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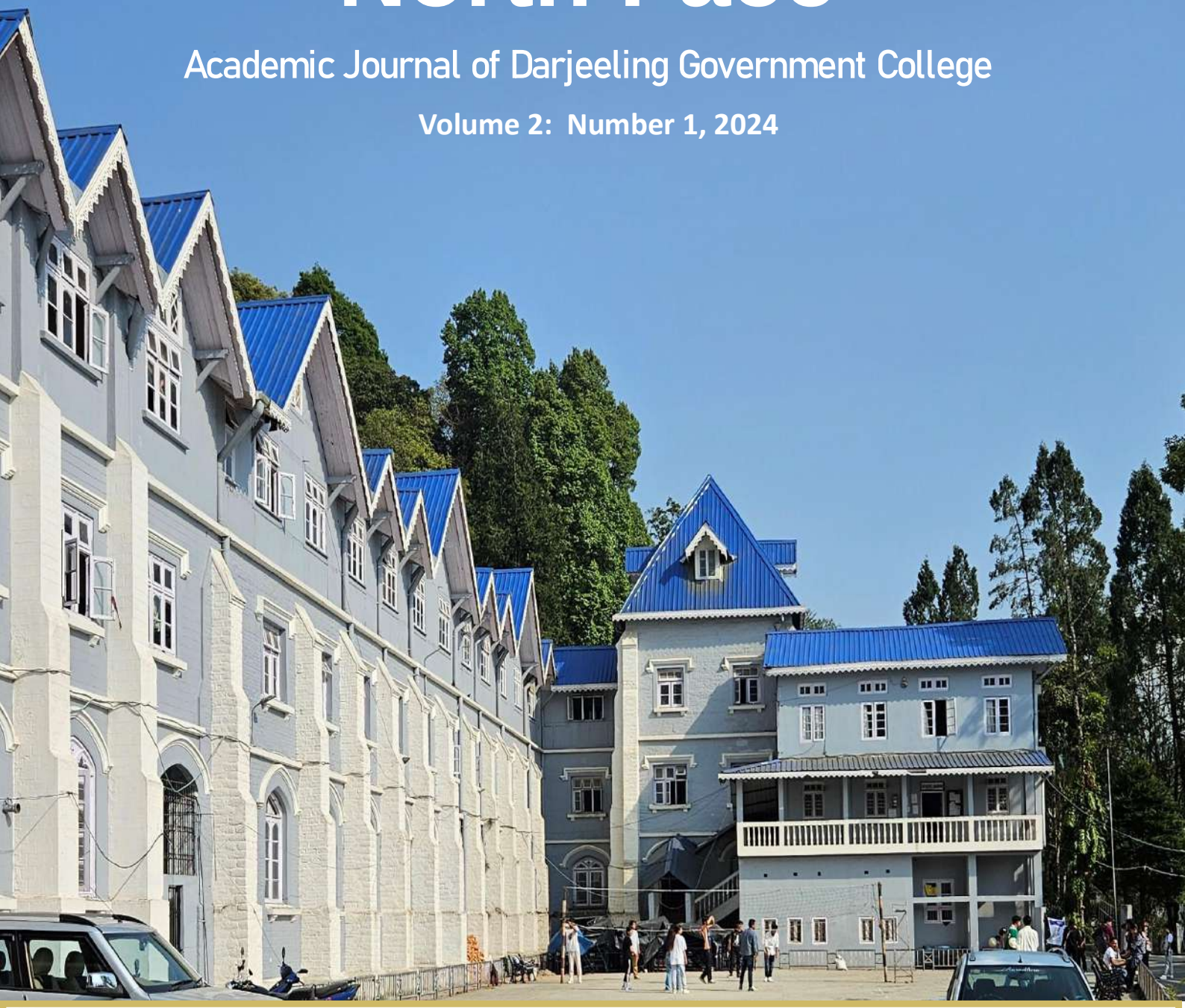

Darjeeling Government College
Lebong Cart Road
Darjeeling- 734101

Founder Editor

About the Journal

The Journal is an interdisciplinary, peer reviewed and refereed academic journal of Darjeeling Government College new noble initiation of the teachers in the academic field. It brings out research based articles/papers on diverse fie comprising of natural science, social science, humanities, commerce and economics, having significant contribution to the development of research and academic activities. Theoretic papers as well as hardheaded papers are welcomed the betterment of academic activities and of research field as well. The essence of journal is to hike up search knowledge with academic rigor.

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From the Desk of Editor-in-Chief

It is with great honor and enthusiasm that I extend my gratitude for being entrusted with the responsibility of overseeing the North Face Magazine, the esteemed academic journal of Darjeeling Government College, for another year.

As we embark on the journey of the 2024 edition of North Face, I am reminded of the rich history and legacy that our college holds in the realm of scholarly publishing. Reflecting on our past endeavors, particularly the publication of the “Journal of Bengal Natural History", under scores our commitment to fostering academic excellence and advancing scientific research in our region.

North Face Magazine continues to be a platform that welcomes scholarly contributions from various disciplines. Shedding light on contemporary issues in Natural Science, Commerce, Humanities & Social Science, and offering insights into the way of life in the northern parts of India. While this volume focuses on peer-reviewed scientific journals, we remain dedicated to embracing diverse perspectives and facilitating meaningful discourse among our readership.

In today’s rapidly evolving landscape of scholarly publishing, it is imperative that North Face adapts to meet the evolving needs and expectations of our authors and readers. As Editor-in-Chief, my foremost objective is to ensure that North Face maintains its flexibility while upholding the highest standards of academic rigor and integrity. By introducing innovative initiatives to our editorial and review processes, we aim to enhance the dissemination of cutting-edge research and contribute to the advancement of knowledge.

The dedication of our renowned editorial board and the commitment of our authors are integral to the success of North Face, I extend my gratitude to all contributors for their invaluable contributions and invite researchers to continue submitting their work for consideration. Our rigorous peer-review process remains steadfast in its commitment to fostering excellence and scholarly advancement.

As we navigate the challenges and opportunities that lie ahead, I am confident that North Face will continue to serve as a beacon of academic excellence, inspiring breakthroughs and shaping the future of scholarly discourse. I extend my best wishes for a promising future to North Face and express my gratitude to all who contribute to its success.

Thank you for your continued support and dedication.

Warm regards,

(Dr. Projjwal Chandra Lama)

Editor-in-Chief

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श्रद्धावान लभते ज्ञानम्

Article No. DGC-NF-24/01

Inhibitory efficiency of phytochemicals against HCK as a therapeutic target for Cancer: Insight of Molecular Docking

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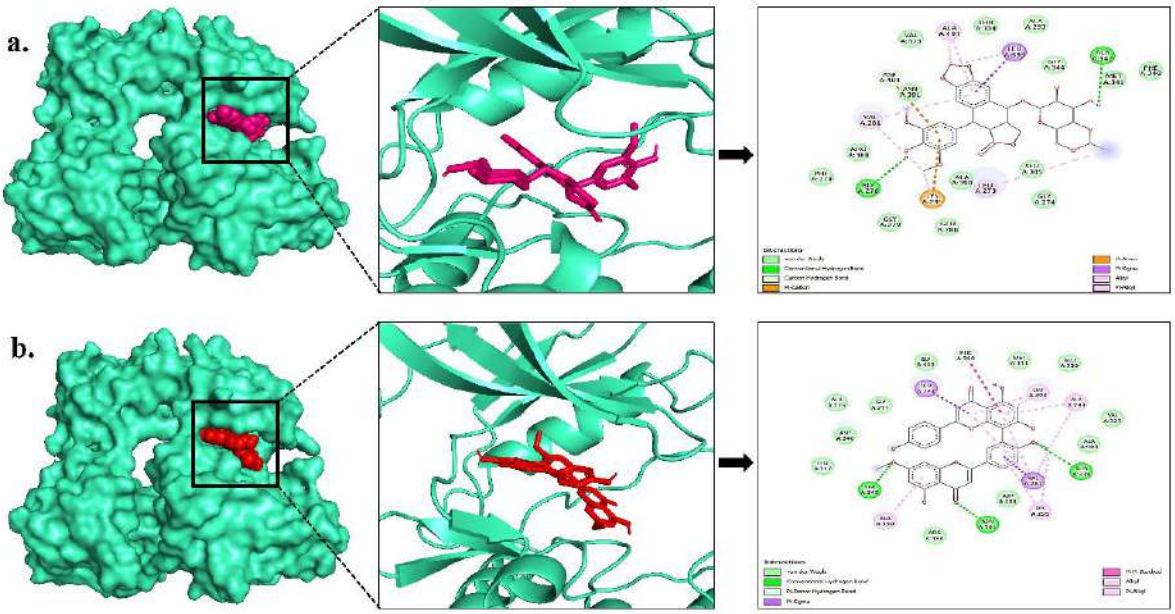
Abstract:

Cancer has thrown a big challenge to the globe by snatching millions of human lives from the world. Hematopoietic cell kinase (HCK), which belongs to the SRC family of non-receptor protein tyrosine kinases (SFK), is largely expressed in B lymphocyte lineages and myeloid cells and is the most abundantly expressed SFK in tumour-associated host stroma. In this study, fifty bioactive compounds were screened against HCK. The molecular docking study reflected that among these compounds, Theaflavin has the highest binding affinity (-11.2 kcal/mol) as compared to Dasatinib (-8.2 kcal/mol), which is available in the market. Theaflavin is predicated form two hydrogen bonds with ALA 342 and GLY 276 and therefore, Theaflavin could bind to the ligand binding site of HCK, inhibiting its essential functions, which in turn could hinder cancer cell proliferation. ADMET profiles of the phytochemicals showed promising and comparable properties to the approved drugs against HCK.

Keywords: HCK, Molecular Docking, Theaflavin, Dasatinib, ADMET properties.



1. Introduction:

Multiple changes in gene expression caused an imbalance in cellular proliferation, leading in cancerous cell growth [1]. Cancerous cells have unique abilities such as survival, immortality, self-sufficiency of growth signals, infinite replication, gene instability, avoiding planned cell death, continuous angiogenesis, and a wide range of mutations [2]. Cancer is now treated using chemotherapy, radiation, immunotherapy, surgery, and anticancer medications, all of which have been shown to be less successful due to the disadvantages of recurrence, drug resistance, influence on non-targeted cells, and other diverse side effects and toxicity. Identifying novel biomarkers and possible therapeutic targets may open up new therapy avenues to enhance cancer outcomes and prognosis [3]. HCK, which belongs to the SRC family of non-receptor protein tyrosine kinases (SFK), is largely expressed in B lymphocyte lineages and myeloid cells and is the most abundantly expressed SFK in tumour-associated host stroma [4,5]. In humans, HCK is composed of the p61HCK and p59HCK isoforms [6]. HCK expression was reported to be elevated in pancreatic cancer, CRC, gastric cancer, and other solid tumours [7–9]. HCK overexpression may have a role in carcinogenesis, cancer progression, and survival [10]. Findings suggest that HCK may enhance the formation and control of cancer biological behaviours, making it a possible target for cancer therapy development. Phytochemicals can stop or reduce cancer development and spread by lowering oxidative stress, slowing cell

proliferation, triggering programmed cell death, stopping angiogenic processes, and arresting the cell cycle [3,11]. Using an *in silico* approach, this study looked for bioactive molecules that might target HCK.

2. Materials and Method

2.1 Selection and Preparation of Ligands

Fifty bioactive compounds from different plant sources collected from public database and published research papers were downloaded from https:// pubchem. ncbi. nlm. nih.gov in SDF format. Also, one standard drug Dasatinib[12] was downloaded in 3D Sdf format for comparison with the phytochemicals.

2.2 Protein Preparation

3D structures of Haematopoetic cell kinase HCK (PDB ID-1AD5) was downloaded from RCSB protein data bank (http:// www. rcsb. org/ pdb/ home/home. do) and the protein was prepared for docking using dockprep in UCSF Chimera [13].

2.3 **Docking Analysis**

After the proteins and ligands preparation, molecular docking was executed using Autodock Vina in PyRx[14]. For binding to take place, the grid box coordinates were, center_x = 29.89, center_y = 47.17, center_z = 102.26, size_x = 36.50 size_y = 32.91, size_z = 40. The structures showing interaction between ligands and proteins were viewed using Discovery Studio Visualizer [15].

2.4 In Silico *ADMET Analysis*

Pharmokinetics parameters such as Absorption, Distribution, Metabolism, Excretion, and Toxicity wasevaluated in the ligands using pkCSM[16].

3. Results and Discussion

3.1 Docking Analysis

Due to the involvement of SRC family kinases in the development and progression of cancer, it is necessary to develop suitable drug candidates that can effectively inhibit them at the binding sites of receptors with little or no effects. The binding energy between ligands and receptors determines binding affinity; the lower the energy, the stronger the binding affinity.

For this purpose, fifty bioactive compounds were screened against Hematopoietic cell kinase (HCK). Commonly used anticancer drug (Dasatinib) that have activities against the target were also docked against the protein and the binding energies were compared. After docking, the binding energy(kcal/mol), number of hydrogen bond, and amino acids involved in hydrogen bonding were noted and presented in

Figure1 and **Table 1**. Fifty phytochemicals used in our study showed binding energy ranging from -11.2 to -5.1 kcal/mol. The top two drugs found in our study are Theaflavin and Ginkgetin, showed different kinds of bonding interactions with the target. Theaflavin is predicated to have a binding energy of -11.2 kcal/mol and Ginkgetin is predicted to have a binding energy of -10.1 kcal/mol as compared to Dasatinib showing to have a binding energy of -8.2 kcal/mol. Theaflavin is predicated form two hydrogen bonds with ALA 342 and GLY 276 and Ginkgetin was found to form three hydrogen bonds with THR 338, ASN 391, and SER 345.

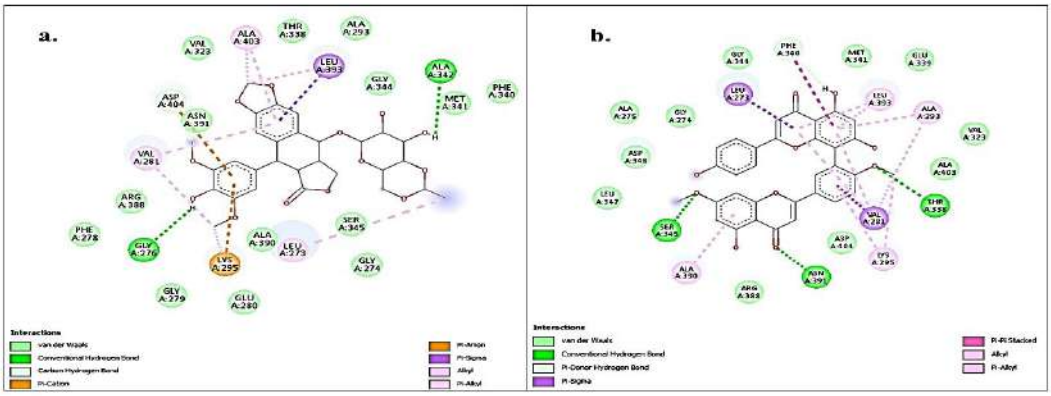


Figure 1. Molecular docking ofTheaflavin and Ginkgetin in ligand-binding site on HCK. (a) Theaflavin is predicated to form two hydrogen bonds with ALA 342 and GLY 276 (b) Ginkgetin was found to form three hydrogen bonds with THR 338, ASN 391, and SER 345.

Table 1. The interaction profiles of the top two drug hits by BIOVIA Discovery Studio Visualizer.

Phytochemicals	Binding energy (kcal/mol)	Residues forming H-bond	Interacting amino acids with Ligand
Theaflavin	-11.2	ALA 342, GLY 276	VAL 323, ALA 403, THR 338, ALA 293 LEU 393, GLY 344, ALA 342, MET 341, PHE 340, GLY 274, SER 345, LEU 273, ALA 390, LYS 295, GLU 280, GLY 279, GLY 276, PHE 278, ARG 388, VAL 281, ASN 391, ASP 404
Ginkgetin	-10.1	THR 338, ASN 391, SER 345	GLY 344, LEU 273, PHE 340, MET 341, GLU 339, LEU393, ALA 293, VAL 323, ALA 403, THR 338, VAL 281, LYS 295, ASP 404, ASN 391, ARG 388, ALA 390, SER 345, LEU 247, ASP 348, ALA 275, GLY 274

3.2 In Silico ADMET Analysis

The purpose of this assay is to offer information that may be used in the drug research and development process. The phytochemicals were evaluated for ADMET qualities using pkCSM, a free online application that predicts and assesses the pharmacokinetic and toxicological aspects of various compounds. *In silico* pharmacokinetics of the top two ligands as shown in **Table 2** reveal that the ADMET profiles of them are comparable to the standard drugs therefore can be further optimised for effective cancer treatment.

Table 2. ADMET profiles of top two ligands predicted by pkCSM

ADMET	Model Name	Theaflavin	Ginkgetin	
Property		**Predicted Value**	**Predicted Value**	**Unit**
Absorption	Water solubility	-2.892	-2.942	Numeric (log mol/L)
	Caco2 permeability	0.141	-0.084	Numeric (log Papp in 10 cm/s)
	Intestinal absorption (human)	65.009	95.376	Numeric (% Absorbed)
	Skin Permeability	-2.735	-2.735	Numeric (log Kp)
	P-glycoprotein substrate	Yes	No	Categorical (Yes/No)
	P-glycoprotein I inhibitor	Yes	Yes	Categorical (Yes/No)
	P-glycoprotein II inhibitor	Yes	Yes	Categorical (Yes/No)
Distribution	VDss (human)	0.679	-1.274	Numeric (log L/kg)
	Fraction unbound (human)	0.2	0.271	Numeric (Fu)
	BBB permeability	-1.577	-1.884	Numeric (log BB)
	CNS permeability	-4.33	-3.266	Numeric (log PS)
Metabolism	CYP2D6 substrate	No	No	Categorical (Yes/No)
	CYP3A4 substrate	No	Yes	Categorical (Yes/No)
	CYP1A2 inhibitior	No	No	Categorical (Yes/No)
	CYP2C19 inhibitior	No	No	Categorical (Yes/No)
	CYP2C9 inhibitior	No	Yes	Categorical (Yes/No)
	CYP2D6 inhibitior	No	No	Categorical (Yes/No)
	CYP3A4 inhibitior	No	No	Categorical (Yes/No)
Excretion	Total Clearance	0.081	0.646	Numeric (log ml/min/kg)
	Renal OCT2 substrate	No	No	Categorical (Yes/No)
Toxicity	AMES toxicity	No	No	Categorical (Yes/No)
	Max. tolerated dose (human)	0.439	0.427	Numeric (log mg/kg/day)

ADMET	Model Name	Theaflavin	Ginkgetin	
Property		**Predicted Value**	**Predicted Value**	**Unit**
	hERG I inhibitor	No	No	Categorical (Yes/No)
	hERG II inhibitor	Yes	Yes	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	2.505	2.733	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	4.008	2.475	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	No	No	Categorical (Yes/No)
	Skin Sensitisation	No	No	Categorical (Yes/No)
	*T.Pyriformis*toxicity	0.285	*0.285*	Numeric (log ug/L)
	Minnow toxicity	6.092	-2.351	Numeric (log mM)

4. Conclusion:

An *in-silico* study is being carried out to investigate the potential of different phytochemicals to suppress cancer development and progression via modulating HCK, a SRC family non-receptor protein tyrosine kinase. Our study showed that promisingphytochemicals such as Theaflavin and Ginkgetin could bind to the ligand binding site of HCK, inhibiting its essential functions, which in turn could hinder cancer cell proliferation. ADMET profiles of the phytochemicals showed promising and comparable properties to the approved drugs against HCK. More research is required to determine the pharmacodynamics and kinetic characteristics of these phytochemicals. Despite the study's encouraging findings, validations such as *in vitro* and *in vivo* experiments should be pursued further to establish them as possible anticancer agents.

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श्रद्धावान लभते ज्ञानम्

Article No. DGC-NF-24/02

Organocatalysis: A Promising Pathway for Medicinal Chemistry and Drug Discovery

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Abstract:

The potency and synthetic versatility of organocatalysis have contributed to the area of organic synthesis since the early 2000s. Organocatalysis field have extended beyond the academia and undergone into scale-up production of natural products, chiral drugs and enantiomerically enriched biologically active molecules. In this chapter, an outline of organocatalytic strategies in medicinal chemistry is highlighted, and the specific applications of the organocatalytic methods in pharmaceutical synthesis are demonstrated with a focus on the synthesis of various medicinally valuable targets such as antimalaria drugs, antiparasitic, neuroprotectors, antipyretic agents, antitumor agents and antiviral agents.

Keywords: Organocatalysis, Drugs, Discovery

1. INTRODUCTION:

Medicinal chemistry and drug discovery aim to design and synthesis of novel and purified bioactive molecules that fulfil certain criterion have the desired properties or features to become harmless and promising drug molecules (1). For numerous pharmaceutical applications, synthesis of drugs molecules starts with the screening of chiral compounds formed from diverse and readily available starting materials (2). In this scenario, the organocatalysis, which generally provides eco-friendly catalysts, effective and generic modes of activation, large scale, high selectivity and relatively mild conditions that avoid the use of a large solvent volume and waste formation are recognised as an

operative tool for the development of medicinal chemistry (3-5). The utilization of small chiral organic molecules such as amino acid derivatives (e.g. proline and arginine), alkaloid derivatives (e.g. cinchona, strychnine, brucine and nicotine) and phosphoric acids as catalysts which have used for large scale production of natural products, chiral drugs, enantiomerically enriched pharmaceuticals and bioactive compounds. Various chiral organic molecules oxalic acid, lactic acid, citric acid, and tartaric acid are commonly used as a catalyst to synthesis of chiral drugs by constructing enantioselective C-C, C-O, C-N, C-S, C-P and C-halide bonds (6). Moreover, the proceeding of bifunctional H-bond directed Diels-Alder reaction via a dienamine intermediate is become a stereo and region control reaction (7). The participation of multiple H-bond in highly enantioselective reaction channels leading to the identical enantiomer of product in a one-step transformation provided an opportunity to develop the drug molecules. There have been a number of successful organic reactions, such as aldol reaction, Michael Addition, Mannich Reactions, Diels-Alder reactions, Knoevenagel reactions and Friedel-Crafts Reaction carried out by organic catalysts to form new covalent bonds (8,9).

Nowadays, organocatalysis provides an alternative toolbox to metal-based and enzyme catalysis with simple building blocks in a convenient manner. Generally, organocatalysis reactions can be occurred at milder pH condition as well as lower temperature as compared to metal-based catalysis reactions (10). Also, the generation of metallic waste and metallic components in the targeted products can be avoided which is one of the prime criteria for new drug synthesis in medicinal chemistry.

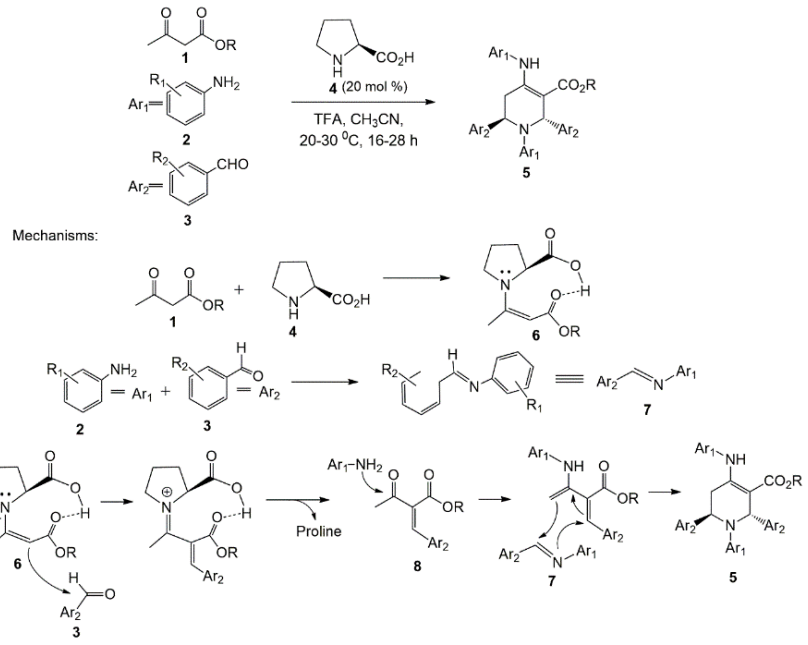
2. Applications of organocatalysis in medicinal chemistry

Organocatalysis is independent synthetic toolbox for the synthesis and design of the chemo-selective multi-functionalization active compounds with diverse structures. Several routes have been developed for drug synthesis, but many are required multiple steps to synthesis which rise the price of drugs in market. The price of the drugs can be reduced by using simple organocatalysis in medicinal chemistry for controlling the following factors- (a) no more than 10 synthetic steps including few separate and purification operations, as possible; (b) overall yield more than 50% and (c) avoiding use of expensive reagents.

2.1 Antimalarial and other antiparasitic agents

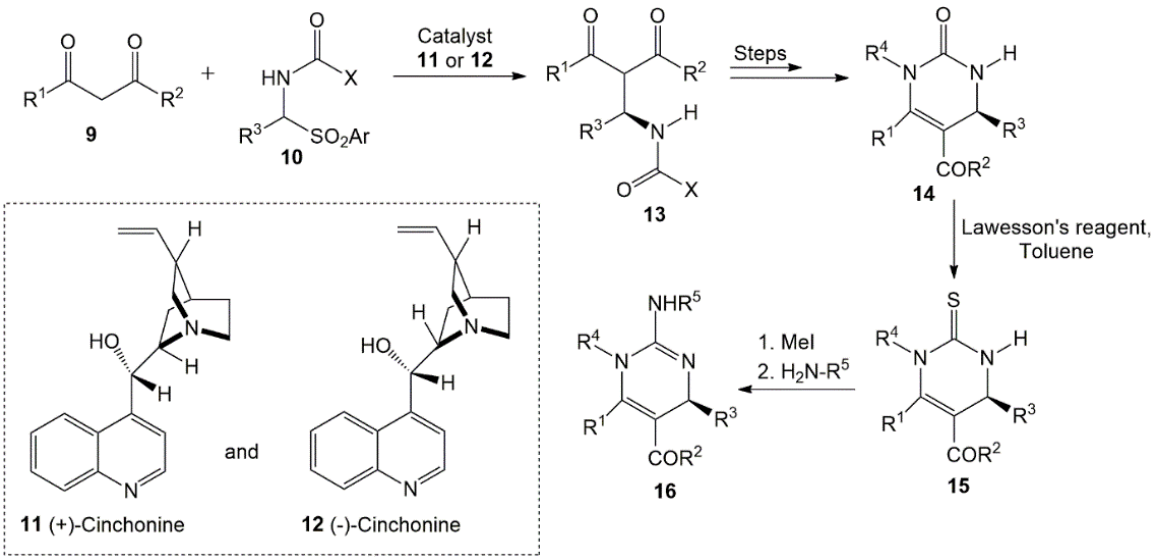
The World Health Organization (WHO) estimates that around 241 million cases and 627000 deaths for malaria globally in 2020. According to the report, 14 million more cases and 69000 more deaths was estimated as compared to 2019. To control the malaria cases, several efforts have been made toward the improvement of organocatalytic enantioselective synthesis of the effective antimalaria drug (11).

Using a target-based drug, tetrahydropyridines **5** have been identified as potent and selective drug against Plasmodium falciparum in vitro (12). The product has been synthesised in a one-pot reaction using a multicomponent organocatalysis (L-proline/TFA). In this synthesis cheap building blocks (β-ketoesters**1**, anilines **2** and aromatic aldehydes **3**) were used which is quite attractive from the environmentally and economic impact. In the first step, the enamine formation **6** was carried out between the organocatalyst proline **4** and the β-ketoester (Scheme 1). With the reaction of generated enamine **6** and aldehyde followed by dehydration, the proline moiety is released and the Knoevenagel condensation product **8** is produced. After condensation with aniline, product **8** gives rise to the 2-aza-diene **8**. Finally, 2-aza-diene **8** is treated with the imine **7** and yielded to the target tetrahydropyridines **5** via an aza-Diels-Alder reaction. Eight derivatives have been screened for blood schizonticidal activity towads P. falciparum 3D7 strain with 100% inhibitory activity at 1.25 µg mL-1.



Scheme 1: Proposed reaction mechanism for the synthesis of tetrahydropyridines **5** by one-pot organocatalysis.

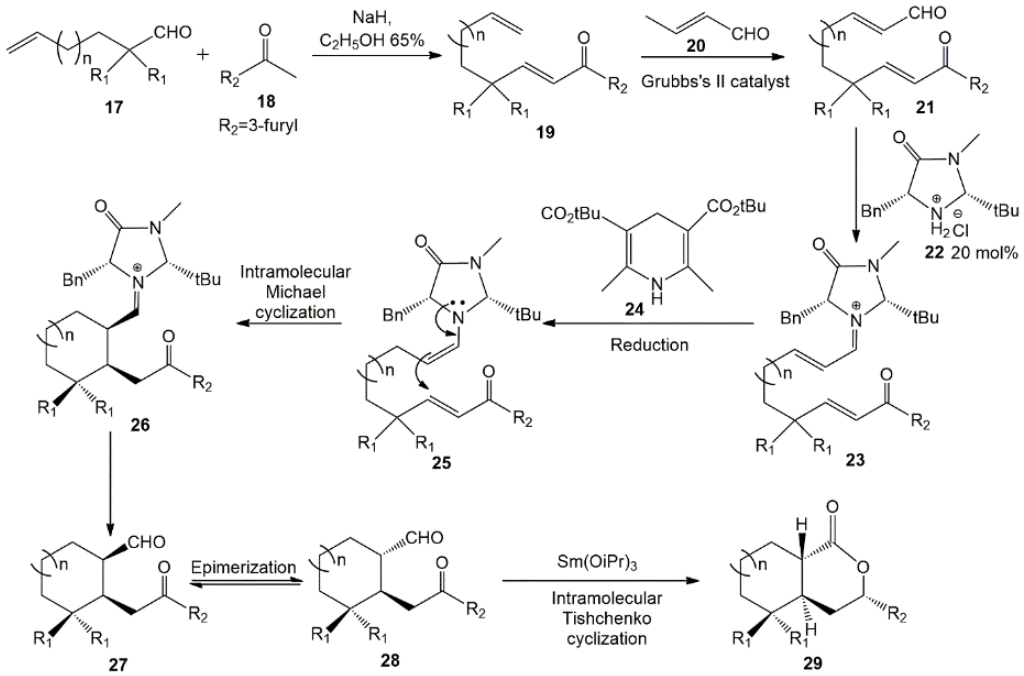
The guanidine derivatives show inhibition capability of the growth of P. falciparum (13). The synthesis of guanidine analogues **16** is carried out via organocatalytic steps catalysed by quinine alkaloid derivatives **11** and **12**. The plausible mechanism for the guanidine analogues **16** synthesis is proposed in (Scheme 2). Initially, the deprotonation of malonate derivative was carried out by the chiral base organocatalyst (cinchonidine or cinchonine) which reacts the imine and leads to in-situ formation of compound **10**, and then compound **10** reacts with compound **9** and give the high enantiomeric excess intermediate **13**. Then the intermediate **13** was cyclized into dihydropyrimidone **14**. The subsequent formation of guanidine derivatives **16** was initiated when dihydropyrimidone **15** reacts with Lawesson's reagent, followed by sulphur alkylation.



