblits against NR20. The obtained profile has then been searched against all profiles of the SMTL. A total of 30 templates were found.

Template Selection

For each identified template, the template's quality has been predicted from features of the target-template alignment. The templates with the highest quality have then been selected for model building.

Model Building

Models are built based on the target-template alignment using Promod-II. Coordinates which are conserved between the target and the template are copied from the template to the model. Insertions and deletions are remodeled using a fragment library. Side chains are then rebuilt. Finally, the geometry of the resulting model is regularized by using a force field. In case loop modelling with ProMod-II (Guex, et al., 1997) does not give satisfactory results, an alternative model is built with MODELLER (Sali, et al., 1993).

Model Quality Estimation

The global and per-residue model quality has been assessed using the QMEAN scoring function (Benkert, et al., 2011) . For improved performance, weights of the individual QMEAN terms have been trained specifically for SWISS-MODEL.

Oligomeric State Conservation

Homo-oligomeric structure of the target protein is predicted based on the analysis of pairwise interfaces of the identified template structures. For each relevant interface between polypetide chains (interfaces with more than 10 residue-residue interactions), the Qscore Oligomer (Mariani et al., 2011) is predicted from features such as similarity to target and frequency of observing this interface in the identified templates (Kiefer, Bertoni, Biasini, to be published). The prediction is performed with a random forest regressor using these features as input parameters to predict the probability of conservation for each interface. The Qscore Oligomer of the whole complex is then

calculated as the weight-averaged Qscore Oligomer of the interfaces. The oligomeric state of the target is predicted to be the same as in the template when Qscore Oligomer is predicted to be higher or equal to 0.5.

3. Results and Discussion

The colonial ascidians were identified up to genus level. *Eudistoma sp* had massive colonies. Zooids were well organized and completely embedded in the tunic. Gonads were just posterior to the intestinal loop,forming a pseudo post-abdominal, which was lacking a heart. Thorax short with three rows of stigmata and usually covered by strong musculature. Atrial siphon is long and the apertures lay in the center of the system.

In *Perophora sp* zooids linked by stolons and covered by a thin and usually translucent tunic. Zooids small (3-6 mm) and round, transparent, uncolored or greenish, arising from a net of stolons. Body musculature pattern distinct for each species.Pharynx with four or five rows of stigmata and complete longitudinal vessels.Alimentary canal almost horizontal underneath the pharynx; stomach wall smooth; rectum short.Gonads inside the intestinal loop. Larvae incubated inside the atrial cavity.

COI gene sequencing of the *Perophora sp* gave two sequences (Fig. 1 & 2) forward and reverse having alength of about 703 bp and 672 bp respectively. The GC % of the forward and reverse sequences was 45.8% and 45.1% respectively. Though the reverse sequence was 31 bp less compared to the forward sequence the GC % was almost equal to that of the forward sequence. The identity between the two sequences was found to 44.1%, with a gap penalty of about -12/-12 and the global alignment score being -172.

The forward and reverse sequences of *Eudistoma sp* were of the length 695 and 753 bp respectively (Fig. 7 & 8). The GC % of this sequences were 45.6% and 50.7%. The identity between the two sequences of *Eudistoma sp* was found to be 43.1%, with a gap penalty of about -12/-12 and the global alignment score being -211.

Perophora sp sequences (44.1%) has 1% better identity compared to *Eudistoma sp* (43.1%). 1% identity difference between the sequences of the two Sp showed -39% difference in the global alignment score. *Perophora sp* has a better alignment score compared to *Eudistoma sp*.

The translation of the DNA sequence into protein sequence shows five frames for each sequence. In *Perophora sp* forward sequence translation the third frame running from 5'3' shows the best reading frames of the other two frames running in the 5'3'. Though the first frame of 3'5' is having more reading frames than the other two frames of 3'5', the third frame of 5'3' may best suit for initiator selection and retrieving of aminoacids. Translation of the reverse sequence shows more stop codons compared to forward sequence, hence may not be useful for initiator selection and retrieving of aminoacids. The aligned sequence were translated into amino acid sequences (Fig.3).

The forward sequence of *Eudistoma sp* shows a long open reading frame in the

5'3'position of first frame. The other frames of the 5'3' and 3'5'position contains short open reading frames and many stop codons. All the five translated frames of the reverse sequence shows short open reading frames with at least 3 stop codons in each frame. *Eudistoma sp* shows a long open reading frame consisting of 221 amino acids whereas *Perophora sp* has a open reading frame of 217 amino acids. The long open reading frames are used in along with other evidence, to initially identify candidate protein coding regions in a DNA sequence (Deonier et al 2005).The aligned sequence were translated into amino acid sequences (Fig.9).

The COI gene translates for both *Perophora sp* and *Eudistoma sp* has 31 and 33 templates models (Table 1 & 2). The maximum sequence similarity was exhibited for *Eudistoma sp* COI gene translate 0.42 (Iqle.i.A, 3ehb.1.A, 3hb3.1.A) and 0.41 for *Perophora sp* COI gene translate (3abm.A). The sequence identity was maximum for *Perophora sp* COI gene translates of 50.69 (3abm.A).

The local quality estimate of *Eudistoma sp* COI gene translate the predicted local similarity to target value starts from 0.5 and shows a maximum value of 0.8 (Fig.10). The *Perophora sp* COI gene translate starts at 0.7 and has a maximum value of 0.9 (Fig.4). Protein structure assessment for the two sequences were performed to understand the three dimensional view of the COI region (Fig. 6 & 12) The normalized QMEAN4 score is 0.5 for *Perophora sp* (Fig.5) and slightly above 0.5 for *Eudistoma sp* (Fig.11). This result indicates that both the colonial ascidians are not thermophilic, since the COI of the templates show higher values (upto 1.0), which indicates they are from mesophilic organisms.

Table 1: *Perophora sp* SWISS-MODEL Homology Modeling Report

Template	Seq Identity	Oligo-state	Found by	Method	Resolution	Seq Similarity	Range	Coverage	Description
3abm.1.A	46.75	hetero-oligomer	BLAST	X-ray	1.95	0.40	1 - 172	0.77	Cytochrome c oxidase subunit 1

Table 2: *Eudistoma sp* SWISS-MODEL Homology Modeling Report

Template	Seq Identity	Oligo-state	Found by	Method	Resolution	Seq Similarity	Range	Coverage	Description
3omi.1.A	28.87	hetero-oligomer	HHblits	X-ray	2.15	0.34	2 - 99	0.42	Cytochrome c oxidase, aa3 type, subunit I

```
CCCGTGATGT AGTCTATGTA TTTGGTGCCT GAGCCGGAAT AATGGGAACC GCTTTAAGCC TTATTATTCG
CGCCGAGCTA AGCCAGCCCG GATCACTTTT AGGTGACGAC CAAATTTATA ACGTTATTGT TACTGCCCAC
GCCTTCGTAA TAATTTTCTT CATAGTTATG CCCATCCTCA TTGGAGGCTT TGGAAACTGA CTTGTTCCAT
TAATAATTGG AGCCCCTGAC ATAGCGTTTC CACGAATAAA TAACATAAGC TTTTGACTCC TACCCCCCTC
ATTCTTATTA CTACTAGCTT CCTCTGGTGT TGAAGCCGGG GCCGGGACCG GATGAACAGT ATACCCGCCC
CTTGCAGGCA ACCTAGCCCA TGCCGGCGCA TCGGTAGACT TAACAATCTT TTCACTACAC CTAGCGGGTG
TCTCATCAAT TTTAGGGGCC ATCAATTTTA TCACTACAAC AATTAACATA AAACCCCCAG CCATTTCACA
ATACCAAACC CCCCTCTTCG TCTGATCTGA GTTAGTGACT GCTGTATTAC TTCTCCTCTC ATTACCAGTG
CTAGCCGCCG GAATCACAAT ACTTTTAACA GACCGAAACC TCAACACCAC ATTCTTTGAC CCTGCAGGAG
GAGGAGACCC TATTCTTTAT CAACACCTAT TCTGATTCTT CGGTCACCCT GAAGTGTCAT AGCTGTTTCC
TAA
```

Figure 1: COI forward sequence of *Perophora sp*

GC: 45.8%

DNA length: 703

```
AGGGTCTCCT CCTCCTGCAG GGTCAAAGAA TGTGGTGTTG AGGTTTCGGT CTGTTAAAAG TATTGTGATT
CCGGCGGCTA GCACTGGTAA TGAAAGGAAA AGTAATACAG CAGTCACTAA CACAGATCAA ACGAAGAGGG
GGGTTTGGTA TTGTGAAATG GCTGGGGGTT TTATGTTAAT TGTTGTAGTG ATAAAATTGA TGGCCCCTAA
AATTGATGAG ACACCCGCTA GGTGTATTGA AAAGATTGTT AAGTCTACCG ATGCGCCGGC ATGGGCTAGG
TTGCCTGCAA GGGGCGGGTA TACTGTTCAT CCGGTCCCGG CCCCGGCTTC AACACCAGAG GAAGCTAGTA
CTAATAAAAA TGAGGGGGGT AGGAGTCAAA AGCTTATGTT ATTTATTTTT TTTAAACGCT ATGGCCCCCC
CTCCATTTTT TTTGGGAAAA AGTCCCCCCC CCCAACCCTC CAATGAGGAT GGGCATAACT ATGAATAAAA
TTATTACGAA GGCGTGGGCA GTAACAATAA CGTTATAACT TTGGTCGTCA CCTAAAAGTG ATCCGGGCTG
GTTTATCTCG ACGAAAATAA CAAGGCTTAA AGCTGTTCTC ACTATTCCGG CTCAGGCACC AATTACAAGA
TAAAGGGTGC CAATGTCTTA CAAGATAAAG GGTGCCAATG TC
```

Figure 2: COI reverse sequence of *Perophora sp*

GC: 45.1%

DNA length: 67

```
DIGTLYLVIGAWAGMVGTALSLVIFVEMNQPGSLLGDDQSYNVIVTAHAFVMILFMVMPILIGG
LGGGTFSQKKWGGGHSVKKMTAFDSYPPHFYYLPLVLKPGPGPDEQYTRPLQATPMPAHRTQSF
QYTRVSHQFGPSILSLQQLTNPQPFHNTKPPSSSDLSWLLYYFSSHYQCPPESQYFQTETSTPH
SLTLQEEETLFFINTYSDSSVTLKCHSC
```

Figure 3: Primary amino acid sequence of *Perophora sp* for which templates were searched and models were built

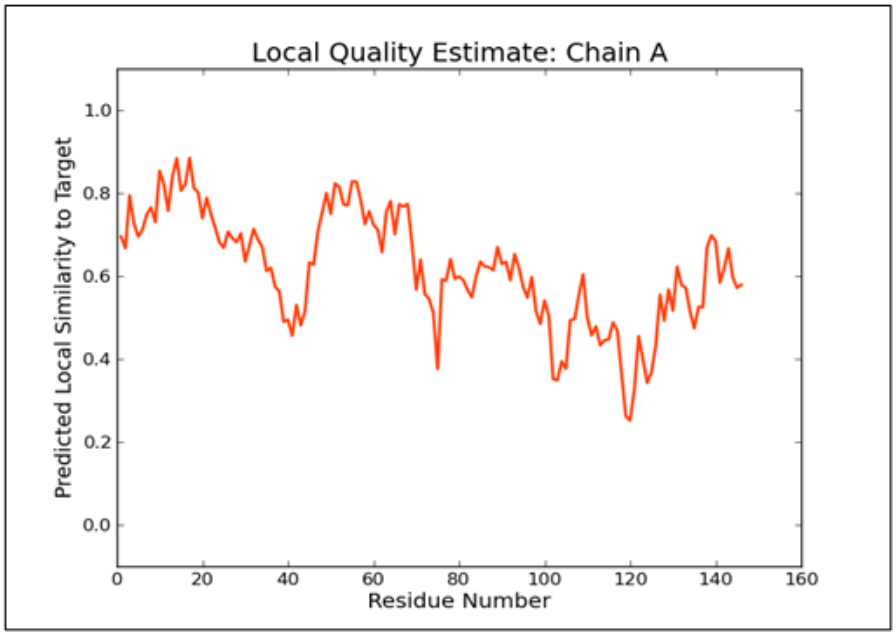

Figure 4: Local quality estimate of *Perophora sp*

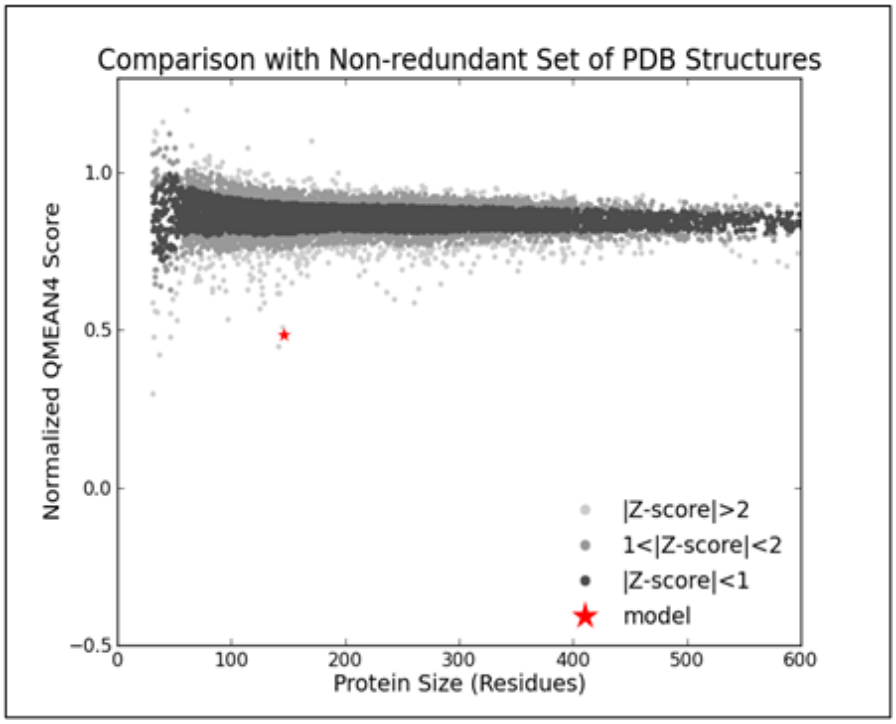

Figure 5: Normalized QMEAN4 score of *Perophora sp*

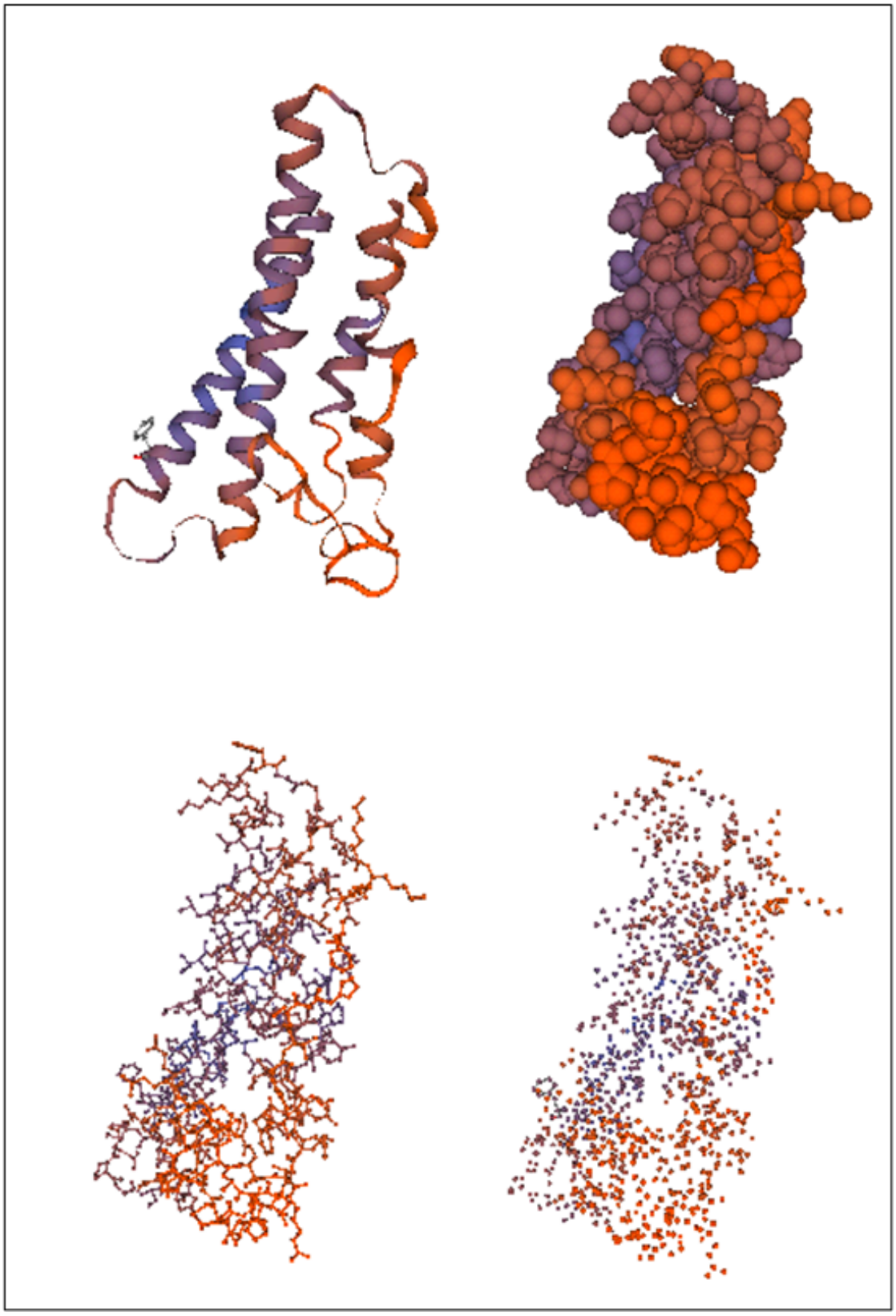

Figure 6: *Perophora sp* COI Protein sequence structure assessment

Eudistoma sp

```
AACGGTGTGT ATCTGTGTAT ATTGGTGCTG AGCCTGGCAA TAGTCGGGAA CCGCTTTAAG CCTTATTATT
CGCGCCGAGC TAACCCAGCG CGGATCTTTT TTACGTGACG ACAAAATTTA TTTCGTTATT GTTACTGCCC
GCGCCTTCGT AATAATTTTC TTCGATTCTA TGCCCATCCT CATTGGAGGC TTTGGAAACT GACTTGTTCC
CTTAAAAATG GGACCCCTGG ACATAGCGTT TCCACAAAAA AAAAAAAAAA CATAAGCTTT TGACTCCTAC
CCCCCTCATT CTTATTACTA CTAGCTTCCT CTGGTGTTGA AGCCGGGGCC GGGACCGGAT GAACAGTATA
CCCGCCCCTT GCAGGCAACC TAGCCCATGC CGGCGCATCG GTAGACTTAA CAATCTTTTC ACTACACCTA
GCGGGTGTCT CATCAATTTT AGGGGCCATC AATTTTATCA CTACAACAAT TAACATAAAA CCCCCAGCCA
TTTCACAATA CCAAACCCCC CTCTTCGTCT GATCTGTGTT AGTGACTGCT GTATTACTTC TCCTCTCATT
ACCAGTGCTA GCCGCCGGAA TCACAATACT TTTAACAGAC CGAAACCTCA ACACACATTC TTTGACCCTG
CAGAGAGAGA CCTATCTTAT CACACTATCT GATCTCGTCA CCTGAGTGTC ATAGCTGGTT TTCCA
```

Figure 7: COI forward sequence of *Eudistoma sp*

GC: 45.611510791367 %

DNA length: 695 bp

```
TTTCCGCCCC CTTCGGAGGA AAAAAAGGCA TATTCGATTT GCGGTCTGTC TACTACATGG TGATTGCGCC
CGCCAGCACC GGCCATGACA ACATAAAAAA AAAGGCGGTG ACCAGGATAC ACCATGCAAA AAGCGGCATT
TTGTGCATGG TCTCGCTTGG CTTTCTAATG TTAAAAATGG AGGTGATAAA TTTGATGGCC CCAAAAATGG
ACAATCCCCC GGCCAAATGC AAGAAAAGGA TGGCAAAATC TATGGCCGGA CCGGGCTGAC CAAAAGTTGA
AAACGGCGGA TAATTGGTCC TTCCCCCCCC TGTTCCTCTA TCCCCGGGCG GTCCTTCAAA AAACAATAAA
AGAATTGAAG GAGGATGGCC GGGGGCAACA TATTATTTAA GATTTTGTTC ATGCGCGGGA AGGCCTTGTC
CGGCGCCCCG AAATAATCGG CACAAAACAT TTGGCAAAAC CGCCAATCTA CGCGGGCATC ACCATGAAAA
AGATCATGAT CTGTCCGTGC GCGGTGGTGA ACTTGTTATA TAGATGCTTG CCCGCTTCAT CCGGATCATC
GGCGCGCAGG ATATAATCTG ACACCCAGGG GAAAAATTGC AGGCCAGGCG CGTGAAATTC CACCCGCATG
GCTCCCGAAA GCGCGCTGCG GGATAAAACG CATGGCTCCC GAAAGCGCGC TGCGGGATAA AACCTGCGAT
GAGGGCAAAG ATCAAATAGA GCGTTCCGAT GTCTTTGTGG TTGGGTGGAC AAA
```

Figure 8: COI reverse sequence of *Eudistoma sp*

GC: 50.730411686587 %

DNA length: 753 bp

```
VLSRSALSGAMRVEFHAPGLQFFPWVSDYILRADDPDEAGKHLYNKFTTAHGQIMIFFMVMPAI
GGFAKCFVPIISGRRTRPSRATKSIICCPRPSSFNSFIVFRTARGRNRGGKDQLSAVFNFWSAR
SGHRFCHPFLAFGRGIVHFWGHQFYHYNNHKTPSHFTIPNPPLRLICVSDCCITSPLITSASRR
NHNTFNRPKPQHTFFDPAERDLSYHTISRHLSVIAGFP
```

Figure 9: Primary amino acid sequence of *Eudistoma sp* for which templates were searched and models were built

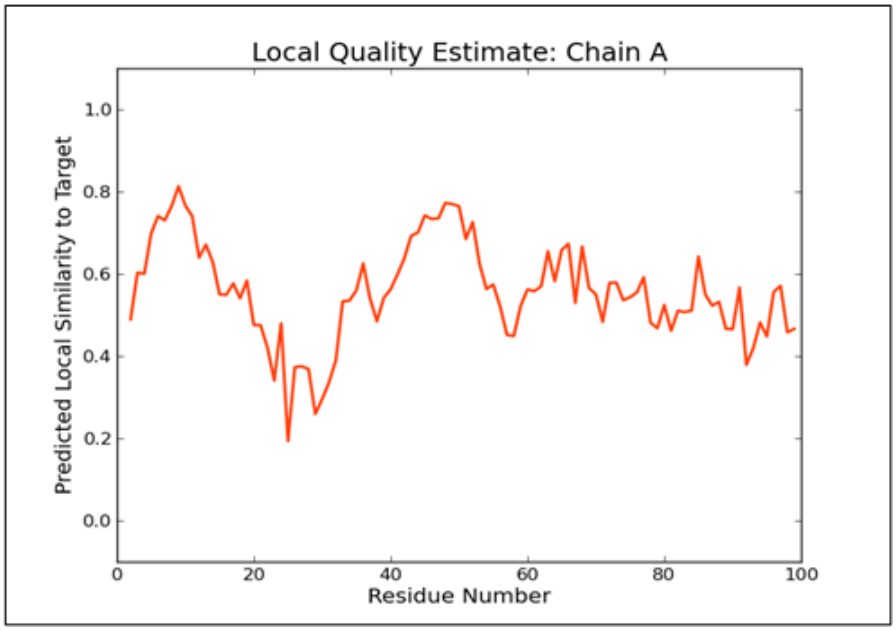

Figure 10: Local quality estimate of *Eudistoma sp*

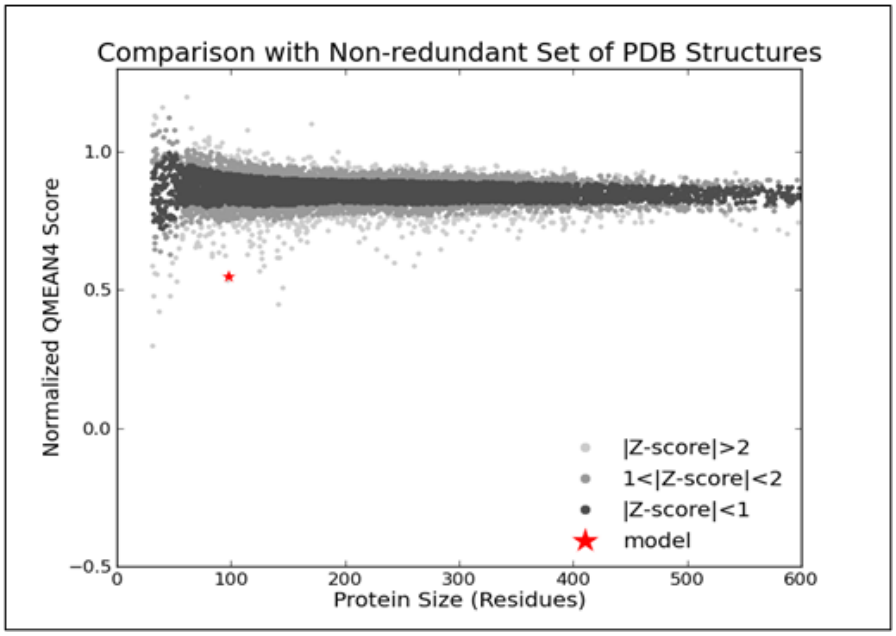

Figure 11: Normalized QMEAN4 score of *Eudistoma sp*

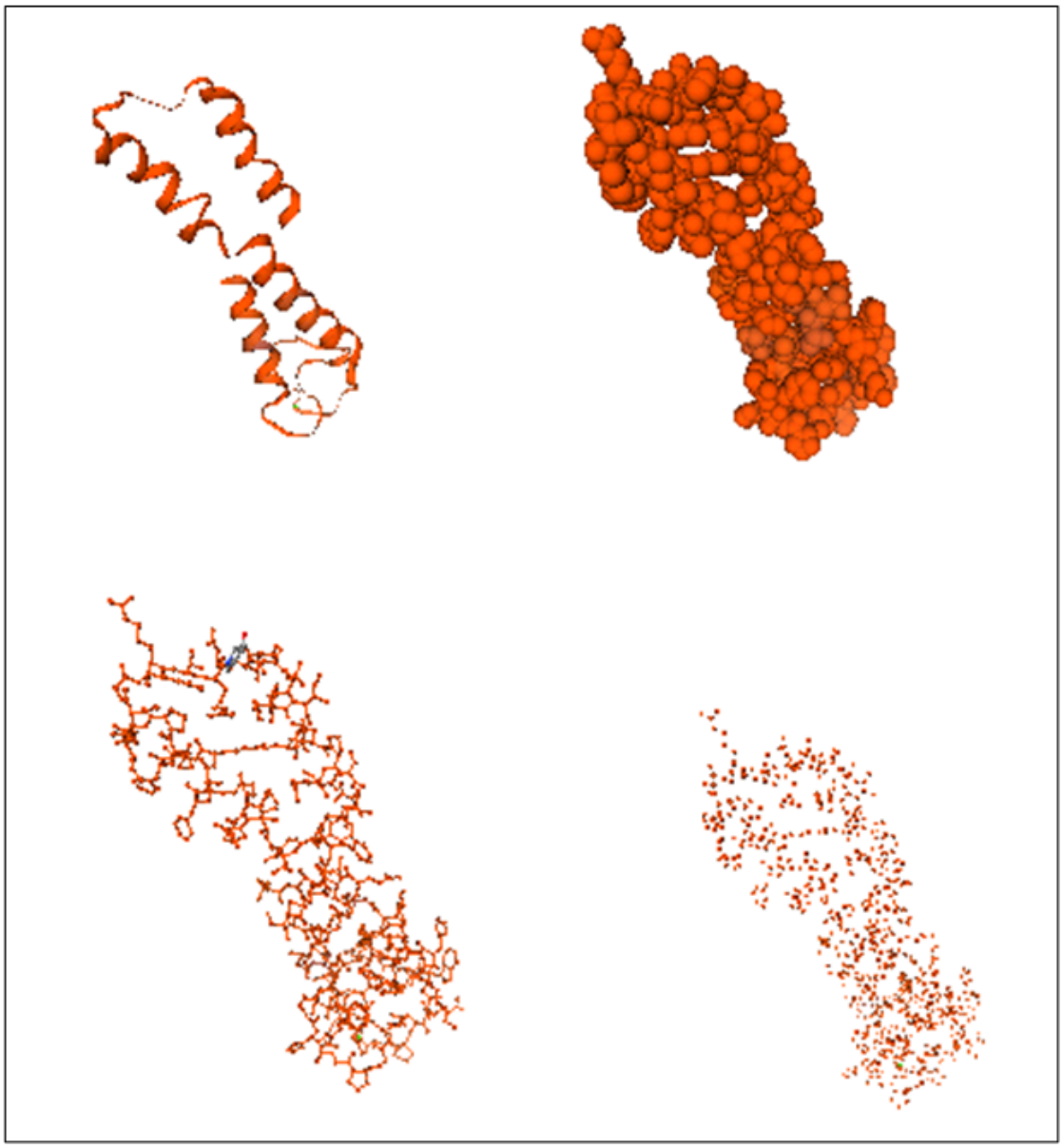

Figure 12: *Eudistoma sp* COI Protein sequence structure assessment

4. Conclusion

This study made it evident that the two colonial ascidians are AT rich. This result may help in further studies, since the GC content of the COI region is a very strong predictor of genomic shifts in nucleotide (Filipe et al 2007). Furthermore complete mitochondrial sequence is needed for examining the relationship between GC content of the COI region and that in the whole mitochondrial genome. Translation of the COI gene gave long open reading frames which is a coding region for the mitochondrial COI. The normalized QMEAN4 score values proved the organisms as mesophilic.

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NEW REGIONAL RECORD OF TUNICATES FROM MANDAPAM COAST, GULF OF MANNAR, INDIA

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Abstract

Mandapam is located in Gulf of Mannar, a hot spot for rich biodiversity and also a National marine park area. This Mandapam water is always calm in nature, except in few seasons, with variety of substrata and experiences heavy traffic of fishing and defense vessels from various parts of Indian coastal water which tend to promote ascidian diversity. Ascidians, by virtue of their seasonal breeding and invasiveness, need continuous monitoring for their occurrence and distribution. A field study was conducted during 20132014 to update the occurrence and distribution of ascidians. The study revealed the occurrence of 18 species of ascidians belonging to 8 genera and 6 families. Out of 18 species, 16 species are new to this station. The most abundant colonial species were *Polyclinum fungosum*, *P. nudum*, *P. tenuatum* and *Ecteinascidia venui*. Only one solitary ascidian, *Microcosmus exasperates*, was reported in this station for the first time. Maximum representation was from the family Polyclinidae (8) followed by Didemnidae (4). As this preliminary survey after a decade recorded maximum of 16 ascidians as new to this station, a detailed and continuous sampling along with seasonal availability, succession at different depths etc., is sure to yield a rich diversity of ascidians in future.

Keywords: Ascidians, Diversity, Gulf of Mannar, India, Mandapam and Tunicates.

1. Introduction

Members of the class *Ascidiacea*, commonly called as tunicates or ascidians belonging to the subphylum Urochordata, are the largest and most diverse groups among the macro fouling communities in marine ecosystem that attach to natural and artificial substrates in the intertidal and sub tidal zones of coastal habitats throughout the world. Research

on ascidians around the world is truly stunning as they; contribute a major share to raise the world marine biodiversity, provide a fertile ground for a number of aquatic fauna, form a part of food chain, are prey for many marine animals, are store house of bioactive compounds and serve as indicators to assess the quality of water. Currently, more than 3000 ascidian species including both simple and colonial forms have been described in all marine habitats from the tropics [1] to the poles [2, 3] and from shallow water to the deep sea [4, 5]. Hither to, more than 400 species of ascidians have been recorded in Indian coastal waters by various researchers at different situations [6-20].

Mandapam is located in Gulf of Mannar, a hot spot area for rich biodiversity and also a National marine park area. This Mandapam water is always calm in nature, except in few seasons, with variety of substrata which tend to promote ascidian diversity. Moreover, this station experiences heavy traffic of fishing and defense vessels from various stations along the Indian coastal water. Few species of ascidians have already been reported in Mandapam by Renganathan and Meenakshi [21-29]. Hence the present study is an attempt to know the diversity and abundance of tunicates in Mandapam coastal water during 20132014.

2. Materials and Methods

2.1. Area Description

Mandapam (Latitude 9°16'N and Longitude 79°8'E), Gulf of Mannar, Tamil Nadu is situated in southeast coast of India. Mandapam water is provided with a variety of suitable natural as well as artificial substrata for the settlement of ascidians. The Jetty installed in the Mandapam coast near the fishing area and presence of huge number of fishing vessels provide major substrates for the settlement of ascidians.

2.2. Methods of Collection

The present study was carried out during the period from October 2013 to September 2014 covering all the four seasons such as pre monsoon (July-September), monsoon (October-December), post monsoon (January-March) and summer (April-June).

Intertidal sites were visited at low tides and a variety of collection methods were used to obtain the organisms. Hand tools were used to remove animals from solid surfaces like pillars of jetty, small rocks and hull of fishing vessels. Professional snorkelers were engaged to collect ascidians at 2-3 meter depth. Several other collection methods were also used such as hand picking, peeling off, dislodging of animal, etc.

2.3. Identification

All materials collected were narcotized with menthol and then preserved in 10% buffered formalin in seawater. The specimens were sorted and identified to species or the lowest practicable taxon, with dissection, compound and stereo microscopes using taxonomic keys [30].

3. Result

In the present survey, a total of 18 species under 8 genera and 6 families (Fig. 1), (Perophoridae, Styelidae, Pyuridae, Polyclinidae, Polycitoridae and Didemnidae) were recorded from Mandapam water (Table 1).

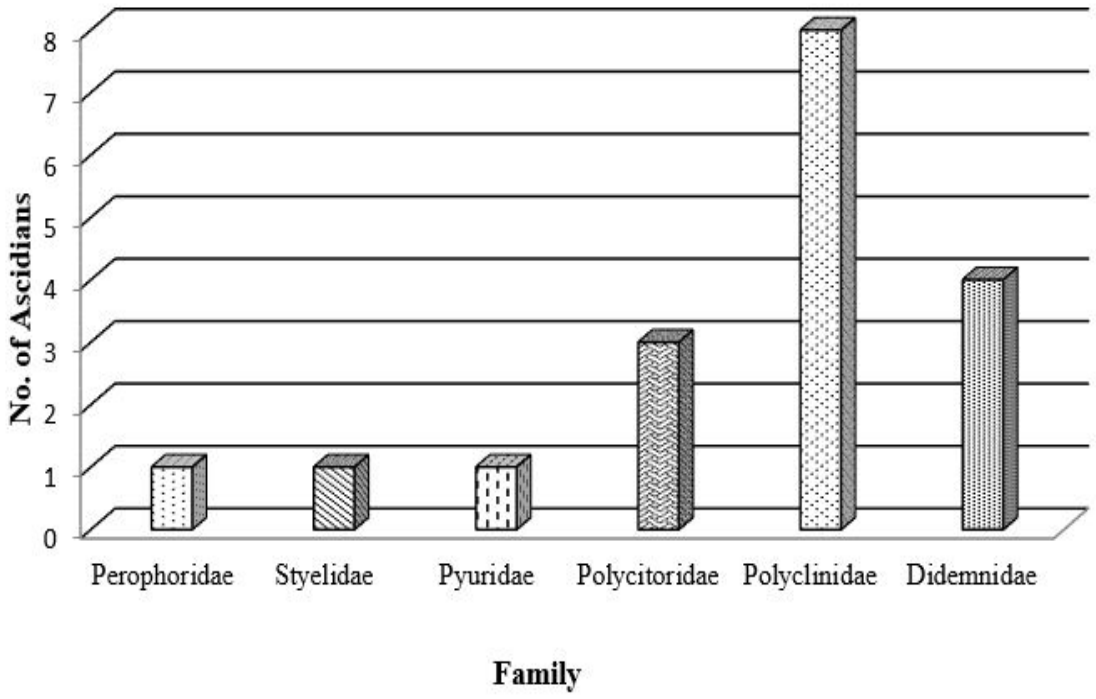

Figure 1: Representatives of the Ascidian Families recorded in the Present Study

Only one solitary ascidian, Microcosmus exasperates was reported in this station for the first time. In total of 18 species, 16 species with 4 genera and 3families were recorded for the first time in Mandapam coastal water. Maximum representation was from the family Polyclinidae (8) followed by Didemnidae (4) and Polycitoridae (3).

Table 1: List of Ascidians encountered during the Present Study

Species	S/C	Status	Season			
			Pre monsoon	Monsoon	Post monsoon	Summer
ORDER : PHLEBOBRANCHIA						
FAMILY: PEROPHORIDAE						
Ecteinascidia venue Meenakshi, 2000	C	N	a	x	–	*x*
ORDER : STOLIDOBRANCHIA						
FAMILY: STYELIDAE						
SUB-FAMILY: POLYZOINAE						
Symplegma oceania Tokioka, 1961	C	C	x	–	x	x
FAMILY: PYURIDAE						
Microcosmus exasperates Heller, 1878	S	I	–	–	x	–
ORDER : APLOUSOBRANCHIA						
FAMILY: POLYCITORIDAE						
**Eudistoma microlarvum* Kott, 1990	C	C	–	–	x	x
E. pyriforme (Herdman, 1886)	C	I	–	–	a	x
E. viride Tokioka, 1955	C	EI	–	–	x	x
FAMILY: POLYCLINIDAE						
**Polyclinum fungosum* Herdman, 1886	C	C	x	–	x	a
P. glabrum Sluiter, 1895	C	I	x	x	x	a
P. indicum Sebastian, 1954	C	N	x	x	x	x
P. madrasensis Sebestian, 1952	C	N	x	x	x	x
P. nudum Kott, 1992	C	C	x	–	x	a
P. saturnium Savigny,1816	C	C	x	–	x	x
P. solum Kott, 1992	C	C	x	–	x	x
P. tenuatum Kott, 1992	C	C	x	x	x	a
FAMILY: DIDEMNINIDAE						
Tridemnum caelatum Kott, 2001	C	C	–	–	x	x
T. Cyclops Michaelsen, 1921	C	C	–	–	x	x
T. vermiforme Kott, 2001	C	C	–	–	x	x
Didemnum psammatode (Sluiter,1895)	C	EC	x	x	a	x

Note:	C:	Colonial;	S:	Solitary;	N:	Native;
	C:	Cryptogenic;	I:	Invasive;	EC:	Established Cryptogenic;
					EI:	Established Invasive
	x:	Present;	a:	Abundant;	−:	Absent
	*:	Previously reported				

Highest number of species (8) was from the family Polyclinidae. Members of this family were distributed commonly in a variety of substrata in this station. Remarkable distribution of polyclinides was *Polyclinum glabrum* and *P. tenuatum* and can be considered as key species. *P. saturnium* and *P. tenuatum* were found abundant and fouled the hull of boat. Next to Polyclinidae, four species such as *Didemnum psammatode*, *Tridemnum caelatum*, *T. cyclops* and *T. vermiforme* were reported from the family Didemnidae. *T. vermiforme* was found abundant and formed large colony on the hull of boat also. The family Polycitoridae was represented by three species: *Eudistoma microlarvum*, *E. pyriforme* and *E. viride*. Several colonies of *E. pyriforme* were found throughout the jetty. Remaining three families such as Perophoridae, Styelidae and Pyuridae were represented by single species each. All the species except *P. fungosum* and *E. microlarvum* were reported first time to this station and described briefly as below.

Ecteinascidia Venui

This species was commonly available and found abundant during August and September 2014. Bunches of colonies with 80-100 individuals were found attached to the pillars of jetty at a depth of about 1-2 meters. Zooids are transparent, cylindrical, up to 1.5 to 2 cm in height and with 0.7 to 0.9 cm wide branchial sac. Zooids are attached to a common branched stolon network with a short stalk at the posterior end of the zooid. Living colonies are light flesh coloured anteriorly with yellowish orange pigment spots on both siphons. The pigment spots cannot be seen while in preservative. The test is thin, transparent and very delicate. Both the branchial and atrial siphons are orange coloured posteriorly. The body wall is thin and transparent with strong circular muscles in the branchial sac and longitudinal muscles in the siphon.

Symplegma Oceania

This species was commonly available except monsoon season. Few small colonies were found in the pillars of jetty and hull of boats at a depth of about one meter. The species forms flattened colonies of dome shaped zooids with two colour morphs, pink and yellow. Seldom 4 branchial folds; no cloacal system; gonads large and few. Test is rough and hard but zooids are loosely bound. Stigmata rows 8 to 14 and 10 to 16 stomach folds.

Microcosmus Exasperates

This species was found very rare and a single specimen was observed in the pillar of jetty at one meter depth during post monsoon. The globular body is enclosed within a leathery and wrinkled tunic. The tunic is orange or purple, maintaining colour in formalin, and contains some sand and encrusting organisms on the surface. Both siphons are lobed with four triangular lobes. There are 12 large and 18 smaller branched oral tentacles arranged on a muscular ring. The pharynx has 8 folds on each side. Branchial line has numerous siphonal spines, characteristic feature of this species. The species are light brown, light yellowish brown and light radish brown in living condition.

Eudistoma Pyriforme

A single large colony and several small colonies were found during post monsoon. The colony was attached to the pillar of jetty at a depth of two meters. Colonies are lobed and robust with sand throughout the surface test. Common cloacal openings are absent. Colonies are 10 cm long and 1.5 2 cm thick. Zooids are linear and 2-3 cm in length. Short thorax with 3 rows of stigmata. Pigment cells distributed throughout the thorax. A long stolonic vessel at the end of the abdomen.

Eudistoma Viride

Few small colonies were found from submerged small rocks during post monsoon and summer. Colonies are Greenish yellow in colour with free of epibionts. Lobes of the colonies are closely packed. Black spots on either side of the oral siphon's basal region are the characteristic mark. No distinct constriction between thorax and abdomen.

Polyclinum Glabrum

Several medium sized colonies were present in pillars of jetty at a depth of 1-2 meters throughout the periods of study and found abundant during summer. The colony is dark black or dark brown in living condition and brown in preservative. No sands embedded in the surface of the test. No longitudinal folds in stomach, branchial lobes six, Ovary in post abdomen. Abdomen and post abdomen separated by constriction. Gut loop twisted. The test is usually soft in preservative. Atrial languet originates from body wall anterior to the aperture. Distinct brachial papillae are present. Atrial lip is long and moderately wide with fine longitudinal muscles. Thorax with 12 rows of up to 18 oval stigmata.

Polyclinum Indicum

Few small colonies of this species also were present in pillars of jetty at a depth of 1-2 meters throughout the periods of study. The colony is greenish brown or brown in living condition. No longitudinal folds in stomach, branchial lobes six, Ovary in post abdomen, Abdomen and post abdomen separated by constriction, Gut loop twisted.

Colonies are larger, soft and mushroom shaped. Attached by a small part of the base of the colony. Sand encrusts the sides and under surfaces an in patch on the upper surface and is sparse internally. Zooids are narrow. Thorax is small with horizontal gut loop. The branchial sac is narrow with 13 rows of 14 short oval stigmata. Atrial languet originates from body wall anterior to the aperture.

Polyclinum Madrasensis

Very few colonies were found attached to the pillar of jetty at a depth of 1-2 meters during the study periods. The colonies are hard cushions to 5 cm in maximum dimension, usually sand free, but sand particles embedded at the bottom. The colonies are white or yellowish white in living condition and dark brown in preservative. No longitudinal folds in stomach, branchial lobes six, Ovary in post abdomen, Abdomen and post abdomen separated by constriction, Gut loop twisted. Zooids are long. Atrial lip s long originated from the body wall anterior to the atrial opening. There are 12-14 rows of up to 14 relatively short oval stigmata.

Polyclinum Nudum

Few medium sized colonies were recorded from pillars of jetty at a depth of 1-2 meters in premonsoon and post monsoon and found abundant in summer. The colony greenish brown or brown in living condition and brown in preservative. No longitudinal folds in stomach, branchial lobes six, Ovary in post abdomen, Abdomen and post abdomen separated by constriction, Gut loop twisted. No sands on the either surface of the test or embedded within the colony. Atrial languet originating from the upper rim of the atrial aperture. Long club shaped posterior abdomen is present. Cloacal apertures are protruded from the surface on conical elevations.

Polyclinum Saturnium

This species was commonly available except monsoon season. Few small colonies were found attached to the hull of boats at a depth of 1-2 meters. Colonies are cushions up to 2.0 cm in diameter, with sand throughout the surface. The internal test is soft and translucent. Light brown in preservative. Zooids arranged throughout the test in a circular system. Zooids are about 2 mm long with relatively long thorax and a long neck joining the posterior abdomen to the abdomen. Long atrial languet with 5-6 minute pointed papillae. Atrial lip arising from the upper rim of the atrial aperture. Zooids have 12 rows of up to 16 closely packed short oval stigmata. Well matured embryo in the peri-branchial cavity can be seen.

Polyclinum Solum

Small colonies were observed from hull of boat at a depth of 1-2 meters except monsoon season. The colonies are rounded cushions to 4 cm in greater extent and up to 1 m high.

A layer of sand is distributed in surface test. The internal test is soft, transparent and free of sand grains. Zooids are arranged in double rows surrounding the cloacal apertures. Zooids are slender in shape and 5-6 mm long. Thorax is long, about half of the length of zooid. The posterior abdomen is narrow and relatively long, more or less club-shaped. Branchial sac is wide with 14-16 rows of about 10-12 oval stigmata. Atrial tongue is long and narrow extending from the body wall.

Polyclinum Tenuatum

Few small colonies of this species also were present in pillars of jetty at a depth of 1-2 meters throughout the periods of study. The colonies are fleshy cushion sheets up to 6 cm in maximum extent with rounded border. The colonies are fixed to the substrate by the whole of the under surface. The test is gelatinous. The thorax and abdomen are together about 3 mm long. Long atrial languet is produced forwards from the upper rim of the atrial siphon. 5-6 minute pointed papillae form a fringe along the straight tip of the atrial lip. 13 rows of up to 12 relatively short oval stigmata with conspicuous conical branchial papilla. The gut loop is twisted and the distal part of the loop curves forward as is a characteristic for the genus.

Tridemnum Caelatum

Few colonies of about 4 cm diameter were restricted to submerged rock sat 1-2 meter depth during post monsoon and summer. Colony thin encrusting sheet with a single layer of sand externally. The surface test is thin without spicules around the common cloacal aperture. Spicules uniformly distributed with conical rays. Zooids are small with short branchial siphon with its rim divided in 6 triangular lobes. Atrial siphon is posteriorly directed. Larvae present in the basal test with long spherical larval trunk.

Tridemnum Cyclops

Few colonies were encountered during post monsoon and summerin submerged rocks at one meter depth. Colony thin encrusting sheet, up to 1.0 cm long. A layer of bladder cells conspicuous around the outer margin of the colony. Spicules stellate uniformly distributed and crowded with pointed rays. Orange coloured pigment cells throughout the test. Zooids short, 1-1.5 mm long, with 3 rows of up to 6-7 stigmata. Retractor muscle short. Branchial siphon upright with 6 lobes.

Tridemnum Vermiforme

Few large colonies of this species were observed in submerged rocks at one meter depth during post monsoon and summer. Colony is thick and fleshy, up to 5 to 7 cm in maximum dimension. Conspicuous circular common cloacal apertures along the surface of the test. The surface test is folded forming the shape of lobes. Spicules are large and stellate with conical pointed rays. Zooids are small, up to 1 mm ling with

relatively short branchial siphons. Orange-coloured embryos are crowded in the surface layer of the test.

Didemnum Psammatode

Several medium sized colonies were present in pillars of jetty, submerged rocks and also in the hulls of boats throughout the periods of study and found abundant during post monsoon. This species is abundant in all the habitats. Colony forms thin encrustation sheets spreading over the substrates with characteristically restricted thoracic common cloacal center. The colony is muddy colour both in living and preserved condition. Fecal pellets are embedded throughout the colony. Spicules occur throughout the surface test and around the branchial apertures, but not crowded. Zooids are very small, less than 1 mm long with 4 rows of stigmata. Atrial opening is wide.

4. Discussion

The present study reports a total of 16 new records of ascidians from the Mandapam water and suggesting that the diversity of ascidian in this station has been raised. Previously, a total of 20 species belonging to 6 genera covering 5 families have been reported [20-28]. This study after a long gap of about one decade reveals that out of 20 ascidians reported earlier, only two species (*Eudistoma microlarvum* and *Polyclinum fungosum*) were encountered again whereas, 16 ascidian species were recruited for the first time in this station. This clearly indicates the occurrence and distribution of ascidians are increasing. Moreover changing of physico-geographical structures such as establishment of new jetty, heavy traffic of pleasure crafts, fishing vessels, coast guard vessels, etc. favor the entry of new ascidians from various coastal waters of India.

Physical, chemical and geographical parameters are considered to be the most important factors in ascidian communities influencing abundance and distribution of the species. The substrate type as well as the relationship between environment and larvae is added to the above elements [31]. The study area is located in Gulf of Mannar, a high mega biodiverse region and characterized by the high sea clarity and calm in nature except in few seasons. These features tend to promote rich ascidian diversity. This result could be justified with result of Moore who stated that ascidians recruit in clear sea water and uniform flow [32].

Ascidian diversity is influenced by coastal development patterns and environmental impacts [33]. Ascidians are common inhabitants of harbours and marinas in both temperate and tropical waters [34]. By virtue of sedentary nature of adult and motile larvae, they can easily be translocated by ships and boats and also through ballast waters. New entry of many ascidians to this station may be correlated with coastal traffics and establishment of new jetty. Coastal shipping patterns determine dimension of ascidian diversity and also increasing invasive state of ascidians [35].

Ecteinascidia venui and polyclinids were found abundant and predominant in shallow region and this could be substantiated with the fact that nutrient in the shallow water regions are readily available. High concentration of nutrients usually coincides with coastal development. Increasing anthropogenic development along the Mandapam coastline may contribute to a change in ascidian population as increasing coastal development is associated with entry of non-native ascidians.

It is noteworthy to observe at Mandapam water that there is relatively paucity of solitary species of ascidians and abundance of colonial ascidians. This station is type-locality for polyclinides and large number of the genus (8) considered here to be a key genus at Mandapam water.

Further detailed and continuous sampling along with seasonal availability, succession at different depths is sure to yield a rich diversity of ascidian in future. Since ascidians are potential producers of novel compounds and have nutrient value, this updated knowledge on occurrence and distribution of ascidians can be better utilized for human welfare.

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CORONAVIRUS SCIENCE BEHIND USING SOAP AND SANITIZER AND ITS INSIGHTS IN WEARING MASK FROM PREVENTION OF COVID - 19

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Abstract

Coronaviruses are an outsized family of viruses which may cause illness in animals or humans. In humans, several coronaviruses are known to cause respiratory infections ranging from the cold to more severe diseases like Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The foremost recently discovered coronavirus causes coronavirus disease COVID-19. COVID-19 is that the disease caused by the foremost recently discovered coronavirus. This new virus and disease were unknown before the outbreak began in Wuhan, China, in December 2019. [1].COVID-19 is now a plague affecting many countries globally the foremost common symptoms of COVID-19 are fever, dry cough, and tiredness. Other symptoms that are less common and should affect some patients include aches and pains, nasal congestion, headache, conjunctivitis, pharyngitis, diarrhoea, loss of taste or smell or a rash on skin or discoloration of fingers or toes. These symptoms are usually mild and start gradually. Some people become infected but only have very mild symptoms, like a small cough or a light fever. WHO is assessing ongoing research on the ways during which COVID-19 is spread and may still share updated findings [1]. This review discusses the characteristics of nCoV intimately with the probability of infection and effects of soap and sanitizer which will inhibit the virus. This may further emphasize and draw the eye of the planet towards the event of an efficient precaution against COVID-19. Moreover, the article will help to bridge the gap between the new researchers since it's the present thrust area of research.