Scheme 2: Synthesis of dihydropyrimidinone guanidine derivatives **16** by organocatalyst.

Organocatalytic reaction have used to the synthesis of drug molecules for antiparasitic activity. List et al. have demonstrated the highly enantio- and diastereoselective synthesis of ricciocarpin A and several ricciocarpin analogues **29** which shown molluscicidal activity towards the snail Biomphalaria glabrata (vector of schistosomiasis) (14). The highlighted features of this synthesis are a one-pot and three-steps reaction including organocatalytic Michael-Tishchenko cascade. First, the aldol condensation of 3-acetylfuran **18** with the aldehyde **17** gave enone **19**. The reaction of olefin **19** in presence of 10 mol% of Grubbs' II catalyst and crotonaldehyde **20** afforded aldehyde **21** with an 84% yield (Scheme 3). Finally, ricciocarpin A or its derivatives **29** were formed by the organocatalytic Michael-Tischenko cascade reaction in the presence of MacMillan catalyst **22** and a

Hantzschester **24**, followed by in-situ treatment with Sm(OiPr)3. In general, the synthesis of ricciocarpin A and its derivatives follows one-pot conjugate cascade reaction of reduction, Michael addition, epimerization and Tishchenko cyclization.



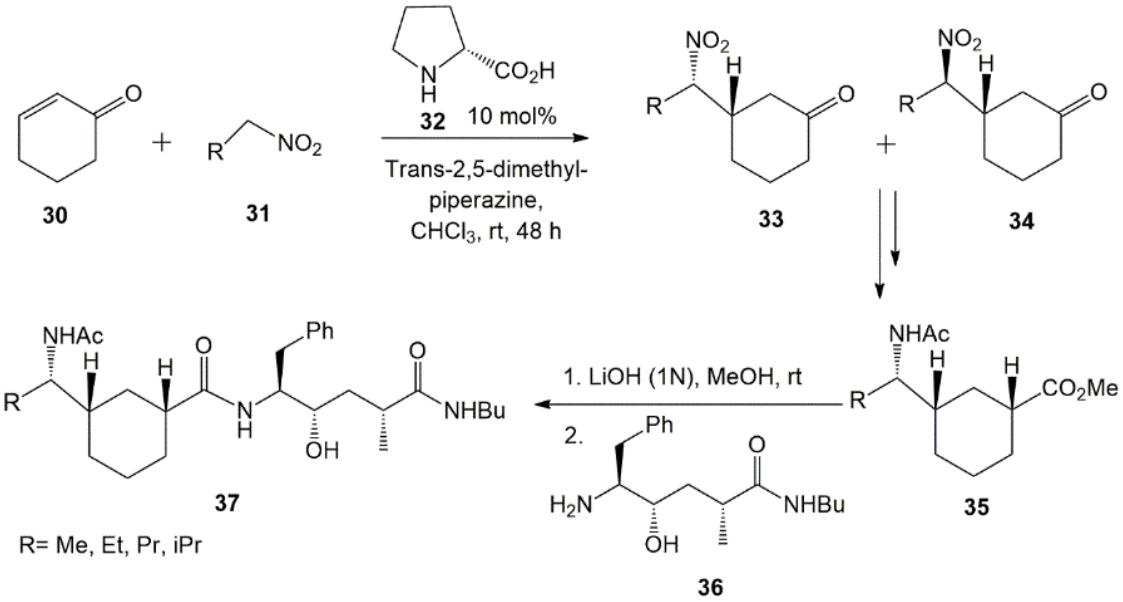
Scheme 3: The organocatalytic route for synthesis of ricciocarpin A and its analogues by the reductive Michael-Tishchenko cascade reaction

2.2 Neuroprotectors

Alzheimer's disease is a progressive brain disorder of the central nervous system that slowly destroys brain memory and thinking skills and provokes confusion, irritability and aggression, trouble with language. This disease is the most common and well-known form of dementia. Several groups are working on finding and development a suitable pathway for synthesis of new drugs for Alzheimer's disease. Many applications of organocatalysis have been reported to the synthesis of Alzheimer's disease inhibitors.

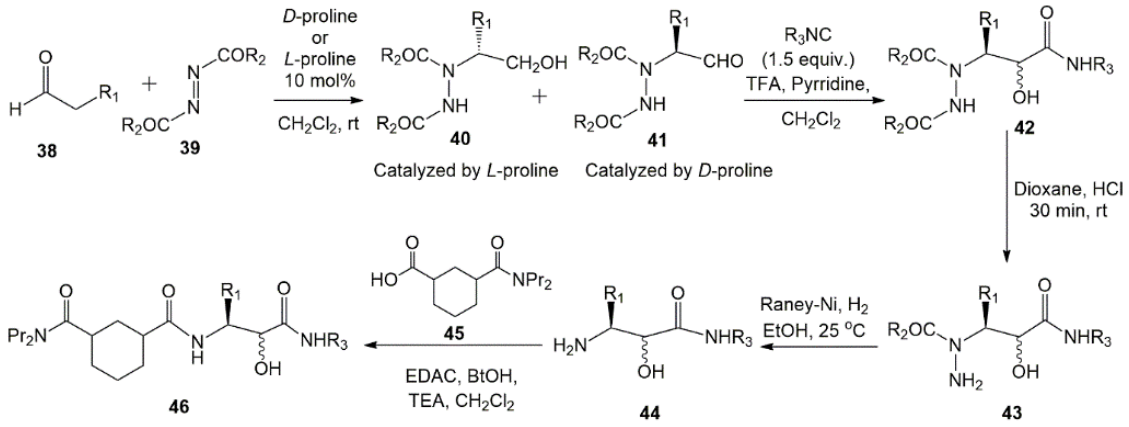
Hanessian et al. reported a stereo-controlled synthesis of carboxylic amino acid dipeptide isosteres via simple synthetic route and the organocatalytic 1-nitroalkane undergoes conjugate addition reaction to 2-cyclohexenone (15). In this synthetic approach, a 1,4-addition is taken place between a nitrioalkane **31** and cyclohexenone **30** through iminium ion formation and activation in the presence of 10 mol% of D-proline **32** as catalyst and trans-2,5-dimethylpiperazine as an additive for the deprotonation of the nitroalkane. (Scheme 4). The reaction proceeds with good enantioselectivity. Excellent

enantioselectivities for the syn-isomer (89% ee) **33** and anti-isomer (74% ee) **34** were observed. Then, the ketone moiety of syn-isomer **33** was converted to the methyl ester and the nitro group was hydrogenated and then acetylated using acetic anhydride. Finally, after hydrolysis of the ester **35**, the resulting acid was coupled with the amine **36** using HOBt/EDC/DACM-H2O to give the final peptidomimetic compound **37** (Scheme 4).



Scheme 4: Synthesis of peptidomimetic compound **37** using a key organocatalytic step.

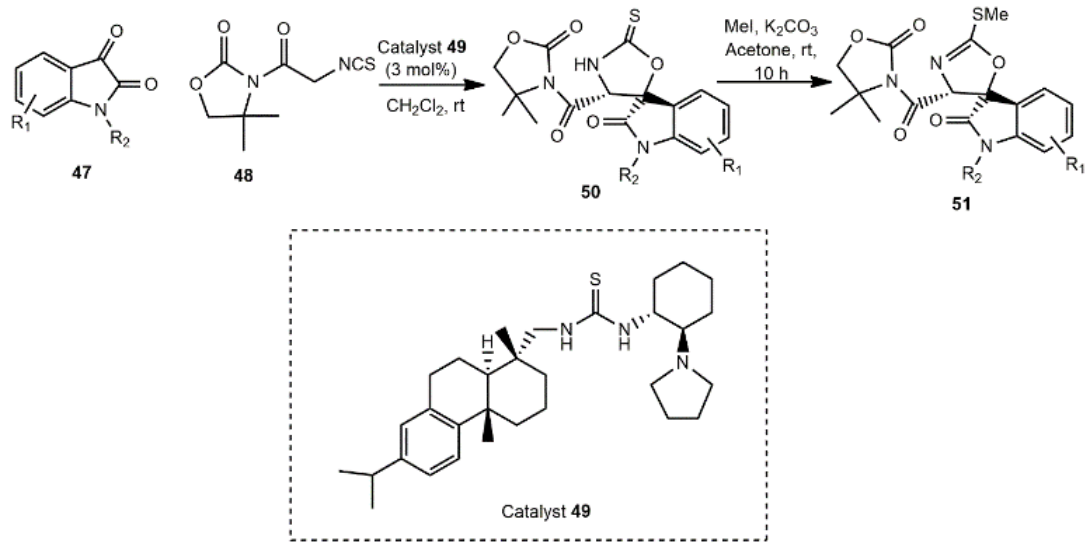
Schmidt et al. reported norstatine derivatives formed in sequential organocatalytic amination of aldehydes by diazodicarboxylates and Passerini reactions (Scheme 5) (16). Initially, common aldehydes **38** were treated with azodicarboxylates **39** and 10 mol% of D-proline or L-proline at room temperature under ambient conditions in dichloromethane solvent and give the α-aminated products **40** and **41**. The reaction of the protected α-amino aldehydes **41** and isonitriles in the presence of trifluoroacetic acid (TFA) and pyridine lead to the diverse α-hydroxy-β-amino amides **42** with no or very little diastereoselectivity. Then, the compounds **42** were treated with HCl in dioxane in order to hydrogenate and cleave the N-N bond followed by addition of Raney-Ni at 34 bar of H2 to give amines **44**. Finally, amines **44** were treated with 3-(dipropylcarbamoyl) benzoic acid **45** using EDAC and BtOH as coupling reagents to give the corresponding norstatine derivatives **46** (Scheme 5).



Scheme 5: Synthesis of norstatine derivatives **46** by organocatalytic a-amination.

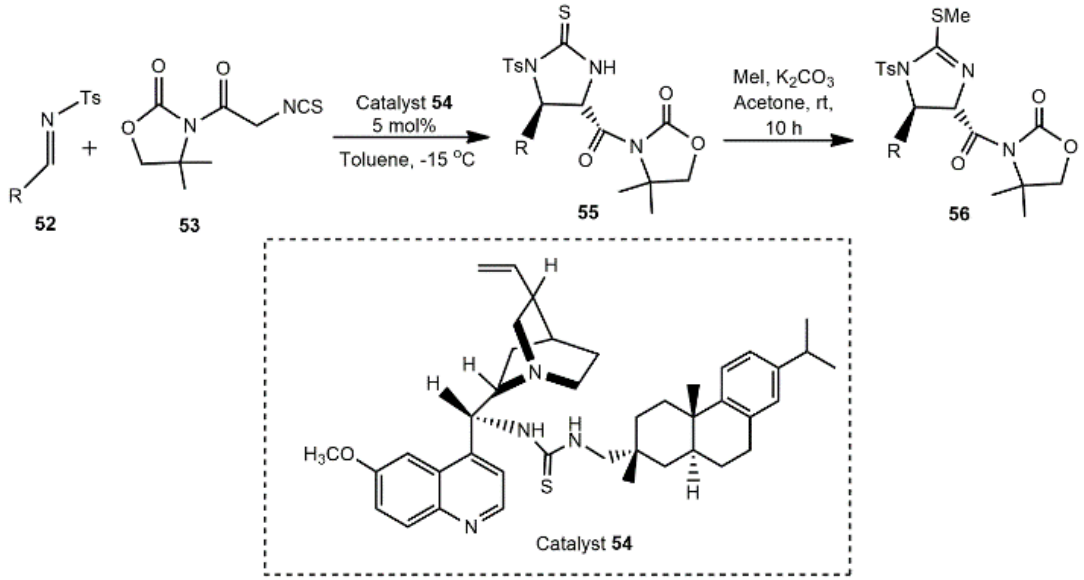
2.3 Antipyretic agents

In 2010, Wang et al. described a convenient strategy that allows the rapid formation of optically active spirooxazolines **51** using an organocatalytic approach which provides an opportunity to discover new antipyretic agents. (17). The authors showed that oxindole-type phytoalexins can be synthesized by the organocatalytic synthesis via an aldol reaction. At first, the reaction between isatins **47** and α-isothiocyanato imides **48** was performed in dichloromethane in the presence of a 10 mol % ligand **49** to give intermediate **50**, which after treatment with MeI and K_2CO_3 leads to the spiroxozolines **51** with high enantio- and diastereoselectivity (83-99% ee) (Scheme 6).



Scheme 6: One-pot organocatalytic synthesis of spirooxazolines **51**.

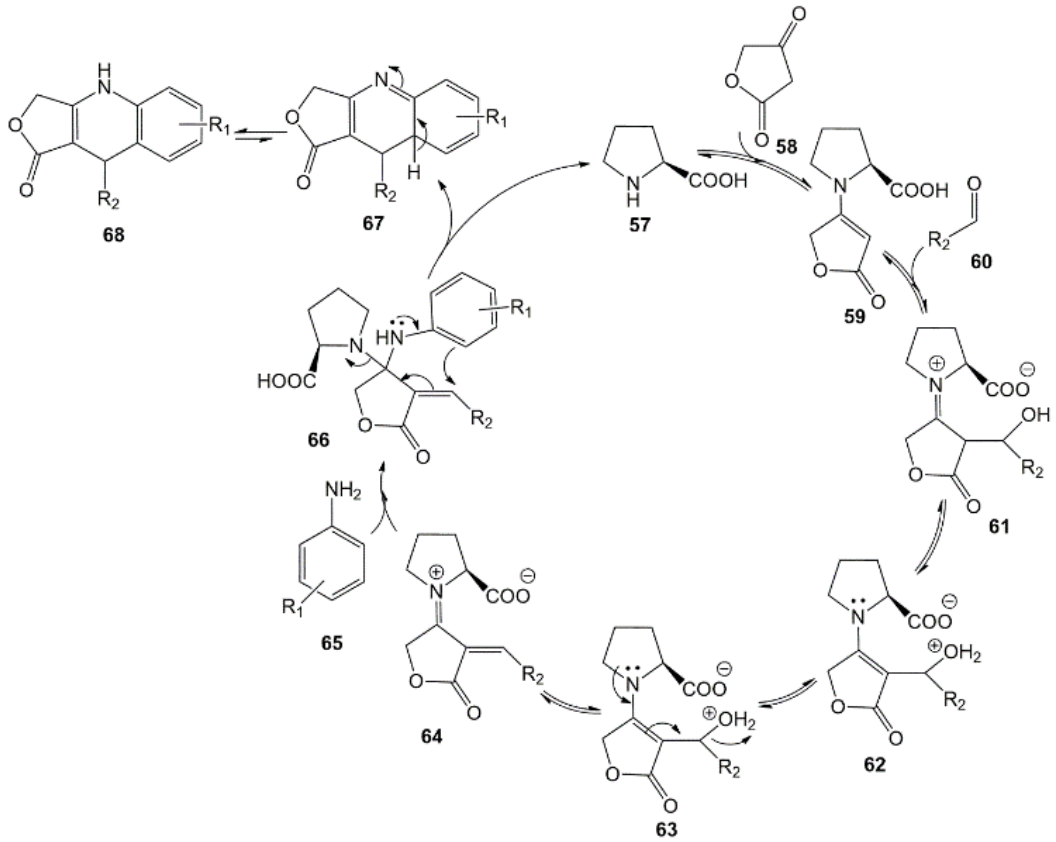
The cyclization thioureas via Mannich reactions leads to an active antipyretic agents (18). In this model reaction, the isothiocyanate **53** react with N-tosylimine **52** and 5 mol% of cinchona catalyst **54**. Finally, the methylthioimidazolines **56** was formed by 'one pot' treatment with MeI in the presence of K_2CO_3 (Scheme 7).



Scheme 7: The route for synthesis of the methylthioimidazolines **56**.

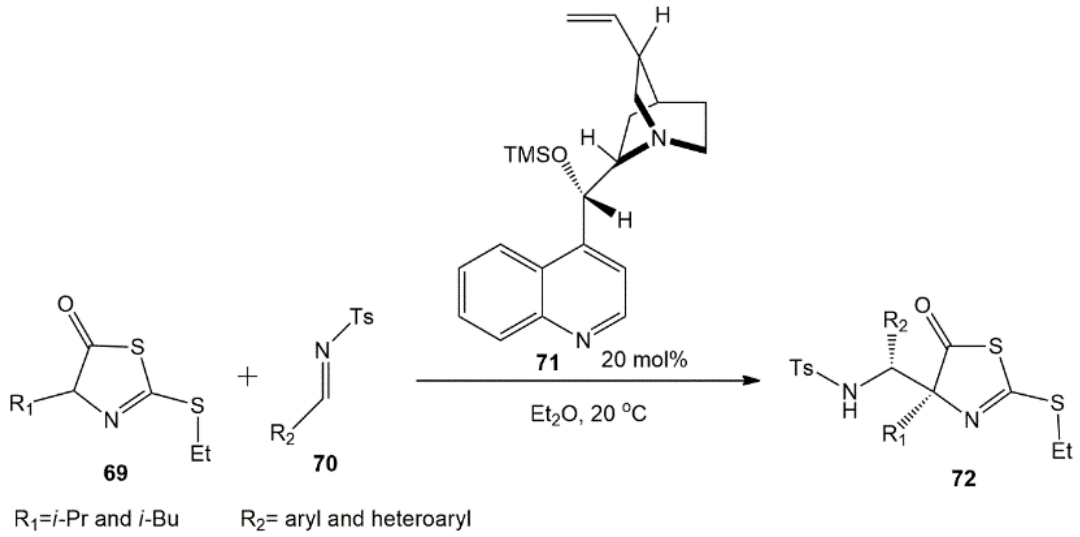
2.4 Antitumor agents

Podophyllotoxin is a good example of a natural product based molecule with antitumor activity. In comparison, the 4-aza-podophyllotoxin **68** and its derivatives have exhibits a higher antitumor activity with lower toxicity than the podophyllotoxin (19). Further, Shi et al. described the design and synthesis of 4-aza-podophyllotoxin derivatives **68** using an multicomponent organocatalysis using aromatic amines, aldehydes, tetronic acid and proline **57** as an organocatalyst (Scheme 8) (20). First, the condensation reaction was taken place between tetronic acid **58** and the organocatalyst **57** to give enamine **59**. When enamine **59** is treated with aldehyde **60**, compound **63** is produced and after dehydration of **63**, the intermediate iminium ion **64** is formed. Then, a nucleophilic attack of the aniline **65** to the iminium ion **64** gives an intermediate **66** followed by successive ring-closure and regeneration of the catalyst. Finally, the generated compound **67** aromatizes to the final compound **68**.



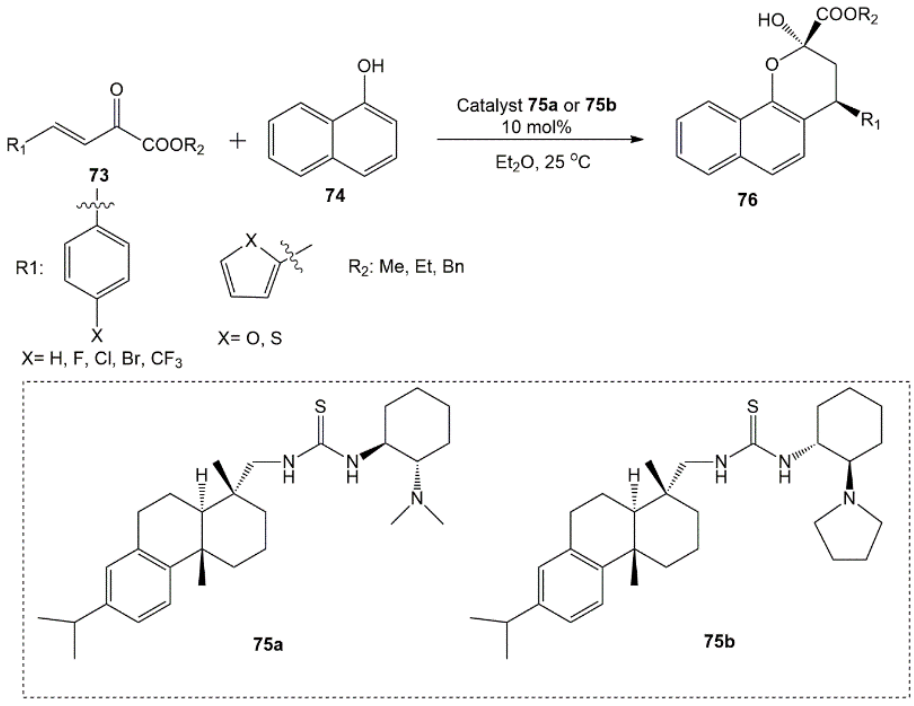
Scheme 8: Proposed catalytic cycle for the synthesis of 4-aza-podophyllotoxin derivatives **68**.

Wang and coworkers reported cinchona alkaloid derivative as an promising organocatalyst for an enantioselective aza-Mannich addition (21). They obtained a series of modified chiral 2-(ethylthio)-thiazolone derivatives **72** by a direct aza-Mannich addition of thiazolones **69** to various N-tosylimines **70** by cinchona alkaloids **71** as an organocatalyst (Scheme 9). The formed derivatives have been shown excellent anticancer activities towards the five different cancer cell lines using the MTT assay. The authors' approach consisted of reaction between 4-isopropyl-2-(ethylthio)-thiazolone and various N-tosylimines **70** in the presence of 20 mol% of cinchona **71** in diethylether, yielded the corresponding target molecules i.e. chiral 2-(ethylthio)-thiazolone derivatives **72** with excellent enantio- and diastereoselectivities.



Scheme 9: Synthesis of thiazolone **72** by organocatalytic aza-Mannich addition.

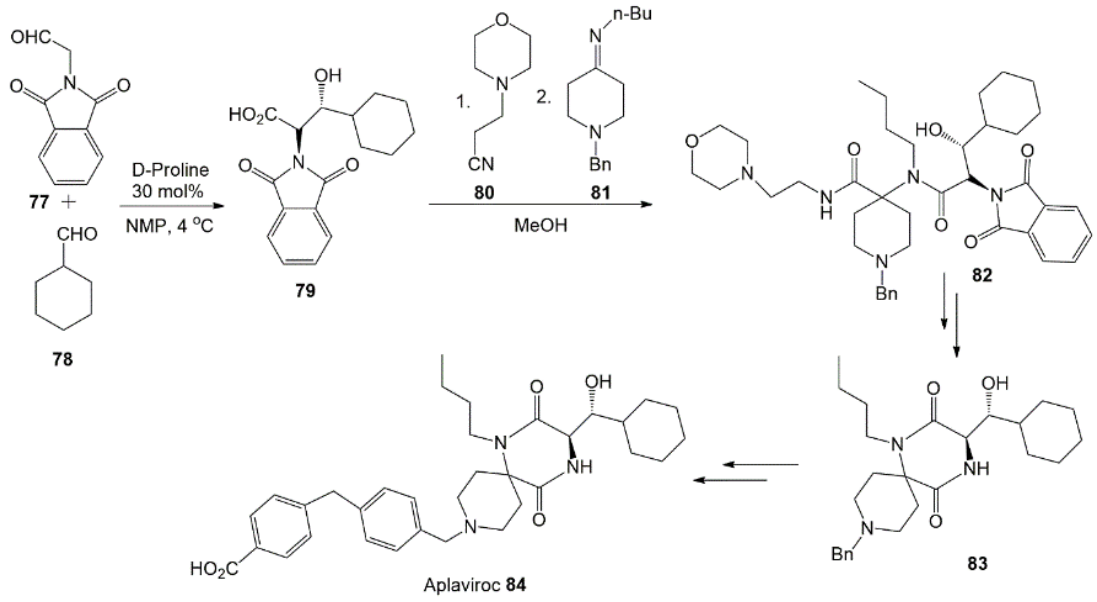
Furthermore, same group have described the synthesis and biological activities of modified chromanes through successful organocatalysis asymmetric Friedel-Crafts alkylation using a rosin-modified tertiary amine-thiourea (22). The targeted chromanes **76** were synthesized by asymmetric alkylation of naphthalene-1-ol **74** with aromatic or heterocyclic β, γ-unsaturated α-ketoesters **73** followed by hemiketal cyclization of the intermediate (Scheme 10). The Bifunctional rosin-modified tertiary amine-thiourea organocatalyst **75a** and **75b** exhibit the best catalytic activities.



Scheme 10: Synthesis of chromanes **76** by organocatalysis **75a** and **75b**.

2.5 Antiviral agents

Aplaviroc is a well-known antiviral medicine which forms an interaction between HIV-1 virus and the receptor CCR5 present on the plasma membrane, blocking the virus approach into the cell. A organocatalytic methodology have been applied to synthesis of a new Aplaviroc as well as anti-β-hydroxy-α-amino acids by only two steps procedure (Scheme 11) (23). The cross-aldol reaction between phthalimidoacetaldehyde **77** and cyclohexane carboxaldehyde **78** in presence of proline as a catalyst obtained anti-β-hydroxy-α-amino acids **79** in good yield with high diastereo- and enantioselectivity. Further, a Ugi-adduct **82** was formed by the three component Ugi reaction using compound **79**, isonitrile derivative **80** and the ketimine **81** (Scheme 11). After cyclization of Ugi-product **82** into piperadinone **83**, Aplaviroc **84** was synthesized.



Scheme 11: Synthesis of Aplaviroc **84**.

Conclusion

In summary, the potential strategies and pioneering efforts of the organocatalysis in medicinal chemistry have been discussed with discussing formation mechanism. The progresses of various modes of the activation in organocatalysis have been afforded to fulfil the demand for promising synthesis of pharmaceutical molecules.

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श्रद्धावान लभते ज्ञानम्

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Organocatalysis for Medicinal Chemistry and Drug Discovery

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Abstract:

Since the early 2000s, the synthetic versatility and efficacy of asymmetric organocatalysis have contributed significantly to the field of organic drug synthesis. With the increasing challenges associated with the effective treatment of life-threatening diseases influences the development of new drug therapies with suitable physicochemical properties, efficiency and selectivity. In this sense, both the academy and the pharmaceutical industries are continuously searching for new compounds having anti-viral, anti-fungal or anti-bacterial activities and in addition, face the challenge of developing greener and more efficient methods to synthesize these drugs. This becomes even more important with compounds possessing stereo-genic centres as highly enantioselective processes are required. In this paper, the advances achieved to improve synthetic routes efficiency and sustainability of important commercial drugs are discussed, highlighting the use of organocatalytic methods.

1. Introduction:

Organocatalysis is the form of catalysis or chemical reaction where the reaction rate is accelerated by an organic catalyst or organocatalyst. These catalysts are small, metal free organic molecules and consists of non-metals such as carbon, hydrogen, oxygen, sulphur, phosphorous, nitrogen etc.[1] These types of catalysts are generally stable and easy to design and synthesize. These are compatible with the functional groups of several reacting molecules which are sensitive to other chemical reactions and reduces the use of protection groups to decrease the total number of reaction steps. Generally, sugars, peptides, amino acids and alkaloids are considered as organocatalysts that can be easily linked to a solid support to produce new drug molecules.

2. Methodology:

Organocatalysts are associated with different chemical reactions like Michael Addition, Aldol condensation reaction, Diels-Alder reactions, Knoevenagel reactions etc. In comparison to the metal-based catalytic reactions, organocatalysis provides eco-friendly and green environment to carry out a stereoselective or asymmetric synthetic reaction. The controlling factors for these stereoselective reactions by chiral organocatalysts include van der Waals, electrostatic and hydrogen bonding interactions. The absence of heavy metal catalysts in these reactions is an advantage and also follows the principles of "Green chemistry". In Biginelli reactions, various organocatalysts such as tartaric acid, oxalic acid, citric acid and lactic acid are very effective. During these drug developmental processes, the formation of enantio-pure chiral drug molecule involving organocatalytic reactions plays a major role (Fig. 1). When these organic molecules are chiral, the catalytic process becomes enantio-selective for the construction of C-C, C-N, C-O, C-S, C-P and C-X (X = halide) bonds.

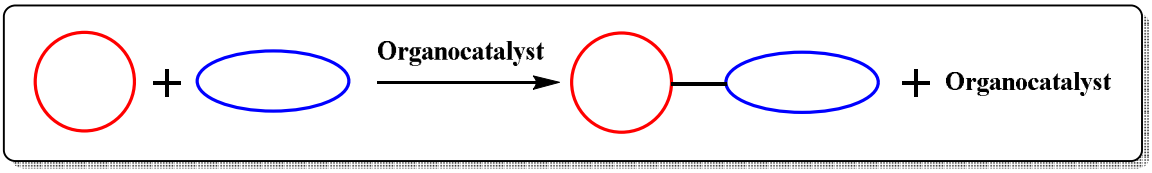


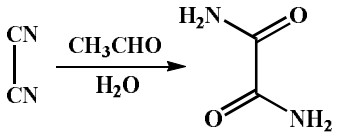
Fig. 1 Role of organocatalyst in chemical reactions

For the stereo and region control dienamine mediated Diels-Alder reactions the bi-functional H-bond directing amino-catalytic system was found to be useful. These catalysts consist of a multiple H-bond donor exhibiting great potential to generate enantio-selective reactions in drug synthesis. Urea or thiourea scaffold is the effective hydrogen bonding organocatalyst to synthesise several compounds. The acidity and strength of the hydrogen bonding interaction of the hydrogen bond donor are mainly considered for the development of efficient organocatalysts. The efficiency and selectivity of various organocatalytic reactions maintain the standards of organic reactions. So, the organocatalytic reactions become powerful for the formation of medicinally active compounds. They avoid the production of metallic hazards and the presence of metallic substances in the final products which is essential criteria for new drug development in medicinal chemistry.

3. History, Advantages and Disadvantages of Organocatalysis:

The first concept of organocatalysis was introduced in 2000, with a publication by

List and Barbas. J. von Liebig's synthesis of oxamide from di-cyan, acetaldehyde and water represent the first organocatalytic reaction. [2,3]

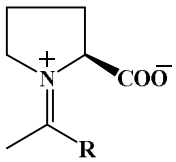


It was found that the use of organocatalyst results in the formation of stereo active molecules with diverse biological activities. Organocatalysts possess several advantages over traditional metal catalysts because they are non-toxic, readily available, stable, efficient, easy to handle, undergo metal free reaction, robust and involve environment friendly reaction or "Green chemistry" (Table 1). As compared to metal-based catalysed reactions, organocatalysis reactions can be carried out at lower temperatures and in milder pH conditions.[4] The metal-based catalysts cause contamination of metals and lead to chemical hazards, which can be removed and minimized by using organocatalysts in various chemical reactions.