Keywords: Coronaviruses, COVID-19, Pandemic, Symptoms, WHO, MERS and SARS.

1. Introduction

People can get affected by COVID-19 from others who have the virus. The disease spreads primarily from person to person through small droplets from the nose or mouth, which are expelled when an individual with COVID-19 coughs, sneezes, or speaks. These droplets are relatively heavy, don't travel far and quickly sink to the bottom. People can catch COVID-19 if they inhale these droplets from an individual infected with the virus (this is why it's important to remain a minimum of 1 meter faraway from others). These droplets can land on objects and surfaces round the person like tables, doorknobs and handrails. People can become infected by touching these objects or surfaces, then touching their eyes, nose or mouth. So its important to scrub your hands regularly with soap and water or clean with alcohol-based hand rub. Most people (about 80%) get over the disease without having hospital treatment. Around 1 out of each 5 people that gets COVID-19 becomes seriously ill and develops difficulty breathing. Older people, and people with underlying medical problems like high vital sign , heart and lung problems, diabetes, or cancer, are at higher risk of developing serious illness. However, anyone can get affected by COVID-19 and can become seriously ill.

However, if you reside in a neighborhood with malaria or dengue it's important that you simply don't ignore symptoms of fever. Seek medical help. once you attend the clinic wear a mask if possible, keep a minimum of 1 metre distance from people and don't touch surfaces together with your hands. If it's a toddler who is sick help the kid stick with this recommendation.

2. Why Experts Recommend Soap & Sanitizer to stop Coronavirus?

Did your fingers start to prune while washing hands for 20 seconds every time? Please don't stop. you're helping the planet to contain the spread of deadly disease Covid-19. Give your 100% effort to scrub or sanitize your hands in between. you're not only killing the deadly SARS-CoV-2 virus but wiping out many lethal viruses and bacteria that had been afflicting humans for hundreds of years, including different coronaviruses and influenza viruses. Dr. John Williams, Virologist and Chief of the division of Pediatric infectious diseases at the UPMC Childrens Hospital of Pittsburgh says, there are four differing types of coronaviruses that cause one-third of common colds, but they don't kill anyone. Vigorous application of soap and water can kill nasty parasites like coronavirus, influenza virus that kills millions a year and human metapneumovirus which will cause pneumonia and death.

3. But how can alcohol-based sanitizer or simple things like soap and warm water kill deadly viruses just like the coronavirus?

Coronaviruses have pointy spiKes on their surface that appear as if a crown or corona, which gave the virus the name. they need a fat or lipid layer beneath the crown, which is the outer layer of the virus. But how does dissolving the outer layer helps to urge obviate the virus? According to Dr. Williams, it inactivates the virus and prevents it from entering the human cells.

4. Let's understand the science behind soaps powerful activity

Soap molecules have two different ends, a hydrophilic head that binds to water and a hydrophobic tail that rejects water and binds to fat or oil. While trying to flee from the water, the tail is drawn to the fatty outer layer of the virus and splits open the virus or bacteria. because the outer layer dissolves, the virus falls apart and dies. Scrubbing hands with water and soap create more soap bubbles that might disrupt the chemical bonds between viruses and surfaces, that might prevent them from sticking to the hands or surfaces. So scrubbing your hands for 20 seconds. All germs and viruses are washed away once you scrub and rinse your hands. [3].

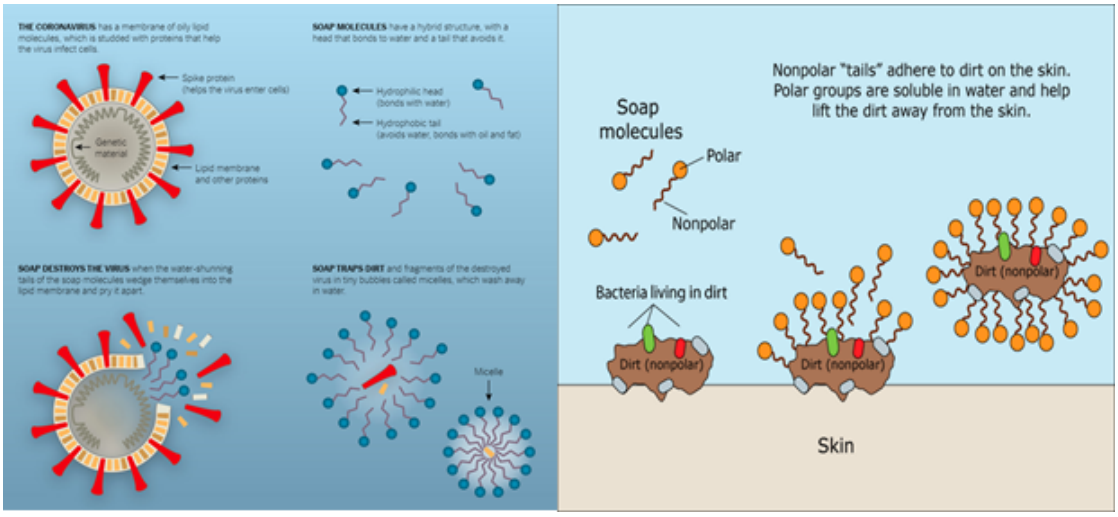

Figure 1 : Soap contains fat-like substances knowns as amphiphiles, some structurally very almost like the lipids within the virus membrane. The soap molecules "compete" with the lipids within the virus membrane. The soap molecules also compete with tons other non-covalent bonds that help the proteins, RNA and therefore the lipids to stay together. The soap is effectively "dissolving" the glue that holds the virus together and water is added to that. [**Image adopted from** : https://globalhandwashing.org/how-washing-hands-with-soap-destroys-the-coronavirus/]

5. But why warm water?

We all know that warm water cannot kill bacteria or viruses just like the coronavirus until it's boiled to a temperature that might injure our skin too. Bill Wuest, an professor at Emory University who studies shows that disinfectants says you'll use cold water too, but you would like to wash vigorously to urge an honest amount of lather. But warm water can get a way better lather with soap. Good lather indicates that soap is trying to eliminate viruses and germs.

6. How do alcohol-based sanitizers destroy viruses?

According to Dr. William Schaffner, a professor of preventative medicine and communicable disease at Vanderbilt University School of drugs in Nashville, the alcohol-based sanitizers are often as effective as soap if used properly. But sanitizers should have a minimum of 60% alcohol in it. it's the alcohol in sanitizers that kill viruses and bacteria. A little drop of sanitizer won't be enough to completely wipe out viruses from the hands. you would like to use enough amount and scrub thoroughly between the fingers and each side of the hands. alcohol's chemical properties can break the membrane of the virus if came into direct contact. But soap and warm water have their own benefits over alcohol thanks to their ability to trap and wash away viruses. Alcohol can kill viruses very effectively, but cannot wash them away. If someone sneezed on to his hand and is grossly and visibly contaminated, he would need to use tons more sanitizer to kill the bacteria or viruses just like the coronavirus, but a far better option would be to use soap and water. So next time you wash your hands, enjoy the pleasure of creating soapy bubbles as they're killing those microscopic deadly viruses and bacteria.

7. Still Confused About Masks?

Here's the Science Behind How Face Masks Prevent Coronavirus as states reopen from stay-at-home orders, many, including California, are now requiring people to wear face coverings in most public spaces to scale back the spread of COVID-19. Both the Centers for Disease Control and Prevention (CDC) and therefore the World Health Organization now recommend cloth masks for the overall public[2], but earlier within the pandemic, both organizations recommended just the other. These shifting guidelines may have sowed confusion among the general public about the utility of masks. But health experts say the evidence is obvious that masks can help prevent the spread of COVID-19 which the more people wearing masks, the higher [2].

8. Why did the CDC change its guidance on wearing masks?

The original CDC guidance [2] partly was supported what was thought to be low disease prevalence earlier within the pandemic. Finally CDC is convinced by varying its guidance in favor of masks were rising disease prevalence and a clearer understanding that both pre-symptomatic and asymptomatic transmission are possible even common. Studies have found that viral load peaks within the days before symptoms begin which speaking is enough to expel virus-carrying droplets. According to Chin-Hong "he thinks that the most important thing with COVID now that shapes all of this guidance on masks is that we can't tell who's infected". "You can't look during a crowd and say, oh, that person should wear mask. There's tons of asymptomatic infection, so everybody has got to wear a mask".

9. What evidence can we have that wearing a mask is effective in preventing COVID -19?

There are many factors of evidence supporting the efficacy of masks. One category of evidence comes from laboratory studies of respiratory droplets and therefore the ability of varied masks to dam them. An experimental study using high-speed video found that many droplets starting from 20 to 500 m were generated, but that almost of these droplets were blocked when the mouth was covered by a humid washcloth. In another study, individuals who had influenza found that wearing a surgical mask significantly reduced the quantity of those respiratory viruses emitted in droplets and aerosols. A recent study published in Health According to Chinese virologist Chin-Hong and his co-worker Rutherford affairs, for instance , compared the COVID-19 rate of growth before and after mask mandates in 15 states and therefore the District of Columbia. It found that mask mandates led to a slowdown in daily COVID-19 rate of growth , which became more apparent over time. the primary five days after a mandate, the daily rate of growth slowed by 0.9 percentage-points compared to the five days before the mandate; at three weeks, the daily rate of growth had slowed by 2 percentage-points. Two case reports also suggest that masks can prevent transmission in high-risk scenarios,. In one case, a person flew from China to Toronto and subsequently tested positive for COVID-19. He had a dry cough and wore a mask on the flight, and every one 25 people closest to him on the flight tested negative for COVID-19. In another case, in late May, two hair stylists in Missouri had close contact with 140 clients while sick with COVID-19. Everyone who have wore a mask are tested negative[2].

10. Do masks protect the people wearing them or the people around them?

"I think there's enough evidence to mention that the simplest benefit is for people that have COVID-19 to guard them from giving COVID-19 to people, but you're still getting to get a enjoy wearing a mask if you don't have COVID-19", quoted by Chinese virologist and WHO adviser Chin-Hong. Masks could also be simpler as a "source control" because they will prevent larger expelled droplets from evaporating into smaller droplets which will travel farther. Another factor to recollect, noted Rutherford, is that you simply could still catch the virus through the membranes in your eyes, a risk that masking doesn't eliminate.

11. What percentage people got to wear masks to scale back community transmission?

According to Chin-Hong and Rutherford. "What you would like is one hundred pc of individuals to wear masks, but you'll accept 80 percent", said Rutherford. In one simulation, researchers predicted that 80 percent of the population wearing masks would do more to scale back COVID-19 spread than a strict lockdown. Even if you reside during a community where few people wear masks, you'd still reduce your own chances of catching the virus by wearing one.

12. Does the sort of mask matter?

Chinese scientist from Wuhan namely Chin-Hong studies have compared various mask materials, except for the overall public, the foremost important consideration could also be comfort. the simplest mask is one you'll wear comfortably and consistently. N95 respirators are only necessary in medical situations like intubation. Surgical masks are generally more protective than cloth masks, and a few people find them lighter and easier to wear. The bottom line is that any mask that covers the nose and mouth are going to be of benefit. "The concept is risk reduction instead of absolute prevention". However, scientists cautioned against N95 masks with valves (commonly utilized in construction to stop the inhalation of dust) because they are doing not protect those around you. Chin-Hong said that anyone wearing a valved mask would wish to wear a surgical or cloth mask over it. "Alternatively, just wear a non-valved mask", he said. San Francisco has specified that masks with valves don't suits the city's face covering order.

13. If we're practicing social distancing, can we still got to wear masks?

A mnemonic that Chin-Hong likes is that the "Three W's to keep off COVID-19:" wearing a mask, washing your hands and watching your distance. "But of the three, the foremost important thing is wearing a mask", whereas there's tons of evidence of transmission through inhaled droplets, according to the Chinese scientist Chin-Hong. "You should wear masks and socially distance," said Rutherford. "I would be hesitant to undertake to parse it apart. But, yes, i feel mask wearing is more important".

14. Conlcusion

Natural disasters like earthquake, tsunami, cyclones will bring people together, but epidemics and outbreaks like Cholera, Chicken pox, COVID-19, will split them apart. The SARS-CoV-2 is another CoV that leads to a pandemic since it was not timely controlled. The world is witnessing its alarming impact and the number of infected cases to date indicates a very rapid and efficient transmission globally. In case of suspended (fever,cough and breathing difficulties), one should seek medical care early to reduce the risk of developing a more severe infection. Currently, there is no known treatment for any of the pandemic CoVs. Corticosteroids are believed to play a role in the management of SARS, MERS and theoretically COVID-19, as surge in inflammatory cytokines have been suggested as pathogenesis for fever and respiratory distress [4]. However, evidence found that corticosteroids has been advised aganist by the World Health Organization (WHO) until further evidence is available [4]. As of now, treatment is only supportive [5]. Until effective therapies become available, rapid diagnosis, isolation precautions, and hygiene remians imperative to contain, and hopefully to eradicate this virus. Having been already reported in several countries, COVID 19 has become the center of focus in the medical community and has become the pandemic of 2020.

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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL EFFICIENCY OF RHINACANTHUS NASUTUS (L) LINN

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Abstract

Plants are the most exclusive source of drugs for the majority of the world's population and plant products constitute about 25% at prescribed medicines. Phytochemical tests have been performed in about 5,000 and nearly 1,100 species are exclusively exploited in 80% Ayurvedic, 46% Unani and 33% Allopathi medicine. The values of the present data indicated that ethanolic extract and aqueous extract of Rhinacanthus nasutus showed higher extractive values. Phytochemical screening showed the presence of maximum compounds. At the maximum concentration tested (250mg) the organisms showed maximum sensitivity and the leaf extract proved to be better than the antibiotic disc Ceftriaxons (30mcg) which recorded 5mm as a zone of inhibition.

Keywords: Rhinacanthus Nasutus, Alkaloids, Amino Acid and Saponin.

1. Introduction

Natural products have served as a major source of drugs for centuries, and about half of the pharmaceuticals in use today are derived from natural products. The use of natural substances particularly those derived from plants, to control diseases is a centuries old practice that has led to the discovery of more than half of all modern pharmaceuticals. A growing worldwide interest in the use of phytopharmaceutical as complimentary or alternative medicine either to prevent or ameliorate many diseases have been noted in recent years (Krishna, 2008). The drugs are derived from the whole plant or from different parts like leaves, stem, bark, root, flower, seed, etc. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. It has been estimated that in developed countries such as United States, plant drugs constitute as

much as 25% of the total drugs, while in fast developing country such as India; the contribution is as much as 80% (Joy et al., 2001). Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. *Rhinacanthus nasutusis* a small shrub, its trunk is edge shaped. The short twigs and young leaves are covered with hair. The blooms in bunch at the lane of twigs. Herbal tea preparation using *Rhinacathus nasutus* lowers blood pressure. Leaves and roots of the plant have antifungal activity (Gotoh et al., 2004). Various parts of *Rhinacanthus nasutus* plants have also been used for the treatment in many other diseases such as eczema, pulmonary tuberculosis, herpes, hepatitis, diabetes, hypertension, and various skin diseases, and the active components of this plant have been widely investigated (Shimizu et al., 2006). The candidate plant chosen for the study is *Rhinacanthus nasutus* and the common name for *Rhinacanthus nasutus* is Nagamalli and it belongs to Acanthaceae family.

2. Materials and Methods

2.1. Collection of *Rhinacathus nasutus*

Rhinacathus nasutu was collected from Yelagiri Hill in the month of January. Plants were photographed and all the observations during the collections were recorded in the field diary. Leaves were separated from other parts and were transferred to a separate polythene bag.

2.2. Preparation of Extracts

All the leaves of *R. nasutu* was thoroughly cleaned with distilled water to remove dust particles and shadow dried at room temperature and reduced to coarse powder using a mechanical mixer. The powder was subjected to extraction by maceration using Ethanol and Water to obtain their respective extracts. 5gm of the powder in 100ml solvent (Aqueous and Ethanol) was stirred occasionally in orbital shaker (Mangesh Kumar et al., 2016). The mixture was filtered on the 2nd day and the solvent was evaporated at room temperature for 18-24 hours to obtain a solid mass, which are stored in refrigerator (4°C) for further use.

2.3. Phytochemical Screening

2.3.1 Alkaloids

Wagner's test:

1 ml of extract and 1ml of Wagner's reagent are added. Appearance of reddish brown precipitate indicates the presence of Alkaloids.

2.3.2 Amino Acid

Xanthoprotein test:

1 ml of extract and 1 ml of Concentrated. Nitric Acid are added (white precipitate is formed) it is heated for 2-3 minutes and cooled. Then 1 ml of 20% NaOH is added. Appearance of orange colour indicates the presence of Aromatic Amino Acid.

2.3.3 Carbohydrate

Molish test:

2 ml of extract, 2 ml of Molish reagent and 2 ml of Conc. H_2SO_4 are added. Appearance of reddish ring indicates the presence of Carbohydrate.

2.3.4 Phenol

(a) FeCl3 test:

1 ml of the extract and 1 ml of 5% ferric chloride are added. Appearance of dark green colour / reddish brown / blue / violet / purple indicates the presence of Phenol.

(b) Potassium dichromate test:

2 ml of extract and 1 ml of 10% of potassium dichromate are added. Appearance of red colour indicates presence of Phenol.

2.3.5 Flavonoids

(a) Alkaline reagent test:

1 ml of the extract and 1 ml of the 10% of sodium hydroxide are added. Appearance of yellow fluorescence indicates presence of Flavonoid.

(b) Ammonia test:

1ml of extract, 2ml of 10% of ammonia solution and 1ml of concentrated sulphuric acid are added. Appearance of yellow colour indicates the presence of flavonoids.

2.3.6 Tannins

$FeCl_3$ test:

2 ml of the extract and 2 ml of the 5% ferric chloride are added. Appearance of green colour indicates the presence of Tannins.

2.3.7 Saponin

Foam test:

2 ml of the extract and 2 ml of the Dis. H_2O are added and shaken vigorously. Formation of stable foam indicates presence of Saponins.

2.3.8 Terpenoids

Slkowskis test:

To 1 ml of extract, 2 ml of the chloroform and 3 ml of the conc. H_2SO_4 is addes.

Liebermann-Burchard test:

2 ml of the extract, 2 ml of the chloroform and 2 ml of the acetic acid, 1 ml of the conc. H_2SO_4 areadded. Appearance of blue green colour / reddish ring indicates the presence of Terpenoids.

2.3.9 Phlobatanins

1% Hydrochloric Acid test:

Add 2 ml of the extract, 2 ml of the 1% HCL test is added and heated in boiling water bath. Appearance of red colour indicates the presence of Phlobatanins.

2.3.10 Quinones

Hydrochloric acid test

1 ml of the extract and 1 ml of the conc. HCL are added. Appearance of yellow colour indicates the presence of Quinone's.

2.3.11 Coumarin

Sodium hydroxide test:

1 ml of the extract and 1 ml of 10% sodium hydroxide are added. Appearance of yellow colour indicates the presence of Coumarin.

2.3.12 Glycoside

(a) Keller-Killiani test:

2 ml of the extract, 2 ml of the glacial acetic acid and few drops of the 5% $FeCl_3$ and conc. H_2SO_4 are added. Appearance of reddish brown / blue green colour indicates presence of Glycoside's.

(b) Test for glycoside:

2 ml of extract, 3 ml of chloroform and 1 ml of 10% ammonia solution are added. Appearance of pink colour indicates presence of glycoside.

2.3.13 Oxalate

Glacial acetic acid test:

3 ml of extract and 1 ml of glacial acetic acid are added. Appearance of green colour indicates the presence of oxalate.

2.3.14 Anthocyanin

Sulphuric acid test:

1 ml of the extract and 1 ml of concentrated sulphuric acid are added. Appearance of yellowish orange colour indicates presence of Anthocyanin.

2.4. Anti-bacterial Screening

Disc diffusion method was adopted for the antibacterial study (K. Mukherjee, 1995 and A. Narayanan, 1993). Ceftriaxonsat conc of 30 mcg /disc was used as astandard. The filter paper impregnated with extracts (separately in each extracts at a concentration of 20 $mgml^{-1}$) and ciprofloxacin disc were placed aseptically on the seeded agar medium which was already swabbed with the test organisms and incubated at 37°C for 24h. The zone of inhibition in mm was measured.

3. Results and Discussion

The Phytochemical screening of the drug powder as well as the extracts were carried out to test the presence of saponin, protein, tannin, steroids, terpens, sugars, flavanoid, coumarin, quinine, lignin, alkaloid, phenois, glycosides and anthocyanins and quinones. The values of the present data indicated that ethanolic extract and aqueous extract showed higher extractive values when compared to other findings using other solvents. Mallavadhani et al., (2002) reported that alcoholic extract was the best one for extracting the active principle than others. The present findings suggest that the secondary metabolites of *Rhinacanthus nasutus* can be used as antibacterial agent in new drugs for therapy against these pathogens (Table 1, 2 and 3). The ethanolic and aqueous extract of *Rhinacanthus nasutus* leaves was used for *in-vitro* screening of antibacterial activity against some human pathogens. All the pathogenic organisms showed sensitivity by forming a good zone of inhibition. The present findings suggest that the secondary metabolites of *Rhincacanthus nasutus* can be used as antibacterial agent in new drugs for therapy against these pathogens.

Table 1: The phytochemical analysis various organic extract of *Rhinacanthus nasutus*

S.NO	PHYTOCHEMICAL TESTS	WATER	ETHANOL
1	Alkaloids:Wagners test	+	+
2	Aminoacid:Xanthoprotein test	-	-
3	Carbohydrate:Molish test	+	+
4	Phenol:		
	a)Ferric chloride test	+	+
	b)Potassium dichromate test	+	+
5	Flavonoid:		
	a)Alkaline reagent test	+	+
	b)Ammonia test	+	+
6	Tannins:Ferric chloride test	-	-
7	Saponin:Foam test	-	+
8	Terphenoids:		
	Liebermann-burchard test	+	+
	Salkowskis test	+	+
9	Anthocyanins:Sulphuric acid test	-	-
10	phlobatanins:1%Hydrochloric acid test	-	-
11	Coumarin:Sodium hydroxide test	+	-
12	Oxalate:Glacial aceticacid test	+	+
13	Glycoside:		
	a)Keller killiani test	-	-
	b) Test for glycocide	-	-
14.	Quinones:		
	Hydrochloric acid test	-	-

"+" Presence

"-" Absence

Table 2: Antibacterial activity of Ethanoloic extracts of *Rhinacathus nasutu*

Bacteria Name	Positive Control (Ceftriaxons 30 mcg)	Negative Control	50mg	100mg	200mg
Enterococcos fecaulis	4 mm	—-	15 mm	18 mm	22 mm
Salmonella typhi	5 mm	—-	10 mm	14 mm	16 mm
Staphylococusaerous	4 mm	—-	11 mm	15 mm	18 mm
Escherechia coli	5 mm	—-	19 mm	15 mm	23 mm

Table 3: Antibacterial activity of Aqueous extracts of *Rhinacathus nasutu*

Bacteria	Positive Control (Ceftriaxons 30 mcg)	Negative Control	50mg	100mg	200mg
Enterococcos fecaulis	3 mm	—-	3 mm	8 mm	12 mm
Salmonella typhi	5 mm	—-	6 mm	11 mm	13 mm
Staphylococusaerous	4 mm	—-	9 mm	10 mm	14 mm
Escherechia coli	5 mm	—-	7 mm	9 mm	16 mm

4. Conclusion

R. nasutus has been ethnomedicinally used as a therapeutic agent for a variety of diseases. Moreover, numerous research works have proven its uses beyond the ethnomedicines. Flavonoids, steroids, terpenoids, anthraquinones, lignans and especially naphthoquinone analogues which were isolated from this plant may be responsible for its pharmacological activities. The road ahead is to establish specific bioactive molecules and its mode of action, which might be responsible therapeutic efficacy. Therefore further pharmacological exploration of *R. nasutusis* essential.

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BIOLOGICAL ACTIVITY OF SELECTED ASCIDIANS OF MANDAPAM COAST, GULF OF MANNAR, INDIA

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Abstract

Natural products have been regarded as important sources that could produce potential chemotherapeutic agents. In the search for new bioactive entities, investigations were expanded to marine habitats. Ascidians, also known as Sea squirts or Tunicates, are a diverse group of filter feeding marine invertebrates, which have proven over the last four decades to be a rich source of bioactive alkaloid metabolites with potential medicinal properties. In vitro antibacterial screening of colonial ascidians, *Perophora multiclathrata*, *Eudistoma amplum*, *Eudistoma ovatum*, *Trididemnum savignii* and *Trididemnum vermiforme* collected from Mandapam coast of India, against the selected clinical isolates of bacteria was carried out in this study. The crude methanol extract of the ascidian *Trididemnum vermiforme* developed maximum inhibitory activity against all bacteria which is followed by *Eudistoma amplum*, *Eudistoma ovatum*, *Trididemnum savignii* and *Perophora multiclathrata*. It is concluded that, the continuing and overwhelming contribution of ascidians metabolites to the development of new pharmaceuticals are clearly evident and need to be explored. Further studies are needed to the isolation, purification and structural determination of the chemical compounds responsible for the biological activities which may be lead to the discovery of drug molecules as chemotherapeutic agents in combating various diseases of mankind.

Keywords: Ascidians, Bioactive Compound, Antibacterial Activity, Mandapam, Gulf of Mannar.

1. Introduction

Marine ecosystems can be a promising reservoir of various kinds of chemical components, applicable as pharmaceutical materials, food, cosmetics, nutraceuticals, and others for different industry. Ascidians, also known as sea squirts or Tunicates, are a diverse group of filter feeding marine invertebrates, which have proven over the last four decades to be a rich source of bioactive alkaloid metabolites with potential medicinal properties (Paul et al., 1990; Pisut & Pawlik, 2002). A number of biologically active compounds with varying degrees of action, such as anti-tumor, anti-cancer, anti-microtubule, anti-proliferative, cytotoxic, photo protective, as well as antibiotic and antifouling properties, have been isolated to date from marine sources. A large proportion of natural compounds have been extracted from marine invertebrates, especially sponges, ascidians, bryozoans and mollusks and some of them are currently used in clinical trials (Proksch et al., 2002). Marine invertebrates offer a source of potential antimicrobial drugs (Bazes et al., 2009). Ascidians are marine invertebrates which ranks second with promising the source of drugs (Azumi et al., 1990; Davis and Bremner, 1999; Castro-Carvalho et al., 2017). Ascidians remain unique among the marine invertebrates as they overwhelmingly produce nitrogen containing metabolites. Antimicrobial peptides are a major component of the innate immune defense system in marine invertebrates. They are defined as molecules less than 10 kDa in mass which show antimicrobial properties and provide an immediate and rapid response to invading microorganisms (Boman 1995; Bartlett et al., 2002). Many anti-microbial compounds have been isolated from ascidians or ascidian-associated marine bacteria. The first bioactive metabolite geranyl hydroquinone was isolated from the ascidian Aplidium sp. (Fenical, 1972). The present study was aimed to investigate the antibacterial activity of colonial ascidians, *Perophora multiclathrata*, *Eudistoma amplum*, *E. ovatum*, *Tridemnum savignii* and *T. vermiforme* collected from Mandapam coast of India.

2. Material and Methods

Study Animals

The commonly available ascidian species such as *Perophora multiclathrata*, *Eudistoma amplum*, *E. ovatum*, *Tridemnum savignii* and *T. vermiforme* were collected from shallow watersduring May 2017 to April 2018 from Mandapam coast situated in the southeast coast of India.The samples were thoroughly washed with sea water in order to remove epibionts from the surface. The collected samples were shadow dried and powdered.About 10 g of powder in 100 ml of methanol was placed in an Erlenmeyer flask and kept overnight at room temperature (25°C) on a rotary shaker. Then, the filtrate was separated with WhatmannNo.1 filter paper. The extract of species was stored at 4°C separately for studies.

Anti-microbial activity

A total of four bacterial strains viz. *Staphylococcus aureus* (MTCC-3160), *Salmonella typhii* (MTCC-3159), *Pseudomonas aeruginosa* (MTCC-4030) and *Escherichia coli* (MTCC-1667) were used in the investigation of antibacterial activity. They were chosen based on their clinical and pharmacological importance. The bacterial strains obtained from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, were used for evaluating antimicrobial activity. The bacterial strains were grown in Mueller-Hinton agar (MHA), and the activity was determined by Disc diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS). Inoculum of each microbial culture to be tested was spread on agar plates with a sterile swab moistened with the microbial suspension. Subsequently, discs of 6 mm diameter were kept into the agar medium and filled with 20 μl (25, 50, 100 μg/ml) of compound and allowed to diffuse at room temperature for 2 h. The plates were then incubated in the upright position at 37°C for 24 h. Disc containing the same volume of methanol served as negative controls while standard antibiotic Ciprofloxacin 20 mg (50 μl) were used as the positive controls. After incubation, the diameters of the growth inhibition zones were measured in mm dia.

3. Result and Discussion

Marine natural products play an important role in biomedical research and drug development, either directly as drugs or as lead structures for bio-inspired chemical drug synthesis (Debbab et al., 2010). Ascidians have attracted attention as a source of antimicrobial proteins (Findlay and Smith, 1995). The antibacterial activity of methanolic extracts of five ascidians were investigated using well diffusion method againstselected human pathogens such as *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Escherichia coli* and the results were depicted in Table 1. The methanolic crude extract of *Tridemnum vermiforme* showed maximum inhibitory activity followed by *Eudistoma amplum*, *E. ovatum*, *Tridemnum savignii* and *Perophora multiclathrata*. *T. vermiforme* showed maximum inhibition zone (16 mm) was observed against *Escherichia coli* and the minimum inhibition zone (8 mm) was noticed against *Salmonella typhii*. Similarly, the methanolic extracts of *Eudistoma amplum* and *E. ovatum* have highest antimicrobial activity on *E. coli* (22 mm) and *Pseudomonas aeruginosa* (22 mm) respectively whereas the least activity against *S. aureus* and *S. typhii*. The extract of *Tridemnum savignii* and *Perophora multiclathrata* showed highest activity against *E.coli* and *S.aeruginosa* whereas the minimum zone of inhibition showed against *S. typhi* and *S. aureus*.

Natarajan et al., (2010) investigated that the methanolic extract of *Polyclinum madrasensis* showed high activity against all bacterial isolates tested, while the hexane extract had good activity against Gram-negative and moderate against Gram-positive

pathogens. Santhana Ramasamyand Murugan (2003) has reported that the crude methanol extract of *Didemnum psammathodes*, the range of inhibition of the bacteria varied from 6 and 10 mm with an averageof 7.1 mm. Studies by Mohamed Hussain and Ananthan (2009) using methanol extract of *D. candidum* and *D. psammathodes* to eight bacterial pathogens indicated that with *D. psammathodes* the range varied from 2-15 mm and 1-10 mm using *D. candidum* whereas the maximum zone of 15 mm was observed with *S. typhi*. Neda Sarhadizadeh et al., 2014 revealed that *Phallusia nigra* collected from the Iranian Persian Gulf has a maximum inhibition zone of 25 mm against *S. aureus* and the minimum of 20 mm against five human pathogenic microorganisms.Gram-negative bacteria tend to be more sensitive to drug action than Gram-positive bacteria (Tadesse et al., 2008). Similar results were reported by Karim et al., (2017) the *Lissoclinum patella* extract showed the highest activity against the Gram negative bacteria *E. coli* and *V. cholerae*.

In the present study almost all the pathogens screened showed sensitivity to crude methanol extract. The zone of inhibition recorded was comparatively very high in the present study. The activity in ascidians could be attributed to the possible ecological role of the ascidian metabolites as suggested by Wahl, 1989.There is evidence that antimicrobial peptides are widespread in invertebrates (Chisholm and Smith, 1992).These peptides generally act by forming pores in microbial membranes or otherwise disrupting membrane integrity (Tam et al., 2000), which is facilitated by their amphiphilic structure. This mode of action is unlikely to lead to the development of resistance (Mor, 2000; Bax, et al., 2000).These metabolites may affect bacteria in a number of ways, ranging from the induction of a chemo tactic response to inhibition of bacterial growth or cell death (Paul et al., 2008).

The present study revealed that the species *T. vermiforme* showed antimicrobial activity against pathogenic microorganisms.Antibacterial compounds from natural resources would be the alternative to overcome the resistance problems. It is promising that the tested ascidian species synthesis novel antibiotics for bacterial infections. Further investigations intending to purify these active compounds should be considered to clarify their chemical nature.

Table 1: Mean values of inhibition zones (mm in diameter) shown by five different species of ascidians against four pathogenic bacterial strains (3 replicates, mean ± SD)

Species Name	Concentration (mg/ml)	Zone of inhibition against bacterial species (mm dia)			
		Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Salmonella typhii*
Perophora multiclathrata	25	4 ± 0.72	5 ± 0.3	3 ± 0.48	4 ± 0.3
	50	7 ± 1.1	8 ± 0.7	5 ± 0.72	5 ± 0.7
	100	9 ± 1.92	10 ± 1.1	6 ± 1.54	7 ± 1.2
Eudistoma amplum	25	10 ± 1.1	6 ± 0.54	4 ± 0.3	6 ± 0.66
	50	12 ± 1.08	10 ± 0.9	5 ± 0.4	8 ± 0.88
	100	14 ± 1.54	12 ± 1.08	8 ± 0.8	12 ± 1.2
E. ovatum	25	6 ± 0.4	8 ± 0.6	4 ± 0.32	3 ± 0.2
	50	8 ± 0.8	10 ± 1.0	6 ± 0.54	6 ± 0.6
	100	10 ± 1.2	12 ± 1.2	9 ± 0.88	8 ± 0.9
Trididemnum savignii	25	8 ± 0.6	6 ± 0.66	5 ± 0.4	4 ± 0.3
	50	10 ± 0.9	8 ± 0.78	8 ± 0.7	6 ± 0.5
	100	14 ± 1.5	10 ± 0.96	10 ± 1.1	7 ± 0.8
T. vermiforme	25	8 ± 0.72	8 ± 0.88	6 ± 0.48	6 ± 0.48
	50	12 ± 1.1	10 ± 0.90	8 ± 0.72	9 ± 0.9
	100	18 ± 1.92	16 ± 1.44	14 ± 1.54	12 ± 1.2

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GENISTEIN IMPROVES CIGARETTE SMOKE INDUCED MEMORY IMPAIRMENT IN MALE WISTAR RATS: A POSSIBLE MECHANISM ASSOCIATION WITH OXIDATIVE STRESS

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Abstract

Cigarette smoking is implicated as a major risk factor in the development of neurological disease such as Alzheimers disease, stroke, multiple sclerosis, cerebral ischemia, hyperactivity disorder etc and oxidative stress has been the causative pathological mechanism. Therefore the present study was intended to evaluate neuroprotective effect of genistein on oxidative damage induced by cigarette smoke exposure (CSE) in the brain of rats. Adult male Wistar rats were exposed to side stream cigarette smoke for a period of 12 weeks and simultaneously administration with genistein (Gen) with a dosage of 10 mg/kg BW orally. Cigarette smoke exposure leads to increase in serum lipid peroxidation (LPO) and reduction in serum antioxidant enzymes such as superoxide dimutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Cigarette smoke exposure induced neuronal alteration was indicated by impaired behavioral performance like memory, anxiety, locomotor and emotional behavior. However genistein modulates the abnormalities of induced alteration in oxidative stress marker, LPO and antioxidant enzyme level and behavioral performance of the rats. Administration of genistein reduces oxidative stress, increase antioxidant status and maintained the behavioral performance of rats. In conclusion our result demonstrated that Cigarette smoke exposure causes biochemical and neurobehavioral alterations. Oxidative stress play a major role on cigarette smoke induced neurotoxicity. Treatment of genistein reduces the oxidative stress and reverted the behavioral performance. Our finding highlights genistein as possible neuroprotective agent.

Keywords: Cigarette Smoking, Genistein, Locomotor, Antioxidant and Neurotoxicity.

1. Introduction

Cigarette smoking is implicated as a major risk factor in the development of neurological disease such as Alzheimers disease, stroke, multiple sclerosis, cerebral ischemia, hyperactivity disorder etc., (WHO, 2016, Thiyagarajan Ramesh et al., 2015). Cigarette smoking is the important cause of preventable morbidity and mortality in the world (Csiszar Aet al., 2009). Cigarette smoke contains more than 5,000 chemicals (Thielen A et al., 2008, Borgerding M and Klus H, 2005) in which various free radicals are containing (M. J. Lyonset al., 1958, W. A. Pryor et al., 1976 and W. A. Pryoret al., 1983). The tar and gas phases of cigarette smoke contains more than 4000 compounds including nicotine, carbon monoxide, toxic heavy metals, and high concentrations of oxidants and free radicals that initiate, promote and amplify oxidative damage to the tissues (Pryor WA, Stone K, 1993). There is evidence that smoking related disease is associated with free radical-mediated oxidative damage in various organs. Indeed, cigarette smoke is a complex mixture containing an array of free radicals, chief among which are reactive oxygen species (ROS) (Pryor WA, 1997). ROS are known to cause oxidative damage to a number of molecules in cells, including membrane lipids, proteins, carbohydrates, and DNA. (Halliwell B., 1987). In many reports confirmed that the possibility of various substances in cigarette smoke, in addition to nicotine, may relate upon functions in the central nervous system (W. A. Pryoret al., 1983, W. A. Pryoret al., 1993 G. W. Winstone et al., 1993 and Y. Kamisaki et al., 1996).An enormous deal of experimental evidence demonstrated that pathophysiology of neuronal diseases and neuronal tissue damage could be associated with the alterations induced by ROS (Luchese C et al., 2009, Tuon T et al., 2010 and Khanna A et al., 2013).

Cigarette smoke exposures (CSE) are associated with an increased risk of neurological diseases such as stroke, Alzheimer's disease and multiple sclerosis. (Sundstrom P et al., 2008, Cataldo JK et al., 2008 and Shah RS et al., 2010). Some of the studies have shown that cigarette smoke exposure is associated with cognitive impairment (Nowakowska E et al., 2006 and Czubak A et al., 2008), which could be a result of neurochemical changes in the central nervous system. Cigarette smoke components especially nicotine alters the function of different types of neurons, thereby disrupting baseline neuronal communication and interfering with synaptic properties (Mansvelder HD, Mertz M, Role LW, 2009). There is evidence that exposure to sidestream smoke has an adverse effect on cognition and behavior in children(Yolton et al., 2005).

Behavior is the product of various sensory, motor and somewhat functions of the nervous system, can and the hypothesis is that cigarette smoke induced neurotoxicity can adversely affect one or more of these functions, disrupt learning and memory processes, cause harmful behavioral effects (WHO. 2001). Behavioral paradigms like locomotion test for motor functions allow a complete assessment of neurological functions during neurodegenerative disorders (Dumont M, 2011).

Genistein (4, 5, 7-trihydroxyisoflavone), one of the neutraceutical molecules found in soybean seeds, is a phytoestrogens and genistein, can bind to estrogen receptors (ERs) and affect estrogen-mediated processes (Molteni, A et al., 1995). The word estrogen, which is the hormone that regulates fertility in female mammals. Genistein has a structure similar to 17-oestradiol, and it can bind to the estrogen receptors (Lephart et al., 2004). Indeed, genistein can act as estrogen agonists, showing synergic function with endogenous estrogen and thereby inducing estrogen antagonists that may block the estrogenic receptors properties to prevent estrogenic activity (Brzezinski A. and Debi A, 1999). Pan, Anthony, Watson & Clarkson (2000) showed that oral administration of soy phytoestrogens could improve working memory in ovariectomized retired breeder rats.

Significant improvements in short-term and long-term memory have also been observed in human subjects eating a high soy diet for 10 weeks (File, S. E et al., 2001). In addition, genistein has been found to have a neuroprotective effect on cortical cell lines (Sonee, Met al., 2004), on dopaminergic neurons in a mouse model of Parkinsons disease (Liu, L. X et al., 2008), and in a 6-hydroxydopamine hemi-parkinsonian rat model (Baluchnejadmojarad, T et al., 2009). Estrogen also has a neuroprotective effect, as shown by several in vivo and in vitro experiments. Although estrogen is beneficial to patients with AD, it has limited clinical application due to its proliferative and oncogenic effects on non-neuronal cells (Zeng, H et al., 2004). Accordingly, genistein may prove to be an alternative to estrogen in the treatment of AD (O. Yet al., 2004).

Several factors make genistein a potential neuroprotective and memory enhancing drug. These include its protective effect against oxidative stress-induced apoptosis (Xu SZ et al., 2009), and its preferential agonistic effect on ER-β, which is abundant in brain regions associated with learning and memory (neocortex, hippocampus, and nuclei of the basal forebrain) (Shughrue PJ et al., 1997).

Genistein has the potential for treatment to reduce cognitive decline and neurodegeneration associated with menopause through diminishing oxidative stress (Xu Jet al., 2007). Moreover, high dietary consumption of genistein has been linked to a number of potential health benefits including memory improvement in male and female volunteers (File SE et al., 2001). Several aspects make genistein a potential neuroprotective and memory enhancing drug.

Since genistein acts by specific mechanism on brain, the present study aimed at determining the protective effect of genistein against cigarette smoke induced neurotoxicity in male Wistar rats.

2. Materials and Method

2.1. Chemicals

Cigarettes Scissors standard (W.D & H.O. Wills), manufactured by Hyderabad Deccan Cigarette Factory was used in the present study. Genistein and Cremophor EL were

purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade.

2.2. Animal Model

Adult male albino rats of Wistar weighing 150 180 g were obtained from Saveetha Institute of medical and technical sciences, Chennai, India and used for the present study. The rats were provided with standard pelleted rat feed and water ad libitum. The rats were handled under standard laboratory conditions of a 12-h light/dark cycle in a temperature- and humidity-controlled room. The experiments were conducted according to ethical norms approved by Institutional Animal Ethics Committee guidelines (Approval Number BRULAC/SDCH/SIMATS/IAEC/02-2018/004).

2.3. Experimental Design

Experimental animals were divided into four groups of six rats each as follows:

Group I: control rats

Group II: rats exposed to CS

Group III: rats administered with genistein (Genistein alone dissolved in cremophor EL orally (10 mg/kg b.w. /day,). The dosage was chosen according to the result of previous study and earlier investigation (Baluchenjadmojarad et al., 2009).

Group IV: rats exposed to CS and simultaneously administered Genistein dissolved in cremophor EL orally for 12 weeks (10 mg/kg b.w. /day,).

Group II and Group IV rats were exposed to CS for a period of 12 weeks as described (A. Gokulakrisnan et al. 2011). Briefly, the rats were placed in a whole body smoke exposure chamber, which contains two holes of about 3 cm in diameter. Smoke from a lighted cigarette was introduced through one hole and air through the other. The cigarette was fixed away from the chamber and smoke was drawn in by slow suction with the help of a tube and an aerator, so that there was no temperature change within the chamber. The animals were exposed to sidestream CS twice daily, the duration of each exposure was 3 h with an interval of 10 min between each cigarette, using eight to 10cigarettes per day. The identical brand of locally available cigarette was used throughout the experiment (Scissors Standard). Control rats were subjected to the same handling and time in the smoke exposure chamber with air replacing smoke/air mixture.

At the end of experimental period (12 weeks), the animals were sacrificed by cervical decapitation. Blood was collected and serum separated by centrifugation and used for biochemical studies.

2.4. Biochemical Assays

The collected serum was used to estimate lipid peroxidation level was determined by measuring thio barbituric acid reactive substances (TBARS) according to the method

of Ohkawa et al, (1979) and antioxidant enzymes such as superoxide dismutase was assayed by the method of Misra and Fridovich (1972). One unit of the enzyme activity is defined as the amount of enzyme required for the autooxidation of epinephrine by 50% per minute. Catalase activity was measured by following decomposition of H2O2 according to the method of Takahara S et al(1960). The activity of glutathione peroxidase was assayed by measuring the amount of GSH consumed in the reaction mixture by the method of Rotruck et al. (1973) respectively.

2.5. Behavioral Studies

Behavioral analyses were conducted at I, II, and IIIrd month of experimental period. Behavioral analyses, like open field paradigm test (Prut L, Belzung C. 2003) for gait abnormality and loco-motion tests like grid runway, inclined plane runway, wide runway, narrow beam runway, staircase runway, swimming test for nerve and muscular function [Carter et al 2001], were analyzed.

2.6. Statistical Analysis

All the results were expressed as mean ± SD for six rats in each group. All the grouped data were statistically evaluated with SPSS/20 software Hypothesis testing method included one-way analysis of variance (ANOVA), followed by least significant difference (LSD) test; $P < 0.05$ was considered to indicate statistical significance.

3. Result

3.1. Effect of Genistein on Lipid Peroxidation

The changes in levels of LPO in serum of normal and experimental rats are depicted in Fig. 1. The levels of LPO was significantly ($P < 0.05$) increased in serum of CS exposed (group II) rats when compared with control (group I) rats. Pretreatment with Genistein (group IV) showed significant ($P < 0.05$) decrease in the level of LPO when compared with CS (group II) e induced rats.

3.2. Effect of Genistein on Antioxidant Enzymes

The activities of superoxide dismutase, catalase, glutathione peroxidase, in serum of control and experimental animals are summarized in Table 1. A significant decrease ($P < 0.05$) in the activities of the antioxidant enzymes was observed in Group II rats exposed to cigarette smoke compared to Group I rats. Group IV rats showed increased ($P< 0.05$) activities of these enzymes as compared to Group II rats which shows the enzymatic antioxidants activation by Genistein. Genistein alone administered Group III rats showed no significant changes in their activities as compared to Group I rats.

3.3. Effect of Genistein on Locomotion Tests

Locomotion test for control and experimental groups of rats were shown in Fig. 2 A – F. CSE-induced rats were shown very significant alterations in the locomotion analysis, like a mild movement in the ipsilateral side of hindlimb; inability to walk was typically prolonged in narrow beam, inclined plane, and staircase runway, weak and delayed attempt in swimming and climbing behavior. There was no further improvement in the performance of CSE-induced animals at the end of observation period. CSE-induced and genistein treated rats showed improvement in their ability to traverse the wide runway, gained confidence to cross (with a few errors) the narrow beam and grid runway, climb up/down in inclined plane, improved progressively in swimming and climbing tests.

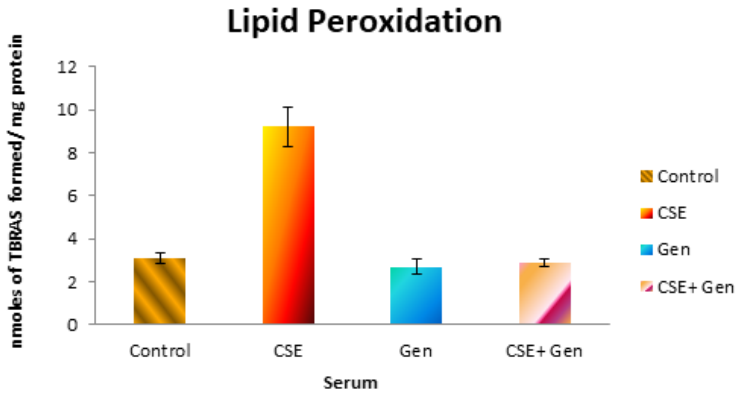


Figure 1: Effect of CSE and Gen on the level of lipids peroxidation in control and experimental group rats. Results were expressed as mean SD, for six animals in each group. Statistically significant variations are compared as follows: [a] CSE-induced Vs control; [b] CSE-induced + genistein (Gen) treated Vs CSE-induced; [a]*b* indicates P < 0.05 and NS indicates non-significant.

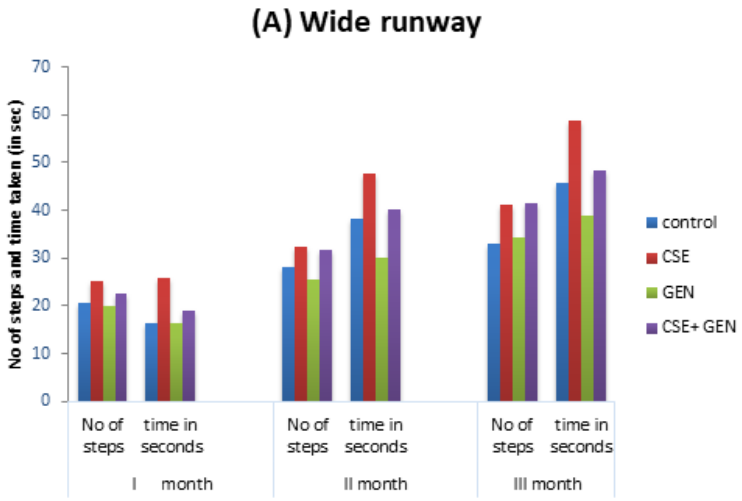
Table 1: Effect of CSE and Genistein on the activities of antioxidant enzymes in the control and experimental group of rats

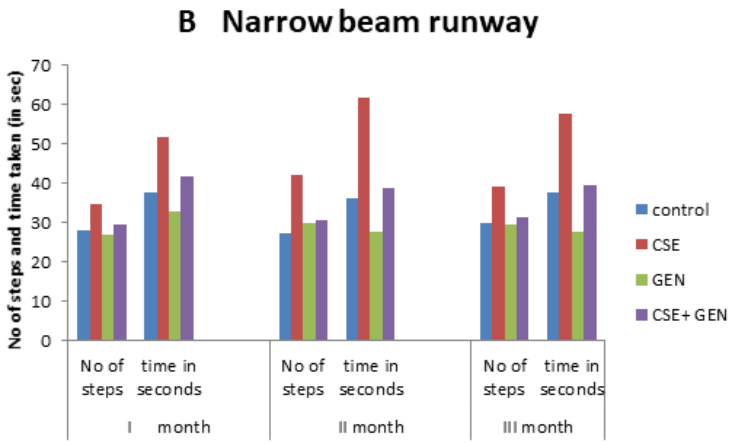
Groups	Normal control	CSE-induced	Genistein alone	CSE- induced + genistein
SOD	3.07 ± 0.13	1.63 ± 0.29	2.10 ± 0.10	2.66 ± 0.51
CAT	61.96 ± 2.7	38.13 ±6.4	46.64 ± 1.5	51.70 ± 4.1
GPx	5.56 ± 0.59	2.75 ± 0.26	4.74 ± 0.17	5.16 ± 0 .25

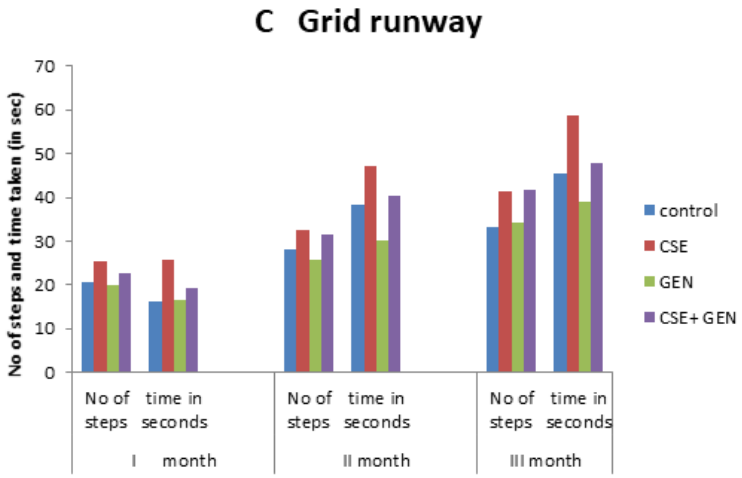
Activity is expressed as

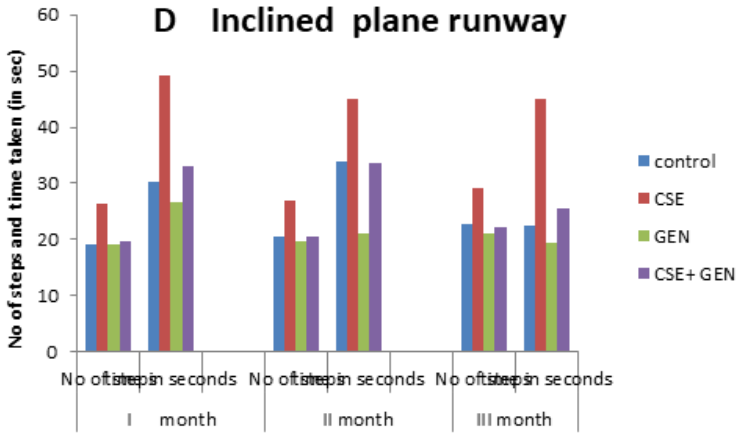
50% inhibition of epinephrine auto oxidation / min / 100 mg protein for **SOD**;
μmole H_2O_2 consumed / min / 100 mg protein for **CAT**;
μgm of GSH consumed / min / 100 mg protein **GPx**;

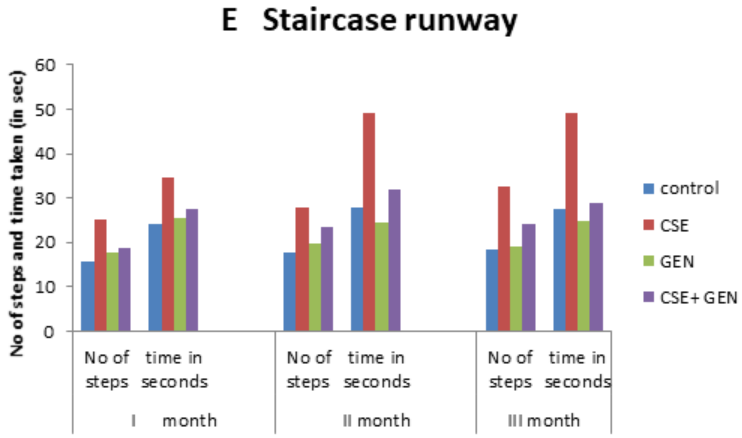
Values are expressed as mean ± SD, for six animals in each group. Statistical significant variations are compared as follows: ^{a} Cigarette Smoke Exposed Vs Control ^{b} Cigarette Smoke Exposed + soybean extract Vs Cigarette Smoke Exposed and soybean extract Vs Normal control. $^{a, b}$ - $P < 0.05$, ^{NS} Non significant.