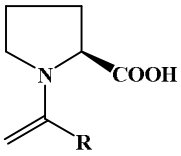
Table 1 Advantages and Disadvantages of Organocatalysis in Comparison with Conventional Catalysis

Catalysis type	Advantages	Disadvantages
Organometallic catalysis	• Choice of substrate is vast • Catalytic activity is higher	• Lengthy process • Risk of metal pollution
Enzyme catalysis	• Selectivity is much higher • Catalytic activity is higher	• Substrate choice is limited • Only a single enantiomer is formed
Organocatalysis	• Enantio-selective product is formed • Cost effective • No risk of toxicity • Catalysts are stable • Monitoring is easier	• Lengthy process • High catalyst loading • Much slower metal catalysis

Organocatalysis is useful to synthesise pharmaceutically active molecules by different modes such as secondary amine catalysis via enamines, secondary amine catalysis via iminium ions, phase transfer catalysis (PTC), nucleophilic catalysis, Lewis acid and Lewis bases catalysis, Bronsted base catalysis and H-bonding catalysis.[5]



Iminium



Enamine

4. Classification of Organocatalysts

Based on their interactions with the substrate or mechanism of catalysis, the organocatalysts can be classified as covalent or non-covalent organocatalysis. In case of covalent organocatalysis, a new covalent bond is formed between the catalysts and the substrate as shown in the case of amino-catalysis and carbenes.[6] While non-covalent organocatalysis involves the interactions between substrate and catalyst where the activation of the substrate occurs via weak binding illustrated by H-bonding or ionic interaction as shown in the case of phase transfer catalysis (Fig. 2).

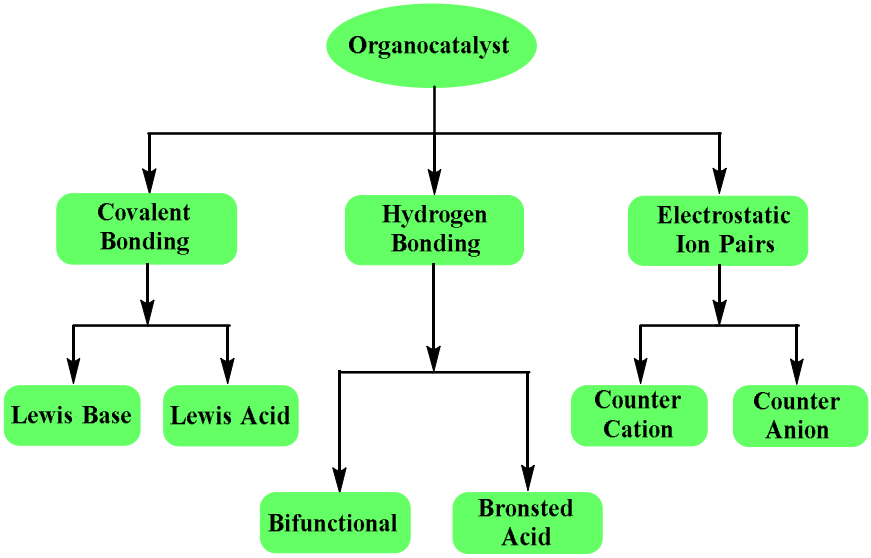


Fig. 2 Classification of organocatalysts

Organocatalysts can be mainly categorised into Lewis acids, Lewis bases, Bronsted acids and Bronsted bases. Lewis acid catalysts activate nucleophilic substrates to produce intermediate complex and the complex undergoes reaction to release the product. Similarly, Lewis base catalysts involve the catalytic reaction via nucleophilic addition to the substrate to produce a complex and finally, the product is generated after reaction of the complex. Bronsted acid and base catalytic reactions are initiated via protonation or deprotonation respectively.[7]

Asymmetric organocatalysis is an eco-friendly and potential synthetic method for the synthesis of enantiomerically pure natural products, chiral drugs, etc. Organocatalysts can be categorised into several types for asymmetric synthesis as follows:

(a) Biomolecules: It includes proline, phenylalanine, secondary amines etc.

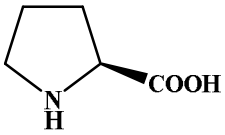
(b) Synthetic catalysts: It is derived from biomolecules.

(c) Hydrogen bonding catalysts: It includes TADDOLS, NOBIN and organocatalysts based on thioureas.

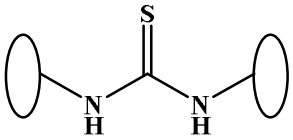
(d) Triazolium salts: It acts as Stetter reaction catalysts.

Among all catalysts, proline is considered to be the most advantageous catalyst due to the following facts.

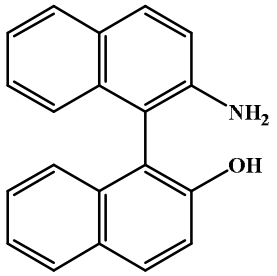
• Non-toxic • Less expensive • Both enantiomers available • Can be used in hydrated solvents and in aerobatic condition • Can be removed from the reaction mixture by aqueous workup.



L-proline



Thioureas



NOBIN

5. Application of Organocatalysis in Drug Synthesis

For the synthesis of various medicinally active organic compounds with different structures and for their chemo-selective functionalization, organocatalysis is the most prominent and safest route. Many organic reactions like Michael reaction, epoxidation, aldol condensation, Diels-alder reaction, Mannich reaction have been chosen to apply these organocatalyst molecules as depicted in Table 2 and Fig. 3.[8, 9] The intermolecular Diels-Alder reaction using organocatalysts have been carried out to perform enantioselective

[4+2] cycloaddition reactions. To establish nitrogen containing chiral drug molecules, Mannich reaction is useful for C-C bond formation. Aza-Michael, oxa-Michael and thio-Michael reactions are applied for the preparation of 1,2-dihydroquinoline, to access functionalized chromenes and to provide functionalized thiochromenes respectively. For the formation of chiral β-hydroxycarbonyl compounds, asymmetric aldol reactions are carried out using different organocatalysts.

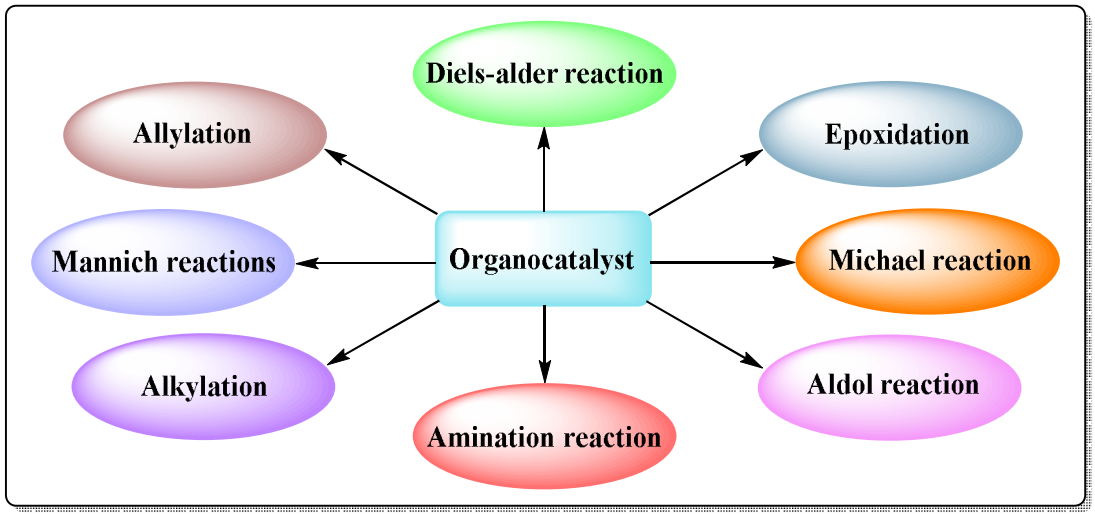


Fig. 3 Organic reactions used in organocatalysis

5.1. Synthesis of Warfarin

Warfarin is used as an anti-coagulant drug that prevents blood clots such as pulmonary embolism and vein thrombosis. Michael addition of 4-hydroxycoumarin to benzylidene acetone is used for the synthesis of warfarin where the iminium ion is catalysed through enone activation.[10]

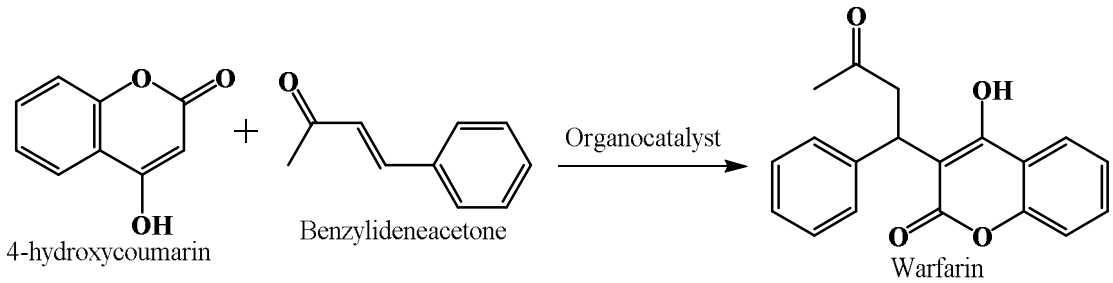
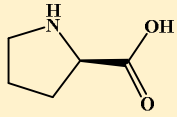
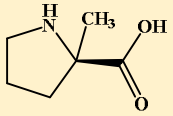
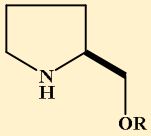
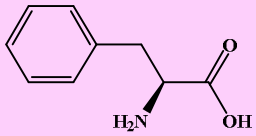
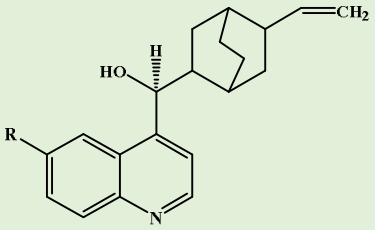
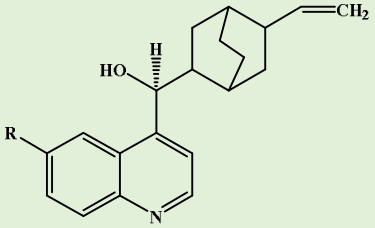
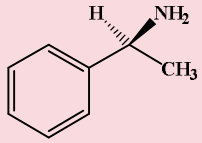
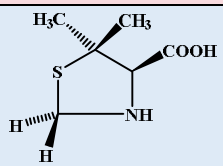
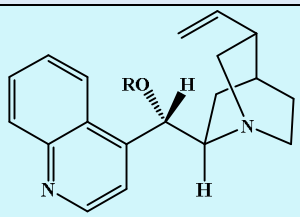
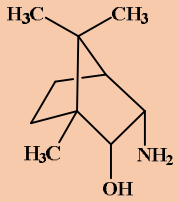


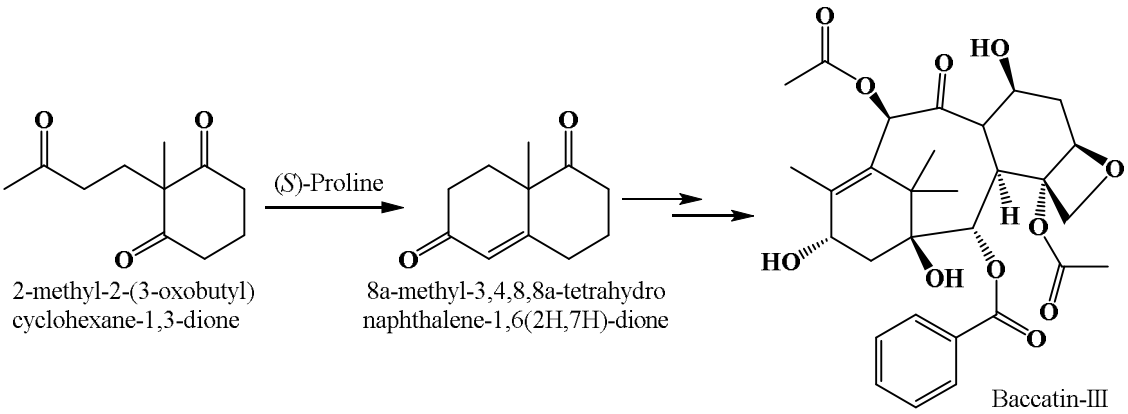
Table 2 Organocatalysts and Their Involvements in Different Reactions

No.	Organocatalysts	Structure	Uses
1.	L-proline		• Intermolecular Michael addition • Aldol reaction • Mannich reaction
2.	α-methyl-L-proline		• Intramolecular α-alkylation of aldehydes
3.	Amino ethers derived from L-proline		• Mannich reaction
4.	L-phenylalanine		• Intramolecular aldol reaction
5.	Quinine R=-OCH_3 Quinidine R=-OCH_3		• Halogenation of carbonyl compounds • Diels-Alder reaction
6.	(-)-Cinchonidine R=-H		• β-Lactam from imines and ketenes • Diels-Alder reaction • Intramolecular Michael addition
7.	(*S*)-1-phenylethanamine		• Intramolecular Michael addition
8.	(*R*)-5,5-dimethylthiazolidine-4-carboxylic acid		• Mannich reaction • Intermolecular aldol reaction
9.	(+)-Cinchonine, R=H		• Intermolecular Michael addition • Diels-Alder reaction • β-Lactam from imines and ketenes • β-Lactone from aldehydes and ketenes

No.	Organocatalysts	Structure	Uses
10.	3-amino-1,7,7trimethyl bicyclo[2.2.1]heptan-2-ol		• Tautomerization of enols

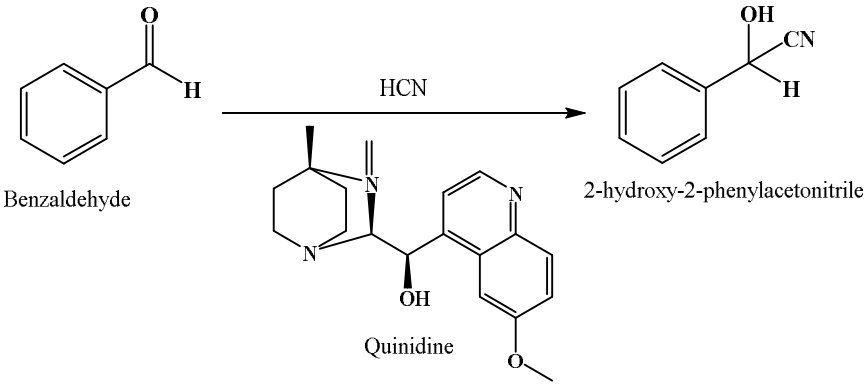
5.2. Synthesis of Taxol

Baccatin III is as a precursor to the anti-cancer drug paclitaxel (Taxol). Baccatin III is synthesised from 2-methyl-2-(3-oxobutyl) cyclohexane-1,3-dione with (*S*)-Proline as catalyst. 8a-methyl-3,4,8,8a tetrahydro-naphthalene-1,6(2H,7H)-dione is an intermediate of this reaction path.[11]

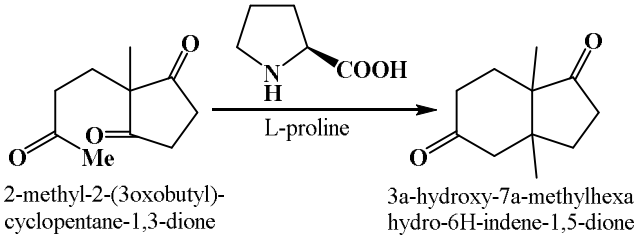


5.3. N-Containing Natural Product Synthesis

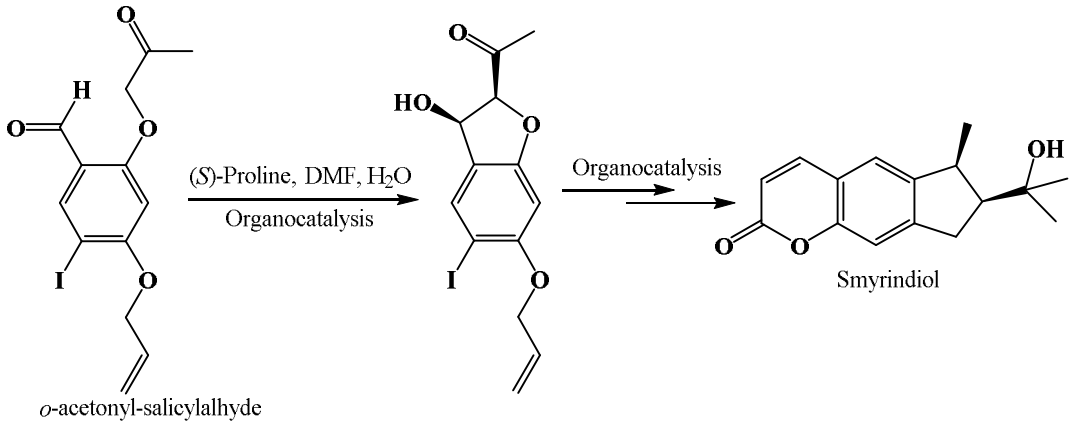
Different kind of alkaloids such as cinchona, nicotine, strychnine, brucine etc. are considered as the first organocatalysts to synthesis nitrogen-containing drug molecules. Wynberg *et. al.* reported that natural cinchona alkaloids act as bi-functional catalysts that activates both hydroxyl and tert-amine groups and helps to orient nucleophile and electrophile respectively to achieve optimum asymmetric catalysis, when cinchona derivatives are used as organocatalysts with primary amine.[12] For the production of L-mandelonitrile, Quinidine is used as an organic catalyst. To stabilize the transition state, both the quinuclidine and the quinoline phenol (C6'-OH) were considered to be involved.



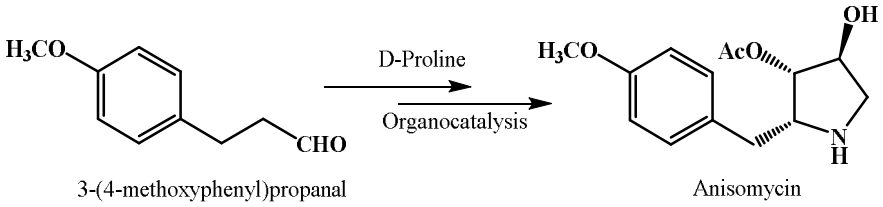
Deng and co-workers (2001) first reported that modified cinchona alkaloids catalyses highly enantioselective cyano-silylation of ketone with ethyl-cyanoformate.[13] Choi *et al.* established that for the trimethylsilyl-cyanation of acetophenone, cinchona alkaloids such as quinine and cinchonine can be used as organocatalysts. For the Aldol-type condensation reactions, Proline is the most prominent natural amino acid catalyst. In the early 1970s, efficiency of L-proline-catalysed asymmetric Robinson annulation was discovered.[14]



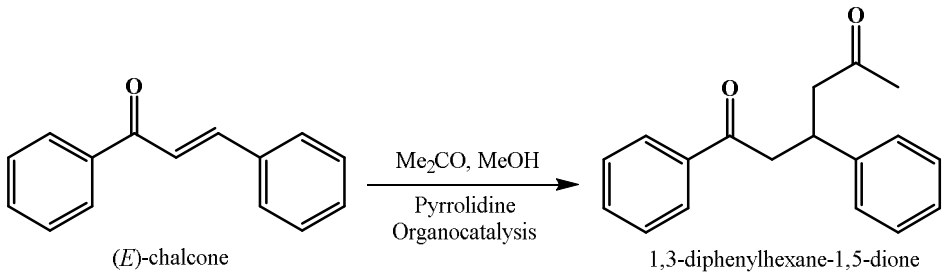
As described by Pizzarello and Weber,L-isovaline (found in the Murchison meteorite) promotes the self-aldol reaction of glycol-aldehyde to generate aldol products like derythrose, L-threose etc in presence of water. Enders *et. al.* (2012) demonstrated asymmetric synthesis smyrindiol [(+)-(2'*S*,3'*R*)-3-hydroxymarmesin] using organocatalysts. By using (*S*)-proline as catalyst, an intra-molecular aldol reaction is performed to isolate smyrindiol from the roots of *Smyrniopsisaucheri* and *Brosimumgaudichaudii*.[15]



Antibiotic (-)-Anisomycin, also known as flagecidin is active against pathogenic protozoa and fungal infections and useful for the treatment of vaginitis and amoebic dysentery. (-)-Anisomycin synthesis is performed by D-proline-catalysed α-amination-olefination.[16]

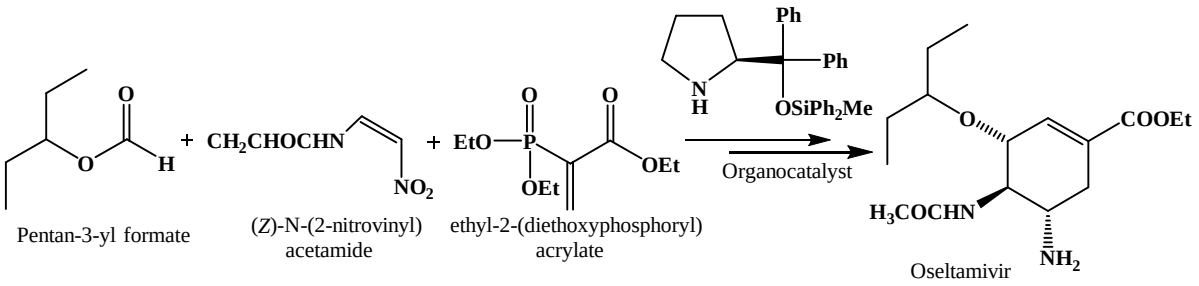


Wagh *et. al.* performed the direct synthesis of 3,5-diarylcyclohexenones from acetone and chalcones using pyrrolidine as catalyst.[17] 3,5-diaryl-cyclohexenones synthesis was also reported from chalcones and ethyl acetoacetate or acetylacetone using piperidine as a catalyst.



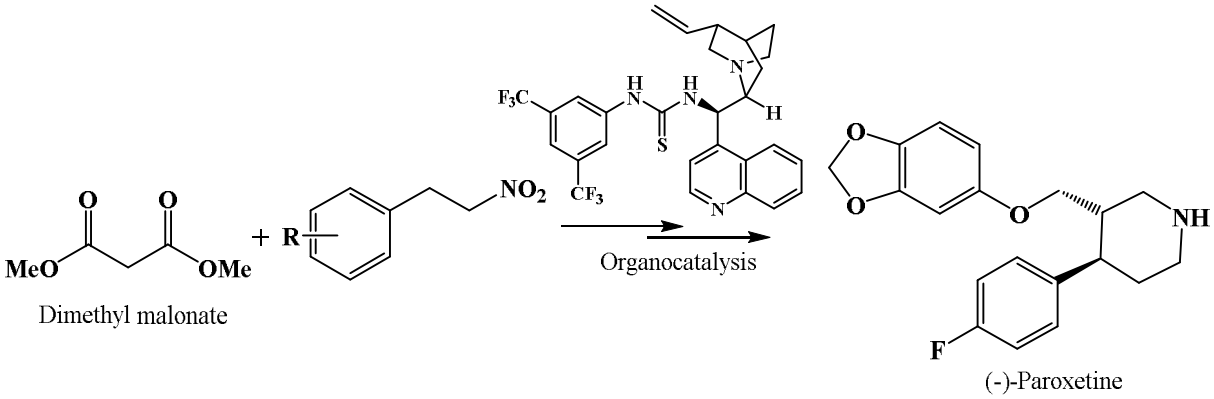
5.3.1. Synthesis of Oseltamivir

(-)-Oseltamivir, brand name Tamiflu is an anti-viral medicine that is used for the treatment and prevension of Influenza A and B. Eamine catalysed transformation is used for the synthesis of Oseltamivir.[18]



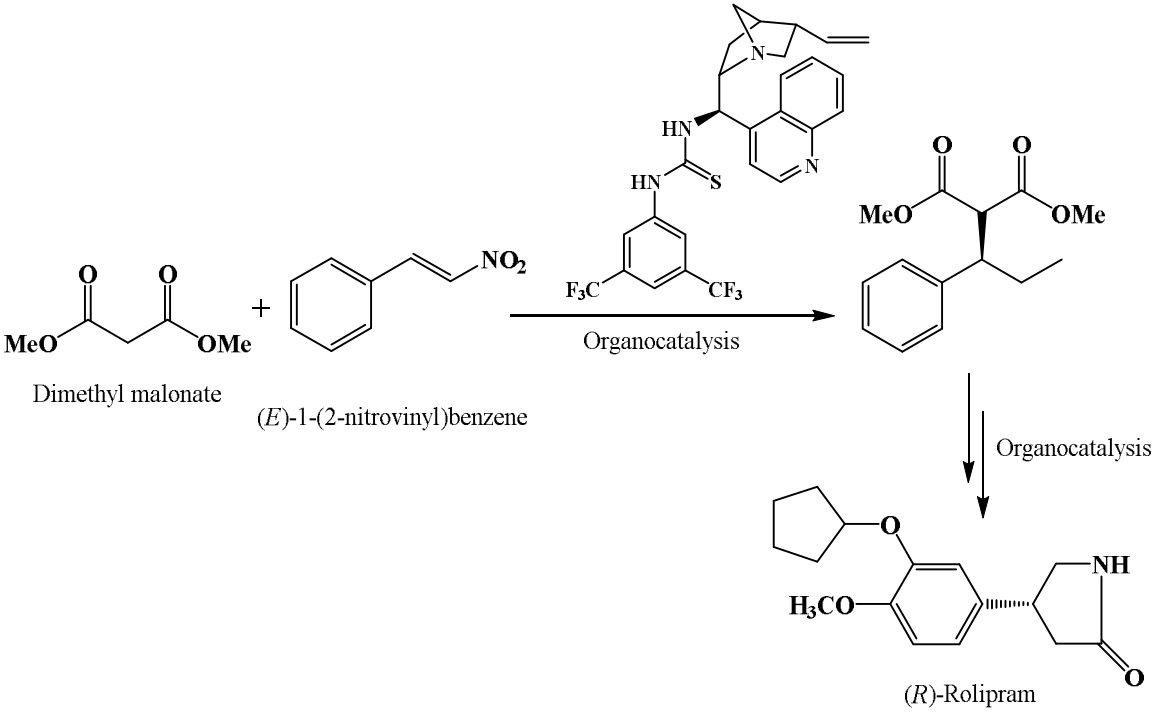
5.3.2. Synthesis of (-)-Paroxetine

(-)-Paroxetine is an anti-depressant that is used for anxiety related disorder treatments. Combined thiourea-cinchona is used as organocatalyst for the synthesis of (-)-Paroxetine.[19]



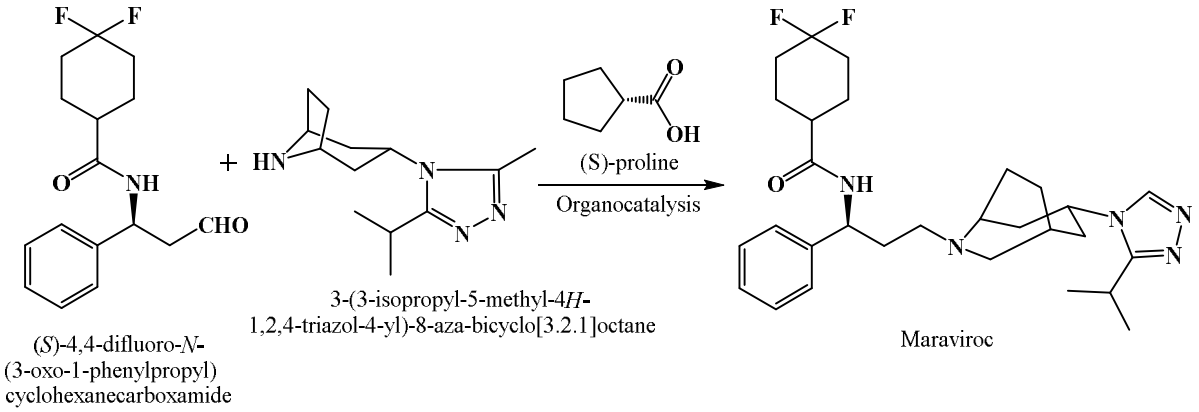
5.3.3. Synthesis of (*R*)-Rolipram

(R)-Rolipram is also an antidepression drug. Enantio-selective Michael addition reaction with the combined use of thiourea-cinchona catalyst is used for the synthesis of (*R*)-Rolipram.[20]



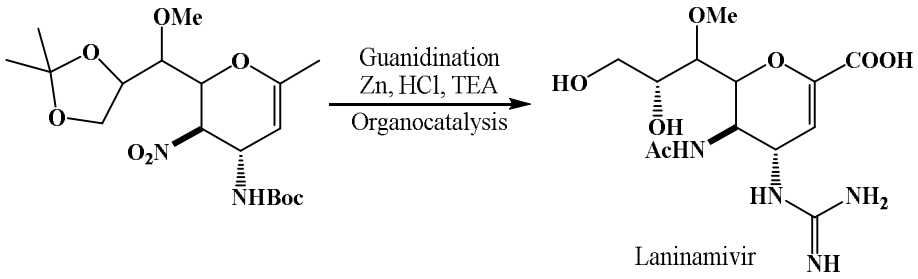
5.3.4. Synthesis of Maraviroc

Maraviroc is synthesised using a (*S*)-proline-derived catalyst. Pfizer reported that Maraviroc is a chemokine receptor-5 (CCR-5) receptor and used for the treatment of AIDS by acting against HIV virus.[21]