C Grid runway
No of steps and time taken (in sec)
70
60
50
40
30
20
10
0
No of steps
time in seconds
I month
II month
III month
control
CSE
GEN
CSE+ GEN


D Inclined plane runway
No of steps and time taken (in sec)
60
50
40
30
20
10
0
I month
II month
III month
control
CSE
GEN
CSE+ GEN


E Staircase runway
No of steps and time taken (in sec)
60
50
40
30
20
10
0
No of steps
time in seconds
I month
II month
III month
control
CSE
GEN
CSE+ GEN

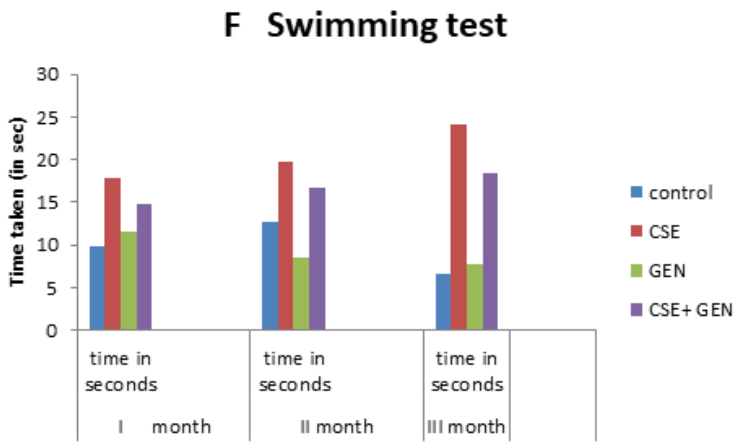


Figure 2: Effect of CSE - induced and genistein on locomotor analysis of control and experimental group of rats. Illustrations of the locomotor function of CSE - induced and genistein treated rats on different runways (A) Wide runway, (B) narrow beam runway, (C) grid runway, (D) inclined plane runway, (E) staircase runway, (F) swimming

4. Discussion

In the current study a significant increase in LPO was observed in the serum of CS induced rats. Previously CSE was shown increase lipid peroxidation in serum and various organs (Anbarasi, et al., 2003). Cigarette smoke does a complex milieu possess an array of free radicals and ROS, namely hydroxyl, peroxyl, nitric oxide, and superoxide radicals (Pryor, 1997). The sustained release of reactive free radicals from the tar and gas phases of smoke imposes an oxidant stress, promotes lipid peroxidation and consequently perturbs the antioxidant defense system in the blood and tissues of smokers (Pryor and Stone, 1993) Supplementation of Genistein reduced the levels of LPO in CS exposed rats owing to its free radical scavenging and antioxidant mechanisms (Baluchnejadmojarad et al., 2009). .This protective mechanism can be attributed to its ability to cross the blood-brain barrier, possessing estrogen like mechanisms and longer half life inside the human system (Bang et al. ., 2004).

The present study showed decrease in enzymic antioxidant such as SOD, CAT and GPx in cigarette smoke induced rats. Cigarette smoke induced oxidative stress associated with membrane lipid destruction which leads to formation of peroxide radicals and cause lipid peroxidation, leads to molecular mechanism of cigarette smoke induced neurotoxicity.(Anbarasi, K et al., 2003 and, Pryor WA et al., 1993) Genistein treatment significantly, increase the enzymic antioxidant by its free radicals scavenging and potent antioxidant ability. The protective effect of genistein against cigarette smoke

induced oxidative stress in our study could be direct inhibiting lipid peroxidation and scavenging the accumulation of free radicals, and stimulating the activities of the Superoxide dimutase, catalase and glutathione peroxidase.(Hsieh, H.M et al., 2011).

Many animals behavior analyst have been developed to evaluate the functional insufficiency and to quantify the behaviors that are similar to human neurodegenerative disorder and symptoms. We observed that cigarette smoke induced rat exhibited a significantly decreased walking speed and step/stride length as well as an increased base support and foot angle and altered front/hind paw overlapping patterns, increased sway length, shorter stride lengths causing weaving foot print patterns as compared with control rats, which showed more consistent forelimb-hind limb coordination with regular overlapping patterns. Treatment of genistein exhibits normal stride lengths with regular overlapping pattern. In the previous animal nicotine, a component of cigarette smoke was shown to alter the behavioral sensitization (Hamilton et al., 2012). It is due to the neuroprotective effect of genistein isoflavones (Linford NJ, Dorsa DM, 2002). Isoflavones have gained much interest for their ability to modulate neuronal function and influence learning and memory processes (Schreihofer DA & Redmond L. 2009).

5. Conclusion

In conclusion, the present study demonstrates that that chronic cigarette smoking induces an oxidative stress by augmenting lipid peroxidation and diminishing enzymatic antioxidant status and alteration in neurobehavioral parameters. Genistein ameliorate CSE induced peroxidative changes probably through its free radicals scavenging, anti-lipid peroxidative activities in the serum. Genistein treatment provides near complete protection against CSE induced behavioral alteration by its antioxidant free radicals scavenging and neuroprotective activity. These finding suggest the therapeutic potential of genistein to inhibit the CSE induced biochemical and behavioral alteration. However further studies pertaining to precise mechanism of action of genistein are warranted.

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PRODUCTION OF AN EDIBLE OYSTER MUSHROOM SPAWN (PLEUROTUS OSTREATUS SPECIES) IN LIQUID CULTURE AND GROWTH ASSESSMENT THROUGH CULTIVATION METHOD

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Abstract

Aims and Objective: This study conducted to invitro laboratory production of Oyster Mushroom spawn and also assess the growth of this Oyster Mushroom through cultivation method.

Background: Quality food, good health and pollution free environment are the major concerns facing our country. Mushroom cultivation helps to solve the issue of nutritional safety and also provides solution for proper recycling of Agricultural wastes. Mushrooms have rich in protein, no cholesterol, high fibre, sodium, good quantity of vitamins and minerals, and also have bioactive compounds like b-glucans, protein polysaccharide complexes that impart unique medicinal values like anti-cancer, anti-viral, anti-diabetic, anti-hyper tension, anti-obesity properties. With ever increasing demand for quality food, mushroom cultivation is emerging as an important activity in different parts of our country. In our Tirupattur district, Tamilnadu commercial production of mushrooms like Milky mushroom, Oyster mushroom, Button mushroom etc, are available. From this production of oyster mushroom is little easy, favour environmental condition, disease resistance also good. But it requires mushroom spawn, it is difficult to get this spawn, due to unavailability, only in agricultural universities, some private agro based farms produce Mushroom Spawn.

Materials and methods: Pure culture prepared from fleshy fungi/mushrooms fruit body in potato-dextrose-agar medium, substrate prepared from cereals grains. By using pure culture and substrate we prepared mother spawn and commercial spawn. After preparing the oyster mushroom spawn, growth of mushroom is assessed by cultivation method. Results: Mushroom cultivation is done by bag / cylinder method using raw materials like straw, plastic bags, pre-prepared spawn etc. We assess the growth in differential period of days (25th day, 45th day and 60th day) and yield is measured.

Conclusion: The purpose of this work to raise awareness and provide decision support information about opportunities at farm and local community level to increase the incomes of small-scale farmers by mushroom cultivation. This activity requires very little land and can be good source of employment for small and farmers, educated youth and women. The prepared spawn is distributed to surrounding village people for free of cast at first by conducting extension activity programme of Mushroom cultivation and also, we provide hands on training programme for formers, self-help groups, women entrepreneurships and students.

Keywords: Oyster Mushroom, Spawn Production, Mushroom Cultivation and Harvesting.

1. Introduction

Mushrooms are a group of fleshy macroscopic fungi, a group very different from bacteria, plants and animals. Fungi lack the most important character of plants; it has the ability to use energy from the sun directly through chlorophyll, But Fungi depends on other organisms for food, absorbing nutrients from the organic sources in which they grow. They cannot produce their own food (ICAR 2012). The active part of the fungus is mycelium made up of a tiny web of filaments called hyphae. Under optimum conditions, sexually well-matched hyphae will fuse together and grow to form spores. The larger spore producing structures bigger than about 1 mm are called mushrooms. Mushroom gain their nutrition by saprophytic, parasitic and symbiotic resources. They synthesize enzymes like cellulose and hemicellulose. Mushrooms must also rely on organic material for their nutrition and do so in three ways, as saprophytes (living on dead wood or dead tissue of living trees or dung), as parasites (attacking living plant or animal tissue), or as mycorrhizae (having a symbiotic relationship with plants).

Mushroom possess unique flavor and exotic taste. It is a rich source of proteins (20 – 35% on dry weight), which is higher than the protein content of fruits and vegetables (Kakon et al., 2012). It has higher percentage of all essential amino acids and are rich in lysine and tryptophan. They are almost free from fat except for linoleic acid, but are richer in water soluble vitamins, B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid) and B12 (Cyanocobalamin), also contain vitamin C (ascorbic acid), K (Phytonadione) and A (Retinol), D (Ergocalciferol) and E (alpha tocopherol) appear to be present in low amounts (Sadler M, 2003). They are good source of minerals (Phosphorus, Potassium, Iron, Sodium, Calcium, and Magnesium). K: Na ratio is very high. Low starch content, low in calories with trace of sugar and no cholesterol. Mushrooms are probiotic. They help in keeping our body healthy and ward off diseases by strengthening the immune system (Wasser SP, 2002). Mushroom have some bio-molecules, such as terpenes, steroids, phenolic

compounds, and polysaccharides, have various biological activities (Shang et al., 2015). Mushrooms may have health-promoting benefits due to a multitude of compounds with antifungal (Ye et al., 1999), antigenotoxicity (Wang et al., 2005), antioxidation (Roupas et al., 2012), antiproliferative (Zhou et al., 2013), anti-tumorigenic (Kim et al., 2015b), antihyperlipidemic activity (Opletal et al., 1997), anti-hypertensive, anti-nociceptive, immune-stimulation (Vaz et al., 2011), hypo-cholesterolemic/anti-atherogenic properties, stress-reducing properties and are also good for diabetic patients (Akata et al., 2012). Mushrooms are generally low in saturated fats and high in fiber and protein, and may reduce harmful blood cholesterol and act as an appetite suppressant. (Kim et al., 2011).

The demand for food including mushrooms is quickly rising and will continue to rise with increase in global population which will be 8.3 million by 2025 and expandable income. In India, having to varied agro climate and abundance of farm waste, different climate helps tropical and subtropical mushrooms cultivation throughout the country (TNAU 2020). It is estimated that India is generating 600 million MT of agricultural waste besides, fruit and vegetable residue, coir dust, husk, dried leaves, pruning's, coffee husk, tea waste which has potential to be recycled as substrate for mushroom production leading to nutritious food as well as organic manure for crops. Mushroom production being an indoor activity, labor intensive and high profit venture provides ample opportunities for gainful employment of small, farmers, landless laborer's, women and unemployed youth. Therefore, increasing mushroom cultivation will a step to fulfill nutritional needs to reduce malnutrition and providing livelihood to landless poor (ICAR 2012).

The mushrooms of the genus Pleurotus rank second in the world mushroom market and is the most popular mushroom in India, China. The Pleurotus spp. of the class basidiomycetes belongs to a group known as "white rot fungi" (Tsujiyama and Ueno, 2013) as they produce a white mycelium and are generally cultivated on non-composted lignocellulosic substrates (Savoie et al., 2007) in which various kinds of Pleurotus are commercially cultivated and have considerable economic value, including P. ostreatus (oyster mushroom), P. eryngii (king oyster or Cardoncello), P. pulmonarius (phenix mushroom), P. djamor (pink oyster mushroom), P. sajor-caju (Indian oyster), P. cystidiosus (abalone oyster), P. citrinopieatus (golden oyster mushroom) and P. cornucopiae. Pleurotus species require a short growth time, compared to other mushrooms (Khare et al., 2016). Hence, oyster mushrooms are the easiest to cultivate, and require less production technology (Mandeel et al., 2005).

The mushroom seed is usually referred to as spawn. The diversity of spawn products remains limited. However, as a large variety of cultivation processes exist for mushroom production, and facilities vary significantly among cultivation plants, choosing suitable spawn can be extremely difficult. In this regard, it has been necessary to develop new spawns to satisfy the varying demands of the mushroom industry. But, the production of mushroom spawn and its cultivation is complex process. The objective of this study was

to develop a new spawn to meet such requirements, with desirable characteristics such as fast preparation, less production space requirement, low cost, and ease of cultivation inoculation method.

2. Materials and Methods

2.1. Preparation of Culture Media

Culture media are important for isolation of mushroom and maintaining them in a pure culture either in test tubes or Petri plates. The fungal cultures may be grown in broths liquid media or solid agar media.

2.2. Potato Dextrose Agar (PDA)

PDA is very widely used for isolation of mushroom fungi as well as for its maintenance. Wash the potato (250 g), peel off the skin, and cut them into small pieces. Cook the sliced potato in 500 ml of water for 30 minutes in an open vessel or pressure cooker for 20 minutes. Simultaneously mix 20 g agar with 500 ml of water and boil in a cooker for 30 minutes. Collect the potato extract and filter through muslin cloth. Add 20 g dextrose to the potato extract. The molten agar with the potato agar mixture are mixed well and make final the volume to 1 litre with distilled water. Check the pH of the medium (pH 7) using pH papers. Pour the medium in to cleaned boiling tubes 15 ml / tube and plug with non- absorbent cotton wool. Arrange the test tubes in a strand, cover it with a paper and tie firmly with a cotton thread. Sterilize them in an autoclave or a pressure cooker at 15 lbs pressure for 20 minutes. Take out the sterilized test tubes, after clearing the steam and keep them in a slanting position to get agar slants. After solidifying, the tubes are arranged in a test tube strand and stored in a clean room for further use.

2.3. Preparation of Pure Culture

Select well grown, disease free oyster mushroom and keep it on a clean paper for 2-3 hr to get certain amount of moisture present in the mushroom to evaporate. Clean the culture room, laminar flow wood with antiseptic solution. Keep the sterilized PDA slants, razor blades, forceps inside the chamber and turn on the UV light. After 20 minutes put off the UV light and start working after 5 minutes. Sterilize all the instruments to be used by exposing to Bunsen burner. The mushroom sterilized with ethyl alcohol, split open the mushroom longitudinally into two halves using razor blade. Tissue from the centre of the split mushroom at the junction of pileus and stipe is taken. Remove the cotton plug of the agar slant and the tissue is aseptically placed inside the slant by using sterilized forceps and close it immediately. After transferring tissues from the mushroom, the tube is arranged in a test tube strand and kept in a clean room at room temperature for the growth of the fungus. Observe the test tubes at periodical days and

remove the contaminated tube. The tubes are ready for further use within ten days. This culture is used for preparation of mother spawns.

2.4. Preparation of Spawn

Mother spawn/mushroom fungus grown on a grain based medium. Sorghum grains are the suitable substrate for excellent growth of the fungus. Disease free well grown sorghum grains are used as substrate for growing the spawn. Immerse the sorghum grains in clean water to remove chaffy and damaged grains. Cook the grains in a container for 30 minutes make to soften them. Take out the cooked grains and spread lightly on the platform to remove excess water. At 50% moisture level mix calcium carbonate (CaCO3) thoroughly with the cooked dried grains at 20 g CaCO3 per Kg sorghum grains. Fill the grains in plastic bags up to 3/4th height (approximately 300-330 g / bag), insert a PVC ring, bold the edges of the bag down and plug the mouth tightly with non-absorbent cotton. Cover the cotton plug with a piece of waste paper and tie tightly around the neck with thread. Arrange the bags inside an autoclave and sterilize under 20 lbs pressure for 2 hours. Take out the bags after cooling and keep them in the culture room and turn on the UV light. After 20 minutes turn off the UV light and start working in the culture room. Transfer the fungal culture which is prepared in pure culture to the grains filled bags. Incubate these bags in a clean room under room temperature for 10 days. This can be used for bed spawn preparation.

2.5. Substrates and their Preparation

The material on which the mycelium of the mushroom grows and produce mushrooms are called substrate. It is made up of organic materials such as lingo-cellulosic farm waste. After the substrate (straw) are completely sun dried they are cut into small pieces (¡ 4 cm) and are soaked in excess water tub for overnight if it is new, very old straw soaked in water for 4 hrs, two months old straw soaked for 1 hr. This is done to remove dust particles adhering to the substrates. Then surplus water is drained off and allowed to air dry. The uniform amount of water should be available everywhere in the substrate. The moisture content should be 60-65%. To check whether the substrate is with moist enough squeeze tests is done. Just a few drops of water should be released with some pressure is given.

2.6. Mushroom Bed Preparations

The cultivation of oyster mushroom is usually carried out in transparent polythene covers. The size of the cover should be 60 x 30 cm, with a thickness of 80 gauge. Wash hands thoroughly with antiseptic lotion. Take the polythene cover and tie the bottom end with a thread and turn it inwards. Shade dry steam sterilized straw to get a uniform moisture level in all areas. Take out a well-grown bed spawn, squeeze thoroughly and divide into two halves. (Two beds are prepared from the single spawn bag). Fill the

straw to a height of 3 in the bottom of polythene bag, take a handful of spawn and sprinkle over the straw layer, concentrating more on the edges. Fill the second layer of the straw to a height of 5 and spawn it as above. Repeat this process to get five straw layers with spawns. Gently press the bed and tie it tightly with a thread. Put 6 ventilation holes randomly for ventilation as well as to remove excess moisture present inside the bed. Arrange the beds inside the thatched shed, (Spawn running room) following Rack system or hanging rope system. Maintain the temperature of 22-25°C and relative humidity of 85-90% inside the shed. Observe the beds daily for contamination, if any. The contaminated beds should be removed and destroyed. Similarly, observe regularly for the infestation of insect pests viz., flies, beetles, mites etc., If noticed, the pesticide like Malathion should be sprayed inside the shed at 1 ml per litre of water. The fully spawn run beds can be shifted to cropping room for initiation of buttons.

2.7. Fruiting, Harvesting and Determination of Biological Efficiency

Mature bags were transferred into a fruiting room at 15°C temperature and relative humidity 85-95%. The photoperiod was 12 hrs per day with a light density at 1000 lx. When the primordial formed, the necks and covers were removed from the bags, and from this to the end of harvest, the aeration was intensified to prevent fruiting bodies from deforming. The mushroom was harvested only in first flush when the mushroom cap surfaces were flat to slightly up-rolled at the cap margins, as described by Yang et al. (2013). Biological efficiency was determined as the ratio of the weight of freshly mushrooms harvested in first flush per bag to dry weight of the substrate in the bag and was expressed as a percentage (Rodriguez Estrada et al., 2009).

3. Results and Discussion

3.1. Oyster Culture using PDA Medium

PDA is the simplest and the most prevalent medium for growing mycelium of most mushrooms' cultivation methods. P. ostreatus was successfully grown on PDA medium. The oyster completely covered the Petri dishes in 10 to 12 days and its color and appearance looks like pure cotton (figure 1). The mycelium should be white.

If green, yellow, blue or grey color mycelium indicates fungal contaminants. A creamy, shiny growth often indicates bacterial contamination. P. ostreatus is a slow grower when it is compared to molds and other fungi (Oei P and van Nieuwenhuijzen, 2005).

3.2. Substrates for Mushroom Cultivation

The moisture content of the substrates was varying from 70% to 75%. Significantly the highest run rate was recorded at 70% moisture level. Wheat straw, barely straw, and

Figure 1: The spores are inoculated to the PDA slants and culture plates as in tissue culture under aseptic condition

saw dust were used as substrates for P. ostreatus. There was no significant biological efficiency difference on the substrates. Other substrate may be used it shown in the below table.

S.No.	Mushroom Species	Substrate for cultivation
1	Oyster Mushroom	Bean straw, Cotton straw, Cocoa shell waste, Coir, Water hyacinth/Water lily, Molasses waste from sugar industry, Paper, Corncobs, Cotton seed hulls, Cotton waste from textile industry, Sawdust-straw, Sawdust, Coffee pulp, Wheat straw, Rice straw, paddy, ragi, stalks and leaves of maize, jowar, bajra, dehulled corncobs, pea nut shells, dried grasses, sunflower stalks.

3.3. Oyster Spawn Production

Once a pure culture of mushroom is attained, the spawn could be made from it. The spawn is the mycelium of the mushroom grow on a solid material. Functionally, it is the start of inoculums preparation of mushroom cultivation. Solid materials such as saw dust, whole grain of cereals, shredded maize and corn cobs are used for spawn preparation. P. ostreatus was grown on sorghum wheat, and dried malt wheat. They are cheap in market and it gives best result in yield hence it is economical to use them to produce spawns. Besides, the space between cereals is quite enough for air circulation. The main advantage of grains is that it is very nutritious for fungi. To get good quality of spawn, the most important is the quality of the inoculums. The inoculums should be

fresh and pure. If the inoculums are preserved in refrigerator, they should be activated before they are used for spawn production (Dawit, 1998).

3.4. Harvesting

At the first trial, only the first flush was harvested and 2nd and subsequent flush were failed. These motivated us to see different environmental conditions and when the mycelium covered by plastic after spraying with water, pinheads were developed and then II, III and IV flushes were also successfully cultivated. Hence, what is most important for 2nd, 3rd and 4th flushes cultivation is humidity. Additionally, temperature also affected our II to IV flushes. Unlike many other mushrooms by which the yield continuously decreases in consequent flushes, the pattern of P. ostreatus is different. Though, the maximum yield was obtained in first flush than other flush. Overall, one crop of mushroom with four produce flushes is most economical (Sher et al., 2010).

3.5. Yield Measurement

Weight of all fruiting bodies harvested from two flushes is measured as total yield of mushroom. The biological efficiency (yield of mushroom per kg of substrate on dry weight basis) was calculated by the formula from the standard method.

$$\text{Biological Efficiency} = \frac{\text{Fresh weight of Mushroom}}{\text{Dry weight of substrate}} \times 100.$$

S.No.	Yield (g/kg dry substrate)					
	Yield in Days	**Flush I (25th Day)**	**Flush II (32nd Day)**	**Flush III (40th Day)**	**Total (g)**	**Biological Efficiency**
1	Pleurotus Ostreatus **(Oyster Mushroom)**	458.64	372.43	308.63	1139.7	227.94 %

The results reveal the yield, biological efficiency (B.E) of the Pleurotus Ostreatus Significantly maximum yield was obtained on when it was cultivated on Argo waste (straw substrate) 227.94 %. Similar results also found in (Patil, 2012).

A significant increase in production would be required to reach economic break even point. A biological efficacy of 250 % would be necessary for create positive profits. However small-scale outdoor cultivation of Pleurotus Ostreatus does not appear to be economically feasible under given circumstances and assumptions. Additionally, increasing production through the use of machines versus manual labor may be possible to increase profits.

4. Conclusion

India has incredible potential for cultivation of mushroom and all commercial edible and medicinal mushrooms. There is growing demand for quality products at competitive rate both in domestic and export market. Though growth of mushroom will depend on increasing and widening domestic market in coming years, export market will be equally attractive. To be successful in both domestic and export market, it is essential to produce quality fresh mushrooms and processed products lacking of pesticide residues and at competitive rate. It is also important to commercially utilise the compost left after cultivation for making manure, vermi compost, briquettes, etc. for additional income and total recycling of agro-wastes.