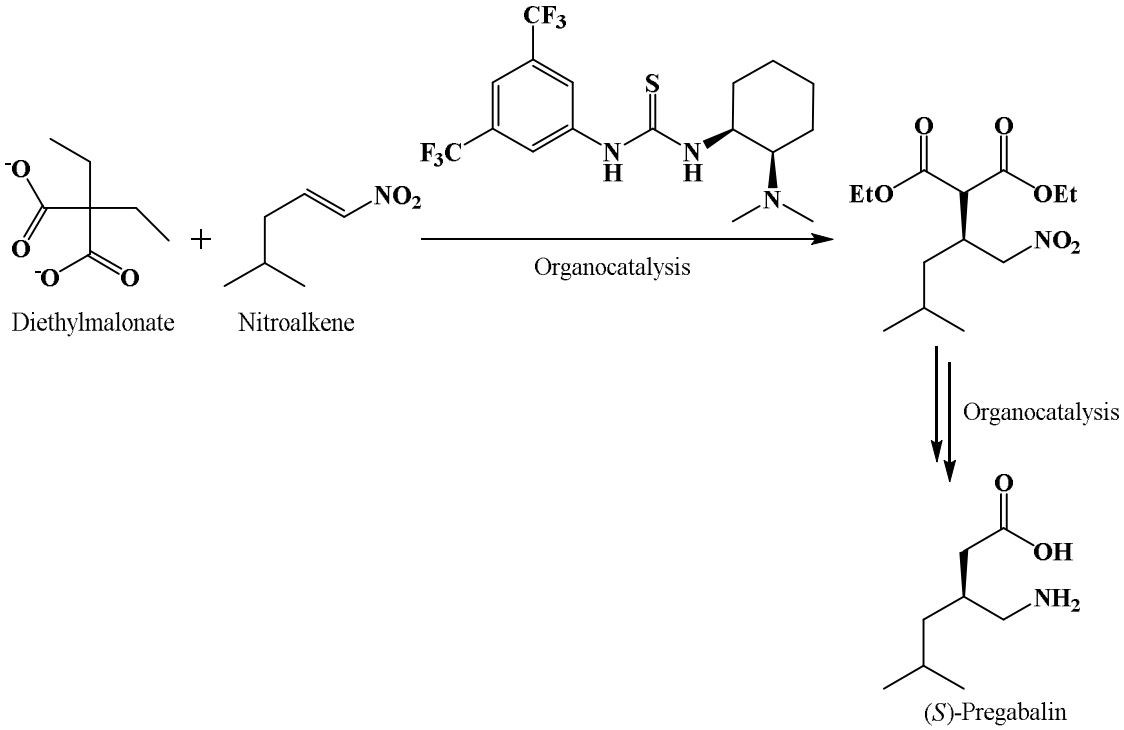
5.3.5. Synthesis of Laninamivir

Laninamivir acts as a neuraminidase inhibitor and useful for the prevention and treatment of influenza viruses. Laninamivir synthesis is carried out by a intramolecular Michael addition reaction.[22]



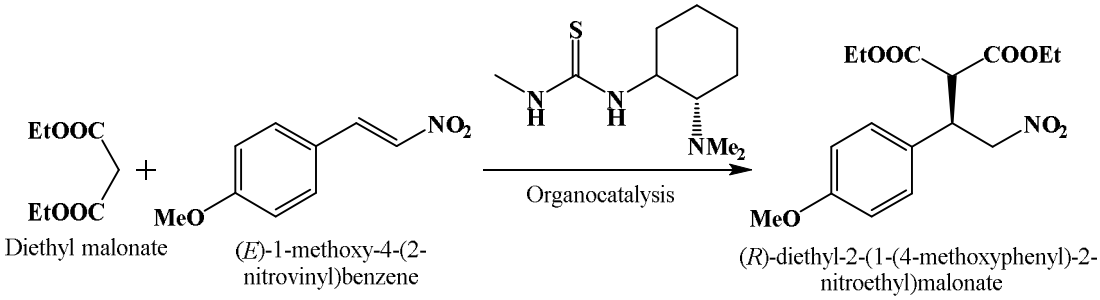
5.3.6. Synthesis of (*S*)-Pregabalin

(*S*)-Pregabalin is an anti-convulsant and anxiolytic medication. Diethyl-malonate undergoes a stereoselective nucleophilic addition reaction with nitroalkene in the presence of chiral thiourea to prepare the intermediate and the reduction of the nitro moiety followed by hydrolysis and decarboxylation produces (*S*)-Pregabalin.[23]



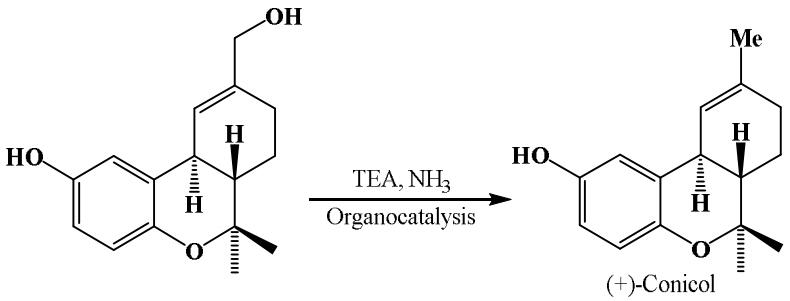
5.3.7. Synthesis of (*R*)-diethyl-2-(1-(4-methoxyphenyl)-2-nitroethyl)malonate

This compound is important for its quorum sensing activity that means the ability to detect and respond to cell population density by gene regulation. It is synthesised by a Michael addition reaction from diethyl malonate and nitro-styrene in the presence of methyl-thiourea catalyst.[24]



5.3.8. Synthesis of (+)-Conicol

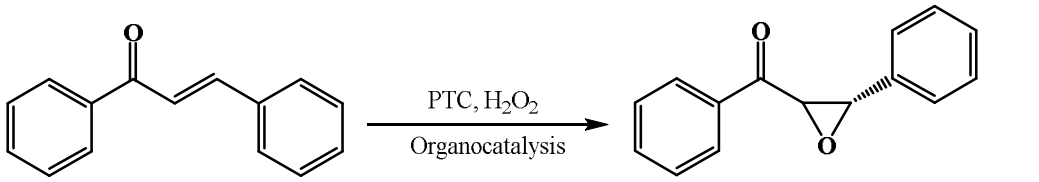
(+)-Conicol is used in the eye-drops and eye-ointments. Hong *et. al.* (2010) reported the enantioselective synthesis of (+)-Conicol via organocatalytic intramolecular Michael addition.[25]



(+)-Conicol

5.3.9. Epoxidation of Chalcones

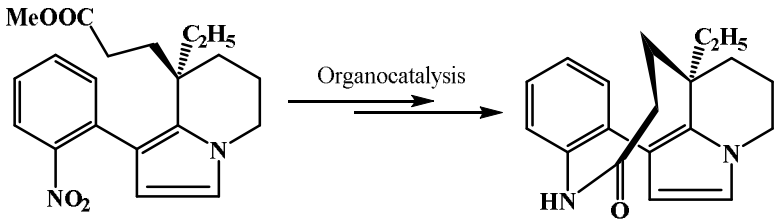
Phase transfer catalysis is used for the epoxidation of chalcones. Chalcone and its derivatives show anti-inflammatory, anti-microbial, anti-fungal, anti-oxidant and anti-tumour and many other biological activities.[26]



(*E*)-chalcone Phenyl((*3S*)-3-phenyloxiran-2-yl)methanone

5.3.10. Synthesis of Rhazinilam

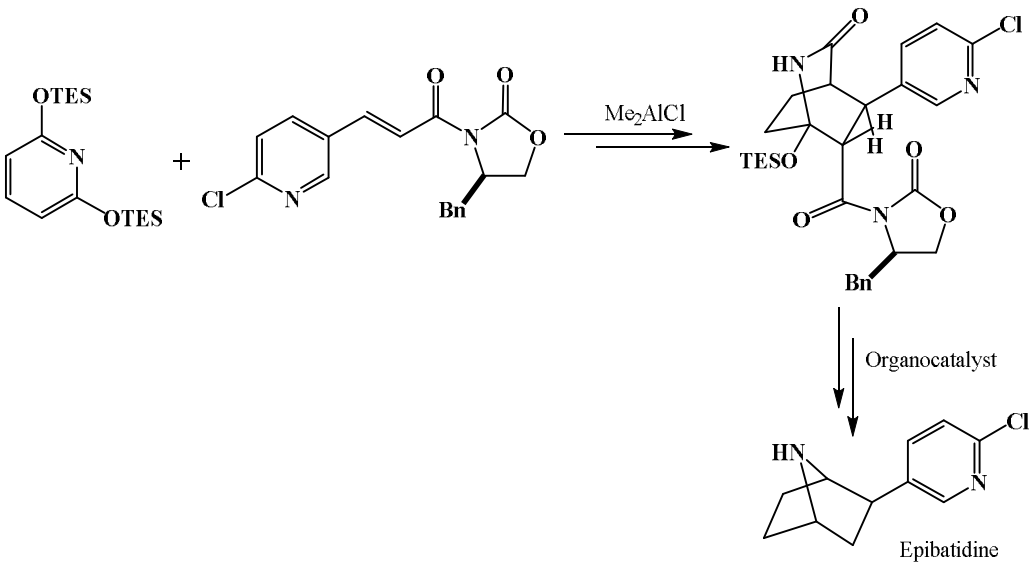
Rhazinilam acts as a spindle drug and has similar activity to that of colchicine, vinblastine and taxol. Zhu *et. al.* reported a catalytic de-symmetrisation method to synthesis enantio-selective Rhazinilam using chiral Bronsted acid as organocatalyst.[27]



Rhazinilam

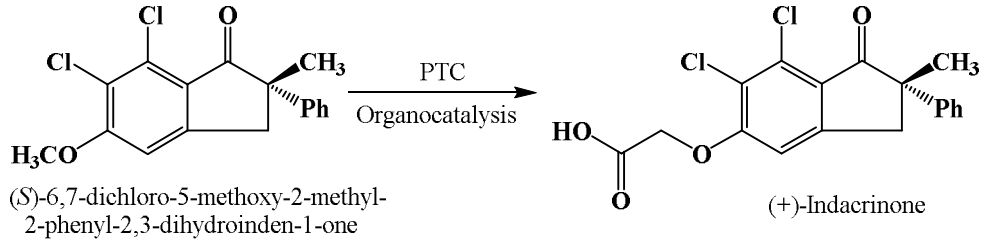
5.3.11. Synthesis of Epibatidine

Epibatidine is used in the treatment of leishmaniasis. An asymmetric hetero Diels-Alder reaction is used for the synthesis of (-)-Epibatidine.[28]



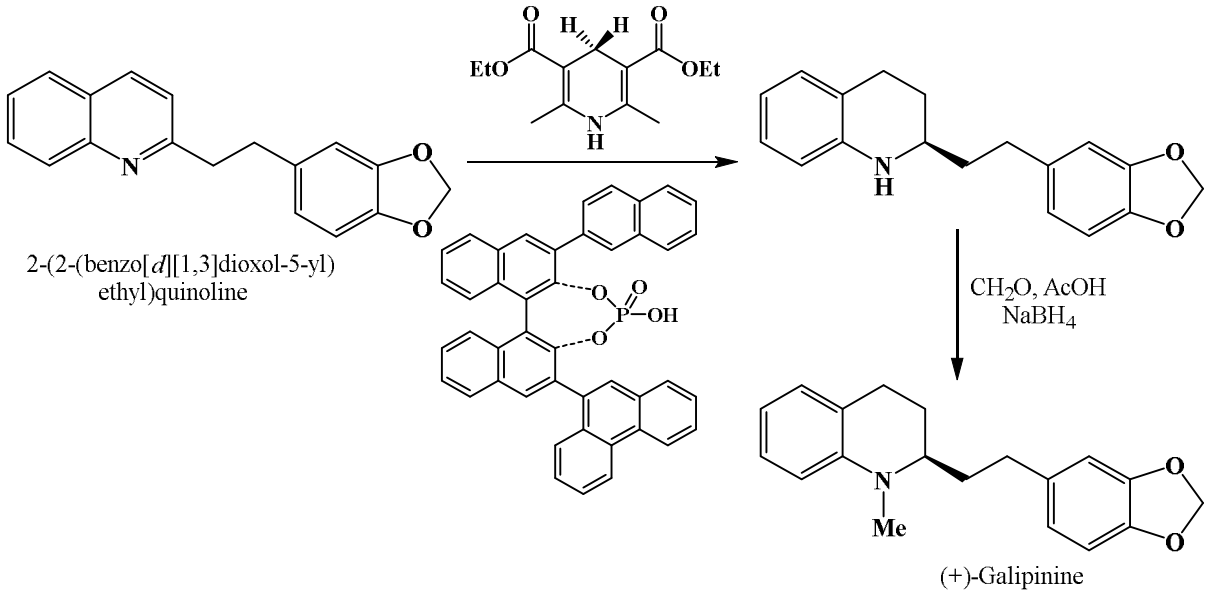
5.3.12. Synthesis of Indacrinone

Indacrinone is useful for the treatment of gout by decreasing re-absorption of uric acid. Merck (1984) established a chiral phase-transfer organocatalysis for (+)-Indacrinone synthesis.[29]



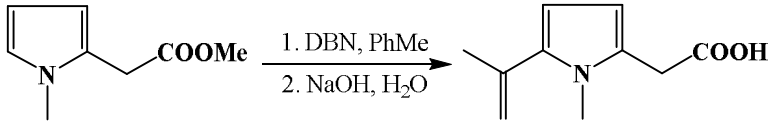
5.3.13. Synthesis of (+)-Galipinine

(+)-Galipinine is an anti-convulsant or anti-epileptic drug. An acid-catalysed enantioselective reduction reaction via binolphosphoric is carried out for the (+)-galipinine synthesis.[30]



5.3.14. Synthesis of Tolmetin

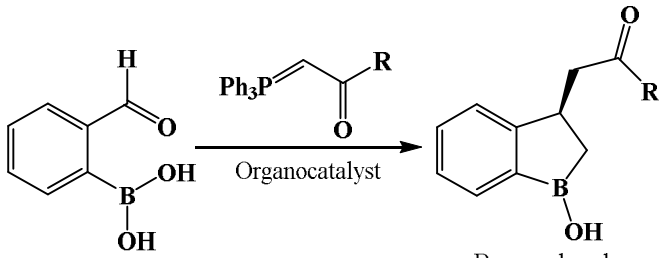
Tolmetin is a non-steroidal anti-inflammatory drug (NSAID) and it is synthesised by a 1,5-diazabicyclonon-5-ene (DBN) catalysed Friedel-Crafts acylation.[31]



Tolmetin

5.3.15. Synthesis of Benzoxaborole

Benzoxaborole exhibits anti-viral, anti-bacterial and anti-parasitic activities and is also a β-lactamase inhibitor. Benzoxaborole is synthesized by using chiral bi-functional organocatalysts.[32]



2-formylphenylboronic acid Benzoxaboroles

6. Conclusion:

Now-a-days, the scientific world has widely appreciated that organocatalysis has many advantages in comparison to other metal catalytic routes because of the stability of catalyst molecules in air and water, less price, availability from renewable resources and less chance of toxicity. Various use of hydrogen bonding catalysts, e.g., Bronsted acid/base, thioureas etc. have been reported to establish the organocatalytic reactions with different modes. Enormous effort has been carried out to design new type of enantioselective organic catalysts to synthesis chiral drug molecules. For faster development of new methods to synthesis enantioselective drugs, enantioselective organic catalysts are a better option as compared to other metal catalysed chemical routes. Chiral organocatalyst like guanidine has tremendous application recently due to its high enantioselective catalytic activities. This paper explains that the successive development of new chiral organocatalysts hold the future of asymmetric drug synthesis.

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श्रद्धावान लभते ज्ञानम्

Article No. DGC-NF-24/04

Ethno- medicobotanical studies of the Ging tea garden and adjoining areas of Darjeeling district, West Bengal, India.

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Abstract:

An ethno-medicinal survey was conducted among the local community residing in Ging areas, Darjeeling district, West Bengal. Darjeeling Himalayan region is characterised by a rich diversity of ethno-botanical plants as well as a rich heritage of traditional medicine practices. Modern medicinal facilities are lacking in the villagers of Darjeeling hills. Therefore people are largely dependent on herbal medicine for their healthcare. Present study was framed to record the ethno-medicinal plants of Ging areas of Darjeeling and field work was conducted during March to June 2022, using questionnaires. Data was collected from local herbal practitioners and senior citizens. The 69 plant species from 65 genera belonging to 43 familieswith their ecological status, vernacular name (Nepali), plant parts used, disease /ailments, distribution within Darjeeling and altitudinal ranges were identified and recorded. 35 of them were herbs, 15 were shrubs, 08 were climbers and rest 11 were trees.

Keywords: Ethno medicinal, medicinal plants, disease, Ging, Darjeeling hills.

INTRODUCTION:

Ethno botany is a multidisciplinary science and defined as the interaction between plant and people. Since ancient times human has used various natural materials as source of medicines. The use of plants to cure diseases and relieve physical sufferings has started from the earliest time in mankind's history (Hill 1989).

The plants used for curing various diseases in humans have been mentioned in ancient literatures like the Rig-Veda, Bible and Quran. In India, medicinal plants form the

backbone of several indigenous traditional systems of medicine.

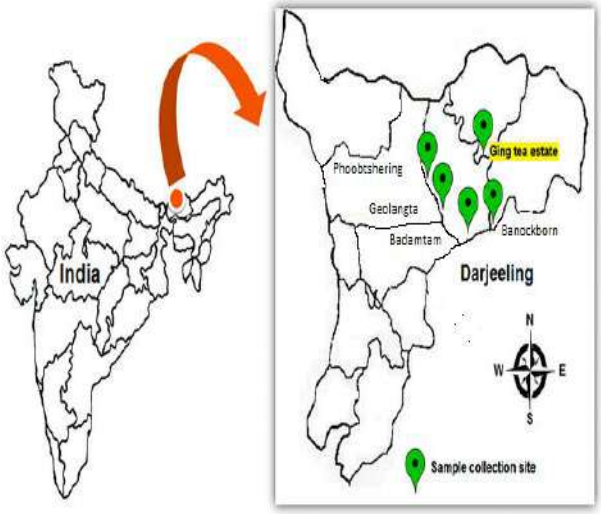
The Darjeeling Himalayan region is distinguished by a rich heritage of traditional medical practises and a vast diversity of ethno medicinal plants (Chhetri et al. 2005). Darjeeling Himalaya is situated between the $87^0$59′ - $88^0$53′E and $28^0$31′ - $27^0$13′N in the Eastern Himalayan region of India. The altitudinal range of this hilly region varies from 130 to 3660 m.

WHO, report depicts that more than 80% of world's population rely on plants-based products to meet their health care needs. Nearly, 25 to 45% of modern prescriptions contain plant derived lead molecules as a basic source in drug formulations. Furthermore, about 42% of 25 top selling drugs marketed worldwide are either directly obtained from natural sources or entities derived from plant products (Jain, 1991).

STUDY AREA:

Generally the Ging area of Darjeeling district is inhabited by tribal population. The tribal communities live in forests, hilly tracts and naturally isolated areas from the urban society. That's why they developed their cultures on their own, in tune with the nature. They depend on the nature, for their food, shelter, and livelihood. Thus the vegetation has much influence on the tribal life. The Ging tea garden is located at 27.0761^0N 88.2996^0E, around 10 km north-east of Darjeeling. According to the 2011 Census of India, the Ging Tea Garden had a total population of 4,089 of which 2,037 (50%) were males and 2,037(50%) were females. The total number of literate people in this area was (75.47%). 10 tribal groups who inhabit this area are, Lepcha, Rai, Tamang, Bhutia, Limboo,Sherpa, Gurung, Dewan, Newar, and Magar.

Figure 1: LOCATION OF THE STUDY AREA



METHODOLOGY:

The Ging forest division encompasses several villages such asGing, Badamtam, Geolangtar, Phoobtshering and Banockborn etc. Thevillages were visited several times during March to June 2022, to collect information on ethno-medicine.Eightinformantswere thoroughly interviewed in this study (Appendix I).Besides, some elderly people also provided some informationon medicinal uses of plants. In this study, questionnaires were used to collect information on the Nepali common names of the plants, plant parts used for preparation of medicine and method of use of the medicine. In the study area, the people prescribing medicine are known as the '*Village Headman'*, the 'Jari-Buti wala' and other traditional knowledgeable healers.

During the period of study, door to door visits were made to identify local people with specialized knowledge on the use of medicinal plants. The Plants were collected along with their local names, the parts used and specific ethno medicinal uses were recorded.

Plant juice is typically obtained by crushing or grinding fresh plant material to release the liquid content. This can be done using a mortar and pestle, a blender or a juicer. The resulting plant material is then strained to separate the juice from the solid parts. The juice can be used immediately or preserved for later use.

Plant powder is made by drying the plant material and grinding it into a fine powder. The drying process can be carried out using

various methods such as air drying, sun drying or using a dehydrator. Once the plant material is completely dried, it can be ground using a mortar and pestle, a mixture grinder. The resulting powder is then stored in airtight containers to maintain its potency.

A decoction is prepared by boiling plant material, such as roots, bark or tougher plant parts, in water for a specific duration. The plant material is usually chopped or crushed to increase the surface area and enhance the extraction process. The mixture is simmered or boiled gently to allow the water to extract the desired compounds. After boiling, the liquid is strained to remove the solid plant material, resulting in a concentrated decoction that can be used as is or further diluted as needed.

plant paste is typically made by grinding fresh or dried plant material with a small amount of liquid to form a thick, semi-solid consistency. The liquid used can vary and may include water, oil or other solvents depending on the plant and its intended application. The plant material is ground using a mortar and pestle or a blender until a smooth paste is formed. The paste can be used directly or stored for later use.

Fresh plant parts that have not undergone any cooking or boiling processes. These parts can include leaves, flowers, stems or other plant organs that are harvested in their natural state. Fresh plant parts are often used for immediate use, such as in salads, teas etc. They may also be used in preparations where the plants active compounds are volatile and can be best utilized in their fresh form.

Boiling plant parts involves the plant material to heat in water for a specific period. This process is commonly used for tougher plant parts like roots, barks or woody stems which require heat to extract their medicinal or chemical components effectively. After boiling, the liquid is typically separated from the solid plant material through straining, resulting in a liquid preparation that can be used topically or orally.

The samples of recorded herbs, shrubs, climbers and trees were identified with the help of previous literature and regional floras. The plants specimens were processed using the standard herbarium techniques andthe specimens were deposited in the Herbarium of the PG Department of Botany, Darjeeling Government College, Darjeeling.

Appendix I: Profile of the informants in the Ging tea garden and adjoining area, Darjeeling district, West Bengal.

Name	Sex	Age	Village	Occupation	Category of Healers
Dhanmaya Manger	Female	58	Ging Tea Garden	Shopkeeper	Traditional old healer
Sunil Subba	Male	65	Badamtam	Farmer	Kaviraj
Suren Tamang	Male	50	Geolangtar	Forest watcher	Traditional old healer
Kalpana Pradhan	Female	58	Phoobtshering	Tea garden worker	Traditional old healer
Gobin Chettri	Male	60	Banockborn	Artist	Village headman
Denesh Bhutia	Male	48	Ging	Farmer	Servant
Bidya Gurung	Female	45	Banockborn	Shopkeeper	Traditional old healer
Anil Telaija	Male	52	Geolangtar	Kavirajstore	Traditional old healer

RESULTS:

The plants used by the people of local community to treat different ailments are given in Table 1, along with all relevant informations.

Table 1: Some of the herbal remedies as used by local people of Ging and adjoining areas in Darjeeling.

Scientific name	Family	Local name (Nepali)	Part (s) used	Habit	Medicinal uses
*Aconitum heterophyllum*Wallich	Ranunculaceae	Bikhuma	Roots	Herb	Stomachache, fever, cough & asthma
Acorus calamus Linn.	Araceae	Bojho	Stem and rhizome	Herb	Hormonal disorder, osteo arthritis
Allium sativum L.	Liliaceae	Lahsoon	Leaves and bulbs	Herb	Antiseptic, anti-cancerous and anti-tuberculosis agent
Aloe vera Tourin.ex Linn.	liliaceae	Ghew kumari	Fresh leaves	Herb	Skin ailments , Burns , piles ,cuts , carminative , digestive

Scientific name	Family	Local name (Nepali)	Part (s) used	Habit	Medicinal uses
Andromeda villosa Wall.	Ericaceae	Lekh angeri	Young twig	Herb	Skin disorder
Artemisia vulgaris (C B Clarke)Pamp	Asteraceae	Titepati	Young twig	Shrub	Sinusitis,nausea,diabetes,cut, wounds menstrual disorder
*Astilbe rivularis*D.Don	Saxifragaceae	Buro okhati	Fresh and dry rhizome	Herb	Tonsillitis, body ache, tonic for post natal women
Berginiaciliata(Haworth) Sternberg	Saxifragaceae	Pakhamvet	Stem	Herb	Boils, diarrhoea, dysentery
Bidens pilosa L.	Asteraceae	Kuro	leaf	Herb	Skin diseases
Brassica nigra (L.)Koch	Brassicaceae	Tori	Seed, Young twig	Herb	To obtain oil, as vegetables, skin ailments like piles.
Buddleia asiatica Lour.	Loganiaceae	Bhimsenpati	Young twig	Shrub	Skin problems
Camellia sinensis (L.)O. Kuntze	Theaceae	Chiya	Leaves	Shrub	Eye trouble, piles and throat irritation
Cannabis sativa Linn.	Cannabaceae	Ganja or bhang	Root,matured leaves and inflorescence	Herb	Indigestion,acidity,epilepsy,gonorrhaea,diuretic antispasmodic and sedative
Carica papaya L.	Caricaceae	Mewa	Fruits , seeds and leaves	Shrub	Fruit used as astringent; seeds anthelmintic, decoction of leaves used for platelets production in blood.
Cedrela toona Roxb.	Meliaceae	Tuni	Bark	Tree	Bark is a powerful astringent, cures dysentery
Centella asiatica (L.)Urban	Apiaceae	Golpata or dhungrijhar	leaf	Herb	Tonsillitis, skin disorder, piles, pneumonia, memory improvement
*Cinnamomum tamala (*Ham.) Nees	Lauraceae	Sinkouli/ Tejpatta	Leaves	Tree	In piles and heart trouble
Costusspeciosus (Koen.)Sm.	Costaceae	Bethlaure	rhizome	Herb	Night blindness,antioxidant, anthelmic, purgative, depurative, indigestion, stimulant
Cucumis sativa L.	Cucurbitacea	Kankra	fruit	Climber	Skin ailments such as pimles, pigmentation
*Curcuma aromatica*Salisbury	Zingiberaceae	Banhardi	Rhizome	Herb	An appetizer, grounded rhizome applied to wounds to heal
Curcuma longa Linn.	Zingiberaceae	Hardi	Rhizome	Herb	As spices, as antiseptic in wounds,diarrhoea,jaundice,ur

Scientific name	Family	Local name (Nepali)	Part (s) used	Habit	Medicinal uses
					inarydisorders,stimulant and carminative
*Cuscutareflexa*Rexb.	Cuscutaceae	Sunauli	Whole plants	Climber	Constipation, liver problem, spleen disease, diarrhea, piles, inflammation
Cymbopogon citrates Stapf.	Poaceae	Kagatayjhar	leaves	Herb	Bronchitis, intestinal worms, gastric chronic, rheumatism fever , dyspepsia
Cynodondactylon (L)Pers	Poaceae	Dubo	Whole plants	Herb	Piles,burning urination, veneral diseases, liver cirrhosis, nose bleeding, wounds
Dioscorea alata L.	Dioscoreaceae	Ghar tarul	Tuber and leaves	Climber	Tubers used in fever; leaves cure rashes and itch; and also against constipation
Drymaria cordata (Linn.) Willd	Caryophyllaceae	Abhijalo	Leaf aerial part	Herb	Sinusitis, nasal congestion, fever, headache
Equisetum debile L.	Equisetaceae	Kurkure jhar	Whole part	Herb	Mouth sore, loss of appetite, gonorrhoea, acidity, urinary trouble
*Eupatorium adenophorum*Sprengel	Asteraceae	Banmara/Kalijhar	Root and leaves	Shrub	Antiseptic and for blood clotting
*Eupatorium glandulosum*Kunth.	Asteraceae	Banmara	leaves	Shrub	External injuries, cuts, wound
*Ficus benghalensis*L.	Moraceae	Bar	Leaves, latex, roots (aerial) and bark	Tree	Ulcer, vomiting, vaginal complaints, fever, inflammation.
*Girardiniadiversifolia*Forsk Gaud.	Urticaceae	Bhangresishnu	terminal young twig and inflorescence	Shrub	Lower high blood pressure and bone fractures
Gloriosa superba L.	Liliaceae	Langaraytarul/ Bikhphool	Tubers, roots and flowers	Climber	Abdominal pain, itching, piles etc.
Glycine max (L.) Merrill	Fabaceae	Bhatmas	Roots and seeds	Herb	Astringent property and nutritional diet
Helianthus annuus L.	Asteraceae	Ghamful	Flower, roots, seeds and leaves	Herb	Flowers to cure ulcers, leprosy, anaemia, asthma
Hypericum	Hypericaceae	Mehandi phul	Leaf twig	Shrub	Boiled as tea and taken in

Scientific name	Family	Local name (Nepali)	Part (s) used	Habit	Medicinal uses
*hookerianum*W &A.					urine infection
Imperata cylindrica (L.) Rausch. Vaughan	Poaceae	Siru	Roots,flower and stem	Herb	Root against fever, cough, internal bleeding, jaundice and kidney problems
Ipomoea batatas (L.) Lamarck	Convolvulaceae	Sakarkhanda	Tuber and plant	Climber	Fever and skin diseases
Kaempfera rotunda L.	Zingiberaceae	Bhuinchampa	Tuber,stem,root	Herb	Bone fracture, joint dislocation, gout, rheumatism and swelling
Litsaea citrate Blume	Lauraceae	Siltimur	Fruit	Small tree	In cough, fever and gastritis
Lycopersicon esculentum Miller	Solanaceae	Rambhera/ Golbhera	Fruits	Herb	Headache and rheumatism
Mentha spicata L.	Lamiaceae	Padina	Leaves and tender shoot tips leaves	Herb	Fever, bronchitis, nausea; astringent, cure rheumatic pains
Momordica charantia L.	Cucurbitaceae	Karela	Leaf, fruit, seed	Climber	Diabetes, Skin ailments
Nasturtium officinale R. Brown	Brassicaceae	Simrayo	Entire plant	Herb	Treats scurvy, antidotes to toxin
Nephrolepis cordifolia (L.) Presl	Nephrolepidaceae	Bhui amala or pani amala	fruit	Herb	Burning urination, diabetes, eye diseases, kidney problem
Oxalis corniculata L.	Oxalidaceae	Chariamilo	Whole plant	Herb	Skin diseases
Pentapanax leschenaultia Seen.	Araliaceae	Chinde	Young twigs	Tree	To lower blood pressure
*Phlogacanthusthyrsiflorus*Nees.	Acanthaceae	Chua	Flower cluster	Tree	To lower high blood pressure
Plantago erosa Wall.	Plantaginaceae	Jibrejhar	Young leaves and rootstock	Herb	Amoebic dysentery, tonsillitis, cut and bruises and fever
Polygonum runcinatum Ham	Polygonaceae	Ratnaulo	Whole plant	Herb	Skin diseases
Prinsepia utilis Royle	Rosaceae	Bhekali	fruit	Tree	Skin ailments
Prunus cerasoides Don	Rosaceae	Paiyun	Bark , leaf	Tree	Skin ailments
Psidium guayava Linn.	Myrtaceae	Ambak	Tree bark, young leaves	Tree	Diarrhoea, dysentery, mouth odour