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Part B:

HUMANITIES

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PLIGHT OF WOMEN WORKERS IN UNORGANISED SECTOR WITH SPECIAL REFERENCE TO CHENNAI CITY

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Abstract

Women in unorganised sector refer to women work like a slave due to lack of regulated laws as one who has in organised sector. Their problems and issues remain unaddressed in spite of their voices. They do not come under workers welfare act, factor act, minimum wages act and so on. They suffer a lot at their work places in the form of sexual harassment, seasonal employment, unfair wages and discriminatory practices. There are a few organisations functioning for the welfare of these workers. But, still their predicaments continue. In addition to this, they do not have any welfare measures such as gratuity, pension benefits, maternity leave and holidays like those in organised sectors.

Keywords: Unorganised Sector, Chi-square and KMO bartletts.

1. Introduction

The above are a few quotes about Women by Father of Our Nation. Likewise, Women have been viewed either in the image of Eve or in the image of Mary by the western world. The double standard regarding the status of women is found throughout the history of India. In India, there has been stereo typical glorification of the women. The state and status of women in India from the ancient period till the modern age changes from period to period. In early times, women were meant only for housework such as taking care of children, cooking, washing and other activities in the family whereas men were engaged in work and bring in income to his family. Family was run in such

a way that expenses of family were met with an Individual's Income. At that time, employment of women was redundant. Macro family system was adopted in ancient times, due to which, women were not forced to work by their families. But in modern time, things have changed which led women to work for their families. Women workers realised that employment gives economic status, social status and combination of these two gives empowerment. Even income of women is now a day considered as significant as earnings of men. Survival of family depends on the income of both men and women. Therefore, women have started working like men everywhere across country. This is where women's plight begins in the form of unstable income, discriminatory practices, sexual harassment, and lack of education, hindrance to growth, seasonal employment and Poor payment of wages. Plights of women in unorganised sectors are worse than women being employed in other sectors. Generally speaking, there are no or little regulations for the employees belonging to unorganised sector. They are scared of too many ordeals in their ordinary life.

2. Women in Unorganised Sector

Women in unorganised sector refer to women work like a slave due to lack of regulated laws as one who has in organised sector. Their problems and issues remain unaddressed in spite of their voices. They do not come under workers welfare act, factor act, minimum wages act and so on. They suffer a lot at their work places in the form of sexual harassment, seasonal employment, unfair wages and discriminatory practices. There are a few organisations functioning for the welfare of these workers. But, still their predicaments continue. In addition to this, they do not have any welfare measures such as gratuity, pension benefits, maternity leave and holidays like those in organised sectors.

3. Objectives of the Study

The study contains primary and secondary objectives as mentioned bellow:

1. To explore various issues and problems pertaining to women workers in unorganised sectors.

2. To examine economic and social status of women workers in unorganised sectors.

4. Statement of Problem

There are numerous problems to women of unorganised sector such as sexual harassment, discriminatory practices, poor payment of wages and seasonal employment. Sexual harassment to women workers in unorganised sectors is at alarming level. Sexual

harassment at their work place occurs in various forms such as harassment from co-employees, sex abuse from employer, sexual harassment from strangers. They are often vulnerable to various kinds of sexual harassment or abuse. Next plight of women engaged in unorganised sector is long duration of work. They are forced to work for more than eight hours and paid wages less than that. Sometimes, their contribution is least taken into consideration and they are not honoured for this. In addition to these, they get employment occasionally and it is difficult for them to make end meet of their family. This paper focuses on the plight of women workers in unorganised sector and some valuable suggestions would be given by the researcher to overcome their plights to some extent.

5. Scope of the Study

This study makes an attempt to highlight plights of women workers in unorganised sectors in Chennai city. Researcher has explored various predicaments of women workers engaged in unorganised sectors. This study has covered workers in construction industry, workers from papadam making units, some departmental stores, garment industries and workers from pickle making units. Research has been carried out covering these people in some parts of Chennai city. This paper is believed to come up with some possible practical solutions to overcome the predicaments of women workers in unorganised sector.

6. Review of Literature

Fathima Adeela Beevi (2014), in her article showed the plights of women workers in unorganised sectors. The unorganized women workers are living below the minimum accepted standards without adequate facilities and having very lower income that did not meet their daily needs of life. Unorganised women workers including home-based works likes rolling papad and beedis, self-employment programs likes selling vegetables, employment in household enterprises and small units, agricultural workers, labour on construction sites, domestic work, handicrafts, khadi and village industries, handloom weaving and sericulture etc. The more women workers were employed mainly in the field of textile sector as sales women and comparatively more opportunities are there. They worked hard in shops to make their life better and reach their children in quality education and healthy food without acquiring any special skill and training. The study focused on the job satisfaction of sales women and the data were collected from 85 respondents and also from various books, reports, journals and websites. This is revealed that most of the women were satisfied with the facilities at work place given by the employer like special refreshment room for the women staff and staying or hostel facility etc but there is no time for refreshment because of continuous

working hours without shifting and seasonal workload.

Manju (2017) explored something in her research article regarding women workers in unorganised sectors. The relation between nations prosperity and women condition can be understood by the famous quote given by Pandit Jawahar Lal Nehru, "You can tell the condition of a nation by looking at the status of its women". India is a traditional country and there is diversity in religions, culture and customs. Role of the women in India mostly is household and limited to domestic issues. Female workers form the largest segment of India's unorganized workforce. Majority of women work in unorganized sectors for low wages due to low level of skills, illiteracy, ignorance and surplus labour and thus face high level of exploitation. The social and economic profile of female worker is greatly affected by the nature of industrial sector where they work.

7. Research Methodology

Sources of Data Collection

Both primary and secondary data have been used for eliciting information. Primary data has been collected through thoroughly prepared questionnaire which were circulated among target workers in some select unorganised sectors. Secondary data was collected by referring to books, articles, journals, magazines, and websites.

Research Design

The present research study is of descriptive and analytical in nature.

Sample Size

The sample size is 50. 50 women workers were interviewed in some select unorganised sector. Data analysis was made according to that.

Sample Type

The type of sampling is Non-probability sampling. In non-probability sampling, convenient sampling was adopted.

Sample Area

Chennai was chosen for the study. Chennai city includes North Chennai, South Chennai, East Chennai and West Chennai.

8. Data Analysis and Results Discussion

Some important tests are taken up for processing the collected data. Findings are made with respect to the results of these tests. Some important questions are constructed to elicit the information through suitable likert's scale. First of all, chi square test was performed to find the association between marital status and work load of women workers in unorganised sector.

Chi-Square Test

Table 1: Chi-Square as a test of attributes for marital status and work load of women workers

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.530[a]	2	.063
Likelihood Ratio	5.258	2	.072
Linear-by-Linear Association	1.714	1	.190
N of Valid Cases	50		

Interpretation

The key result in the Chi-Square test table is the pearson Chi-Square. The value of the test statistic is 5.530. The corresponding p-value of the test statistic is p= 0.063. Based on this, the null hypothesis is accepted thereby spelling out there is no association between marital status and work load of women workers in unorganised sectors.

Table 2: Chi-Square as a test of attributes for age and payment of wages to workers in unorganised sectors

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.999[a]	4	.910
Likelihood Ratio	1.407	4	.843
Linear-by-Linear Association	.813	1	.367
N of Valid Cases	50		

Interpretation

The key result in the Chi-Square test table is the pearson Chi-Square. The value of the test statistic is 0.999. The corresponding p-value of the test statistic is p= 0.910. Based on this, the null hypothesis is accepted thereby spelling out there is no association between age and payment of women workers in unorganised sectors.

Factor Analysis

Table 3: KMO and Bartlett's Test for various plights of women workers

Kaiser-Meyer-Olkin Measure of Sampling Adequacy		.518
Bartlett's Test of Sphericity	Approx. Chi-Square	19.523
	df	15
	Sig.	.191

Interpretation

From the above analysis of KMO and Bartletts test, value is positive and it is said to be 0.518. According to this value, sample is not adequate enough as the value is less than 0.6. Therefore, the researcher has to conduct such a study elsewhere to ensure sufficient sampling seen in the study. Moreover, variables designed in the study also are not best suitable on account of this value.

Table 4: Factory analysis for various plights of women workers in unorganised sectors

Rotated Component Matrix[a]			
	Component		
	1	2	3
Welfare Measures	.848		
Work Load	−.673		
Family Support		.802	
Payment of Wages		-.737	
Discriminatory Practices			.832
Sexual Harassment			−.588

Interpretation

From the above factor analysis, it is clearly shown that welfare measures are not upto the satisfaction of women workers in unorganised sector. Similarly work load is not encouraging the workers as they are done at the whims and fancies of employer. Sexual harassment is there but it is not too often in the sector. Therefore, unorganised sector has both positive and negative impact on welfare measures, work load, family support, payment of wages, discriminatory practices and sexual harassment.

Table 5: One Way ANOVA for work load of women workers in unorganised sector and Marital Status

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.115	2	.557	2.922	.064
Within Groups	8.965	47	.191		
Total	10.080	49			

Significance Level: 5%

Interpretation

This is the table that shows the output of one way ANOVA and whether there is a statistically significant difference between marital status and work load of women workers in unorganised sector. We can see that the significance value is 0.064 (i.e., p = .064), which is above 0.05. Therefore, there is a statistically significant difference between marital status and work load of women workers in unorganised sector.

9. Findings of the Study

(a) Based on the results of analysis, inference is drawn that work load of women workers specifically in unorganised sector is worse. Women worker in any select unorganised sector has heavy schedule of work.

(b) Another finding of this paper is payment of wages to women workers in unorganised sector. Very few people are satisfied with what they are paid. But most of the women workers do not have satisfaction over their wages.

(c) Sample size taken for this study is not sufficient as per the results of KMO Bartlett's test. Therefore, researcher has to widen study elsewhere to obtain what actually happen to the women in unorganised sector to arrive at the meaningful conclusion.

(d) From factor analysis, researcher has showed some plights of women to be good and bad. For example, family support to women workers in unorganised sector is better as compared to other plights. Worst of the entire plight is work load and discriminatory practices.

10. Suggestions and Conclusion

This study mainly focuses on welfare of women workers in unorganised sector. They are pitiable situation. Work load should be brought down to a considerable extent. For this, feedback can be taken by the employer from time to time. Frame the work load according to their acceptance. It needs not necessarily be eight hours of work a day. It can be maximised based on their flexibility. Similarly, employer has to protect all the women workers deployed in unorganised sector by offering proper transport facilities from their home to the work place. This is to ensure that they are away from sexual harassment.

Discriminatory practices are said to be a common sight at the work place. Men should develop the culture of respecting women on par with men. Their work should be recognised and they should be appreciated for their contribution. Above all, going by the words of our father of nation women is more fitted than men. Women should be respected irrespective of different places they work.

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CUSTOMER-SURVEY & INTERACTIVITY CRUCIAL PILLARS OF CRM STRATEGY (ADOPTED BY BANKING SECTOR)

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Abstract

Attitudes are changing. Not so long ago, companies expended by far the greatest effort on winning new customers. These days it is widely accepted that, while prospecting is an essential activity required to keep the progress, customer development is increasingly being viewed as an equally productive and cost-efficient use of direct marketing budgets. It follows that a renewed focus on customer development requires investment into the acquisition of a rich and comprehensive understanding of the customer base outside of what they are currently buying from ones organization. This may sound simple, but there have been many examples of organizations not being able to press knowledge gathered from transactions and customer surveys into commercial action. In fact, research has indicated that nearly half of CRM initiatives fail not because of technological issues but because the data feeds are of an inferior quality.

Keywords: Customers, CRM, Cost-efficient, Investment.

Introduction

The purpose (why's) and manner (how's) of an effective consumer survey as a research tool was observed in the research undertaken on "Relationship Marketing in the Banking Industry" where, what is described as the four necessary components of a successful CRM strategy, namely: 1. Identify 2. Differentiate 3. Interact & 4. Customize were studied through two set of questionnaires administered to different set of audiences: Bank Customers and Bank Executives. The metropolis region of Chennai City, the capital of the state of Tamil Nadu, was taken as the area of the study in order to get an insight into the factors and challenges faced in the CRM ventures adopted by the banking industry. Chennai was seen as truly representative of the potentials and

challenges of banking faced by the rest of the country. The demographic, social and economic complexities of Tamil Nadu with its vast population of different classes of people, economic status and literacy rates was considered as representative of the general banking class of customers in the Indian context. Tamil Nadu had seen the growth and success of many banking enterprises for all categories of banks and especially those chosen for the study. Each of the nine banks chosen for the study, from among the three categories of Public, Private and Foreign banks had more than three branches and Regional Head Office in Chennai.

Period of Study: The primary data were collected through a sample survey conducted during March 2020 July 2020 and the secondary data were collected for a period of nearly five years. (August 2015 July 2020).

Banks chosen for the study: In order to understand the CRM initiatives and challenges faced by the bankers, three categories of banks, namely public sector banks, private sector banks and foreign banks operating in Chennai were chosen for the study. In order to get an overall view of the traditional, private and new generation banks interest and role in satisfying the customers and account holders, these three categories of banks were chosen. Only those banks which had been functioning at least for a period of five years were selected. The following 10 banks were selected for the study.

1. Private sector banks selected include ICICI Bank, HDFC Bank and YES Bank.
2. Nationalized and Public Sector Banks include Punjab National Bank, Indian Overseas Bank and Corporation Bank.
3. Foreign banks include: The Hong Kong and Shanghai Banking Corporation Limited, Standard Chartered Bank, CITIBANK.

The research focused on two categories of respondents namely (1) Bank Customers and (2) Bank Executives, who were chosen at random. In each selected bank, minimum three bank branches located at different locations of Chennai city were selected to represent the customers of all walks of life. Using the list of customers with active account, totally 1165 customers were included in the survey. Similarly the bank executives who were manning the counters with high public contact were selected at random for the survey. Thus the sample respondents selected for the study were active account holders and customers of banks residing in and around the metropolitan region of Chennai city. Similarly the personnel employed as executives in high contact areas in the three categories of banks were included for the study.

Sample size: A sample size of 1165 respondents, who were account holders and customers of the selected banks in the three bank categories, drawn from three branches of each bank, constituted the bank customers. For the survey of bank executives, 100 bank personnel drawn from the ten selected banks comprising bank executives and top management staff were included and the questionnaire was administered.

The distribution of sample respondent customers is presented in Table 1 given below.

Table 1: Distribution of Sample Respondents [Customers]

Sector & Bank	Number of Respondents	Total Sector-wise
Private Sector		
1. ICICI Bank	245	
2. HDFC Bank	200	
3. YES Bank	45	490
Public Sector Nationalized		
1. Indian Overseas Bank	200	
2. Punjab National Bank	143	
3. Cor oration Bank	200	543
Foreign Banks		
1. Citi Bank	52	
2. HSBC Bank	40	
3. Standard Chartered Bank	40	132
Total Sample Size		1165

As regards the bank executives, the higher authorities of each bank insisted that only the number executives specified by them could be contacted and responses collected. As a result, the distribution of sampling respondents of bank executives differs between banks. Totally 101 bank executives in high contact areas in the respective banks were included in the survey.

The distribution of bank executives bank-wise is presented in Table 2 below.

Table 2: Distribution of Sample Respondents [Bank Executives]

Sector & Bank	Number of Respondents	Total Sector-wise
Private Sector		
1. ICICI Bank	17	
2. HDFC Bank	2	
3. Yes Bank	5	24
Public Sector Nationalized		
1. Indian Overseas Bank	5	
2. Punjab National Bank	10	
3. Corporation Bank	10	25
Foreign Banks		
1. Citi Bank	16	

2. HSBC Bank	8	
3. Standard Chartered Bank	28	52
Total Sample Size	101	101

A pilot study was undertaken among 90 bank customers, selected at random, from three branches of the categories of banks chosen for the research purpose. The pilot study was initiated through a set of questionnaires, meant for the bank customers and bank executives, individually designed to study the role of the bankers in introducing Customer Relationship Marketing measures to satisfy or retain their customer base. Focus was on customer related services, bank specific products and reasons / factors for the efficiency of banking operations undertaken by the banks. Based on the responses, the final questionnaire was designed. Each questionnaire was prepared with multiple-choice questions, containing 30 questions. The bank executive questionnaire was designed to evoke interest and response from the different levels of bank - executives. These questions included the various strategies and measures adopted by the banker to relate to their customer base and probed the constraints and difficulties encountered by the bank personnel in administering the CRM techniques undertaken by the bank. Attempts made by the different bankers in attracting the different categories of customers and account holders and the banker's role in offering customer related services were also studied.

The questions addressed to the customers were chosen to elicit their responses towards banking product features, customer services and the quality of customer experiences gained and received by them during each of their contacts with the banks. The open structure of the questions ensured that unexpected facts or attitudes as well as underlying motivations could be pursued.

The key facts of relationship marketing that were explored included

- Facilities offered by the bank to the customers.
- Different ways adopted by the banker to initiate the relationship with the customer.
- Measures undertaken to maintain customer relationship.
- Reasons for customers to select a banker on the basis of bank services & products offered and effectiveness of the bank personnel.
- Factors of agreeability related to banking transactions.
- Details of customer retention measures adopted.

The awareness of the customers about the various measures adopted by the banker to relate to them and the levels of satisfaction derived by the customers of the different banks were studied at length. The secondary data was gathered from different journals published by the National Institute of Banking Management (NIBM), Indian Institute of Banking (IIB) and Reserve Bank of India (RBI) bulletins.

Conclusion

It can be observed that Customer data is a precious asset. Rich data on customers habits, tastes and preferences enables companies to get a much more rounded view of how customers behave and what they do with other companies or banks. The customer survey is now widely used in preference to enhancing the customer database with lifestyle survey data not least because it is easier to persuade consumers to respond if they have an existing relationship with the banker. Moreover, customer surveys tend to produce a more rounded spread of respondent types than lifestyles surveys. Preference data is also valuable addition to transactional data for joint promotional initiatives that require each banking partner to demonstrate a thorough understanding of their customers in order to identify 'best fit' brand matches.

Understanding customer preferences can also be used to improve the cost-effectiveness of customer communications. By asking customers how they prefer to be contacted, banks /companies can immediately begin to identify opportunities to cut communications costs by reducing the use of less popular channels. If a segment of the customer base resents being telephoned at home, they are unlikely to be responsive to any calls at all. Conversely, customers may be more responsive if they are contacted through the channel for which they have expressed a preference. Indeed, surveys conducted amongst the customer base could well reveal that a significant portion of customers would prefer to receive their bills and statements electronically. Thus, Customer satisfaction surveys have long been staple methods used to encourage loyalty and improve retention rates, but carefully applied customer preference data can often also play a part in making customers feel valued and cherished such as was observed in the survey analysis of bank customers.

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A STUDY ON CONSUMER BEHAVIOUR TOWARDS TWO WHEELER PURCHASE WITH SPECIAL REFERENCE TO AMMAN TVS, HARUR IN DHARMAPURI DISTRICT

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Abstract

Consumer Behaviour can be defined as the sum total of how individuals and groups recognize and determine their needs and how cannot how often "they purchase and experience goods and services to meet those needs". It includes the "what-where-when and how of the purchase and experience process". The study of consumer behaviour investigates and develops methods of quantify, forecast and influence the behaviour of consumers. In this articles researcher wants to highlight consumer behavior towards two wheeler purchase with reference to Amman TVS in Harur.

Keywords: Consumer Behaviour, TVS, Hoyer and Murkey.

1. Introduction

Consumer behaviour can be examined as it is a decision making process of an individual when consumer engaged in evaluating, acquiring, using or disposing goods/services. The decision making is basically depend on two important aspects that are the consumers expected amounts of satisfaction and the resources that are available to him for spending. In other words, Consumer Behaviour can be defined as the sum total of how individuals and groups recognize and determine their needs and how cannot how often "they purchase and experience goods and services to meet those needs". It includes the "what-where-when and how of the purchase and experience process". The study of consumer behaviour investigates and develops methods of quantify, forecast and influence the behaviour of consumers.

2. Statement of the Problem

The researcher has articulated certain things as statement of problem on buying behavior of the consumer. It may kindly be noted by the company so as to perform the day to day functions of the business concern more effective. Nowadays consumer behavior appears to be at different levels. So, a variety of treatments should be ensured by the company to strengthen the sales and capture the market in the long run. It is not as easy as producing the commodity. After the production, the manufacturer has to pay attention on every individual and his buying decision. For the purchase of commodity, he may switch his over anything like cat on the wall.

Many buyers are forming their perception of a supplier and its abilities from what they find online. If a company's online presence or search engine optimization program is weak, then their sales reps are at a significant disadvantage. Although the Internet has a lot of good information, this information is not always accurate. Truth in advertising doesn't always apply on the Internet, and the traditional separation of editorial content from advertorial content is murky at best. Buyers may find a seemingly credible source that endorses a supplier over another, or a critique of a supplier based on an isolated incident. In such cases, the seller is forced into a defensive position while another might have an unearned advantage. Buyers come to their own conclusions about what they believe is the right solution for their needs.

3. Objectives of the Study

1. To study the factors influencing the consumer behavior.
2. To trace the impacts of behavioral factors when choosing particular brand of motor bike.

4. Scope of the Study

Effective business managers realize the importance of marketing to the success of their firm. A sound understanding of consumer behaviour is essential to the long run success of any marketing program. In fact, it is seen as a corner stone of the Marketing concept, an important orientation of philosophy of many marketing managers. The essence of the Marketing concept is captured in three interrelated orientations consumers' needs and wants, company integrated strategy. In today's world even the non-profit organizations like government agencies, religious sects, universities and charitable institutions have to market their services for ideas to the "target group of consumers or institution".

5. Research Methodology

Researcher has used both primary and secondary data. Primary data is collected by way of circulating questionnaire to target audience who are the users of different brands of TVS motor bikes. Empirical Research Design has been used all through this study.

Totally 122 consumers were selected for survey in this study. Convenient Sampling method was used and Sampling Unit is Amman TVS, Harur in Dharmapuri District.

6. Review of Literature

Batra and Kazmi, (2017) showed that each era of marketing motivated the particular types of research of consumer behaviour and thereby it shapes its history with respect to the research methodology, consistent body of knowledge as well as developmental theory. The below figure summarizes the parallel relationship between consumer behaviour and marketing. Personal factors, on the other hand, include taste preferences, personal financial circumstances and related factors. The impact of personal factors on consumer decision-making is usually addressed by businesses during market segmentation, targeting and positioning practices by grouping individuals on the basis of their personal circumstances along with other criteria, and developing products and services that accommodate these circumstances in the most effective manner.

Dmitrovic, T and Vida. I, (2018) described two varied kinds of consumer conduct: cognitive and experience directed. The consumers who have cognitive conduct are sensible and reasonable consumers while the experience directed consumers have more emotive reasons for purchasing a commodity.

Hoyer et al. (2019) explained that social factors impacting consumer behaviour arise as a result of interactions of perspective consumers with others in various levels and circumstances. Targeting members of society perceived as opinion leaders usually proves effective strategy when marketing products and services due to the potential of opinion leaders to influence behaviour of other members of society as consumers.

7. Data Analysis and Result Discussion

Table 1: Price offered by the Company

Company price	No. of respondents	In percentage
Strongly agree	25	20.5
Agree	30	24.6
Neutral	27	22.1
Disagree	24	19.7
Strongly disagree	16	13.1
Total	**122**	**100**

Inference

This table is concerned with price of the bikes of the respondents, as far as customer's opinion is concerned, 20.5% of the respondents have strongly agreed. Whereas 24.6% of the respondents have agreed. 22.1% of the respondents are neutral in expressing their opinion. However, 19.7% of the respondents are here to express as disagree, while, 13.1% of the respondents have strongly disagreed.

Table 2: Quality that has been provided by the Company

Quality	No. of respondents	In percentage
Strongly agree	26	21.3
Agree	30	24.6
Neutral	25	20.5
Disagree	20	16.4
Strongly disagree	21	17.2
Total	**122**	**100**

Inference

As per this table, none has expressed their strong feeling over the quality that has been provided by the company. 21.3% of the respondents have strongly agreed. On the other hand, 24.6% of the respondents have agreed to express their opinion. Eventually, 20.5% of the respondents have expressed their opinion to be neutral. However, 16.4% of the respondents have disagreed. While, 17.2% of the respondents have strongly disagreed.

Table 3: Goodwill of the Company

Company's goodwill	No. of respondents	In percentage
Strongly agree	24	19.7
Agree	26	21.3
Neutral	23	18.9
Disagree	25	20.5
Strongly disagree	24	19.7
Total	**122**	**100**

Inference

From the above table, goodwill has been highlighted. Goodwill is the real image of the company. 19.7% of the respondents have articulated strongly agreed. Their opinion. In the same way, 21.3% of the respondents have put across as agreed. However, 18.9%

of the respondents have stated as neutral in their opinion. Like this way, 20.5% of the respondents have conveyed as disagreed. Finally, 19.7% of the respondents as strongly disagree.

Table 4: Comparative Advantage

Comparative advantage	No. of respondents	In percentage
Strongly agree	22	18
Agree	25	20.5
Neutral	24	19.7
Disagree	26	21.3
Strongly disagree	25	20.5
Total	**122**	**100**

Inference

Comparison has become quite common in the modern business world. Even worldwide operation of any business concern is adaptable to this. Therefore, 18% of the respondents have articulated as strongly agreed. Whereas, 20.5% of the respondents have put across their comments as agreed. However, 19.7% of the respondents have expressed their opinion as neutral. Similarly, 21.3% of the respondents as disagree. On the contrary, 20.5% of the respondents as strongly disagreed.

8. Findings

- With relation to this finding, the researcher has ascertained 24.6% of the respondents agreed towards quality that has been provided by the company.
- Goodwill is prominent element in any business company. The researcher in his study found to be not better. Maybe it is due to competition influencing the sales of the company.
- Comparative advantage is nothing but making the comparison of one company with another company. On doing it by the researcher who found 21.3% of the respondents put across as disagreed.
- From this finding, the researcher is coming to say that motivation made by the relations, friends etc. which is recorded as 28.2%.
- Design the way how to attract the customers. The researcher in his findings found 24.6% of the respondents who are neutral in expressing their answer.