Scientific name	Family	Local name (Nepali)	Part (s) used	Habit	Medicinal uses
*Rhododendron arboreum*Smith	Ericaceae	Laliguras	Flowers & young leaves	Tree	Fresh and dried leaves and flowers used in dysentery and diarrhoea
*Rubia manjith*Roxb.Ex Flemming	Rubiaceae	Majito	Whole parts	Climber	Jaundice,boils, menstrual disorder, skin diseases
Rubus ellipticus Smith	Rosaceae	Ainselo	Root	Shrub	Tonsillitis, gastritis
*Rumex nepalensis*Sprengel	Polygonaceae	Hal haley	Roots and leaves	Herb	Leaf infusion given in colic, applied to syphilitic ulcer; root paste applied to wounds
Salvia sp.	Labiatae	Babarephul	Leaf	Small shrub	Allergy
Smilax sp.	Liliaceae	Kukurdaine	Young twig	Climber	Skin diseases
Spinacea oleracea L.	Chenopodiceae	Palang sag	Aerial part	Herb	Anti-oxidant,anti-cancer,anti-obesity,inflammation
Swertia chirayita(Roxb. Ex. Flem.)	Gentianaceae	Chirawto	Whole plant	Herb	Eye problem, blood purifier , malarial fever , skin problems
Tagetes patula L.	Asteraceae	Sayapatri	Leaves, petals	Shrub	Skin problem, removes mouth odour
Tectaria coadunate (J.Sm.)C.Chr	Aspidaceae	Kalo uniu	Frond	Herb	Acts as antiseptic on cuts, wounds
Thysanolaenaima xima(Roxb.) Kunth	Poaceae	Amliso	Root, young shoot	Shrub	Skin problems, tonsillitis, boils , abortion
Urtica dioica L.	Urticaceae	Patley sishnu	Young twigs, inflorescence	Small shrub	Hypertension, cholengitis, gout, heart disease
Urtica parviflora Roxburgh	Urticaceae	Sishnu	Whole plant	Small shrub	Hemorrhages, jaundice and high blood pressure
*Vanda undulata*Lindl.	Orchidaceae	Sunakhari	Pseudo-bulb	Epiphyte on tree	In Muscle cramp bulb is mixed with milk.It is rich in calcium
Viscum album L.	Santalaceae	Harchur	Leaves,root	Shrub	Bone fractures, body ache, joint dislocation
Zingiber officinale Roscoe	Zingiberaceae	Aduwa	Rhizome	Herb	Used as spices, bronchitis, asthma, tonsillitis, fever
*Zingiber rubens*Roxb.	Zingiberaceae	Fachyang	Rhizome	Herb	Nausea, vomiting, gastritis, gout, limb swelling, bone fractures

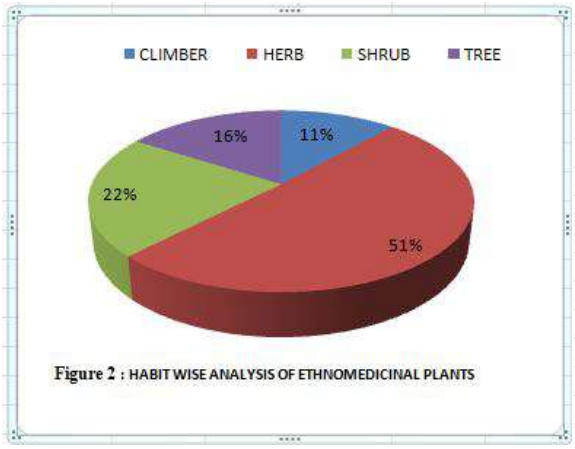


Figure 2 : HABIT WISE ANALYSIS OF ETHNOMEDICINAL PLANTS

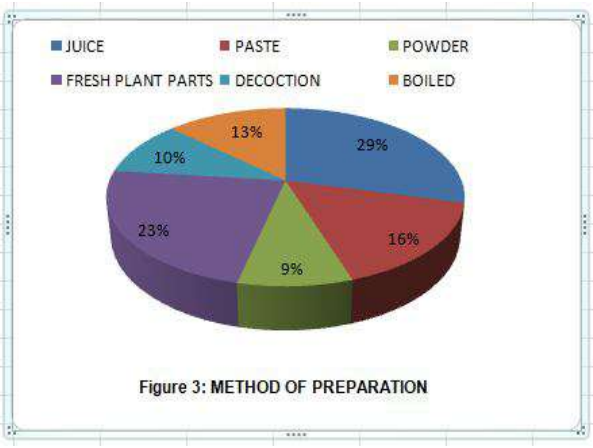


Figure 3: METHOD OF PREPARATION

DISCUSSION:

Darjeeling Himalayan region is one of the prospective places as sources of medicinal plants in the nation due to its extremely great biodiversity. Local healers harvest free medicinal plants from the forest, and as a result, the locals can engage in some traditional medicine practices. Local healers are paid to some extent in this line of work. Some of the information in this study are novel, because it hasn't been reported before. The majority of medications used in the region are made from fresh-foraged ingredients and are typically consumed in the form of juice, paste, or powder. Oral administration was the common practice.The plant parts used for medicine preparation were bark, flowers, rhizomes, roots, leaves, seeds and whole plants. Some plant materials were collected during the period of availability, stored and used in time of necessity. The traditional medicines prepared in this study were mostly from a single plant or a combination with other plants.*Artemesiavulgaris* (C B Clarke) Pamp and *Swertia chirayita* (Roxb. Ex. Flem.) were recorded for the highest use value.The traditional knowledge has been restricted to elder people and With the advancement of the society, this valuable knowledge are also disappearing at an alarming rate that needs immediate attention for conservation for the greater interest of mankind not only that the protective measures should be taken in order to conserve precious multipurpose species that are facing overexploitation.

Urtica dioica, is utilized by the Lepcha tribe for curing diarrhoea and cough and the soup prepared from it is given to the pregnant women which helps is easy delivery of child

A recent research by Rajbhandari et al. (2007) has shown that methanolic extract ofthe rhizome of a *Bergenia ciliata* has antiviral properties against influenza virus A.

Artemisia vulgaris L., a perennial aromatic shrub with bitter taste, is consider valuable medicinal plant. *Artemisia vulgaris* leaves are used to treat skin diseases.

Leaves of *Centella asiatica* are used to cure throat pain. The people of Chakma communities in Bangladesh use juice of *Centella asiatica* as a remedy of syphilis, and ulcer.

Rhizomes of *Zingiber officinale* and *Curcuma longa* were simply chewed to get relief from fever, diarrhoea, vomiting, and cough and this practice was also documented by Idrisi et al. (2012).

During the present Ethnobotanical study 69 plants were used to cure varies ailments, reported by the informants for the 43 families, out of the 43 families, 34 are dicots, 7 are monocots and two are Pteridophytes. Out of the 43 families Asteraceae have (6) plants, Zingiberaceae have (5) plants, Poaceae have (4) plants, Liliaceae,Urticaceae, Rosaceae have (3) plants, Lauraceae, Cucurbitaceae, Brassicaceae, Araceae, Ericaceae, Polygonaceae, Lamiaceae, Saxifragaceae have (2) , and remaining families each one have single species. These 69 plants were used to cure varies ailments, i.e., Irregular menses, urinary problems, diarrhoea, dysentery, malarial fever, asthma, diabetes, fever, jaundice, burns, cough, cold, rheumatism, wounds, ophthalmic problems, ulcers, bone fracture, abdominal pain, heart diseases, skin ailments, piles, muscular pain, tonsillitis, body swellings and arthritis. Among the 69 plants that are recorded, herbs include (35), followed by shrubs (15), trees (11) and climbers (8) as shown in the figure 2.

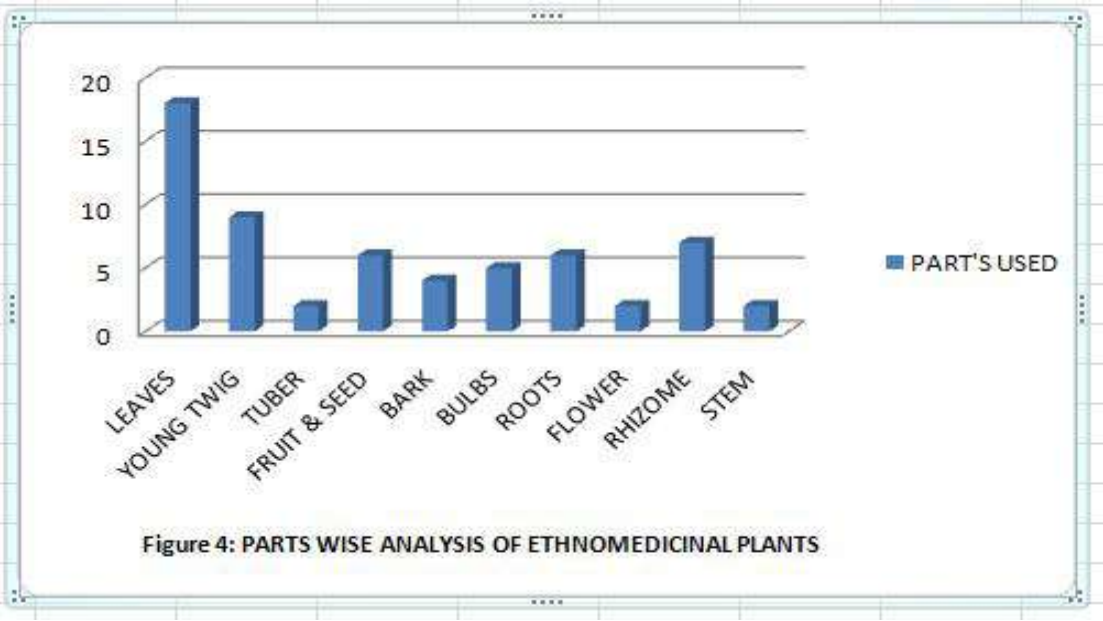


Figure 4: PARTS WISE ANALYSIS OF ETHNOMEDICINAL PLANTS

CONCLUSION:

Most of these medicinal plant species can be found in their natural habitats. Some plants are disappearing at a rapid rate for a variety of reasons, including landslides, construction work, urbanisation, etc. Therefore, precautionary measures are required to ensure the continued supply of these plants. Farmers should be involved in the cultivation of medicinal plants in their gardens, since many residents of the Darjeeling district rely on these plants to treat a variety of minor illnesses, including cough and cold, skin diseases, tonsillitis, and dysentery. In order to ensure the sustainable and long-term conservation of the region's natural resources, it is necessary to promote the local cultivation of medicinal plants and other economic species. Since they are the greatest judges of the area, it is important to actively involve locals in the appraisal, planning, execution, and monitoring of the activities.

Proper research should be carried out in the hills to develop cultivation techniques of medicinal plants on priority basis. The old healers pass the information in oral form to their offsprings and near relation which is not documented.Thegrowing disinterest in the use of the folk medicinalplants and its significance among the younger generation of the local people may lead to the disappearance of this practice. Educated younger generation of the different community should be encouraged by the Government to protect and cultivate these valuable herbal plants before they get

extinct due to the impact of modernization and urbanization and also due to deforestation.

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श्रद्धावान लभते ज्ञानम्

Article No. DGC-NF-24/05

Retention of Seed Vigour and Enhancement of Plant Yield of a Local Rice Seeds by Chemical Manipulation under Ambient Storage

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Abstract:

Rice (Oryza sativa L.) is the most important food crop and life for thousands of millions of people. Rice seed vigour is a key element of seed quality and high vigour seeds give uniform plants stand and higher yields. Viability of most seeds normally reduces with the storage period, Storage temperature and relative humidity. Seed storage and seed vigour are the major problem in agricultural production of Darjeeling hill. Keeping this problem in mind, an attempt was made to enhance the yield of three rice varieties of Darjeeling hill viz. Adday. Kataka. Arya which are pre-treated with chemicals ASA, SADH and NaDK and kept undergo forced ageing under adverse storage environment/ long term accelerated ageing treatment (95% relative humidity. RH) for 365 days. Prolongation of ageing led to deterioration of both germinability and seed production. Thus the major, objective of this work was to test the efficacy of chemicals ASA. SADH and NaDK on the alleviation of total yield attributes after prolonged storage. However. remarkable diminution of germinability and seed production was noted in the chemically pretreated seed lots with Ascorbic acid (ASA) with concentration of 250 and 500µg/ml, Succinic acid 2.2-dimethylhydrazide (SADH) with 150 and 300µg/ml, Sodium dikegulek (NaDK) with1000 and 2000µg/ml compare to distilled water (Control). The study concludes that accelerated ageing showed effect on seed quality of all the varieties of rice. All the test experiments performed concluded that the deleterious effect of yield attributes *i.e.* total seed weight/ panicle and 1000 seeds weight was substantially alleviated and ameliorated best by NaDK.

Keywords: Yield attributes, Acceleated ageing. NaDK, SADH, ASA, Darjeeling hill.

Introduction:

The people of Darjeeling Hill have a wealth of knowledge useful to the neighbourhood in cultivating the local environment because of their familiarity with natural farming. The villagers engage in traditional hill cultivation, where fields are rain-fed and cultivating frames are constructed using various indigenous techniques. Maize, corn, and vegetables such as cabbages, potatoes, squash, coriander, and chillies are among the crops planted. In the hills, cultivation systems are inseparably linked to usability in terms of the comparison of usability and use of assets (Mukherjee. 2012).

Rice has been grown in Darjeeling for many years in a very small field. Indigenous methods of agriculture and innate scientific competence are part of a particular community's culture and history. Farmers rely on biodegradable waste in indigenous agricultural systems, which are effective in helping to maintain soil quality. Wet rice terrace agriculture, finger millets/black lentils, and forest area management by eco-friendly ecosystems and bioregional farm activities such as bio-waste recycling are all part of the rice-based farming scheme, which requires upgrading the advancement process to benefit from these populations (Mukherjee, 2010). In this area, the rice varieties are extremely short and thin-grained. Each variety is expensive in the area where it is produced, and it lacks a well-established market. Farmers consider rice as auspicious and basically cultivate rice for personal domestic consumption and utilisation for festivity and auspicious ceremonial purposes.

Due to extremely high relative humidity in Darjeeling, which promotes the growth of microorganisms, the question of maintaining seed vigour in Darjeeling and the territories surrounding it is far more serious. As most crop seeds probably need storage for either one or a few planting seasons, farmers and horticulture professionals from this area are often impeded from maintaining standard seed vigour in an environmentally friendly storage environment. The moisture content of seeds and the storage temperature deeply affected the deterioration rate (Rai. et al., 2000; Chettri *et al*., 1993). Seed degradation has been shown to have several physiological and biochemical indicators (Saraswathy *et al*., 2017; Kapoor *et al*., 2010; Jatoi *et al*., 2004; McDonald, 1999).

In recent years, some effective physical and chemical manipulative techniques have been developed by seed technologists to get rid of such climatic as well as biotic hazards which conducive to earlier deterioration of stored seeds. There are some reports that

hydration- dehydration treatment as well as treatment of seeds with chemicals of diverse nature (salts. phenols, organic acids, essential oils, plants growth regulators, bio products) can favourably influence the viability status of seeds (Chhetri *et.al*. 1993; Bhattacharjee *et al*., 1999; Pati, 2019 & Kanap*et al*., 2021). Accelerated ageing tests enable testing the vigor of stored seeds by subjecting the seeds to a particular temperature and relative humidity over time and then performing standard germination tests. Accelerated ageing is considered an excellent option as a vigor test when compared to seedling emergence and index of emergence speed because of the shortest time of acquisition and efficient results.

After 365 days of Accelerated ageing, field test were carried out using three rice varieties each. The following plant yield attributes were recorded from accelerated aged seeds with three local varieties of rice seeds: total seed weight per panicle and 1000 seed weight per plant. The yield data that was presented was an average of three results.

MATERIALS AND METHODS

Rice Seed lots of 3 varieties Addey, Kataka and Arya were collected from local farmers of Bijanbari. Darjeeling, West Bengal, India. After collection, the seed lots were separated from husk and healthy, undamaged seeds were selected for experimental purposes. During the experimental period the environmental conditions of Darjeeling were as follows: Temperature:16-22^0 Celsius, Relative humidity: 90-95±2%.

Experimental condition and seed pre-treatment:

After the surface sterilization with 0.1% mercuric chloride ($HgCl_2$) for 90 sec, all the seed varieties were separately pre-soaked with aqueous solutions of Ascorbic acid (ASA) with concentration of 250 and 500µg/ml, Succinic acid 2,2-dimethylhydrazide (SADH) with 150 and 300pg/ml. Sodium dikegulek (NaDK) with 1000 and 2000µg/ml or distilled water for 6 hours and then dried back to original weight of seeds. After 6 hours intervals such soaking drying treatments were repeated 3 times to make the total duration of pre-treatment of 18 hours. This mode of pre-treatment enabled maximum pre-treatment of the chemical while avoiding the commencement of germination. After complete pre-treatment of seed lots, the pre-treated seed lots (20g) each were put into separate cloth bags and thus stored in a desiccators in which an environment of 95% relative humidity was pre imposed by keeping 250 ml 5.96% Sulphuric acid

(vol/vol) within it. This experimental setup was kept allowing the seeds to experience forced ageing treatment and Sulphuric acid was changed periodically to restore the desired relative humidity within the dissector throughout the experimental period. After 365 days of pretreated seeds, seeds were sown in the field and allow to germinate and grow into fully grown individual plants. All of the field experiments for this study were conducted in a rice field in Sunsari, lower Goke Busty, Jamuney. Darjeeling. The following plant yield attributes were recorded which include total seed weight per panicle and 1000 seed weight per plant. The yield data that was presented was an average of three results. Statistical analysis of the data was done in terms of Least Significant Difference (LSD) which was calculated at 95%confidance limits (Panse and Sukhatme, 1967).

RESULTS AND DISCUSSION

Studies have shown that artificial ageing tests are an effective technique to contemplate rice seed vigour rather than natural ageing (Henga, *et al*., 2019: Zhou, *et al*., 2020). According to findings in this experiment under long-term accelerated ageing, seed vigour decreased as ageing time increased, indicating a negative relationship. Seed vigour was impaired drastically with an increase in days of treatment. The data showed that a high relative humidity treatment increased the forced ageing process and this decrease vigour and viability of seeds, which was considerably checked by seed pretreatment with NaDK, ASA and SADH respectively. However, a significant alleviation of the injurious effect was noted best in seeds that underwent pretreatment with NaDK.

Results showed that pretreatment of three varieties Adday. Kataka and Arya local rice seeds with Ascorbic acid (ASA 250 and 500µg/ml). Succinic acid 2,2-dimethylhydrazide (SADH 150 and 300µg/ml). Sodium dikegulek (NaDK 1000 and 2000µg/ml) significantly alleviated the ageing-induced enhanced field attributes capacity under accelerated ageing environment in capture to control. Reduced field emergence are considered to be the important visible criteria for the evaluation of poor seed vigour (Rai 2000). Data indicated that when plants were grown from pretreated and accelerated aged seeds. Yield attributes such as overall yield per panicle and 1000 seed weight were improved significantly in compare to control, resulting in a significant improvement in crop yield. In contrast to control samples, seed pretreatment with Na- dikegulac showed a significant improvement in crop yield.

Conclusion

In this investigation, the chemical-induced arrestation of rapid loss of seed deterioration is indicative of strengthening the defense mechanism by the chemicals under ambient storage condition. To overcome the vigour and viability status of rice seeds under ambient storage, the chemicals NaDKmaintains metabolic activity leads to better seed health. Thus, it can be concluded from the results of this investigation that chemical Na-dikegulac is effective in enhancing seed viability and thus showed better yield attributes than rest of the samples. Thus, invigoration property of the present seeds pretreating agent seems to be apparent from these experimental results.

TABLE

Effect of seed pretreatment with ASA, 250 and 500µg/ml, SADH, 150 and 300µg/ml and NaDK, 1000 and 2000µg/ml followed by 365 days of accelerated ageing treatments of the seeds on yield attributes of rice plant types Adday, Kataka and Arya.

Seeds were pretreated with test solution or distilled water for 6h and then sundried. This was repeated three times in close succession and the seed lots were kept in 95% RH. Plants were raised from accelerated aged seeds in the experimental field and data were recorded after harvest.

Pre-treatments (µg/ml)	YIELD ATTRIBUTES					
	ADDAY		KATAKA		ARYA	
	Seed wt./ Panicle	1000 seeds wt. (g)	Seed wt./ Panicle	1000 seeds wt. (g)	Seed wt./ Panicle	1000 seeds wt. (g)
Control (H_2O)	3.824	21.12	4.641	20.564	3.88	25.52
ASA (250)	5.963	24.465	6.885	22.54	5.938	27.752
ASA (500)	6.838	25.19	7.983	23.932	6.067	28.268
SADH (150)	5.556	23.78	6.593	22.372	5.753	27.012
SADH (300)	6.784	24.92	7.357	23.49	5.882	28.752
NaDK (1000)	7.966	26.83	8.937	23.748	6.882	30.492
NaDK (2000)	7.872	25.78	9.116	24.036	6.004	29.194
LSD (P = 0.05)	0.191	1.05	0.23	1.208	0.194	1.276

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श्रद्धावान लभते ज्ञानम्

Article No. DGC-NF-24/06

The Story of Our Universe

Part I: The Origin

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Abstract:

The origin of our universe is discussed in the light of modern cosmology without any non-trivial mathematical expressions. Different models are discussed which were tried to explain the creation of everything such as the steady state theory, eternal inflation theory, oscillating universe theory, and the Big Bang theory. Among these theories, the Big Bang theory is discussed in detail as it supports observational evidence. Some limitations of this theory have also been discussed here.

1. INTRODUCTION

Ever since humans start to think about the nature in which they lived, they questioned themselves about how this world was created, how it was before, and how it will be in the future. Many human civilizations tried to answer the sequestions. Wherever we look around us, we see something is happening; it has a beginning as well as an end, which is the same as our lives do. For the past hundreds of years, human has been looking at night under the starlit sky and wondering how all of these has formed, how big it is and what will happen to it. What about the beginning and end of our universe?

In the quest for answering these questions, cosmology, a branch of natural science has developed over the years. Cosmology is one of the oldest branches of all known natural sciences. It is not only the study of planets, stars, galaxies, and the cosmic background, it is an effort that could lead us towards the origin of our universe, how it has evolved

over the years and what will be its future. Since the time of Aristotle, our understanding of the universe has gone through a continuous state of variation based on our research and scientific progress. In the late twentieth century, scientists invented sophisticated instruments that have been used to view the macroscopic and the microscopic domain of our universe. To understand the outer universe, we invented the Hubble Space Telescope, James Webb Telescope, etc., whereas giant machines like the Large Hadron Collider (LHC) have been constructed to understand the inner universe. We perceive that our knowledge about the universe is constricted to our endeavors.

All of us here are nothing but tiny observers, whirling about in space on a small planet, the Earth, which is soaked for part of each day by the light and warmth of a nearby star, the sun. We are voyaging around 12 million miles in a day around the center of the Milky Way galaxy, which is also whirling in a universe having more than 100 billion galaxies, each of which consists of approximately 100 billion stars. This universe is believed to start at a single point around 13.7 billion years ago when something exploded and directed the creation of everything. Since then, it has been expanding rapidly with time whereas its temperature is steadily decreasing. Today we know a lot of things about our universe. Over the last few decades, none of us have anticipated that the universe is so vast, gigantic, and beautiful as well as mysterious. Today we can perceive a universe, which is at least 93 billion light years across in diameter.

There is a lengthy list of cosmic lovers who have devoted their lives, to trying to build scientific instruments and unlock the mysteries of our universe. Johannes Kepler and Isaac Newton helped to understand the very first transparent picture of the universe by discovering the concept of gravitation. After that, we were able to understand the workings of our solar system such as why the moon does not fall to the Earth. Despite that, lots of unresolved questions remained. Many years later, Albert Einstein successfully refined our view with his famous theory of special and general Relativity. Even a century later, we are continuing his work.

In the next sections, we shall discuss the origin of our universe from the beginning without any mathematical jugglery. We have tried to convey the basic information to the general audience without assumming any prior understanding of modern physics.

I. BEFORE BIG BANG

As we stated earlier, our entire universe existed in a minuscule singularity about

13.8 billion years ago, when something exploded and creation happens. This is known as the “Big Bang” and space-time came into existence. The Big Bang scenario is one of the great milestones of science as it describes an event that looks like the origin of the universe, but it still doesn’t tell us what we want to understand, where all the matter and energy in the universe come from in the first place? What lies beyond the part of the universe that we can observe? How did time begin if time even had a beginning? Why was the universe born with this particular building plan and not some other? No one yet knows.

So, when we ask ourselves ‘what existed before the Big Bang?’ then we are asking the wrong question as there was no time before the Big Bang. Even, less than thirty years ago, this question was ruled out as politically incorrect. Even Stephen Hawking stated that these questions were like asking ‘what lies north of the North Pole’. He also once wrote, “Events before the Big Bang are simply not defined because there’s no way one could measure what happened at them. Since events before the Big Bang have no observational consequences, one may as well cut them out of the theory and say that time began at the Big Bang.” The terms before and after the Big Bang are bounded by the strict laws of time. So, the beginning was the Big Bang, and “before” make no sense; there was no “before”. Time itself was T = 0 at the instance of the explosion and started counting as the explosion occurred. The human brain is constructed in such a way that, it is always compelled to imagine the existence before the Big Bang. Our brain is accustomed to three-dimensional space and time but intelligence can not grasp any thing beyond that. Although mathematics can extrapolate things before the Big Bang but it is an almost impossible endeavor for a human brain to comprehend it.

In the past thirty years, we have seen a change of paradigms that leads to a new view of the world regarding the stage before the Big Bang. Some theories are prescribed although these are still speculative and certainly neither accepted nor admired by all scientists, although that permits us to look before the formation four universe.

II. THE BIG BANG

The Big Bang is not merely a theory or hypothesis or a wild guess. It was a wise conclusion, supported by many scientific pieces of evidence painstakingly accumulated over the last centuries. Albert Einstein’s model of gravity prophesied this phenomenon, which has survived against every test thrown at it to date.

The Big Bang model narrates how our universe came into existence, but it could not help to describe the region of space, which is embedded in the darkness, beyond our observable universe. There are three potential possibilities of what could be beyond our observable universe.

1. The first possibility is that space-time may be different in that region. The laws of physics and mathematics that we know in this universe do not prevail there. There may be some different sets of rules beyond our observable universe.

2. The second possibility is that our 4-dimensional (3-dimensional space and time) space is expanding into a much bigger fabric of space-time. This idea stems from the fact that, the universe is expanding and it must be expanding into something bigger universe, producing more and more of itself at every bit of second.

3. The third possibility is that if we want to observe beyond our observable universe, we might need to open new dimensions. Our universe is limited to three physical dimensions and one dimension of time. We might have to get rid of these limited dimensions and observe ourselves from higher dimensions. In that way, we will be able to see if we are expanding into something bigger, or if the space is only creating more of itself.

Now a days, we have many different theories which respond the same question. Most cosmologists address the creation and evolution of the universe, but only a few of them could explain the origin and the evolution of our universe. Today, the most accepted and influential theory, which can explain the origin of the universe, is the Big Bang Theory. If we have a theory that can narrate the origin of the universe, it must be consistent with the hundreds of observations and theoretical explanations. The Big Bang theory is so far the only theory that could explain observational data. Before we discuss many facets of the Big Bang theory, we must talk about some of the early competitors of the Big Bang Theory. These theories not only challenged the Big Bang theory, but also enlightened us with a different perspective of the universe. Among many rivals of the Big Bang theory, we will only discuss three of them in the next three sections.

III. STEADY STATE THEORY

One of the early competitors of the Big Bang Theory is the Steady State Theory, which was first put forward by Sir James Jeans in the 1920s and further revised by Hermann Bondi, Thomas Gold, and Fred Hoyel in 1948. This theory suggests that the

universe looks the same in all directions, no matter where your point of observation is. All the different laws of physics occurring at different places are likely to be the same. This theory constructs a map of theuniverse, which is infinite in extent with no beginning or end and does not change with time. Steady State Theory proposes the continuous creation of matter throughout the universe where there was no beginning, and there will be no probable fate of the universe. The universe continuously creates newmatter by itself and that's why the universe goes through a continuous expansion. Although the universe is expanding, its overall density remains the same. This theory offers an initial picture of the expanding universe by suggesting that matter is being created inequal proportion to the expansion. As a result, the overall density always remains unchanged, which is quite different from the observational evidence. This theory was popular in the 1950s, but it is not accepted by most scientists at the present time. Evidence found in the mid of 1960 concluded that this theory is incorrect and our universe is anticipated to have a finite age. This theory also offers no explanation for the Cosmic Microwave Background Radiation, which maps the entire cosmos. As we look further into the universe, it shows that the universe is not the same as it was a few billion years ago, which quite contradictory to the arguments is proposed in Steady State Theory.

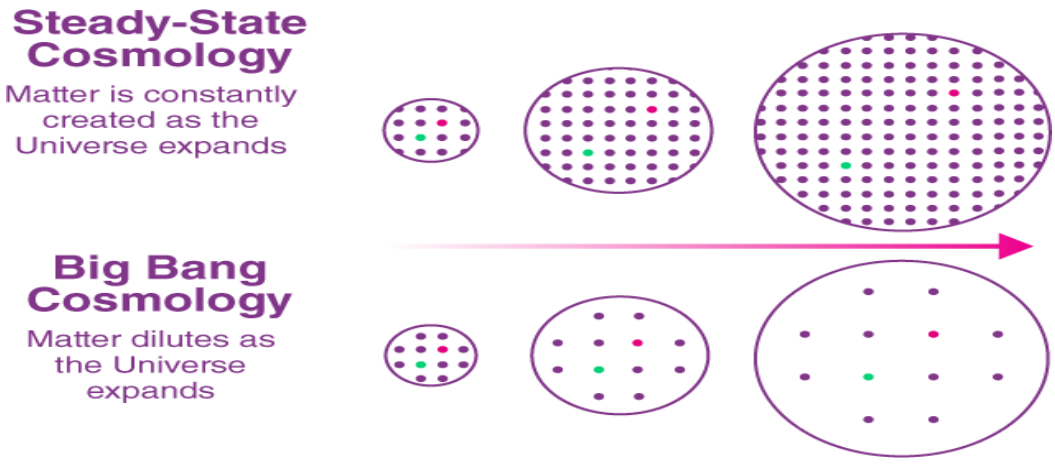

FIG.1: Comparison between the Steady State Theory and the Big Bang Theory

IV. ETERNAL INFLATION THEORY

Eternal inflation is a hypothetical inflationary model, which looks like the extension of the Big Bang theory of our universe. This theory says that the universe went through a rapid expansion for a brief period of time after the Big Bang, called

"inflation". This inflation did not stop and it never will and go for an infinite period of time. We can witness this inflation in the form of expanding universe. This expansion is driven by the forces of so called 'dark energy'. It also talks about the possibilities of multiple universes by predicting new universes, which are coming into existence in a complex model called, the 'Multiverse'. Our universe is just one of the infinite numbers of such universes. Those different universes could have different laws of physics and different properties, different from our known universe. The laws of science that we know in this universe might break down completely in those universes.

V. OSCILLATING UNIVERSE THEORY

If we hold a spring in our hand and if we stretch and release it, it will oscillate. Once it is released, it will contract and reach a minimum amount of tension, and then it will again expand, which will build more tension and the process will continue for some time. This is the idea of the oscillating model of the universe. This theory states that our universe is going through an endless sequence of Big Bangs followed by 'Big Crunches' that reinstate the same cycle once again. This theory backs the idea of the Big Bang and its manifestation, but it also points out a glitch in the Big Bang model, which does not allow an endless streak of universes. The Steady State Theory has been ruled out your current understanding of the universe but the Eternal Inflation Theory and the Oscillating Universe Theories are still accepted among scientists, although neither of these theories are as successful as the Big Bang theory. So, let us have an inside view of the Big Bang Theory.

VI. WHY DID IT BANG?

We have some strong shreds of evidence that the Big Bang explosion happened, but we are not certain about the reason behind its occurrence. We still do not know what caused the instability in the space-time singularity, which resulted in the explosion. Today we have a handful of ideas explaining the origin of Big Bang from the singularity, but we do not know what started this process in the first place. One way of knowing is to go back in time 13.8 billion years ago and observe the singularity although it seems impossible in practice. Even if we could travel back intime, we would never be able to observe the space-times ingularity itself as time and space did not exist back then. Multiverse theory discusses it to some extent but provides almost no practical evidence. The theory says that there is an infinite ocean of foams made of pure energy. These foams create the universes like an inflating soap bubble,

each of which has its different laws. Out of these, some die at the very instant of time they are formed, while the others are stable like our universe. Although we do not know the real cause of the Big Bang, we hope to unveil the mystery in the future.

VII. FOUNDATION OF THE BIG BANG MODEL

The Big Bang theory is the most accepted theory which can explain the birth of our universe. This theory rests on two critical theoretical pillars.

1. General Theory of Relativity (GTR): Albert Einstein formulated this mathematical theory back in 1916 as a new theory of gravity. He put forward a very different picture of our universe. We know that Newton's gravity is valid only when the bodies are either at rest or moving very slowly. These assumptions restrict us to explain other phenomena beyond these limits and necessitate the discovery of the GTR. The central concept of GTR is that gravity must not be considered as a gravitational field, rather it is the distortion of space-time itself. Physicist John Wheeler famously said, 'matter tells space how to curve, and space tells matter how to move.' GTR successfully explained many phenomena, which were unexplained previously such as the bending of light around the planets, and the orbit of various planets. Albert Einstein stated that the matter (m) can be converted into pure energy (E) and vice versa through his most famous equation $E = mc^2$, where c is the speed of light in vacuum. After the Big Bang explosion, an enormous amount of pure energy was released. According to Einstein's equation, this energy later turned into matter as the universe cooled down. This connection between the Relativity and the Big Bang backs the Big Bang model of our universe.

2. The Cosmological Principle: After the introduction of GTR, the application of the new gravitational dynamics to our universe was a strenuous task. The distribution of matter in the universe seems to be homogeneous because it seems to appear the same in every possible direction. This is called the "Cosmological Principle". This assumption is tested as the distribution of galaxies, which are observed on larger scales. In addition, the cosmic microwave background radiation, (which gave the footprints of the Big Bang) showed that the temperature was highly uniform throughout the entire universe. This entails the uniform distribution of gases after the Big Bang explosion.

VIII. EVIDENCE OF THE BIG BANG MODEL

A. Hubble's Law: The red shifts

In 1929, astronomer Edwin P Hubble published the results of a series of observations, which are made with a 100-inch diameter reflecting telescope located at Mount Wilson, near Los Angeles. This work continued the earlier contributions of Vesto Melvin Slipher, who had shown that the light from several galaxies was shifted towards the red end of the visible light spectrum in 1914. When an object moves away from us, its wavelength appears longer, and the light is shifted towards the red end of the spectrum. We call this phenomenon as "redshift". Hubble examined the light from more distanced galaxies and also estimated the distances to both Slipher's and his galaxies. As a result, he was able to demonstrate that the 'redshift increases with distance'. This can be expressed through a roughly linear relation, known as "Hubble's law". Its discovery paved the way of modern observational cosmology. Hubble's law strongly supports the Big Bang model, the universe has gone through a rapid expansion after the explosion.

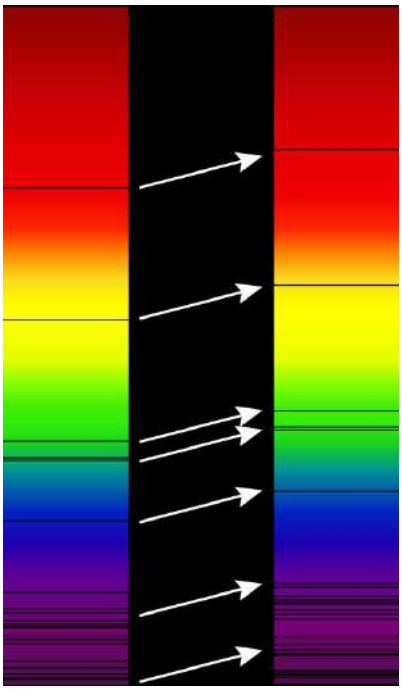

FIG. 2: The black straight lines are the absorption lines in the visible spectrum of a super-cluster distant galaxies (right), as compared to the absorption lines in visible spectrum of the Sun (left). Arrows indicate red shift. Wave length increases up (frequency decreases), towards the red end and beyond.

We know that the Stars and galaxies emit a wide range of wavelengths in the electromagnetic spectrum. In the central cores of stars, the atoms interact with one another to such an extent that the normal line spectra stem from the isolated atoms are merged into a continuous band. This light needs to pass through the outer layers of star to reach us. But the outer layers are less dense (hence the atoms act individually as in a gas) and much colder than the core. So, the atoms will be tending to absorb light rather than emitting it. Hence, the continuous spectrum of light produced in the core of stars is modified by dark lines cut into it where atoms in the outer layers have absorbed some wavelengths. By looking at the patterns of the wavelengths that are absorbed, astronomers can conclude which atoms are present in the outer layers of the star. These dark lines are called 'Fraunhofer lines', which was named after a German optician Joseph von Fraunhofer, who discovered them in the sun's spectrum. This allows us to estimate the extent to which an electromagnetic spectrum has been shifted. Ordinarily, if a spectrum is shifted towards the red wavelengths, then the longest wave lengths become longer, that is they become infrared and hence invisible, but at the other end, the ultraviolet wavelengths become violet and hence visible. The visible part of the spectrum is therefore unchanged. The Fraunhofer lines are produced in unique wavelength patterns characteristic of each atom. If they are shifted, the astronomer can see by how much they have been moved along the spectrum. Without the Fraunhofer lines, we would not be able to tell that a continuous spectrum had been shifted. Let us consider that λ is the wavelength of a line within the spectrum and λ' is the measured the wavelength of that line in the light from a galaxy, then we define the "redshift" of the galaxy as, $z = (\lambda - \lambda')/\lambda$. Hubble's observations suggest that the size of the redshift, z, is proportional to the distance to the galaxy, d: $z = H' d$. In this equation, H' is related to a very important constant in modern cosmology, known as the "Hubble constant" H; $H' = H/c$ or $H = H' c$. Accurate measurement of the Hubble constant would help us to estimate the current age of the universe as well as its ultimate fate. However, H is notoriously difficult to measure (basically, the galaxies are a very long way away, so rather indirect means have to be used to estimate the distances involved). However, techniques are constantly improving and the data are certainly far more convincing now than it was when Hubble first published his famous results. Why should the redshift of distant galaxies be proportional to their distance from our galaxy? The Big Bang theory provides a pleasing answer to this question

as well as describes the physical mechanism for the redshift.

Despite the uncertainties, we can be sure that, some 14,000 million years ago, the universe was much smaller and much more congested than it is now. This inference has led to the current Big Bang Theory for the origin of the universe, accepted by most physicists. According to this theory, at some instant in the past, all the energy in the universe was condensed at a point, a point with no volume, which the scientists refer to as a 'singularity'. It implies that at the instant the universe did not come into existence, space nor time existed! Starting from this singularity, the universe expanded and the space-time is created.

B. Abundance of Lighter Elements:

Much of our understanding of the Big Bang came together in the year 1948 through a rapid series of research papers by George Gamow, his student Ralph Alpher, and Gamow's colleague Robert Herman. They were first able to sketch out a consistent description of the first few minutes after the Big Bang. At the very early stage, the universe was expanding extremely fast. The infant universe was extremely hot and dense. Gamow originally thought that all types of chemical elements had been created in the Big Bang, starting with the simplest element hydrogen. But Alpher estimated that the universe will be expanding too fast and cooling too quickly if it is formed with higher chemical elements. He anticipated that, after the universe cooled, the composition would be about three-quarters hydrogen and one-quarter helium, which is the next heaviest chemical element. Only a little amount of the next two elements, lithium, and beryllium, would be created as well. Alpher's calculations were supported by observations. The predicted abundances are the same as the observed amount of gas that composes much of the galaxies and that constitutes the bulk of most stars. For example, the Sun is about three-quarters hydrogen and one-quarter helium by weight. This agreement between theory and observation constitutes the second pillar of evidence of the Big Bang.

C. Cosmic Microwave Background Radiation (CMBR)

Cosmic microwave background radiation is the electromagnetic the radiation left over from the early stages of our universe. This radiation gives us the correct picture of the very early universe and informs us how the radiations were diffused through the entire universe after the Big Bang. CMBR is one of the most fundamental pillars on which the Big Bang model is based. CMBR is the mapping of thermal electromagnetic radiations or the after glow radiations

scattered over the entire universe after explosion happened. In general, the study of CMBR is the study of residual radiations left over just after the Big Bang when the universe was around 380,000 years old and the seare still circulating in the universe. It is one of the most remarkable discoveries we have seen in the modern era to enhance our understanding of the universe.

We know that rapidly expanding gas cools down, likewise, the continuous expansion of our universe resulted in a lower temperature. The temperature became low enough to form atoms instead of breaking them apart, which happens at high temperatures. So, this cooling led to the transition from a foggy universe to a transparent one. Thus, CMBR gave a perfect picture of our universe when the temperature dropped enough to facilitate electrons and protons to form hydrogen atoms. When these elements combined in the form of hydrogen atoms, photons, and other radiations were emitted, which we refer them as CMBR. This era, when the universe took it searly shape, is popularly known as the Recombination Era. The CMBR radiations are the earliest radiations that we could detect with the help of detectors. CMBR radiations can be detected every where no matter which direction we point our telescope. We cannot measure or observe these background radiations with the naked eye due to the limited vision of our eyes. The discovery of CMBR is considered the landmark test of the Big Bang theory. Although, Alpher and Herman predicted CMBR in the late 1940s, Penziasand Wilson took another 15 years to prove the existence of the seradiations. They stated that if the Big Bang theory is correct, there must be some background radiation

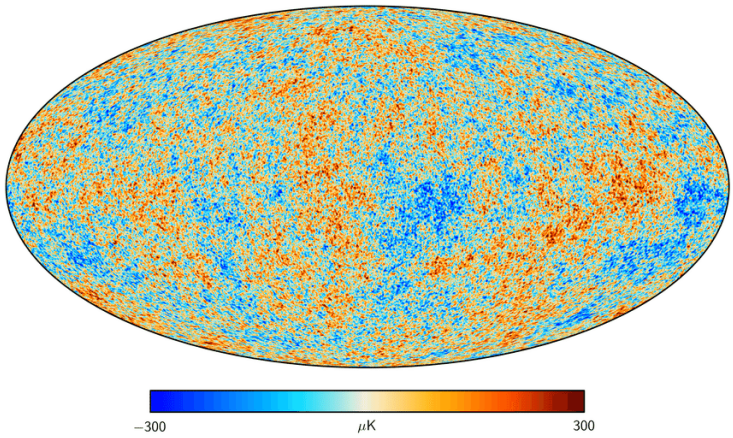

FIG.3: The cosmic microwave background radiation is a snapshot of the old estlight in the universe, from when the cosmos was just 380,000 years old. The colors of the map represent small temperature fluctuations that ultimately resulted in the galaxies we see today.

Left over from there combination period that we are unable to find. When the CMBR radiations are figured out, it boosted the picture of the Big Bang theory. At that moment, the early rival to the Big Bang theory, i.e., the Steady State Theory, lost its credibility as this could not explain the CMBR. In 1971, both Penzias and Wilson were awarded the Nobel Prize for there discovery of this ancient light. In summary, CMBR is there sidual heat of creation itself that dates back to about 13.8 billion years. We estimated the current average temperature of the universe to be about 2.725K, which is the temperature of the CMBR. However, in the beginning, this temperature was very high, trillions of degrees. On Earth, we hunt for fossils to explain what the Earth might have looked like millions of years ago. But in space, we search for these radiations with the same objective. The CMBR helps us to perceive the shape, size, and geometry of our universe. The different spots on the map correspond to the different photon energies released during the recombination period. The blue spots represent the high-energy are as while the low-energy areas are in red. This is similar to a burning candle, the hottest region is the blue region whereas, the coldest and least energetic part is red. It has been found that the red spots (cold regions) are denser then the blue spots. As the photons take a long time to free from the Sun's gravitational shackles, likewise, more gas cloud sand space dust was pulverized, which would not permit the radiation to reach us, while the blue spots are the less dense regions. For the cold esttemperature, the density is maximum and vice-versa. When the CMBR graph was plotted for the Milky Way galaxies, it gave a long red line along its center.

These three lines of evidence, the expansion of our universe, the abundance of hydrogen and helium and the light coming to us from the infant universe, the cosmic microwave background radiation, are three solid pillars of evidence for the Big Bang.

IX. FAILURE OF THE BIG BANG THEORY

No theory is perfect. Almost every theory which describes the beginning of our universe has its limitations. The Big Bang model of the universe also has some significant flaws, such as:

1. The Big bang theory states that the universe came from a space-time singularity, which is a point of infinite mass and density. This singularity and infinite energy densities are the foundations of this theory, which are mathematical concepts only.

2. The singularity that exploded at the time of the Big Bang, was once completely stable. Big Bang theory could not describe

what happened within singularity which in turn made it unstable. Physicists are still trying to understand the possible reasons behind it. It could not answer why doesn't the singularity of Black Holes also become unstable and explode in a similar manner as the Big Bang.

3. The universe is going through an expansion, whose rate was very rapid soon after the Big Bang. The Big Bang model could not explain what changed the rate of expansion of our universe.

4. The Big Bang model fails to explain what persists before the singularity although it gives a good picture of what happened after the Bang. It could not explain the structure of the universe before the explosion.

X. WHAT HAPPENED IN THE BIG BANG?

Scientists believe that all the energy of the universe jammed into a very minuscule point in space. This extremely dense point shattered with unimaginable force, creating matter and propelling it outward to form the billions of galaxies in our vast universe. Scientists termed this gigantic explosion as the Big Bang.

We did not experience any explosion like Big Bang on earth. In a hydrogen bomb explosion, the temperature in the central part can go upto approximately 100 million degrees Celsius. The debris in this explosion can move through the air at nearly 300 meters per second. But in the big Bang, cosmologists believed that energy could be traveled in all directions at the speed of light in a vacuum (300,000,000 meters per second, which is a million times faster than the debris coming out after the explosion of the hydrogen bomb). The temperature of the entire universe was 1000 trillion degrees Celsius just after a tiny fraction of a second after the explosion. This is an enormous temperature. Even we cannot see the cores of any stars as hot as it. Also, when a hydrogen bomb or any man-made bomb explodes, the energy of the explosion and debris expands through the air, but the Big Bang did not expand through the air, because there was not medium at the beginning of time. This also makes Big Bang a unique event. Rather, scientists believe the Big Bang created and stretched space itself and it resulted in the expansion of our universe.

As the universe rapidly expanded, the energy emitted after the Big Bang became more and more "diluted" in space. It helped the universe to cool down from a hot state. This is like the expansion of gas, once confined in a container, spreads into the air, and its temperature drops. This rapid cooling permitted matter to form the universe. Around one ten-thousandth of a

second after the Bang, protons and neutrons are created. Then with in a few minutes, these particles cemented together to form atomic nuclei of hydrogen and helium, in general. Then hundreds of thousands of years have been taken by the electrons to stick to the nuclei to make complete atoms. About a billion years after the Bang, gravity caused these atoms to congregate in huge clouds of gas, forming collections of stars, which are known as galaxies. Over billions of years, the stars "cook" hydrogen and helium atoms in their hot cores to make heavier elements like carbon and oxygen. Large stars explode over time, blasting these elements into space. This matter then condenses into the stars, planets, and satellites that make up solar systems like our own. In the next issues, we shall discuss the evolution of our universe in more detail.

XI. CONCLUSION

In this short article, we have tried to discuss the origin of our universe from the beginning of time with out any complicated mathematical equations. Different theories were proposed for that purpose such as the Big Bang Theory, the Steady State Theory, the Eternal Inflation Theory, the Oscillating Universe Theory, and many more. Most of these theories are not accepted by scientists as they contradict experimental evidence except for the famous Big Bang Theory. According to this theory, the universe has expanded after a gigantic explosion from a tiny point in space, where all the energy was condensed. Just after the explosion, time has been started and the universe evolved to its current state. This theory supports modern experimental evidence such as the red shifts, abundance of lighter nuclei, and cosmic micro wave background radiation. We have discussed these in detail. We also have discussed some limitations of this theory, but what we have not discussed is the evolution of our universe after the Bang. This will be discussed in the next issues.

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श्रद्धावान लभते ज्ञानम्

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A short review on the anti oxidant and anti cancer properties of *Tinospora cordifolia*

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Abstract:

The diverse array of bioactive molecules harboring in the plant has an important contribution in developing new therapeutic drugs. Nowadays, ethnobotanical studies of medicinal plants are gaining more importance in clinical research because of their better pharmacological attributes with no side effects as compared to allopathic drugs. *Tinospora cordifolia* commonly called Guduchi or Giloy, is one of the important medicinal plants which is known for its application in the treatment of various diseases acting as one of the primary sources of new pharmaceuticals and health care products. This review aimed to provide an updated literature study on the pharmacological properties of giloy plant, focusing on its anti-oxidant and anti-cancer potential.

Keywords: Bioactive molecule, Medicinal Plants, Giloy, Anti-oxidant.

Introduction

Among ancient civilizations, India has been considered the richest source of medicinal plants which are being explored for different therapeutic applications. Medicinal plants are safer to use with no or minimal side effects as compared to allopathic drugs. According to WHO (World Health Organization) reports, around 80% of people worldwide depend on herbal medicine for their health benefits (1). Among the diverse group of medicinal plants, *Tinospora cordifolia is* widely used in the native system of ethnomedicine. Commonly known as giloy, this plant is a deciduous climbing, large shrub growing at

higher altitudes with heart-shaped leaves that belongs to the family Menispermaceae (2, 3) as depicted in figure 1.This plant is widely distributed in India, Burma, China, Myanmar, and Sri Lanka (4). It has multiple synonyms in different languages such as in Arabic (Gilo), Assamese (*Amarlata*), English (*Tinospora*), Chinese (*K'uanchuHsing*), French (*Culancha*), Hindi (*Giloe, Gulbel, Gurcha*), Gujerati (*Galo, Gulo*), Nepali (*Garjo*), Punjabi and Kashmiri (*Gilo*), Sanskrit (*Amrita, Guduchi*) and Sikkim (*Gurjo*) (5).

Figure 1: A photo of Tinospora corifolia plant taken at Baiguney, West Sikkim. It is deciduous climbing, large shrub with heart-shaped leaves.

Recently, this plant is gaining considerable interest in research because of its medicinal properties including anti-periodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, hepatoprotective, immunomodulatory, and anti-neoplasticism activities (6). Various phytoconstituents of *Tinospora cordifolia* play an important role in its therapeutic application which includes diterpenoid lactones, alkaloids, steroids, glycosides, phenolics, aliphatic compounds, and polysaccharides present in different parts of the plant like leaves, stems, roots, etc (7). Traditionally, this plant is used in different forms such as whole plant, powdered form of root and stem bark, juice form of root, juice or paste form of leaves and extract form of roots and stem which helps to cure various health issues such as fever, jaundice, asthma, skin diseases, eyes disorders, etc (8). Compared to leaves, the stem is an extensively used and beneficial part of the plant (9). It helps in improving digestion and getting relief from the problems like abdominal pain, excessive thirst, vomiting, and even liver disorders like hepatitis. Due to the presence of antioxidants, this plant is very effective in

reducing dark spots, pimples, fine lines and wrinkles, and skin diseases (10). Some studies have also been successful in demonstrating the anti-cancer property of giloy implicating its use in cancer treatment with fewer or no adverse side effects unlike chemotherapy (11, 12). Apart from medicinal purposes, *Tinospora cordifolia* is also taken as a nutritional supplement for important minerals i.e. copper, manganese, zinc, iron, phosphorus, and calcium (13). However, consumption of this plant can be risky to an individual with diabetes who is taking medicines to lower blood glucose (7).

Tinospora cordifolia has immense potential to be used in various fields. In this review we have discussed two important properties viz antioxidant property and anticancer activity as summarized in figure 2

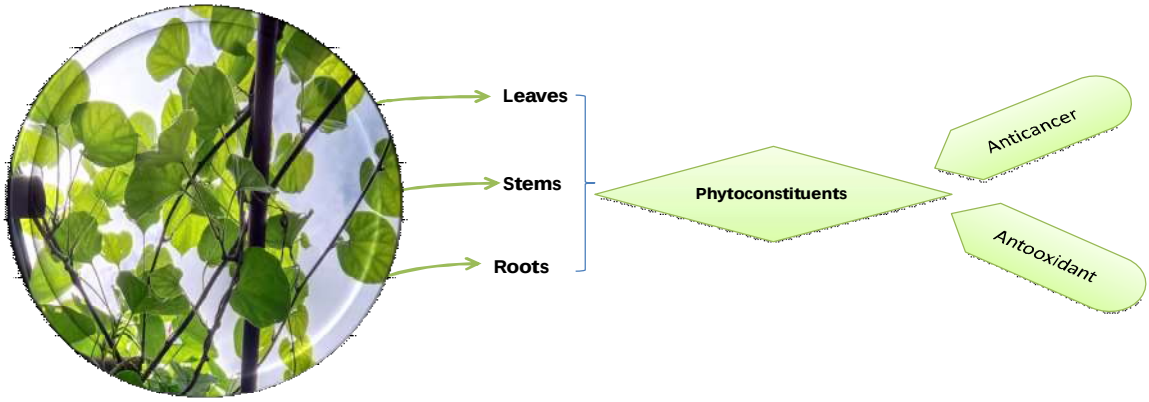

Figure 2: Different parts of *Tinospora cordifolia* such as leaves, stem, and roots are used due to the presence of various phytoconstituents such as terpenoid, alkaloids, steroids glycosides, phenolics. Phytoconstituents play a role in controlling free radical-mediated oxidative damage (antioxidant property)and in inducing lipid peroxidation and apoptosis-mediated cell death in tumor cells (anticancer activity).

Anti-oxidant property of *Tinospora cordifolia*

Recent studies suggest that reactive oxygen species (ROS) and reactive nitrogen species (RNS) are highly involved in lipid peroxidation, oxidative DNA damage, and protein oxidation causing injuries to cells and tissues which ultimately lead to several degenerative diseases like cancer, asthma, arthritis, and cardiovascular problems (14, 15). Though the naturally occurring bodily antioxidant systems (enzymes and antioxidant nutrients) can control the free radical-mediated oxidative damage, its long time exposure may lead to irreversible oxidative damage. Thus, there is an immense need for antioxidants mainly from dietary sources for therapeutic applications as they are safer and more effective than synthetic antioxidants (16, 17). Several studies have reported the antioxidant potential of *Tinospora cordifolia* considering it a potential alternative to

synthetic preservatives in the food system (18, 19). Tyagi et al. (2020) reported the presence of a good amount of crude fiber, minerals, and bioactive phyto-ingredients in the root, stem, and leaf of giloy exhibiting antioxidant and antimicrobial effects (20). They reported high antioxidant potential in GSP (giloy stem powder) extract in terms of higher total phenolic content (3.883 gallic acid equivalent/g of dw), total flavonoid content (67.532 rutin trihydrate equivalent/g of dw), and 2′-diphenyl-1-picrylhydrazyl (DPPH) (127.33 quercetin dehydrate equivalent/g of dw). Nile and Khobragade (2009) have also reported the high antioxidant potential of giloy stem (21). In a study, Ilaiyaraja and Khanum (2011) compared the anti oxidant potential of the solvent extracts of leaf and stem of giloy plant where the stem extract was found to show higher antioxidant potential than the leaf concerning all the radical scavenging activities (22). Similarly, antioxidant property was also observed in giloy squash and juice which could add variability to its commercial exploitation (23).

Anti cancer property of *Tinospora cordifolia*

The anti-cancer activity of giloy was first reported in the year 1998 when different solvent extracts of giloy were used to check its cytotoxic effects on human cervical cancer cells (HeLa) (24). It was observed that giloy extracts could kill HeLa cells in a concentration-dependent manner where dichloromethane extract was found to be most effective at a lower concentration as compared to other extracts of giloy. As DNA damage plays a major role in cell death, giloy extracts were efficient in producing DNA damage in the form of micronuclei. Treatment with these extracts produced multiple micronuclei in a cell indicating their ability to produce complex multiple sites of DNA damage thus limiting the chances of tumor cell survival. The dichloromethane extract of giloy can reduce glutathione concentration accompanied by an increase in lipid peroxidation and lactate dehydrogenase activity, thus leading to antineoplastic mechanism (25). A similar observation was made in mice transplanted with Ehrlich ascites carcinoma where the cytotoxic effect of dichloromethane extract of giloy occurred in a dose-dependent manner (26). The ethanol extract of giloy has also been reported to reduce the proliferation of glioblastomas (C6 glioma cells) with an increased expression of senescence marker, mortalin and its translocation from perinuclear to pancytoplasmic spaces (27). The active phytochemical present in the giloy extract which participates in the anti-cancer activity was found to be berberine chloride (BCL), an isoquinoline alkaloid rendering its

cytoxicity in HeLa cells (28). Another active constituent was found to be Palmatine, an alkaloid that was able to reduce tumor incidence in the 7,12–dimethylbenz(a)anthracene (DMBA) induced skin carcinogenesis in mice (29). Patil et al. (2021) could successfully demonstrate the inhibitory activity of giloy against oral cancer cell line AW13516 by inducing apoptosis-mediated cell death (30).

CONCLUSION:

Giloy has been used traditionally by the tribal and ayurvedic regimes as a medicinal plant due to its multipotent role in curing many diseases. The phytoconstituents of this plant contribute to its advantageousness in the management of several diseases and discomfort including pyrexia, dyspepsia, syphilis, gonorrhea, diseases of the urinary tract, gout, viral hepatitis, anemia, general weakness, urinary tract infections, dermatological diseases, loss of appetite, asthma, etc. Due to its bitter taste, direct consumption of giloy is generally unacceptable. Thus it can be incorporated into various value-added food products like RTS, squash, bakery, beverages, etc. It is also used extensively in other nonfood products like wound healing creams and health supplements. Through this review we were able to explain the therapeutic applications and health benefits of giloy emphasizing on its anti-oxidant and anti-cancer potential, thereby providing a way for the exploration of cancer preventive strategy and also in the management of other malignant disorders. However, more studies are to be done for explaining the mechanistic approach of the phytochemicals of giloy towards different diseases.

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श्रद्धावान लभते ज्ञानम्

Article No. DGC-NF-24/08

Diversity of Birds in and around Birch Hill Area, Darjeeling

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Abstract:

Among many animal groups, birds are reliable and widely used as indicators for conservation planning and monitoring ecological conditions. A survey was conducted at Birch hill in Darjeeling, West Bengal for the last two years. A total of fifty-nine species belonging to twenty-eight families and eight orders were encountered during the study period. That small natural habitat faces numerous challenges including habitat loss and fragmentation, unregulated tourism and global climate change. It is suggested to conserve this tiny enclave through strengthening protection and reduction in forest encroachment and habitat destruction, conservation awareness program, and comprehensive bird surveys with long-term monitoring to protect its rich biodiversity.

Introduction

Birds have been widely considered an important tool in biodiversity conservation planning and monitoring (Kremen 1992; Chettri *et al.* 2001; Bregman *et al.* 2014) and for identifying conservation actions. Birds and their diversity provide strong bio-indication signals (Vielliard 2000; Bhatt and Joshi 2011; Urfi 2011; Bregman *et al.* 2014), and stand as surrogates for the health of the ecosystem and status of biodiversity overall (Chettri 2010; Pakkala *et al.* 2014; Pierson *et al.* 2015). Anthropogenic threats have fomented large-scale habitat destruction, fragmentation, and degradation as well as climate change, necessitating an assessment of the impacts of such change on birds (Wiens 1995; Chettri *et al.* 2001; McLaughlin 2011; Hazra *et al.* 2012; Bregman *et al.* 2014). Understanding the diversity of bird communities in different habitats is essential to understand the community structure and niche relationships, as well as to delineating the importance of regional or local landscapes for avian conservation (Kattan and Franco

2004; Chettri 2010; Singh *et al.* 2013; Khan *et al.* 2016).

A study on avifaunal diversity was conducted in the Birch Hill area, located in the district of Darjeeling, West Bengal, India. Although present within the overcrowded and congested town of Darjeeling, dense forests of both deciduous and evergreen trees in and around Birch Hill still persist. Apart from deciduous trees such as birch, oak, and elm, many wet alpine trees are also grown here. Rhododendron too is a common site in these hills. Moreover, these hilly slopes are home to many medicinal plants such as *Aconitum ferox,Adhatodavasica, Artemisia vulgaris, Berginiaciliata, Hedychium spicatum, Sapindusmukorossi, Taxus baccata, Zingiber cassumunar*etc. Sadly, deforestation due to rapid urbanization is now eating away the green cover. Birch Hill is also home to varied types of birds such as woodpeckers, orioles, sunbirds, flycatchers, finches, etc. During the migrating season, one can also witness a varied number of migratory birds making their way to and from the plains. Other than birds, one can also find several species of small mammals such as martens, civets, mongoose, and badgers in this part of the hills. The present study was conducted from January to May in 2021 and 202, and during that time it has been found that this tiny natural habitat is a potential avian abode for both residential and altitudinal migratory species.