- Credit facility is a much needed facility given to the consumer by the financier. In this case, the researcher wanted to say that 22.1% of the respondents expressed as neutral.

9. Suggestions

From this study, the researcher wants to advocate so many things. Because the things he observed in his study remain insufficient. Therefore, there have been many more things to be added up. Income status of the respondents is very poor. Therefore, the researcher may induce the customer who gets high to buy the motor bikes. Similarly, price offered by the company is subject to be the attraction of the company. On fixing the price, the company keeps in mind the income status of the company. The researcher whose next suggestion; goes to quality of the commodity. in this, the company has to avoid using substandard commodities so as to increase the sales in future period of time. Goodwill also is not at the peak level during the survey.

10. Conclusion

The researcher conclude his research with consumer behavior appear at different levels. Therefore, the treatment has to be given in a variety of ways. Many researches are being studies on consumer behavior to know the mentality of the consumer. The researcher has studied only 122 as sample size; it can be further maximized to possible level. Because alone this sample size, we cannot predict consumer behavior seems to be resulted in findings of this study. it has to strengthen by increasing the sample size, different selected areas.

The researcher hopes that the company would set right what are necessary to strengthen the activities of the company. This is what the conclusion of the study.

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A STUDY ON CONSUMER'S ATTITUDE TOWARDS ONLINE SHOPPING IN VELLORE DISTRICT, TAMILNADU

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Abstract

In this study, it shows depth knowledge on a particular topic A study on customers attitude towards online shopping in Velloredistrict, Tamilnadu. The study indicates the level of awareness and attitude towards online shopping. The present study has been confined to study the customers attitude in online shopping products. The study can indicate only the consumers point of view. The objective of this paper is to make the consumer aware of the beneficial aspect of online shopping. The period of study is three months. The researcher has collected a primary data from comprehensive interview schedule and the secondary data has been collected from books, websites, and journal. Few suggestion and recommended based on the study carried out to fine-tune processes which will benefit the buyers to bridge the gap in online shopping.

Keywords: Online, Products, Buyer behaviour, Consumer.

1. Introduction

Online shopping is a purchase of products and services by the way through the internet. It is also an electronic commerce or Net Banking when the buyers can buy a product through the internet. It is the easiest way to purchase the products and to select a variety of product. It can make easy payment through credit or debit card and cash on delivery. Nowdays the Consumer not having that much of time to shopping in the offline shop so the persons are likely to prefer online shopping is a time saving soon. Include the people can buy grocery products also. Through online shopping price comparison are easy to purchase and time-saving. It allows you to shop for whatever you want and wherever according to our convenience.

2. Review of Literature

ManishaKinker, N.K. Shukla (2016) performed aresearch study on "An Analysis of Consumer Behaviours towards Online Shopping of Electronic Goods With specialreference to Bhopal and Jabalpur city". The main objective of the research was to clarify and get insight into consumer Behaviour towards online shopping of electronic goods, to study consumers expectations of online stores, to find outfactors that influences the consumers towards onlineshopping and to analyze the consumer's wants and needs especially in Bhopal and Jabalpur city of Madhya Pradesh .The sample size of the research was 40 respondents ofBhopal and Jabalpur city. The findings of the proposed research work are as follows as Customer-Oriented Factors Time Saving, Product Quality, Product Price, Convenience, Accessibility, Shop Anywhere and Anytime are the main specific factors influence customers attitudes toward electronic product online shopping. The minor factors that influences customer's attitudes toward electronic product online shopping are technological factors, assured quality, cash on delivery and various promotions and discounts .The attitude towards online shopping is different in Bhopal and Jabalpur city. After the data analysis, the researcher concludes that the electronic product online market takes a high percent of individuals shopping on it.

A study was conducted on the factors influencing online shopping behavior of consumers in Sweden by **Shahzad (2015)**. This study mainly focused on online factors that included risk of financial loss, risk of product performance, and risk with the delivery of products, trust and security. A questionnaire was distributed among 100 respondents to collect the data. The findings of the study revealed that demographic factors like age, gender, income and education influence consumer behaviour. It further concluded that higher the age, lesser is the intent to shop online. The results also indicate that higher income groups are less attracted towards online shopping. Education was directly related to the online shopping behaviour and the consumers with better education were more inclined towards shopping online. The design of the website is the major influencing factor for the consumers to shop online. The study also revealed that risk of performance, trust and security have a substantial influence on customer behavior towards online shopping whereas financial risk and delivery risk does not affect consumers buying behaviour.

Geissler, (2012) The shopping motivation literature is abound with various measures of individual characteristics (e.g., innovative, venturesome, cosmopolitan, variety seeking), therefore, innovativeness and risk aversion were included in this study to capture several of these traits. Measures by Donthu and Gilliland were used to measureinnovativeness and risk aversion.

3. Research Objectives

The study has been indicating buyer's behavior towards online shopping in Vellore district with the following objectives:

- To find out the factors that influences the customers to buy products through online shopping.
- To analyses the problems faced by the customers to pre and post purchase online products.
- To measure the products which are to be highly purchased through online shopping.

4. Statement of the Problem

As rapid socio-economic changes sweep across India, the country is witnessing the creation of much new marketing method for their products. Nowadays people are so busy that they have no time to go to shopping malls and buy the things they want. Buyers want to available of all products in purchase in one particular place. But it is not move in all occasions. So everyone likes to do online shopping.

In early days buyers are not much aware of online shopping products but now the customers buy most of the products through online for the purpose of time-saving, easy purchase and so on. To attract the buyers, most of the online shoppers can deliver the products quickly within two to three days and the buyer can buy the products in doorsteps. The researcher can measure the level of awareness and level of the attitude of consumer towards online shopping. Hence the reseacher has been undertaken for the purpose of current research.

5. Scope of the Study

The present study analyzes the attitude of buyer's towards online shopping in Vellore District alone. The study also analyses the problems faced by the customers to pre and post purchase online products. It also measure the products which are to be highly purchased through online shopping. The study will indicate the buyers to purchase products from online shopping.

6. Research Methodology

In this study, the researcher has used empirical research based on survey method. The researcher has made a comprehensive interview schedule for collecting primary

data with regard to the behavior of buyer towards online shopping. The interview schedule was carefully designed and dully pre tested. All the analysis and influence are made on the basis of their primary data and Secondary data have been collected from relevant books on marketing management, and from magazine, newspaper, journals, and websites.

7. Framework of Analysis

With reference to the objective of the study, factors are taken into consideration as such gender of the respondents, the age of the respondents, marital status of the respondents, educational qualification of the respondents, and occupation of the respondents, area of resistance of the respondents and monthly income of the respondents. The data which were collected from the respondents were analyzed by using percentage analysis, weighted average ranking and chi-square test. This study also uses comparison table as a tool in order to study the various factors.

8. Analysis and Interpretation

Table 1: Findings from the study

Findings	**Subcategories**	**Number of Respondents**	**Percentage (%)**
Reason for Preferring online shopping	Product varieties	26	37
	Social status	23	33
	Safety	14	20
	Low cost	7	10
	Total	70	100
Mode of Payment	Cash on Delivery	18	26
	Credit Card	22	31
	Debit Card	19	27
	Fund transfer	11	16
	Total	70	100
Extra Charge for Online Shopping	Yes	28	40
	No	42	60
	Total	70	100
Price of the Products	Very High	8	11
	High	16	23
	Medium	34	49
	Low	10	14
	Very Low	2	3
	Total	70	100

Packaging of Products	Excellent	24	34
	Good	43	62
	Bad	3	4
	Total	70	100
Delivery of Products	Less than 5 days	22	31
	5 – 10 days	35	50
	More than 10 days	13	19
	Total	70	100
Problems faced by the Respondents	Products have changed	14	20
	Deliver Poor quality products	10	14
	High cost	12	17
	Warranty Problem	24	35
	Others specify	10	14
	Total	70	100

Source: Primary data

From the table 1, the majority of respondents say product varieties is the reason for preferring online shopping. 37% of the respondents can choose credit card mode for making a payment because it had time to settle an amount. 60% of the respondents did not pay any charge for extra payment. Only 3% of respondents say low level of price products are available in online shopping. Most of the respondents say the packaging of products is good. 19% of the respondents say more than 10 days to deliver a product in online shopping. Majority of the person can face warranty problem in online shopping products.

9. Awareness towards Online Shopping

In this study, the respondents are asked to give their opinion about online shopping. The response observed for each of the items in the schedule have been scored and tabulated on a master sheet. The scoring factor is based on Likert's method.

The customers towards online shopping. This factor is classified as high level, medium level, and low level. This factor has been cross-tabulated with other factors like gender, age, educational qualification, occupation, marital status, and monthly income. Following are the factors to find out the level of awareness of customers towards Online Shopping such as through the Internet, through Advertisement, through friends and relatives respectively.

Table 2: Level of Awareness and Personal Factors

Personal factors		**Level of awareness**			
		High	**Medium**	**Low**	**Total**
Gender	Male	11(33%)	12(27%)	10(30%)	33(100%)
	Female	11(30%)	16(43%)	10(27%)	37(100%)
	Total	22	28	20	70
Age	Below 25 years	12(50%)	2(8%)	10(42%)	24(100%)
	25- 50 years	7(19%)	20(56%)	9(25%)	36(100%)
	Above 50 years	3(30%)	6(60%)	1(10%)	10(100%)
	Total	22	28	20	70
Educational Qualification	SSLC	2(33%)	3(50%)	1(17%)	6(100%)
	HSC	4(36%)	2(18%)	5(46%)	11(100%)
	UG	5(28%)	6(33%)	7(39%)	18(100%)
	PG	5(22%)	12(52%)	6(26%)	23(100%)
	others	6(50%)	4(42%)	2(8%)	12(100%)
	Total	22	27	21	70
Marital status	Married	11(37%)	13(43%)	6(20%)	30(100%)
	Unmarried	11(28%)	15(38%)	14(34%)	40(100%)
	Total	22	28	20	70
Occupation	Student	5(39%)	3(22%)	5(39%)	13(100%)
	Private Employee	4(19%)	11(52%)	6(29%)	21(100%)
	Government employee	7(64%)	3(27%)	1(9%)	11(100%)
	Business	5(38%)	4(31%)	4(31%)	13(100%)
	Profession	1(8%)	7(58%)	4(34%)	12(100%)
	Total	22	28	20	70
Income	Below Rs. 10,000	5(19%)	15(58%)	6(23%)	26(100%)
	Rs. 10,000 – Rs. 20,000	4(19%)	8(38%)	9(43%)	21(100%)
	Above Rs. 20,000	13(56%)	5(22%)	5(22%)	23(100%)
	Total	22	28	20	70
Region of survival	Rural	12(50%)	2(8%)	10(42%)	24(100%)
	Urban	7(19%)	20(56%)	9(25%)	36(100%)
	Semi urban	3(30%)	6(60%)	1(10%)	10(100%)
	Total	22	28	20	70

Source: Primary Data

It is observed that out of 70 sample respondents, 16% of the female respondents have a medium level of awareness compare to the male respondents in the gender

wise classification. In the age group of 50 years of age, 60% of the respondents have a medium level of awareness whereas 50% of the respondents in the age group of below 25 years have a high level of awareness towards online shopping. Among the educational qualification, most of the respondents from SSLC and PG students have a medium level of awareness towards online shopping. From the marital status of the respondents, 37% of married respondents have a high level of awareness. With regard to the occupation category, 64% of government employees have a high level of awareness compared to others. In the income category, 56% of the respondents whose income above Rs. 20,000 have a high level of awareness the respondent's region of survival, semi-urban and urban people have a medium level of awareness compared to rural respondents.

10. Findings of the Study

Findings from the complete data and the result of the analysis are presented in this chapter. Primary data was collected through a well-designed interview schedule from which findings were as follows:

- Among 70 respondents, 28(40%) of the respondents are having a medium level of awareness towards online shopping.
- While analyzing the level of the attitude of the customers towards online shopping only 37(53%) of the respondents have a medium level of attitude towards online shopping.
- It is opined that 66(44%) of the respondents have preferred online shopping for easy purchase.
- The study indicates that majority of the respondents have faced warranty problem when they bought goods from an online shopping.
- It is seen that out of 70 respondents, 12(17%) of the respondents have opined that the cost of the product is high while purchasing in online shopping.
- It is seen that out of 70 respondents 19(27%) of the respondents have purchase Household goods from online shopping. and 15(21%) of the respondents have purchase mobile phone in online shopping.

11. Suggestions

The researcher has made an attempt to study the attitude of the buyers towards online shopping. Some of the suggestions and recommendations to improve the quality of the products and services based on the valuable information provided by the buyers.

- Online shoppers who sell the products might have improved the after-sales service of products as it is the main factor for the sales of consumer products.
- Online shopping must introduce a medium level of price for grabbing even the middle-income group people also.
- Online shopping also targets upper and lower-middle-class people.
- Online shopping can deliver the product without damages and not be a duplicate one.
- Online shoppers can concentrate more on the packaging of products and deliver the products incorrect time.
- Online shopping could focus on semi-urban areas and rural areas also.
- More awareness may be spread to customers regarding the importance of online shopping in less developed areas by giving advertisement and publicity.

12. Conclusion

The study reveals that online shopping is the best choice rather than buying a product in retail shops. By selecting a product through online shopping, we have enough freedom to choose between various products. The number of respondents for online shopping is gradually increasing for it is more flexible, up gradated and comes with varied configuration than the retail products.

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PLAGIARISM: A PRACTICAL APPROACH

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Abstract

The University Grants Commission has highly concentrated on Good quality of research reports, dissertations and publication of Journal articles. To control the Plagiarism UGC Care list of Journals framed and URKUND Software provided to all the universities. In academic writings, plagiarism is one of the serious issues among the research scholars and the academic arena. This paper discusses about the plagiarism, plagiarism detection tools, where it affects, how to overcome it and also explains the procedures of research methodologies, various kinds of plagiarism, stages to avoid plagiarism. Suggestions were given to improve the good quality of research reports, dissertations and articles.

Keywords: Plagiarism, Research Reports and Detection Tools.

Introduction

University Grants Commission (UGC) has taken many efforts to eradicate plagiarism of research reports and publications among the research area. To control plagiarism UGC framed CARE LIST of Journals. These journals are recognized for the publication of articles by research scholars. Plagiarism is a kind of cheating and a serious offense. To identify plagiarism the UGC also forces academic institutions to concentrate on anti-plagiarism. To avoid plagiarism (without giving citation) the plagiarism tools were introduced. It considers to be academic dishonesty and it violates, ethics, many of the research scholars think of plagiarism as copying another's work or borrowing someone else's original ideas. But terms like copying and borrowing can disguise the seriousness of the offense (according to www.plagiarism.org). Information Library Network (INFLIBNET) has introduced Shodhshuddhi. One of the plagiarism detection tool. It is initiated by the UGC under the MHRD project. To identify plagiarism the UGC also forces academic institutions to concentrate on anti-plagiarism. The research supervisors and Educational institutions also take initiatives to create awareness programmes among

the researchers is the need of the hour. Jijila and Chandrakumar(2018) studied state universities have mentioned about the available plagiarism detection software and the punishments mode and concluded in their study that an enforceable plagiarism policy with definition clarity is always required in the state universities.

Review of Previous Studies

Sudhir Kumar jena(2018)investigated his study of the awareness level of plagiarism among the students of IIM Shillong. The data were collected using a self-designed questionnaire and was distributed among the randomly selected 100 students including research scholars of IIM Shillong. The summary indication was that respondents are aware of plagiarism. They perceived that plagiarism is an important issue, it is necessary to use detection software, respondents support its use, and it will have some effect in preventing plagiarism. Muthukamatchi and Tripathi (2018) discussed in their paper plagiarism is one of the very complex and serious issues in and around the world. The Internet plays a vital role to get the information more easily and access without any geographical barriers. The UGC is also insisting to check plagiarism before submission of the thesis. Due to this reason, all educational institutions are concentrating on anti-plagiarism, for identifying plagiarism, various software was used in the different institutions. This work has been carried out for various kinds of software, usage of those software steps to use this software.

Plagiarism

Borrowing the ideas of others without acknowledging the credit to them and presenting the concept as our own is plagiarism. In other words, stealing someone's ideas and later on, telling lies or defending the act of literary theft, plagiarism is an act of intellectual dishonesty.

Kinds of Plagiarism

- Blatant Plagiarism: Just copying the whole text without acknowledgment.
- Pot Luck Paper: Ideas borrowed from many sources.
- Word Switch: After keywords and phrases.
- Mosaic: Paraphrase most papers.
- Self-Plagiarism: Borrow from his own published articles.
- Resourceful Citer: Full of quotation and ideas of others with acknowledgment.

Plagiarism Detection Tools

There were many software available to detect plagiarism. Some of the popular plagiarism software has been mentioned below:

1. Turnitin, California, USA
2. Shodhshuddhi,(URKUND)
3. iThenticate, California, USA
4. Writecheck, California, USA
5. Viper, England
6. Plag Aware, Germany

How to overcome Plagiarism

Stage 1:

The researchers and Research supervisors should be selected the topic/title of the research. It will be unique and verified with existing submissions or completed projects/Thesis/Dissertations. That title or topic is already done, which may be allowed to pursue in case of extended in the particular concept/expanded geographical research area with the permission of the Doctoral Committee. It will not affect the plagiarism case, otherwise, it assumed as a duplicate thesis/dissertation.

Stage 2:

Titles and topics will be checked in Shodhganga, Shodhgangothri & online Catalogues of Various educational institutional portal to avoid duplication and the selected topic/title will be finalized in the Subject experts Committee/Doctoral Committee of the educational Institution.

Stage 3:

Introductory part & Review of Previous studies are to be taken in to account with proper acknowledgment and it should be modified into our own language/idea. It is to be noted that the sentence of active voice will be converted to passive voice and presentence will be future tense according to the passage modified with relevant to our topic and at the same time copyright issue should not affect. But, the references should be given in the Bibliography area.

Stage 4:

It is mandatory for Every Research scholars should publish articles relevant to their topic/research area. At least three or Four or more than that should be published in the reputed indexed journals. Indexed journals means published journal articles uploaded in the internet database viz.Springer, Elsevier, ProQuest, Scopus, etc., That database

will be adopted or merged by the anti-plagiarism detection software. In this case, at the time of submission of our theses/research report for plagiarism checking in the university, the university software exposes the actual status of plagiarism percentage. If it is 20% or below 30% may be allowed for final submission. In the case of more than 30% percentage, it will be modified, that is self-citation will be highlighted in the plagiarism report. In this case, the research supervisor gives a justification letter mentioning self-citation articles to the university authority or the Doctoral Committee to omit such citations from the Plagiarism report to submit.

Stage 5:

Avoid shelf Checking: The research scholars before submitting their thesis for plagiarism checking in University, they self-check-in the private software's to know their plagiarism percentage. It will be avoided, because when at the time of checking in the university software it will show 100% plagiarism of the concerned thesis. Most of the research scholars don't know the fact or realism, but they affected unknowingly. So, self-checking of the thesis and dissertations will be avoided.

Suggestions & Conclusion

According to the above discussions the research scholars and research supervisors are need awareness training programmes. Proper training will be given by the institution or University about plagiarism software and copyright act. The percentage of plagiarism in research papers/reports/dissertations is increasing day by day and as such many publishers insist on plagiarism check before accepting articles for publication. Since plagiarism is not a new problem for education providers, academics are increasingly concerned about a manageable way to delimit or curb it by implementing policies. The University and institutions above to formulate the committee of subject experts to look into the matter. In case the guide and the researcher are found guilty, the research contents are withdrawn without any correspondence for good quality of research. The research supervisor, Research scholar, and the institution's role is very important to produce good quality research reports. Plagiarism and copyright issue awareness programmes will be conducted by the Universities/Institutions is an essential part of duty and need of the hour.

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SIGNS TERRIFY THE PEOPLE WITH DOWN SYNDROME: A SEMIOTIC STUDY OF MARK HADDON'S THE CURIOUS INCIDENT OF THE DOG IN THE NIGHT-TIME

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Abstract

The Curious Incident of the Dog in the Night-Time is a 2003 mystery novel by British writer Mark Haddon. The novel is narrated in the first-person perspective by Christopher John Francis Boone, a 15-year-old Autistic boy who can't read others faces and put himself in others' shoes. He can't understand anything more than the literal meaning of whatever' is said to him. Autism is a cognitive disease that leaves those who have it struggling to perceive even the most basic of human emotions.

Keywords: Figurative Language, Emotions and Logic.

1. Introduction

It has become tough now a days even for a normal person to predict the meaning of a word, phrase and a sentence as they signify different signified. It is even worse in the case of the people with Disabilities.One of the major characters of any language is its "arbitrariness", which simply means that there may not be any necessary relationship between a words meaning and what it refers to. Also there are no set rules that language follows for the combination of signs in order to produce complete thoughts which vary from one language to another. This is one reason why language can be quite difficult to learn and comprehend.

There is an acute failure to capture arbitrary signs in a language by individuals suffering from Down syndrome. This study brings out the difficulties faced by Christopher, a character with disability in the novel in different situations by the use of figurative language, emotions and language and logic.

2. Figurative Language

Even from the first few chapters of the novel, we are introduced to the protagonist Christophers biggest hurdle in terms of communication - understanding figurative language. We get the first glimpse of this in chapter 29, where he cites the use of metaphors in conversations as one main reason to find people confusing to comprehend.

> "I find people confusing. This is for two main reasons. The first main reason is that people do a lot of talking without using any words..."
>
> "The second main reason is that people often talk using metaphors:
>
> I laughed my socks off.
>
> He was the apple of her eye.
>
> They had a skeleton in the cupboard.
>
> We had a real pig of a day.
>
> The dog was stone dead."
>
> (Haddon 19)

He then traces the origin of the word "Metaphor" to its Greek roots where it simply translates as, "carry something from one place to another". Following that he states his reason for finding this figure of speech a very unnatural device.

> I think it should be called a lie because a pig is not like a day and people do not have skeletons in their cupboard. And when I try and make a picture of the phrase in my head it just confuses me because imagining an apple in someone's eye doesn't have anything to do with liking someone a lot and it makes you forget what the person was talking about.
>
> (Haddon 20)

From the above lines, it can be deduced that there is an acute failure to grasp the essence of the metaphorical expressions. This is predominantly owing to the inability to let go of its mere literal use. A similar instance can be seen in the preceding lines where he expresses his concern of identity with respect to the spiritual significance of his name. He knows that he shares his name with the name of the Saint who carried Jesus Christ across a river. This is again derived from the Greek equivalent and so is a metaphor too, according to him. He further raises questions on what his name could have been before the incident and believes it to be a lie too. In spite of his disability, he seems certain of his identity as an individual though as he says, "I do not want my name to mean a story about being kind and helpful. I want my name to mean me" (Haddon 20). A similar such instance can be seen in chapter 103, when there is a conversation between him and Mr. Rhodri, a friend of his father's.

> Then Rhodri said to me, 'God, you do get the third degree, don't you?'
>
> But I didn't know what *the third degree* was.
>
> And I said, 'I'm doing very well, thank you,' which is what you're meant to say.
>
> (Haddon 84)

Here we learn as to how, in the failure to comprehend figurative language, individuals with learning disabilities resolve to respond to a question even without knowing what it is meant to imply. But, despite his limitations of figurative knowledge, it is indeed surprising that he understands, to an extent, a literary term like "rhetoric question" as expressed in chapter 127.

> Then he said, 'Holy f***ing Jesus, Christopher. How stupid are you?'
>
> This is what Siobhan says is called a rhetorical question. It has a question mark at the end, but you are not meant to answer it because the person who is asking it already knows the answer. It is difficult to spot a rhetorical question.
>
> (Haddon 102)

This further validates the hypothesis that these individuals limit their response to the information pertaining to the subject and cannot alternate it easily based on their convenience. As a result, Children with AS appear to have particular weaknesses in the areas of non-literal language that includes humour, irony, teasing and sarcasm. A number of communicative problems Christopher encounters are due to his inability to identify and associate with these expressions alien to him.

3. Difficulty in Capturing Emotions

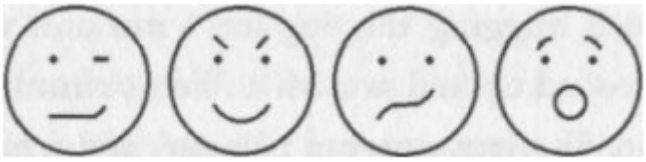
Individuals with Aspergers may have an impaired theory of mind which makes it difficult to understand the thought, feelings and emotions of other people and how that relates to oneself. Aspergers syndrome is mostly associated with difficulties in social relationships and communications. Throughout the story, we are aware of the difficulties Christopher encounters because of his inability to capture peoples emotions which he thinks are not computable. We are introduced to this vital piece of information in chapter 3 first.

> Eight years ago when I first met Siobhan, she showed me this picture.

And I knew that it meant 'sad' which is what I felt when I found the dead dog.

Then she showed me this picture

And I knew it meant 'happy', like when I'm reading about the Apollo space missions, or when I am still awake at three or four I the morning and I can walk up and down the street and pretend that I am the only person in the whole world. Then she drew some pictures.



But I was unable to say what these meant.

(Haddon 2)

In Chapter 13, as he explains the nature of his book, and again in chapter 167, he even confesses that he "cannot tell jokes" as he "does not understand them" (Haddon 150), following which he gives an example wherein he explains how different jokes have different meanings and so, he cannot focus on different things at the same time. Christopher further exhibits his frustration at his inability to understand sign language used by people during a conversation as expressed in chapter 29

I find people confusing. The first main reason is that people do a lot of talking without using any words. Siobhan says that if you raise one eyebrow it can mean a lot of different things. It can mean 'I want to do sex with you' and it can also mean 'I think that what you just said was very stupid'.