Materials and Methods:

Study area:

Birch hill (27.0566°N & 88.258°E) is located in the district of Darjeeling, West Bengal, India at an elevation of 1,975 meters (6,480 feet). It is adjacent to Padmaja Naidu Himalayan Zoological Park and The Raj Bhavan of Darjeeling (Fig. 1).

Fig 1: Birch Hill (the study area is demarcated by yellow line)

Bird census:

Bird counts were performed on foot and by point transect method. During each census, counts were begun at 8 h IST and continued until 17h IST covering the entire area of Birch Hill. Binoculars, spotting scopes and camera were used for counting and identifying birds.

Data Analysis:

The census data for the period of study were processed for avifaunal diversity using the program PAST(PAleontologicalSTatistics) (version 2.10) (Hammer *et al*, 2001; Sinha *et al*, 2011) and the taxonomic distributions were analyzed by Microsoft Excel.

Results and Discussion:

A total of 2063 individuals of birds belonging to fifty-nine species, twenty-eight families, and eight orders were encountered during the study period. Among these species, 48 are resident to the eastern Himalayas, while the rest are local or altitudinal migrants. The checklists of the birds are given below:

Order Galliformes

Family Phasianidae

Sl. No.	**Name**	**Status**	**No.**
1.	Kalij Pheasant (*Lophuraleucomelanos*)	Resident	4

Order Falconiformes

Family Falconidae

Sl. No.	**Name**	**Status**	**No.**
2.	Common Kestrel (*Falco tinnuunclus)*	Resident	2

Order Accipitriformes

Family Accipitridae

Sl. No.	**Name**	**Status**	**No.**
3.	Black Eagle*(Ictinaetusmalayensis)*	Resident	2
4.	Himalayan Buzzard(*Buteo refectus*)	Resident	2

Order Columbiformes

Family Columbidae

Sl. No.	Name	Status	No.
5.	Oriental Turtle Dove (*Streptopellaorientalis*)	Resident & migrant	46

Order Cuculiformes

Family Cuculidae

Sl. No.	Name	Status	No.
6.	Common Hawk Cuckoo (*Hierococcyxvarius*)	Widespread resident & partial migrant	18

Order Strigiformes

Family Strigidae

Sl. No.	Name	Status	No.
7.	Asian Barred Owlet (*Glacidiumcuculoides*)	Resident	4

Order Piciformes

Family Ramphastidae

Sl. No.	Name	Status	No.
8.	Great Barbet(*Megalaima virens*)	Resident	28

Family Picidae

Sl. No.	Name	Status	No.
9.	Darjeeling Woodpecker(*Dendrocoposdarjellensis*)	Resident	2
10.	Lesser Yellownape(*Picuschlorolophus*)	Resident	3

Order Passeriformes

Family Oriolidae

Sl. No.	Name	Status	No.
11.	Maroon Oriole(*Oriolustraillii*)	Resident	11

Family Rhipiduridae

Sl. No.	Name	Status	No.
12.	White-throated Fantail(*Rhipidura albicollis*)	Resident	12

Family Corvidae

Sl. No.	Name	Status	No.
13.	Yellow-billed Blue Magpie(*Urocissaflavirostris*)	Resident	8
14.	Grey Treepie(Dendrocittaformosae)	Resident	14
15.	Large-billed crow (*Corvus macrorhynchos*)	Resident	52

Family Paridae

Sl. No.	Name	Status	No.
16.	Green-backed Tit(*Parus monticolus*)	Resident	86

Family Aegithalidae

Sl. No.	Name	Status	No.
17.	Black-throated Tit(*Aegithalos concinnus*)	Resident	142

Family Hirundinidae

Sl. No.	Name	Status	No.
18.	Barn Swallow(*Hirundo rustica*)	Widespread Resident	18

Family Pycnonotidae

Sl. No.	Name	Status	No.
19.	Striated Bulbul(*Pycnonotus striatus*)	Resident	27
20.	Himalayan Bulbul(*Pycnonotusleucogenys*)	Resident	52
21.	Mountain Bulbul(*Ixosmcclellandii*)	Resident	23
22.	Black Bulbul(*Hypsipetes leucocephalus*)	Resident	38

Family Sylviidae

Sl. No.	Name	Status	No.
23.	Smoky Warbler(*Phylloscopusfuligiventer*)	Local migrant	22
24.	Sulphur-bellied Warbler(*Phylloscopusgriseolus*)	Local migrant	18
25.	Blyth's Leaf Warbler(*Phylloscopusreguloides*)	Local migrant	12
26.	Grey-hooded Warbler(*Phylloscopusxanthoschistos*)	Resident	16
27.	Chestnut-crowned Warbler(*Seicercuscastaniceps*)	Resident	144

Family Timaliidae

Sl. No.	Name	Status	No.
28.	Pygmy Wren Babbler(*Pnoepygapusilla*)	Resident	2
29.	Rusty-cheeked Scimitar Babbler(*Pomatorhinuserythrogenys*)	Resident	1
30.	Coral-billed Scimitar Babbler(*Pomatorhinusferruginosus*)	Resident	1
31.	Black-faced Laughingthrush(*Garrulaxaffinis*)	Resident	28
32.	Chestnut-crowned Laughingthrush(*Garrulax erythrocephalus*)	Resident	136
33.	Red-billed Leiothrix (*Leiothrixlutea*)	Resident	214
34.	Blue-winged Siva(*Siva cyanouroptera*)	Resident	16
35.	Bar-throated Siva (*Siva strigula*)	Resident	28
36.	Red-tailed Minla (*Minlaignotincta*)	Resident	72
37.	Rufous-winged Fulvetta(*Pseudominlacastaneceps*)	Resident	120
38.	White-browed Fulvetta(*Fulvettavinipectus*)	Resident	13
39.	Rufous Sibia(*Malaciuscapistratus*)	Resident	240
40.	Whiskered Yuhina(*Yuhinaflavicollis*)	Resident	74

Family Sittidae

Sl. No.	Name	Status	No.
41.	White-tailed Nuthatch(*Sittahimalayensis*)	Resident	12

Family Certhiidae

Sl. No.	Name	Status	No.
42.	Hodgson's Treecreper(*Certhiahodgsoni*)	Resident	8

Family Sturnidae

Sl. No.	Name	Status	No.
43.	Common Hill Myna(*Gracularegigiosa*)	Resident	32

Family Turdidae

Sl. No.	Name	Status	No.
44.	Blue Whistling Thrush(*Myophonuscaerulus*)	Resident	28
45.	Plain-backed Thrush(*Zootheramollissima*)	Resident	6
46.	Scaly Thrush(*Zootheradauma*)	Local migrant	2
47.	White-collared Blackbird(*Turdusalbocinctus*)	Resident	2

Family Muscicapidae

Sl. No.	Name	Status	No.
48.	Himalayan Bluetail(*Tarsigerrufilatus*)	Resident	5
49.	Large Niltava(*Niltava grandis*)	Resident	4
50.	Grey Bushchat(*Saxicola ferreus*)	Resident	2
51.	Verditer Flycatcher(*Eumyiasthalassinus*)	Local migrant	84
52.	Pale Blue Flycatcher(*Cyornisunicolor*)	Local migrant	12
53.	Grey-headed Canary Flycatcher(*Culicicapaceylonensis*)	Resident	34

Family Chloropseidae

Sl. No.	Name	Status	No.
54.	Golden-fronted Leafbird(*Chloropsiscochinchinensis*)	Resident	2

Family Dicaeidae

Sl. No.	Name	Status	No.
55.	Fire-breasted Flowerpecker(*Dicaeumignipectus*)	Resident	4

Family Nectariniidae

Sl. No.	Name	Status	No.
56.	Green-tailed Sunbird(*Aethopyga nipalensis*)	Resident	14

Family Passeridae

Sl. No.	Name	Status	No.
57.	House Sparrow(*Passerdomesticus*)	Widespread resident	21

Family Prunellidae

Sl. No.	Name	Status	No.
58.	Maroon-backed Accentor(*Prunellaimaculata*)	Local migrant	8

Family Fringillidae

Sl. No.	Name	Status	No.
59.	Dark-breasted Rosefinch(*Carpodacusnipalensis*)	Resident	32

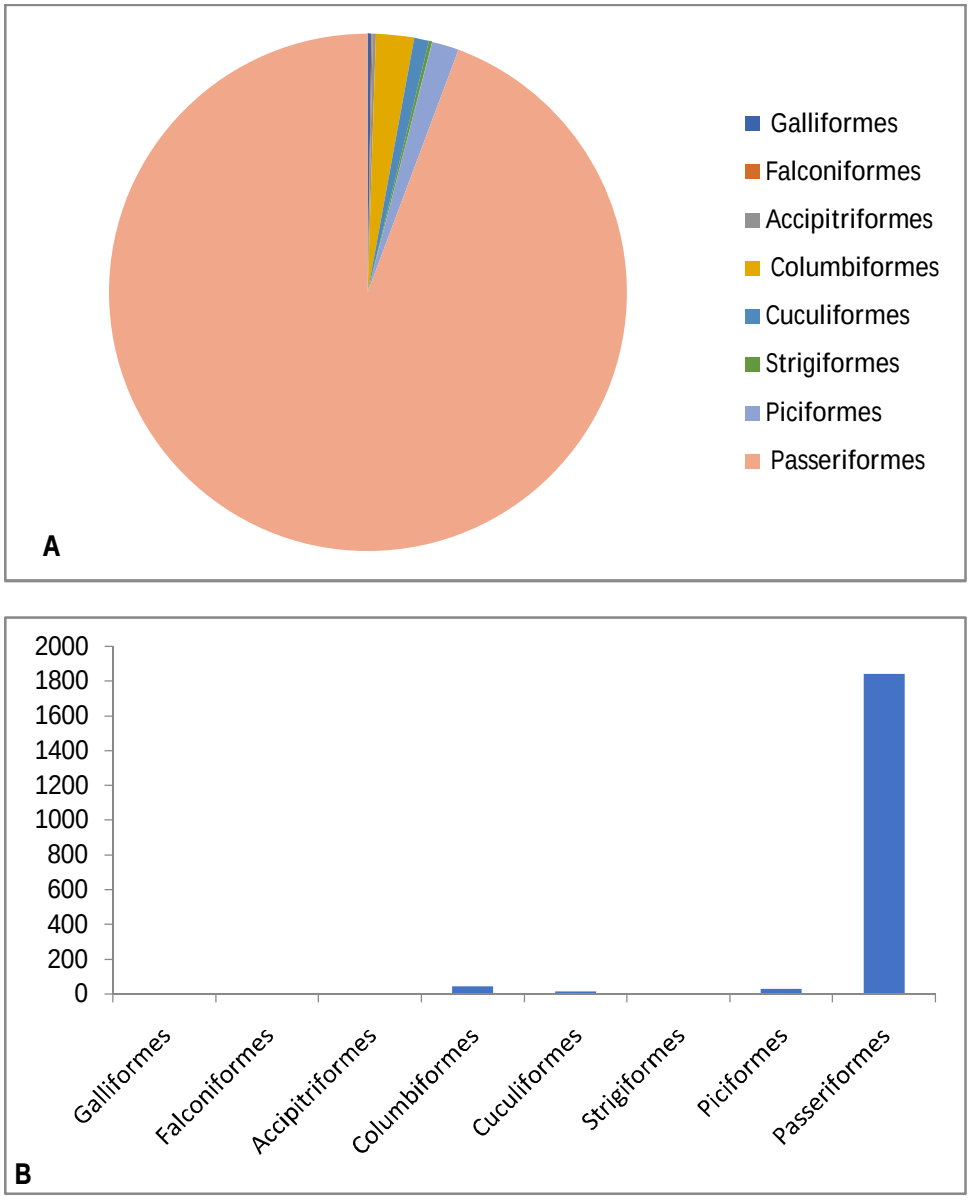

Fig 2(A & B): Taxonomic distribution of the birds in the Birch Hill area by order.

The census data of the study period were processed for avifaunal diversity using the program PAST (version 2.10) and the interpretations of outputs are given below:

Components of diversity	PAST Output	Data interpretation
Dominance	0.05	Ranges from 0 (all taxa are equally present) to 1 (one taxon dominates the community completely). So, the value indicates no taxon is dominant in the community.
Simpson index	0.95	Measures 'evenness' of the community from 0 to 1. The value of Simpson index in the study area is 0.95. That indicates the

Components of diversity	PAST Output	Data interpretation
		evenness is high.
Shannon index	3.34	A diversity index taking into account the number of individuals as well as number of taxa. Varies from 0 for communities with only a single taxon to high values for communities with many taxa, each with few individuals. In real world ecological data, the Shannon index usually ranges between 1.5-3.5, it hardly reaches 4. The value of Shannon index in our study indicates rich Avifaunal diversity in this area.
Brillouin's index	3.28	Both the Brillouin and Shannon indices tend to give similar comparative measures. The value obtained rarely exceeds 4.5. Although Brillouin's index gives weightage to both species richness and evenness, so may have some advantage over the Shannon index and preferable in conservation biology. Hence, the Avifaunal diversity is Birch Hill area is moderately high.
Margalef's richness index	7.6	The value usually ranges above 1.0 and in normal ecological communities, it is above 1.5. And the higher value in the study denotes higher species richness.
Equitability (Pielou's evenness)	0.82	The value usually ranges between 0-1. Hence, in this study the relative abundance is high.

The Eastern Himalaya is a meeting ground for the Indo- Malayan, Palaearctic, and Sino-Japanese biogeographical realms. The area is known for diverse ecological and altitudinal gradients (CEPF 2005, 2007) and provides habitat for a rich diversity of flora and fauna, including birds of the Oriental region (Crosby 1996). The Eastern Himalaya has been identified as a Priority I Endemic Bird Area (Birdlife International 2001), supporting 22 restricted-range bird species of which 19 are endemic to the region (Stattersfield*et al.* 1998; Jathar and Rahmani 2006; Acharya and Vijayan 2010). The region also represents one of the largest concentrations of globally threatened birds in Asia (Acharya and Vijayan 2010).

The study of avifaunal diversity in Birch Hill, adjacent to Darjeeling town, shows the

potential of the area as an important avian abode. The lower value of dominance, a high value of Simpson index, and Pielou's evenness indicate no taxa is dominant in the avian community of this region; at the same time, evenness in this community is also high. High species richness (in Shannon and Margalef's index) and moderately high value of Brillouin's index also shows significant stability of the community. As the natural vegetation and food base are still remaining in this tiny natural enclave, it can harbor a safe denizen for not only common but also rare and threatened bird species. Moreover, the protection of this region can also support the existence of other small wild animals and may also provide natural resources for the local people. Despite the global biological significance, this region, like many other parts of Darjeeling hills, faces numerous challenges including habitat destruction, fragmentation, unsustainable extraction of natural resources, invasive alien vegetations (e.g. *Cryptomeria japonica*),and unregulated tourism activities. So, proper actions must be taken in an urgent basis for conservation and sustainable development in this degrading but still potentially rich biodiversity habitat.

Conclusion

The study indicates rich avifaunal diversity in Birch Hill as well as its potential to support a variety of other wildlife. However, anthropogenic threats are a serious issue and may cause a great toll on the biodiversity of this area. Necessary protection measures and regulation of anthropogenic activities can not only save this natural habitat but also give potential benefits to the locals by providing natural resources.

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श्रद्धावान लभते ज्ञानम्

Article No. DGC-NF-24/09

PAS: The versatile domain

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Abstract:

Hierarchically domains are interim configuration between super secondary and tertiary structure of an inclusive protein. PAS is one such multifunctional omnipresent domain occurring in all kingdoms of life. PAS is the abbreviated form of *Drosophila* period clock protein (PER), vertebrate aryl hydrocarbon receptor nuclear translocator (ARNT) and *Drosophila* single-minded protein (SIM). PAS domain functions as crucial signaling module via monitoring changes in light, redox-potential, oxygen, small ligands and cellular energy level approximately encompassing a region of 100 to 120 amino acids, being typically located in the cytosol. The PAS fold is constituted of a centrally located anti-parallel β-sheet with five analogous strands Aβ, Bβ, Gβ, Hβ and Iβ besides several flanking α-helices designated by Cα, Dα, Eα and Fα with the strands of the β-sheet arranged in the topological order of B-A-I-H-G that is in 2-1-5-4-3 configuration. Most PAS domain binds corresponding cofactors within their core for ensuring precise coordination and specificity in a thermodynamically favourable manner transmitting signals to downstream effector domains in the transduction pathway. PAS genes have been depicted to be highly expressed owing to their translational efficiency possessing a high degree of codon homogeneity. However, it is still an early phase for complete understanding of the multidimensional aspects behind the overall functioning of PAS domains awaiting further elucidation.

Keywords: PER, ARNT, SIM, Protein, Circadian rhythm, Adaptation.

Introduction:

Various proteins contain compact units within the folding pattern of a single chain that appear as if they should have independent stability. These are referred to as domain. In a hierarchical perspective, domain lies between super secondary and

tertiary structure corresponding to the configuration of a complete protein monomer (Lesk, 2005). Living organisms possess diversified domains with specialized roles across varying species. One such multifunctional domain is the PAS/PAC domain.

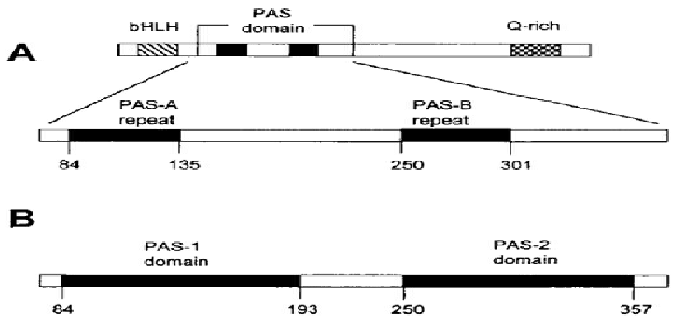
PAS is an acronym derived from the names of the proteins in which imperfect repeat sequences were first recognized through sequence homology namely the *Drosophila* period clock protein (PER), vertebrate aryl hydrocarbon receptor nuclear translocator (ARNT) and *Drosophila* single-minded protein (SIM) (Hoffman *et al*., 1991; Nambu *et al*., 1991; Borgstahl *et al*., 1995) being acknowledged as widespread components of signal transduction proteins in addition to functioning as universal signal sensors and interaction hubs. PAS domain have been attributed to occur in all kingdoms of life (Finn *et al*., 2006) and regulate diversified processes initiating from Nitrogen fixation in *Rhizobia* (David *et al*., 1988); phototropism in plants (Christie *et al.,* 1998); circadian behavior in insects (Nambu *et al.,* 1991) to gating of ion channels in vertebrate species (Morais Cabral *et al.,* 1998). PAS domain is modular, a feature common with other signal transduction systems (Pawson and Nash, 2003); denoting the ability of PAS sensor (input) domain to perceive a wide variety of physical and chemical stimuli followed by response via regulation in the activity of effector (output) domain through catalysis and DNA binding. Summatively PAS domain serve as imperative molecular signaling module that monitor changes in light, redox potential, oxygen, small ligands and overall cellular energy level with small molecules including other proteins being associated through binding of the PAS domain (Liu *et al*., 2015).

Prequel of PAS domain

The initial investigations identified the eukaryotic PAS domain as an expanse of roughly 270 amino acids comprising of two 50 residue conserved sequences defined as PAS-A and PAS-B repeats (Figure 1.1.A) (Crews *et al.,* 1988; Hoffman *et al.,* 1991). Though, recent research exploration advocates PAS domain to encompass a region of approximately 100 to 120 amino acids with some being even longer (Taylor and Zhulin, 1999; Chakraborty *et al*., 2016). The PAS-A and PAS-B repeats essentially correspond to the N-terminal half of the respective PAS domains (Figure 1.1.B) with unlike most other sensor components, PAS domain being located in the cytosol (Taylor and Zhulin, 1999). It is quite archetypal to locate paired PAS domains in eukaryotic transcriptional activators viz. SIM in contrary to microbial proteins containing

single, dual to multiple (upto six) PAS domain units.

Figure 1.1.: Comparison of earlier (A) and current (B) definition of PAS domain illustrated in the *Drosophila* SIM Protein. (A) One PAS domain containing two PAS repeats as described primarily. (B) Two individual PAS domains being identified in the SIM protein i.e. Q-rich and glutamine-rich activation domain.



Lagarias*et al.,* (1995) reported a motif almost similar to a PAS repeat in an algal phytochrome including twenty other proteins from both prokaryotic and eukaryotic origin besides suggesting that this 40 amino acid motif represents a common fold that might be similar to the N-terminus of the Photoactive yellow protein (PYP) of *Halorhodospira halophila* in respect of which the crystallographic structure had been determined for illustrating the three dimensional (3-D) structure of PAS domain (Borgstahl *et al.,* 1995). Subsequently, it was recognized that the identified motif happened to be the most highly conserved block (S1-box) of a larger PAS domain entity. The motif being extended in the carboxyl direction by defining the S2-box (PAC motif) aided in understanding the complete structure of the PAS domains (including S1 and S2 boxes or PAS-PAC motifs). Eventually PAS-PAC motifs were identified in more than two hundred proteins across varied organisms throughout the phylogenetic tree (Ponting and Aravind, 1997; Zhulin*et al.,* 1997). In due course the entire 125 residue of the Photoactive yellow protein (PYP) (Pellequer*et al.,* 1998); the N-terminal domain of the eukaryotic Potassium channel HERG protein (Morais Cabral *et al.,* 1998) and the heme domain of the FixL protein (Gong *et al.,* 1998) were projected as structural prototypes of the three dimensional (3-D) fold of the PAS domain super-family. PAS domain has been reported in *Arabidopsis* too with stated occurrence in ZTL and NPH1 genes being almost similar to the PAS domain been characterized in *Neurospora* circadian rhythm associated protein WC-1 (Somers *et al.,* 2000). Chakraborty *et al.* (2016) too reported the stress encountering ability of PAS domains with respect of a high percent of PAS domains in extremophilic Actinobacteria indicating their role in adapting to the manifold prevailing environmental stress.

Configuration of PAS domain and PAS fold

PAS motifs were originally identified as homologous regions of approximately 50 amino acids in length in the N-terminal region of corresponding proteins PER, ARNT and SIM (Nambu *et al.,* 1991) with additional conserved residues instantaneously C-terminal to the region being subsequently recognized as PAC motifs (Ponting and Aravind, 1997) or S2 boxes (Zhulin*et al.,* 1997). The first three dimensional (3-D) structure of the PAS domain depicted in the Photoactive yellow protein (PYP) from *Halorhodospira halophila* (Borgstahl *et al.,* 1995) exhibited that the PAS and PAC motifs espouse a single globular fold of ≈100 residues, currently referred to as the modern view point of PAS domains (Hefti *et al.,* 2004).

Novel PAS domains have been routinely identified and annotated through sequence homology followed by an addendum to the existing literature of previously described PAS domains (Finn *et al.,* 2006). Distant domain relatives can be detected employing sensitive profile method search tools (Taylor and Zhulin, 1999) as implemented via the well known PSI-BLAST (Altschul *et al.,* 1997) or HMMER (Eddy, 1998) based programs. Identification is problematical by the moderately low degree of sequence homology among PAS domains with the pair-wise sequence identity being below 20% on average (Finn *et al.,* 2006). As a result some PAS domains would not be recognized as such (false negatives) while other domains would have a probability of being misannotated as PAS domains (false positives). The structural conformations of forty seven number of PAS domains being deposited in the Protein Data Bank (PDB) till April 2009 essentially show identical folding pattern as in Photoactive yellow protein (PYP), the PAS fold. As portrayed in Figure 1.2.A, comparable to the PAS-A domain of *Azotobacter vinelandii*NifL (Key *et al.,* 2007), the canonical PAS fold is comprised of a centrally located anti-parallel β-sheet with five analogous strands Aβ, Bβ, Gβ, Hβ and Iβ in addition to several α-helices designated by Cα, Dα, Eα and Fα correspondingly flanking the sheet. The strands of the β-sheet are arranged in the topological order of B-A-I-H-G that is in 2-1-5-4-3 configuration (Figure 1.2.B). The region comprising α-helical and β-strand based secondary structural elements from Aβ to Iβ is generally referred to as the PAS core along with the N- or C- terminal extensions to the core region as flanking regions. Multiple PAS domains within a single protein are sequentially labeled in an alphabetical order from the N to the C terminus as exemplified by PAS-A and

PAS-B.

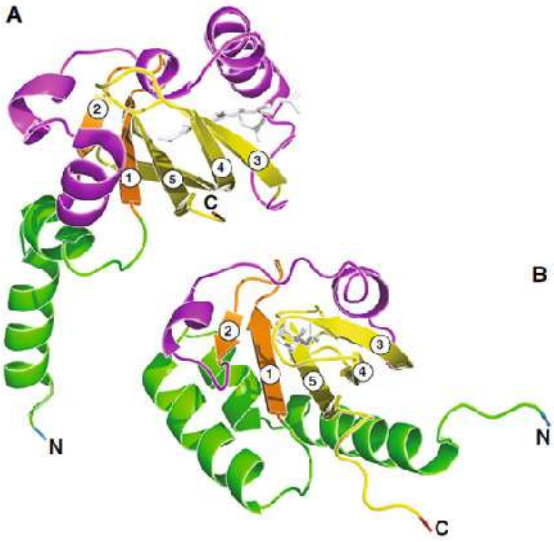


Figure 1.2.: 3-D structure of PAS (A) and PDC (B) domain. The structures are based on (A) *Rhizobium meliloti* oxygen sensor FixL protein attached to the ligand heme [UniProt ID: P10955; position: 122–251 and PDB ID: 1D06] (Miyatake *et al.*, 2000) and (B) the ligand binding domain of sensor kinase CitA protein in *Klebsiella pneumoniae* with its ligand citrate [UniProt ID: P52687; position: 5–135 and PDB ID: 1P0Z] (Sevvana*et al.*, 2008). In respect of both the structures, core β-strands are sequentially labeled from 1 to 5. The schematic models were generated employing PyMol-Schrödinger. The colour patterns in each region are as follows: amino terminal ends with blue; α-helical segments with green; initial β-strands with orange; inter-domain α-helical expanses with magenta; termini β-strands with yellow and the carboxyl ends with red besides ligands being represented in white stick models.

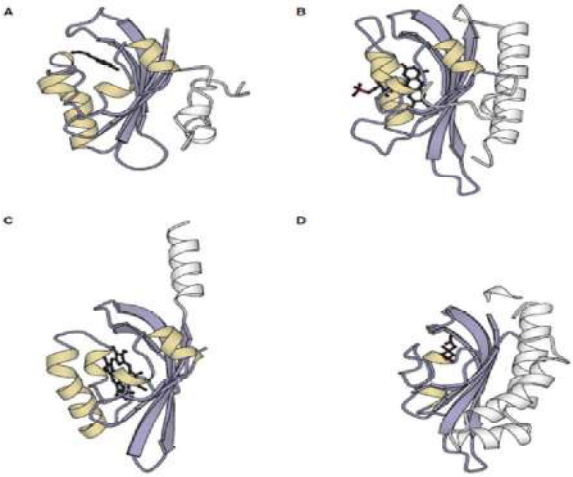


Figure 1.3.: Structural diversity of PAS domains. Three-dimensional structures of the PAS domains of: (A) *Halorhodospira halophila* PYP (1MWZ), (B) *Avena sativa* phototropin-1 (2VOU), (C) *Bradyrhizobium japonicum* FixL (1XJ3), (D) *Klebsiella pneumoniae* CitA (2J80).

Structural diversity of PAS Domains

The forty seven PAS domain structures documented in the PDB are derived from proteins with contrasting effector domains possessing an ability to respond against diversified chemical signals viz. metabolic concentration in the cell or occurrence of a particular physical stimuli such as light. Structural superposition discloses the central β-sheet to be the most conserved region with the average root-mean-square deviation (rmsd) values of the β-sheet backbone atoms in between two corresponding PAS domain structures being deduced to be 1.9±0.6A°.

In comparison to the constancy of β sheet, the orientation, length and count of intervening α-helices vary significantly; as noted in the PAS-A domain of *Vibrio harveyi*LuxQ (2HJE) lacking the Dα and Eα helices (Neiditch*et al.,* 2006). Therefore, the signature structural characteristic of the PAS core is the five stranded anti-parallel β-sheet organized in the topological order of 2-1-5-4-3 (Figure 1.3). Even though the length of β-strands is well-conserved among the PAS domain pool; loops and the interim region in between strands Bβ and Gβ, comprised of the core Cα, Dα, Eα and Fα helices differ distinctly in length and structure.

Liaison with other signaling domains

The terminology called light-oxygen-voltage (LOV) domain was introduced for referring to two tandem PAS resembling domains evident in plant phototropins (Hualа*et al.,* 1997; Crosson *et al.,* 2003). Since, LOV domains are precisely classified among PAS domains through sequential and structural likeness, the term LOV domain is presently restricted to a meticulous subset of PAS photosensors that binds flavin nucleotides besides displaying phototropin like photochemistry.

In recent times, certain PAS like domains were reported to adopt a distinct PDC fold (PhoQ-DcuS-CitA) (Cheung *et al.,* 2008). Although, the corresponding configuration superpose well with genuine PAS structures in respect of the defining characteristics of PAS fold including the central β-sheet (Figure 1.3). The resultant rmsd values for the β-sheet atoms in between structures from the PDC batch and other PAS structures were estimated to be 2.1±0.5A° being not so significantly higher in comparison to the values obtained for all other PAS domains. Structural dissimilarity is largely restrained to a few extra helical elements with such variations being observed in other apparent bona fide members of the PAS family as well. Therefore, it can be concluded that to some extent, a distinct PDC fold is obvious which may essentially be deemed to be a subclass of the PAS fold.

Another sort of domain, the Cache domain was initially recognized as typical extracellular domains of diversified origin functioning chiefly as prokaryotic and animal signaling proteins. On the basis of sequence similarity, it was imperatively suggested that Cache domains might presume a fold analogous to PAS domains (Anantharaman and Aravind, 2000) with the assumption being lately confirmed by the deduced structure of a Cache domain identified in the bacterial chemotaxis protein (2QHK). Sequential analysis revealed additional conserved regions positioned C-terminal to the inventive Cache motif

(Anantharaman and Aravind, 2000), which were also strictly identical to those in PAS domains, explicitly at the end of strand Iβ (Möglich*et al.,* 2009). Therefore, based on structural and sequential similarity, it seems that Cache domains too constitute a subset of the PAS fold.