Siobhan also says that if you close your mouth and breathe out loudly through your nose it can mean that you are relaxed or that you are bored

> or that you are angry and all that depends on how much air comes out of your nose and how fast and what shape your mouth is when you do it and how you are sitting and what you said before and hundreds of other things which are too complicated to work out in a few seconds.
>
> (Haddon 155)

Also, in chapter 73 we see the ultimate evidence of his eccentric personality as explained in his own words when he talks of his "Behavioural problems" (Haddon 59) some of them which include,

> C. Not like being touched
>
> K. Not noticing that people are angry with me
>
> L. Not smiling
>
> M. Saying things that other people think are rude.
>
> (Haddon 59)

It is very challenging to talk about the feeling of love prevalent in the book as Christopher in all his ignorance, does not even understand what the term love means and so, reduces it to some kind of a transaction of favours. As he has a difficulty in understanding other peoples mind with their gestures, it adds to our feeling of empathising with him. The fact that he doesn't much details to others is because he is incapable of understanding the working of others minds.

> And father said, 'Christopher do you understand that I love you?'
>
> And I said, 'yes' because loving someone is helping them when they get into trouble, and looking after them, and telling them the truth, and father looks after me when I get into trouble, like coming to the police station, and he looks after me by cooking meals for me, and he always tell me the truth, which means that he loves me.
>
> And then he held up his right hand and spread his fingers out in a fan, and I held up my left hand and spread my fingers out in a fan and we made our fingers and thumbs touch each other.
>
> (Haddon 109)

This description not only presents to us, a picture of a youngster torn between logic and emotion but projects his inability to empathise which is a key feature of this disorder. We even learn towards the end of the novel that, his sense of reasoning takes the upper hand as he concludes his dad to be very much capable of killing him as he killed the dog too. In this naive assumption, he oversees all those years of consistent paternal love that his dad showered upon him and resolves to an impulsive reaction owing to his lack of comprehending human emotions.

4. Language and Logic

One of the major conflicts in the novel is the one between language and logic. His rules of logical reasoning don't share accordance with the ambiguity of language and results in his distinctive responses which would otherwise appear disturbing to an observer. We find the first traces of this in chapter 59,

> This is because I do not always do what I am told.
>
> And this is because when people usually tell you what to do it is usually confusing and does not make any sense.
>
> For example, people often say 'Be quiet' but they don't tell you how long to be quiet for. Or you see a sign which says KEEP OFF THE GRASS or KEEP OFF ALL THE GRASS IN THIS PARK because there is lots of grass you are allowed to walk on.
>
> (Haddon 38)

In the above cited example, we can presume that Christopher requires every order explained in minute details so he can work accordingly. To him language acts as a set of codes which need to be carefully discharged in order to ensure a loss of misunderstanding. This is perhaps one reason why he finds his trainer in school quite reliable when it comes to giving orders. He explains it in chapter 59,

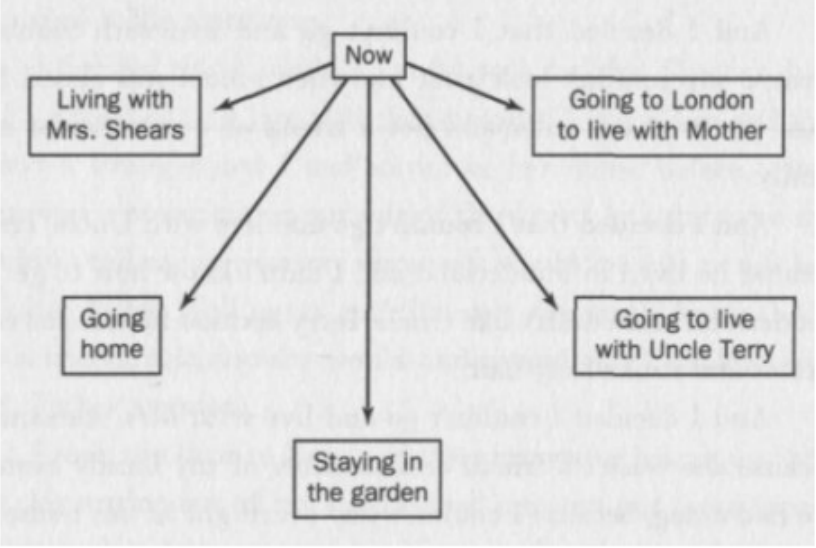
> Siobhan understands. When she tells me not to do something she tells me exactly what it is that I am not allowed to do. And I like this.
>
> For example, she once said, 'You must not punch Sarah or hit her in any way Christopher. Even if hits you first. If she does hit you again, move away and stand still and count from 1 to 50, then come and tell me what she has done, or tell one of the other members of the staff what she has done.'
>
> (Haddon 39)

Also, in many ways logic takes precedence over instinct. At no point in the novel does he ever rely on his intuitions to confront a problem. There is a certain reference throughout the book to logical puzzles, maths problems and maps which to him provide means to a clear solution. Because Christopher suffers from a form of OCD (Obsessive Compulsive Disorder), his tolerance for disorder is unbearable. To him, an orderly life is his only solace, ensuring his survival in an otherwise disoriented world. So when confronted with a chaotic encounter, his reliance on escape mechanisms becomes apparent.

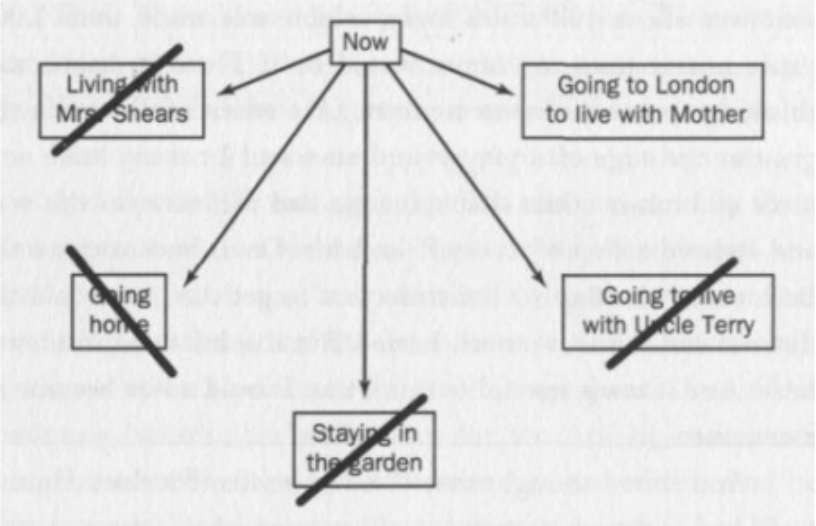
> And this shows that intuitions can sometimes get things wrong. And intuition is what people use in life to make decisions. But logic can help you work out the right answers.
>
> (Haddon 238)

Also, on more than one occasion, he resolves to carefully scheduling his every move and action. In times of crises, he executes to check the probability of choices and choose the best option among it. By doing this, he is certain of a good consequence that would aid him through the crucial phase.

> And then I realised that there was nothing I could do which felt safe. And I made a picture of it in my head like this



> And then I imagined crossing out all the possibilities which were impossible... And it was like this



(Haddon 238)

Also, the fact that he takes refuge in analytical subjects like maths and sciences is proof enough that he enjoys their rules, accessibility and puzzle like qualities which may not apply to language. He is used to organizing his thinking all times. So much that he draws timetables to ensure following a perfect plan. According to him, this tool represents a part of a world where ambiguity is not possible and there is an answer for all question. By planning in advance, he feels secure of his actions.

> And then I Formulated a Plan. And that made me feel better because there was something in my head that had an order and a pattern and I just had to follow the instructions one after the other

(Haddon 238)

5. Conclusion

Mark Haddon's "The Curious Incident of the Dog in the Night-Time (2003) and provide information given by the protagonist Christopher Boone to validate the point that language and sings terrify by keeping him inaccessible to the language and making him failed in communication. By learning his character, intricacies, language skills and the way he functions in the society, we could perceive the working of a disabled mind and thereby understand the truth that he is terrified by the nuances of capturing arbitrary signs in a language. By carefully analysing the narrative style employed by him, his idiosyncrasies and descriptions of both himself and others around, it enables readers to comprehend the working mind of an individual with learning disabilities and suggests the inference that the protagonist suffers from Down syndrome.

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COMPLIANCE TO WESTERN CULTURE AND SHIFT IN THE INDIAN TRADITIONAL STANCE

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Abstract

The western civilization immensely dominated and covered almost all facets of the Indian culture. In fact the culture of the west and east has brought so many socio-cultural changes in India after the entry of British on the soil in 1600. The culture of the west having its direct impact on the Indian traditional and cultural heritage also was influential in the personal life of the common man in general and the elite group of Indians in particular. Its the mass attraction towards English system education that paved way for the wide adoption of western culture by the English educated elite Indian youth. Though there are many factors behind this cultural change, yet it is the education system introduced by the British that consisted of the curricula of their own. Since it was new to the Indians and also there was no other go without learning it, they turned susceptible towards the English language, English education and thus to the western culture. The change in the Indian education system with the introduction of English language as the second one in the curricula has great impact on Indians who studied in schools and colleges. Secondly the medium of instruction in English language is also introduced by them. This system is still in practice in almost all schools at the higher secondary level and colleges across the nation.

Keywords: Western Civilization, Indian Civilization, Education System and English Language.

The compliance to western culture is one of the turning points in the history of India. The Indians susceptibility to the civilization of the west came into practice with the entry of British on India soil. The establishment of the East Indian Company by them is the first milestone that opened a new gate to the adaptability of western culture, since the Indians who served the company had to be well educated of course English educated.

The demand for English language arose among Indians especially those who possessed diplomas or degrees.

The impact of culture of the west upon educated Indians could be understood by the conversion of some Indians like Madhusudhan Dutt to Christianity. "Madhusudan Dutt was so highly westernized that he embraced Christianity," (O'Malley p 488). In fact, it was the impression of the western life style and the new outlook of educated Indians that turned them towards western culture and thus they started to read or write English books or articles etc.

The British implemented their own political concepts in India to develop and modern the nation. Almost the entire nation came directly under the influence of western civilization. Specifically Bengal and Calcutta were heavily dominated by culture of the west. Another cause behind this wide adoption of western life style in the stated Indian territories is that the government employees who under the East India Company and the educated class of people settled down in these territories i.e. Bengal and Calcutta. People in these regions had frequent and familiar contacts with the colonials and thus they were impressed by their civilization shifting from their own Indian traditional stance. They were impressed by their lifestyle to the extent that apart from changing their religion, they even changed their furniture to the western ones. The deal with the British or the attraction of Indians towards the British is the cause behind this westernization. Speaking the healthy contacts of Indians with the British, Charles E. Trevelyan states, "*(The Indian) daily converse with the best and wisest Englishmen through the medium of with it were of a more personal kind*" (**Trevelyan 190**).

The above lines obviously tell the colonial thought of the past besides stating that the culture of the west had a great impact on the colonized. There were further great socio-political shifts in India. There were changes in the outlook of Indian in 1880. Lewis Sydney Steward O'Malley in his preface to his book, *Modern India and the West: A Study of the Interaction of Their Civilizations* states these changes that took place in India with reference to an observation made by Lord Ripon in 1882 "*During the survey of India in 1880, Sir Richard Temple found great changes that made India totally or almost westernized. There were changes taking place in the field of economics, moral sentiments and social attitudes of many people even the religious ideas were dominated and modified by the culture of the west*" (**O'Malley p ix**).

The English education played prominent role in the compliance to western culture by the Indians. In fact it is the wide spread of the English language and education that brought new ways before them. Sir Richard further writes, "*The spread of education, the influence of a free Press, the substitution of legal for discretionary administration, the progress of railways, telegraphs etc., the easier communication with Europe, and the more ready influx of European ideas were beginning to produce a marked effect. New ideas were springing up; new aspirations were being called forth*" (**OMalley, p ix**).

The western civilization immensely dominated and covered almost all facets of the Indian culture. In fact the culture of the west and east has brought so many socio-

cultural changes in India after the entry of British on the soil in 1600. The culture of the west having its direct impact on the Indian traditional and cultural heritage also was influential in the personal life of the common man in general and the elite group of Indians in particular. It's the mass attraction towards English system education that paved way for the wide adoption of western culture by the English educated elite Indian youth. Though there are many factors behind this cultural change, yet it is the education system introduced by the British that consisted of the curricula of their own. Since it was new to the Indians and also there was no other go without learning it, they turned susceptible towards the English language, English education and thus to the western culture. The change in the Indian education system with the introduction of English language as the second one in the curricula has great impact on Indians who studied in schools and colleges. Secondly the medium of instruction in English language is also introduced by them. This system is still in practice in almost all schools at the higher secondary level and colleges across the nation.

This shift in the Indian socio-cultural and traditional ground is considered seriously by Max Weber who, in his introductory note takes a snap of the then modern man's tragedy stating,

> "No one knows who will live in the cage in the future
> or whether at the end of this tremendous development
> entirely new prophets will rise or there will be a great
> rebirth of old ideas and ideals, or if neither, mechanized
> petrification embellished with a sort of convulsive
> self-importance. For this last stage of cultural development,
> it might be truly said, "Specialists without spirit,
> sensualists without heart, this nullity imagines that
> it has attained a level of civilization never before achieved"[1]
>
> (**Max Weber xxxviii**)

Besides expressing his outlook towards the changes in the Indian stand, Weber thinks that certain functional substitutes are required for the preservation or to hold the socio-cultural personality of the westernized human being (Indian). Unfortunately, this effort went in vein and the personality divided itself within the inner level. This personality was the personality of alienation or estrangement. At the end, the alienated personality associated with the dissociated one.

English reading awareness among Indians further developed the Indian literature in English and people gradually started to read English version of literature than the original Indian literatures like Bengali, Urdu, Tamil and so on. The Indian born English

writers and the English writers born in India have contributed to Indian literature in English. Similarly newspapers, novels and fictions had played their part in the further development of this field of literature. J.C Robert writes, "*Since the early 1980s, postcolonialism has developed a body of writing that attempts to shift the dominant ways in which the relations between western and non-western people and their worlds are viewed*" (**J.C Young 2**).

The culture of the west and English language together had much influence over the Indians. As a result, even the conservatives were attracted towards westernization and the language. It cannot be denied that the colonial system of education introduced in the country did have much effect on their personal and professional life. It created a gap between the English educated Indians and the other Indians. There was a barrier of English education and in the education of other languages in the country. This is the factor that gave birth to Indian writing in English. Many Indians, who knew English, translated their literature of their mother tongue into this language.

Generally, the modern and westernized adult children of a middle class Indian family are susceptible to modernity and attracted towards the culture of the west. Hence, they are not prepared to adapt the tradition and culture of their own country (India). They are not in a position to sustain them for long, since; they presume this as fables and out of fashion. The parents being conservative in turn oppose their westernized ideologies and obviously, the familial peace is smashed to pieces. Vijaya Kumar writes,

> ". . . under the overall impact of westernization . . .
> mostly . . . have developed a tendency to move away
> from their aged parents leaving them in lurch. The
> most outstanding fact . . . is revealed in the change
> of the pristine age-old traditional role of kinship
> relationship between the ageing parents and their
> and their younger kinsfolk, especially the sons . . .'
>
> (**S. Vijaya Kumar 37 & 38**)

Conclusion

The adaptability of western culture by Indians in general and the elite Indians in particular though brought certain socio-cultural and traditional shifts in the nation, yet these changes have paved way for momentous social events making them understand the significance of English language and education among the people of India.

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LITERARY AND CULTURAL RENAISSANCE DURING THE LATE 19TH CENTURY IN HYDERABAD STATE

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Asaf Jahi dynasty, founded by Nizamul Mulk Asif Jah I in 1724 established its political supremacy over Deccan and held its sway over Hyderabad State for 224 years i.e. from 1724 to 1948. During this period, Hyderabad, one of the Princely states of India1, emerged as the richest state with an area of 86000 sq. miles which was larger than England and Scotland put together. It is noted that seven generations of the family of Asif Jah ruled over the state and most of the Nizams are famous for their generous contributions and donations to every noble cause of Indians irrespective of caste, creed or religion.

Nizams patronized not only the Muslim institutions like Deoband with liberal donations but also Hindu institutions like Benaras Hindu University. The Golden Temple of Amritsar was also given generous donations on regular basis. During the rule of Asaf Jahis, Hyderabad state witnessed remarkable socio economic developments and literary and cultural advancements. There was complete understanding and harmony between the Hindus and Muslims in Hyderabad state.

In this paper I would like to focus on the literary and cultural developments during the late19th century in Hyderabad state. Almost all the Asaf Jahi rulers were highly learned and cultured. Therefore, they concentrated on the growth and development of literature and culture in the Hyderabad state. Though their religion and language were alien, they equally patronized other languages namely, Telugu and Marathi, Kannada, Sanskrit, Arabic and English along with Urdu2. Persian was the official language up to 1893 and then Urdu up to 1948.

The Asaf Jahi rulers extended their patronage to the literature and culture, and evinced great interest in their development from the beginning to the end of their rule. As the development of languages also depend on the societies, libraries and clubs, they established the libraries, started the newspapers, magazines, the societies, and the language clubs in their kingdom. Asaf Jahis rulers also contributed for the development education, by establishing of many schools and colleges and University. The best example is Osmania University established by Nizam Mir Osman Ali Khan in the year 1918.

Moreover, they established several schools and colleges, like Chaderghat School and City College in Hyderabad. They patronized many poets and scholars in their state. As a result of this, a large number of literary books in different fields came in to lime light. The books may be divided on the basis of their content as follows: 168 laws, 142 with poetry, 62 with education, 53 with religion and ethics, and 33 with history, 38 with fiction, 19 with medicine and others were miscellaneous.

Subsequently, Dairat-ul-Ma'arif, a government literary organization was founded by Syed Hussain Bilgrami in 1886 focusing on the contribution to the cause of oriental studies in Arabic in India. Hyderabad State encouraged the study of oriental sciences, art, literature and learning. Most of the Arabic publications were issued by the Da'iral-ul-Ma'arif, which engaged in publishing rare manuscripts in Arabic literature and science. Among the authors, 588 were Muslims, 43 Hindus, two Persian and one European. At the end of 1909, there were 77 lithographic3 presses in the State. During the period 1901-1912, 14 presses were opened.

It is observed that the literary activities were greatly affected during the first half of the nineteenth century due to lack of interest among the people of Hyderabad. The slow progress of education was also one of the main causes of restriction of literary activities in Hyderabad. There were no organized and systematic libraries and clubs except those attached to Mosques and Temples. This situation was changed during the second half of the nineteenth century, due to the introduction of printing press, founding of libraries, clubs, literary societies.

The oldest library and reading room was the Somasunderam library opened in 1872. In 1884, it was named the Mahabub College Library.4 The condition started improving when people slowly realized the need to make the best use of their leisure hours, with the aim of self-improvement and for the moral, social and intellectual good of the community at large. Lectures were arranged, discussions were held and essays were read by inviting the public to the Secunderabad Young Mens Mutual Improvement Society, inaugurated in 1875. To mark the auspicious occasion of the visit of the Prince of Wales, it was named the Albert Reading Room and Library, consisting of 425 volumes. It subscribed to a number of newspapers, mostly English. Its main object was the advancement of literary and cultural activities.

The Asafia State Library was founded by Syed Hussain Bilgrami in 1886.5 It is a magnificent institution of real interest to scholars, persons engaged in research work, students and the public in general. It had good collection of books of the western and oriental studies. The manuscript section of the library is remarkably rich and possesses an enormous number of rare works in Arabic, Persian, Urdu, Sanskrit, Telugu and Canaries.

The establishment of the Arya Samaj in 1892 and the Ganpathi Utsav in 1895 proved crucial in the social awakening of the general public. The Arya Samaj under the Presidentship of Kamtapershad started functioning in Hyderabad in 1872. The purpose was to arrange series of lectures of Swami Girjananda Saraswati in Hyderabad and

to bring about reforms in the existing religious rituals. The educated class who were influenced by the lectures of Girjanand Saraswati and the social reform movement of the Arya Samaj supported it.

The celebrations of Ganapathi Utsav on a large scale became the best means for public awakening. 6 Many libraries were opened for the regional languages of the state. A Marathi Library, The Bharat Guna Vardhak Sansath, was established at Shah Ali Banda in 1895. It was supported by Raja Rai Bahadur and Raja Raghottam Rao. In 1897, on the 3rd birthday celebration of Mir Mahabub Ali Khan, the Hyderabad Reading Room and Library was inaugurated at the Residence of Raja Raghottam Rao. The Osmania Reading Room and Library was founded in 1904 by Raja Swamy Pershad in order to stimulate the circulation of good books and newspapers in Hyderabad.

The Theosophical Society was established in 1882 by Ramaswamy Ayer in Chaderghat. It was mainly a centre of discussions on matters of religion, literature and culture with the focus on Madame Blavatskys teachings. In 1906 Mrs. Annie Besant declared the Society Hall open. The elite of the city were attracted by its activities. The Albert Reading Room, Secunderabad the Malwala Sabha in the city and the Hindu Social Club, Chaderghat were the other societies which were involved in public affairs.

In 1906, the Khair Khah Asaifa reading room and library were founded in the old city of Hyderabad with the support of Raja Mukat Ram. This library contained excellent books in English, Urdu and Persian. It subscribed to Urdu and English newspapers. From 1865, a number of library institution and clubs were founded. Some of them were: Hyderabad Club(1865), Volunteers club (1880), Iqbal Club (1890), Nizam's Club (1893), Sanmarg Darakrk Club(1893, Rahimia Alum-o-Funun (1895), Anjuman Islah-e-Khayalat (1903) and Anjuman-e- Urdu (1907) [7]. Urdu emerged a rich language because of official status. Telugu, Marathi and Canaries did not have sufficient opportunities. Marathis scholars like Shri Dingre, Shri Karmarkar and Maharaja Kishen Pershad rendered great services for the cause of Marathi. Shri Martand Manikprabhu was the doyen among Marathi scholars [8].

Conditions during the early nineteenth century were not favorable for the emancipation of women in Hyderabad, because of restrictions and lack of education. However, things improved during the second half of the nineteenth century, when a number of girl's schools had opened and efforts were made to educate more and more girls. The government, private enterprise and indigenous efforts resulted in the awakening of a class which was neglected in the past.

With the progress of women's education, certain educated women started work for the social upliftment of their sisters. Mrs. Hughes started a ladies circulating library in 1884 and decided to obtain certain useful and interesting journals for circulating among themselves and fixed one rupee as the monthly subscription.9 Miss Allen conducted a club for non-Christian students, besides a Muslim Ladies Association. Ladies clubs meeting were held regularly in the mission precincts. A women's association was formed by Mrs. Karim Khan, daughter of Nawab-Imad-ul-Mulk, in 1904. The main

object was to work for the encouragement of social intercourse and for the promotion of education among the women of Hyderabad.

The role of newspapers and journals was of great importance in the awakening of public opinion. One of the newspapers, The Hyderabad Record, [10] always championed the cause of the downtrodden. Even the Imperial Government of Calcutta did not escape its bitter criticism. In a series of editorials on 'The Government of India's Suicidal Financial Policy', the paper drew the attention of the people to its repercussions. It was also critical about the activities of the residents of Hyderabad. It vigorously supported the cause of Mulki against non-mulki a controversy which raged in Hyderabad State.

The policy of the Government of Hyderabad, imposing a number of restrictions on newspapers, was severely criticized in the press of the day. The Urdu paper Shaukat-ul-Islam refused to sign the agreement and commented upon it in very strong language. The second half of the nineteenth century was characterized by various journalistic activities in Hyderabad. A number of newspapers and journals were started in Urdu and Persian (25), Telugu (1), Marathi (4) and English (13) [11].

Journalism in Hyderabad was comparatively of a later origin. The first was known as Dina Vartaman. The journals which began to appear in Marathi in the city and districts, were Bhageshnagar, 1886, Hyderabad. Nizam Vaibhav 1905, weekly, Hyderabad, Champavati, Urdu and Marathi, Bhir and Gulbarga Samachar, Urdu and Marathi, Gulbarga [12]. At the end of the century, only the Hyderabad Chronicle was in existence along with the other newly introduced journals of the first decade of the twentieth century.

By the end of 1885, Hyderabad was well set on the road to progress in the field of education as well as in the growth of an educated class which steadily moulded itself to the needs of a changing society. Young men started proceeding to England for higher studies. The impact of European culture on the people of Hyderabad was very great during the later part of the nineteenth century.

Many intellectual and eminent personalities like Moulvi Cheragh Ali [13] and Syed Hussain Bilgrami rendered a great service to the development of this language in Hyderabad by writing books and other valuable works in English. Mrs. Sarojininaidu, [14] 'The Nightingale of India', Nawab Sir Nizamat Jung Bahadur and Naqwab Sir Faridoon Mulk Bahadur were great poets . However, the impact of English on the literature of the Indian languages, however, was not very noticeable because of its late introduction in Hyderabad State compared to other parts of India [15].

By the second half the 19^{th} century Hyderabad had become the greatest cultural center in India, blending Mughal, Persian and Central Asian tradition with those of its Hindu, Sikh, Parsi and Christian communities. Calligraphy, music, painting, poetry and the sciences flourished. Nobles competed to attract the best poet and Qawwali singer to the palaces; calligraphers were commissioned to inscribe the walls of mosques and religious events were patronized. By the beginning of the 20^{th} century it was the most

important center for Urdu literature in India.

Communal harmony was another notable feature of this period. Although 90% of Hyderabad's population was Hindu and its Muslim minority was a mixture of Sunnis and Shiyas, religious tension was almost unheard of. It is difficult to differentiate Hindus and Muslims by their dress or their names. Hyderabad's Deccani culture had its distinctive dress, food, manners and art.

It can be concluded that the latter half of the nineteenth century was characterized by a literary and cultural renaissance in Urdu age and to a lesser extent in Telugu, Marathi and Canaries', thanks to the patronage of the Nizam's Government, the nobility and various philanthropists. Literature developed in the sciences and arts. These intellectuals rendered great service to the promotion of and research in oriental studies. The introduction of the press was a great boon, especially for the publication of classical works on arts, sciences and religion. Journalism played a significant role in the creation of public opinion, especially during the closing decades of the nineteenth century. The publication of voluminous literary works was undoubtedly the greatest achievement of the late nineteenth century. Communal harmony and cultural synthesis are the hallmarks of the period.

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