In spite of restricted sequence homology (Finn *et al.,* 2006), PAS domains share astonishingly similar three-dimensional folding pattern with GAF domains (Ho *et al.,* 2000). The core of GAF domains is typically constituted of a six-stranded anti-parallel β-sheet configuration with strand topology in the order of 3-2-1-6-5-4, analogous to that of the PAS β-sheet; including an additional strand positioned in between the strands 3 and 2. Noticeably, quite a few GAF core domains also have five-stranded anti-parallel β-sheets; viz. the structural conformations of 3CIT and 2VZW (Podust*et al.,* 2008). The α-helical entities lie amidst strands 3 and 4 with additional α-helices customarily lying in between strands 4 and 5 (Ho *et al.,* 2000). Furthermore, the GAF domain annotation encompasses α-helical N and C terminal segments flanking the core including the GAF core being of a size and fold almost similar to the PAS core. PAS and GAF domains have been linked to allied classes of effector domains (Galperin, 2004); which further emphasizes their relatedness implying to the fact that they share a common evolutionary origin (Ho *et al.,* 2000; Anantharaman *et al.,* 2001). PAS and GAF domains can be distinguished from each other with reference to sequence based domain annotations available in the Pfam database (Finn *et al.,* 2006); though caution needs to be observed owing to possibility of erroneous outputs in some cases. Since, it has still not been established whether PAS and GAF domains utilize similar signaling mechanisms; therefore retention of a separate domain classification system may be considered advantageous.

Binding of cofactors to PAS domain

A number of PAS domains bind cofactors either non-covalently or covalently. In a few PAS sensors these represents the signal to which the protein acts in response, as reported in the citrate sensor CitA (Sevvana*et al.,* 2008); whereas in respect of other PAS domains the cofactor mediates the signal detection mechanism directly being cited in flavin cofactors absorbing blue light (Crosson *et al.,* 2003) or the heme cofactor aiding in binding of oxygen (Key and Moffat, 2005). Some PAS domains have also been characterized to bind a range of chemically distinctive non-natural ligands with high affinity (Scheuermann *et al.,* 2009). Promiscuous binding of discrete ligands might be integral for accurate

physiological functioning of certain PAS domains, such as ARNT (Hoffman *et al.,* 1991).

Möglich*et al.* (2009) scrutinized a representative subset of PAS domains that allows binding of flavin nucleotide, heme, p-coumaric acid and varied Carbon metabolites. Regardless of the wide range in chemical diversity among these ligands, the majority are vaulted in a spatially conserved cleft shaped by the inner surface of β-sheet along with involvement of Eα and Fα helices respectively. Interestingly, the protein stretch around helices Eα and Fα happens to be amongst the structurally least conserved component of the entire PAS core. Thus, the fraction of structural diversity among PAS domains must have aroused through accommodation of diversified cofactors of different proteins. Quite a similar cofactor binding site has been depicted in GAF domains, which further validates the intimate relatedness of PAS and GAF domains (Ho *et al.,* 2000; Wagner *et al.,* 2005). The PAS-B domain of NCoA-1/SRC-1 is composed of a peptide ligand being bound on the surface of the core domain positioned in between the strand Bβ and the helices Cα-Dα (Razeto*et al.,* 2004). Fascinatingly, in the crystal structures of *Drosophila melanogaster* PER (Yildiz *et al.,* 2005) and *Bacillus subtilis* KinA (Lee *et al.,* 2008) protein loops of one PAS molecule are slotted in between the helices Eα and Fα of a different PAS molecule. Numerous PAS domains of bacterio-phytochromes (Wagner *et al.,* 2005) and *Vibrio harvey*iLuxQ (Neiditch*et al.,* 2006) do not directly bind cofactors instead associate with additional sensor domains that do so.

Flanking regions of PAS domain

Most of the PAS domains happen to be part of larger proteins that are covalently connected to effector and other domains. The majority of such proteins, particularly those of prokaryotic origin are featured by linkers between the PAS core and other domains; which are short, generally 20–40 amino acids in length (Finn *et al.,* 2006). Such linkers when analyzed within a large cluster of PAS and Histidine kinases portrayed merely low levels of sequence homology (Möglich*et al.,* 2009). Although, linker lengths could be categorized into discrete classes based on the variation in number of constituent residues; occurring in multiples of seven (i.e. 7, 14 or 21). Additionally, hydrophobicity exhibited a significant heptad residue periodicity, demonstrating the capability of these linkers to form amphipathic α-helices and coiled coils (Möglich*et al.,* 2009). This analysis was extended to PAS domains being linked N-terminally to Guanylate cyclase

(GGDEF) domains (Pei and Grishin, 2001), which also revealed interesting outputs. The linkers in between PAS and GGDEF domains too show the distinctive heptad pattern of hydrophobicity, archetypal of α-helical coiled coils (McLachlan and Stewart, 1975). Noticeably, in about 85% of the 2074 proteins investigated, the linkers between the PAS and GGDEF domains exhibited matching length, signifying that structural requisites for the linker are more rigid in case of PAS-GGDEF domains than those for PAS and Histidine kinases. The left over 15% of PAS-GGDEF proteins customarily have linker sequences extendable by multiples of seven residues. A heptad prototype of hydrophobic residues has also been observed for the linkers in between tandem PAS domains. Contrary to PAS-Histidine kinase and PAS-GGDEF linkers; these linkers are either shorter or longer by multiples of three to four amino acids being consistent with α-helical linkers but not as anticipated with a coiled coil. Summatively these facts imply that some short linker's connecting PAS sensor and effector domains are soundly structured adopting α-helical conformations, while the remaining form coiled coils.

The question that now arises is whether the PAS domain structures tender direct confirmation in respect of helical to coiled coil linkers or they do not? In the beginning structural studies principally focused on PAS core domains employing miniature protein constructs devoid of any flanking regions. Recently, several more structures of longer constructs illustrate well explicated extensions to their cores. Contrary to the common PAS fold being shared by their core solitary PAS domains vary in structure with respect to the flanking regions. Noticeably, greater majority of the flanking regions assume an α-helical conformation. Flanking helices as such occur both at the N-terminal region of the PAS core as in NifL PAS-A domain (2GJ3) of *Azotobacter vinelandii*(Key *et al.*, 2007) including the C-terminus of Jα helix in the phototropin-1 PAS-B (LOV 2) domain (2V0U) of *Avena sativa* (Halavaty and Moffat, 2007). Habitually, the sequences of these flanking helices are amphipathic, in accordance with the above analysis. Flanking helices are either extended from the PAS core or remain packed on the external surface of the β-sheet being stabilized chiefly through hydrophobic interactions. Therefore residues in the β-sheet organization alternate between those that make cofactor contacts through its inner surface besides those which make contacts with flanking α-helices by means of its outer surface. Residues positioned in helical or extended regions of the PAS core generally do not participate in contact formation with flanking helices. α-helices too occur in the N-terminus of

several effector domains being regulated by PAS domains viz. GGDEF domains (Chan *et al.,* 2004); helical DHp sub-domain of histidine kinases (Marina *et al.,* 2005) besides the methyl accepting chemotaxis proteins (Alexander and Zhulin, 2007). Consequential direct fusion of these helices to the C-terminal linker helix would generate a singly long signaling helix (Anantharaman *et al.,* 2006), a helical bundle or a coiled coil (Möglich*et al.,* 2009).

Protein oligomerization in relation to PAS domain

PAS domains promote development of dimers and higher order oligomers in many proteins (Huang *et al.,* 1993; Pongratz *et al.,* 1998; Taylor and Zhulin, 1999). While prokaryotic PAS domains and proteins have been reported to form homo-oligomers, eukaryotic PAS domains have been deduced to form hetero-oligomers; such as in white-collar protein of *Neurospora crassa* (Froehlich *et al.,* 2002). The occurrence of PAS domain could determine the associative specificity of their effector domains; as for example the fundamental helix-loop-helix domain of ARNT homo-dimerizes as an isolated domain, although forming a hetero-dimer with the aryl hydrocarbon (dioxin) receptor, whilst being covalently linked to its PAS domain (Pongratz *et al.,* 1998).

PAS monomers can associate together in varied ways leading to the formation of dimers (Ayers and Moffat, 2008). Numerous PAS domains form parallel dimers, as a result the N-termini of each monomer gets proximal (Kurokawa *et al.,* 2004; Key *et al.,* 2007; Ma *et al.,* 2008); whilst others form anti-parallel dimers (Fedorov *et al.,* 2003; Nakasako*et al.,* 2008) with the remaining ones adopting intermediate orientations (Ayers and Moffat, 2008). PAS domains like PAS-B in *Bradyrhizobium japonicum* FixL (Ayers and Moffat, 2008) and PAS-A in *Bacillus subtilis* KinA (Lee *et al.,* 2008) adopt several diversified quaternary structures under similar solution conditions suggesting that the interface in between PAS monomers is plastic. A number of relative monomer orientations differ merely in free energy level; or, an alternative explanation is that a minor change in free energy of stabilization at the dimer interface would generate an immense quaternary structural change.

Regardless of portraying a wide array of potential monomer orientations, residues comprised in the dimer interface are chiefly conserved in structural position overlapping with those associated in forming contacts to flanking helices. Homodimers and heterodimers are characteristic of most PAS

domains being formed through a patch of hydrophobic residues positioned on the exterior surface of their β-sheet. Quaternary structure can be influenced through electrostatic interactions between charged residues of opposing β-sheets (Card *et al.*, 2005). Flanking helices N-terminal and C-terminal to the PAS core in several prokaryotic PAS dimer structures also contribute to the interface (e.g. 1D06, 1V9Z, 2GJ3, 2J80, 2P04, 3B42, 3B47, 3BQ8, 3BY8 and 3E4O). The flanking helices often associate with each other into α-helical bundles including compact packaging of the β-sheets, resulting in the formation of intramolecular and intermolecular contacts as depicted in *Sinorhizobiummeliloti*DctB (3E4O) (Zhou *et al.*, 2008).

Oligomerization is an integral component for functioning of various PAS sensor proteins such as in histidine kinases which dimerizes to attain phosphorylation in *trans* form (Yang and Inouye, 1991). Accurate orientation of monomers might also be crucial for regulation and functioning of PAS protein sensors. Mutation of residues within the PAS β-sheet in addition to the N- or C- terminal flanking regions could modulate functional aspects besides maintenance of the oligomeric state (Miyatake *et al.*, 2000).

Möglich*et al.* (2009) proposed a general role of PAS domains in modulating the affinity of proteins for a similarly identical protein (homo-oligomerization) or a different protein (hetero-oligomerization). In respect of PAS domain subset serving as sensors, modulation of affinity is signal dependent. Structural promiscuity, oligomerization including the capability of PAS domains in homo- and heterodimerization bestow specificity attributes including accommodation of multifaceted spatial and temporal parameters in regulating cellular signaling networks. Signal induced alteration in quaternary configuration might play significant roles in downstream transmission to effector domain via apt transduction pathway.

Thermodynamics of signaling machinery in PAS domains

Signaling is intrinsically thermodynamic in nature. The occurrence of signal either modifies the intramolecular affinity in one side of the protein or domain relative to the other part via an alteration in tertiary structure and dynamics; or through alteration in intermolecular affinity of an individual protein or domain in respect of another via change in its quaternary structure and dynamics; or by means of both the methodology. This simplified system could be explained in analogy to the induced fit model (Koshland*et al.*, 1966); or relative

to oligomeric proteins in which quaternary structural transformations like domain rearrangements or association reactions is accompanied in due presence of the signal.

Mechanism of signal detection by PAS domains

Almost each and every PAS domain binds corresponding cofactors within their core as a step towards ensuring precise coordination, specificity besides enhancing longevity of the signaling complex. Explicit structures of several PAS domains in presence and absence of the stimulatory signal disclosed the fact that signal induced conformational changes are diminutive being concentrated only in the cofactor binding spot and its vicinity; as illustrated through the light absorption mechanism in certain photo-sensory PAS domains consequently resulting in chromophore isomerization, viz. PYP (Genick*et al.*, 1997); or via formation of a covalent adduct in between the chromophore and the protein such as in phototropin PAS (LOV) domain (Salomon *et al.*, 2001; Crosson and Moffat, 2002; Fedorov *et al.*, 2003). Conformational changes are induced in residues structuring the cofactor binding pocket of certain PAS domains through binding of di-atomic ligands to the cofactor heme (Key and Moffat, 2005). PAS domain of the protein Vivid in *Neurospora crassa* has been reported to cumulatively respond against light and redox potential integrating the effect of respective stimuli (Zoltowski *et al.*, 2007). Interestingly, most PAS domains that have been delineated in detail is characteristic of signals being transmitted apparently via the central β-sheet to spatially remote effector domains in due course; which ultimately leads to modulation of biological activity. Time-resolved crystallography technique revealed structural alterations in the PYP β-sheet following absorption of blue light (Rajagopal *et al.*, 2005). Conformational transformations propagate to a conserved cap region on the Cα helix being sequentially propagated to a N-terminal expanse consisting of short pairs of helices within a timeframe of some nanoseconds to seconds. Since, signal receptors remain packed on the exterior surface of the β-sheet; they are also prone to undergo small conformational changes, with signal induced configuration and dynamic alteration in the β-sheet structure been reported for the PAS domains in Vivid plant phototropins of *Neurospora crassa* (Harper *et al.*, 2003; Halavaty and Moffat, 2007; Yao *et al.*, 2008); *Bacillus subtilis* YtvA (Möglich and Moffat, 2007); *Bacillus japonicum* FixL (Key and Moffat, 2005) and *Klebsiella pneumoniae* CitA (Sevvana*et al.*, 2008). The β-sheet of PAS domains have been

characterized to be malleable being depicted in ARNT PAS-B domain where a solitary mutation induces an alternative conformation with a triple-residue slip in strand Iβ (Evans *et al.,* 2009). The fundamental role of β-sheet in signal promulgation corresponds with its distinctive significance in binding of associative cofactors along with PAS domain dimerization.

Mode of signal transduction to effector domains

The question that now arises is how signals are being further propagated to effector domains besides understanding its role in modulation of biological activity. Realistic answers look forward to high resolution structures of the complete length of protein entities being constituted of both PAS sensor and effector domains mutually in the existence and nonexistence of signal. Apparently due to their intrinsic flexibility the full length PAS signaling proteins have fundamentally eluded efforts for determination of their structure at atomic resolution. The tertiary structural homogeny of PAS core domains is in severe contrast to the wide sequential diversity among effector domains, which in turn assume exceptionally dissimilar tertiary structures. This instantaneously argues against signaling methodology that relies on explicit recognition of tertiary structural conformations ranging between PAS core and effector domains. A key evidence to comprehend regulation of protein activity is based on the assessment that known effector domains function as homo- or hetero-oligomers, predominantly dimers. Prominent examples take account of histidine kinases (Szurmant*et al.,* 2007); serine/threonine kinases like plant phototropins (Christie *et al.,* 1998); phosphodiesterases viz. *Escherichia coli* DOS (Kurokawa *et al.,* 2004); transcription factors such as the *Neurospora crassa* white-collar proteins (Froehlich *et al.,* 2002) and the chemotaxis receptors in *Escherichia coli* Aer (Taylor and Zhulin, 1999). Signal modulates the association equilibrium of monomers and individual domains in oligomeric proteins which further influences their quaternary structure. Signal-induced quaternary conformational changes have been identified in several PAS proteins (Kurokawa *et al.,* 2004; Neiditch*et al.,* 2006; Möglich and Moffat, 2007; Ayers and Moffat, 2008; Nakasako*et al.,* 2008; Sevvana*et al.,* 2008; Zhou *et al.,* 2008; Scheuermann *et al.,* 2009) with quaternary structural rearrangements via association, piston, pivot and rotational movements (Matthews *et al.,* 2006) being compatible with signaling mechanisms across transmembane proteins too.

Conclusion

PAS domain sequences are comparatively divergent being expected to instill varied functions owing to contrasting selective pressures on organisms. They are imperative signaling modules involved in monitoring extracellular and intracellular changes with reported occurrence in every kingdoms of life. PAS containing genes are an integral element of prokaryotic signaling machinery that plays a significant role in two component system aiding in adapting to the immense abiotic and biotic stress prevailing in the environment. Regardless of their lowly conserved sequence homology, PAS genes are highly expressed rendering them to be regarded as translationally efficient with an elevated degree of codon homogeneity. In eukaryotes the majority of PAS domains act as transcriptional activators besides operating in voltage sensitive ion channels while the others being involved in regulatory systems concerning phosphorylation of serine and threonine residues. Greater the stress encountered by the organism, higher is the PAS domain percentage and vice versa. The interception of signal by PAS domains and its sequential relay to downstream components of the signal transduction pathway is still not clearly understood. Although, protein-protein interaction; specifically via heterodimer formation with a different PAS protein might be a fundamental aspect of signaling. Recognition of this synopsis led to the current progress in concluding the loop of circadian clocks. However, even in respect of clock proteins been extensively studied; the sensory role of PAS domains still remains elusive being factual for most of the investigated eukaryotic PAS domains. We are still at a very early phase in understanding the overall functioning of PAS domains and therefore the comprehensive lineage for a PAS-induced signaling system awaits further elucidation.

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श्रद्धावान लभते ज्ञानम्

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A review on Plastic Degrdation by Bacteria: A sustainable approach

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Abstract:

Since last few decades the uncontrolled use of plastics for various purposes such as packaging, transportation, industry and agriculture in rural as well as urban areas, has elevated serious issue of plastic waste disposal and its pollution. Excessive use of plastics poses a serious threat to the ecosystem and human life on the planet. The efficient decomposition of plastic bags takes about 1000 years. Plastic causes pollution when it is dumped onto landfills and when burned release CO_2 and dioxins. There is a need to use adequate biodegradable methods in order to reduce plastics burden from the environment. In order to overcome plastics associated environmental problems, understanding of the interaction between microbes and polymers is of prime importance.Microbial degradation of plastic has shown an eco-friendly way to solve this problem. However, the biodegradation efficiency is not high. The present review outlines the recent advances made in the microbial degradation of synthetic plastics.

Keywords: Plastic degrading bacteria, different types of plastics, degradation pathway.

Introduction

The word plastic comes from the Greek word plastikos, which means ‘able to be molded into varied shapes (Joel 1995). Due to its various desirable characteristics petro-plastics hasbecomepivotalinourlifestyle. Generally, plastic materials are derived from petrochemicals except biodegradable bioplastic (Akmal et al., 2015; Getachew and Woldesenbet, 2016). Plastic consists of chloride, oxygen, hydrogen, carbon, silicon and nitrogen. Polyethylene consists of 64% of total plastic and its general formula is

C_nH_{2n} (Kale et al., 2015; Elahi et al., 2021) Environmentalpollutionbyplasticwastewasfirstreportedin the 1970s (Carpenter and Smith, 1972). At present plastics have become an inseparable need of our life. Plastics play an important part in every sector of economy all over the world. Plastics, the backbone of many industries, are used in manufacturing of various products that are used in our daily life i.e., agricultural films formation, defense materials, sanitary wares, tiles, plastic bottles, artificial leather, fishing net plastics and different other household items. Plastics are also used in packaging of food items, pharmaceuticals, detergents and cosmetics (Thakur, 2012; Piergiovanni and Limbo, 2016; Elahi et al., 2021). Therefore the, production of plastics is rapidly increasing which further increasing amounts of left-over plastics into the environment becoming a big threat to our ecosystem.

There are advantages and disadvantages of plastics. Plastics are strong, durable, and light weight. On the other hand, they are harmful to the natural environment, resistant to degradation and leading to environmental pollution. On our planet, plastics pose a serious threat by accumulating in large quantities (Ahmed et al., 2018; Yang et al., 2020; Al-Thawadi, 2020)

Plastics can be differentiated into degradable and non- degradable polymers on the basis of their chemical properties. (Ghosh et al., 2013). Plastics that are obtained from renewable resources are biodegradable plastics. These are naturally degradable, as a source of cellulose, starch and algal material, an important component in plants, animals and algae. These polymers are also produced by microorganisms. Non-degradable plastics, typically known as synthetic plastics, are derived from petrochemicals and are higher in molecular weight due to the repetitions of small monomer units (Imre and Pukánszky, 2013; Elahi et al., 2021) These plastics in form of micro plastics (size of < 5 mm) are also entering the human food chain causing many health hazards which leads to potential ecotoxicological effects (Zhang et al., 2017; Chen et al., 2020a; 2020b; Wong et al., 2020). Fibrous MPs may be inhaled, may persist in the lung, and along with associated contaminants including dyes and plasticizers could lead to health effects like carcinogenicity and mutagenicity (Gasperi et al., 2018; Wong et al., 2020; Elahi et al., 2021). Plastics can be degraded in the environment by 4 mechanisms i.e., hydrolytic degradation, photodegradation, thermo-oxidative degradation and biodegradation (Webb et al., 2013; Elahi et al., 2021). Disposal of plastics such as Polyethylene, Polypropylene, Polystyrene, Polyvinyl chloride, Polyethylene terephthalate etc. is a matter of concern due to its non-biodegradable nature (Macarthur et al., 2017). These methods are not

environment friendly and do release contaminants in the environment. There is an urgent need to overcome this challenge of plastic waste degradation. This can be fulfilled in an eco-friendly manner with plastic degrading microorganisms. These microbes are also capable of producing enzymes which can degrade plastic. Microbes proclaimed to be efficient in plastic degradation are *Pseudomonas* sp., *Proteobacteria* sp., *Bacillus* sp., *Stenotrophomonas* sp., *Staphylococcus* sp., *Rhodococcus* sp.,*Arthrobacter* sp. *Ideonella*sp., *Comamonas*sp., *Streptomyces* sp. and others. These microbes have been noted to degrade and decompose all types of plastic as polyethylene, polypropylene, polystyrene, polyurethanes and polyvinyl chloride (Thompson et al., 2009)

2. Classification of Non-Degradable plastics

a. Polyethylene (PE)

It is the most abundant non-degradable plastic waste. Microorganisms capableofhydrolyzingPEhavebeenisolatedfr omsoil,seawater,compost andactivated sludge (Montazer et al., 2020a). It is classified into various types based on density of polymer and their degree of branching, such as High-density polyethylene (HDPE), Ultrahigh-molecular-weight polyethylene (UHMWPE), linear low-density polyethylene (LLDPE) and Low density polyethylene (LDPE).This synthetic plastic is used in mostly all known things of routine life ranging from plastic bags, water packing, milk bottles, food packaging, toys etc. attributed to its good processability, water resistance and low oxygen barrier properties (Mirza et al.,2021). Polyethylene contributes to 36% of total non-fiber plastics production. The highly recalcitrant hydrophobic backbone and inert nature of PE makes it hard to decompose and nearly non degradable (Manjula et al., 2017).

Table No.1: *Bacteriain Polyethylene(PE) Biodegradation*

Bacteria	Plastictype	Incubation time,d	Weightloss,%	References
*Pseudomonas knackmussii*N1-2			5.95 ± 0.03%	Houetal., 2022
Bacilluslicheniformis SARR1	LDPE	30	32.15%± 2.27%	Ranietal., 2022
Anoxybacillus flavithermus	PE(50and150 µm)			Akarsuet al.,2022
Pseudomonas aeruginosa	LDPE	60	0.17%	Mefoetal.,2022
		60dwith30% cassavastarch	51.03%	

Lysinibacillus speciesJJY0216		26	9%	Jeonet al.,2021
*Halomonas*sp.	LDPE	90	1.72%	Khandareet al.,2021
Streptomycessp.	LDPE	-	46.7%	Sound2019
Brevibacillusborstelensis	30micron polyethylenesheets	112	20.28±2.30	Muhonjaet al., 2018
*Bacilluscereus*strain A5,a (MG645264)	LDPE	112	-	Muhonjaet al., 2018

Recently many works had been done to increase the rate of biodegradation by using UV radiation (Kalia & MS 2022), enggine oil (Kalia & MS 2022), cassava starch (Mefo et al.,2022).Amongthesecassavastarchhasmostsuccesswith51.03%decreaseinweight.

b. Polystyrene(PS) degradation

Polystyrene (PS) has been the most abundant plastics produced worldwide and largely manufacturedintopackagingmaterialsforfoodanddisposabledishware(PlasticsEurope, 2017). Styrene is a homopolymer made of phenylethane. It is also known as vinylbenzene or phenylethene. Styrene is a colorless, aromatic monomer with a sweetish odor. Styrene is an important mono-aromatic compound which is produced industrially in a very large scale. In 1851, M. Berthelot, a French chemist introduced the production of styrene by catalytic dehydrogenation of benzene with ethylene. Styrene occurs naturally in liquid form and therefore is an essential component used in making variety of strong, light-weight and flexible products. They are mostly found in gasoline and other fuel constituents. They are used in the production of plastics such as polystyrene (PS), Acrylonitrile butadiene styrene (ABS), Butadiene (SB) and Styrene acrylonitrile (SAN)

Table No 2: *Bacteriain Polystyrene Degradation*

Nameofthemicrobe	**Incubation time,weight loss**	**source**	**References**
*Brevibacterium*sp.EDX	-	*Zophobasatratus*larvae	Arunrattiyakornetal.,2022
*Pseudomonas*liniJNU01	-	soil	Kimetal.,2021
*Acinetobacter johnsoni*JNU01	-	soil	Kimetal.,2021
Bacillusparalicheniformis	34%,in60days	soil	Kumaretal.,2021
*Acinetobacter*sp		FromTriboliumcastaneum	

c. Polypropylene(PP) biodegradation

Recently, Jainetal.,(2022) five *Bacillus* strain isolated from composted sample are studied. These results suggest that Bacillus species enhances degradation of polymeric blends a by FTIR and TGA analysis. In 2021 Jeon et al., reported 4 and 9% of degradation in weight of polypropylene without any physicochemical pretreatment by Lysinibacillus sp.

Table No 3: *Bacteria Degrading Polypropylene*

Strain	TestedPP	Incubationtime, d	Gravimetric Weightloss, %	References
*Lysinibacillus*species		26	4%	Jeonetal.,2021
Aneurinibacillusaneurinilyticus; Brevibacillusagri; *Brevibacillus*sp.; *Brevibacillusbrevis*	PPfilmandpellets	140	22.8–27.0	Skariyachan *etal*.,2018
Bacilluscereus	PP &poly-L-lactide(PLLA)in 80:20	45	12.25%,	Jai et al.,2018
Bacilluslicheniformis			9.3%	
Bacillusthuringiensis			9.0%	

d. Polyvinylchloride (PVC) biodegradation

Among all main kinds of synthetic plastics, PVC possesses the highest proportion of plasticizer (up to 50%). In 2022, Yadav et al., reported significant degradation of PVC by species of Bacillus and Micrococcus. Bacillus and Micrococcus species have both proven the biodegradability of PVC based on the mean weight decrease, which was 0.873 for Bacillus species and 0.916 for Micrococcus species after a period of around six months. (Yadav etal., 2022).

e. Polyurethanes(PU)

Polyurethanes (PU) are a family of versatile synthetic polymers intended for diverse applications. Otto Bayer, a German professor, in 1937 produced the first PUR by a polymerization reaction of polyisocyanates and polyester diol. Polyurethanes are monomers of urethane groups. PUR obtained from different sources have variation in their di-isocyanates polyols side chain, as different poly-ol type provides variation in properties and result into different type of polyurethane such as rigid polyurethane foam, flexible polyurethane foam, thermoplastic polyurethane, and

polyurethane ionomers etc. that are suitable for different application (Akindoyo JO et al., 2016). It is synthesized easily because of its varying nature and degree of cross-linking. For example, polyurethanes or polyether which contains hydroxyl groups can be synthesized using polyester or polyether resins (Schmidt J et al., 2017). The strain, identified as Pseudomonassp. By 16s rRNA gene sequencing and membrane fatty acid profile, was able to grow on a PU-diol solution, a polyurethane oligomer, as the sole source of carbon and energy. (Espinosa et al., 2020; Roy et al., 2021)

In 2020 Guna wanetal confirmed that a cholesterol esterase from Pseudomonas species depolymerized 38% of the PU back into monomers and oligomers over 24h of Enzymatic treatment. Comamonas acidovorans is a gram-negative, aerobic, non-spore forming, rod-shaped bacterium belonging to the genus Comamonas and family Comamonadaceae, mostly found in soil and water (Gilligan PH et al, 2003).

f. Polyethylene terephthalate (PET) degradation

Polyethylene terephthalate (PET) widely known as polyester is a thermoplastic polymer made from polymerization of ethylene glycol and terephthalic acid in presence of certain catalysts. It is generally stiff and has high strength attributed to aromatic rings present in its monomer units. First synthesis of PET was carried out by DuPont (North American Chemist) in mid 1940s, whose method was further modified in 1950s resulting in the formation of thin extruded sheets (Koshti R et al., 2018). Among different types of plastics, synthetic ones such as polyethylene terephthalate (PET) are more difficult to degrade. This is due to the high ratio of aromatic terephthalate units in the PET structure.

Table No 4*:* *Bacteria Degrading PET*

Bacteria	PETtype	Weightloss	References
*Priestiaaryabhattai*VT 3.12	PETsheet	40%	Dhakaetal.,2022
Bacilluspseudomycoides VT3.15	PETsheet	-	Dhakaetal.,2022
*Bacilluspumilus*VT3.16	PETsheet		Dhakaetal.,2022
Pseudomonas aeruginosa RD1-3		3.62 ±0.32%	Houetal.,2022
Ideonellasakaiensis	Low-crystallinityPETfilm	_	Yoshidaetal., 2016

Mechanism of Biodegradation

Biodegradation of polymers consists of three steps; (a) microorganism attachment on the surface of polymer, (b) utilization of polymer as a source of carbon, and (c) polymer degradation. Microorganisms attach to the surface of polymers and degrade these polymers by secreting enzymes in order to obtain energy for their growth (Danso et al., 2018). Large polymers degraded into monomers and oligomers that are low molecular weight molecules. Some oligomers may be assimilated in the internal environment of microorganisms after diffusing inside them. After biodegradation end products like CO_2, H_2O and CH_4 are produced (Alsheri et al.,2017).

Various types of methodologies are currently available for measuring the biodegradability of polymeric materials (Yang et al., 2005). Several test methods to assess the potential biodegradability of plastics have been developed by International Standard Organization (ISO) and American Society for Testing and Materials (ASTM) (Piergiovanni and Limbo, 2016) including gas chromatography/- mass spectrometry (GC–MS), stereomicroscopy and micro-Fourier transform infrared spectroscopy (m-FTIR) (Lomonaco et al., 2020; Corami et al., 2020). Biodegradation can be characterized with loss of weight, change in tensile strength, change in dimensions, change in chemical and physical properties, carbon dioxide production, bacterial activity in soil and change in molecular weight distribution (Kathiresan, 2003; Sivan, 2011; Kumar and Maiti, 2016; Chen et al., 2020a; 2020b).

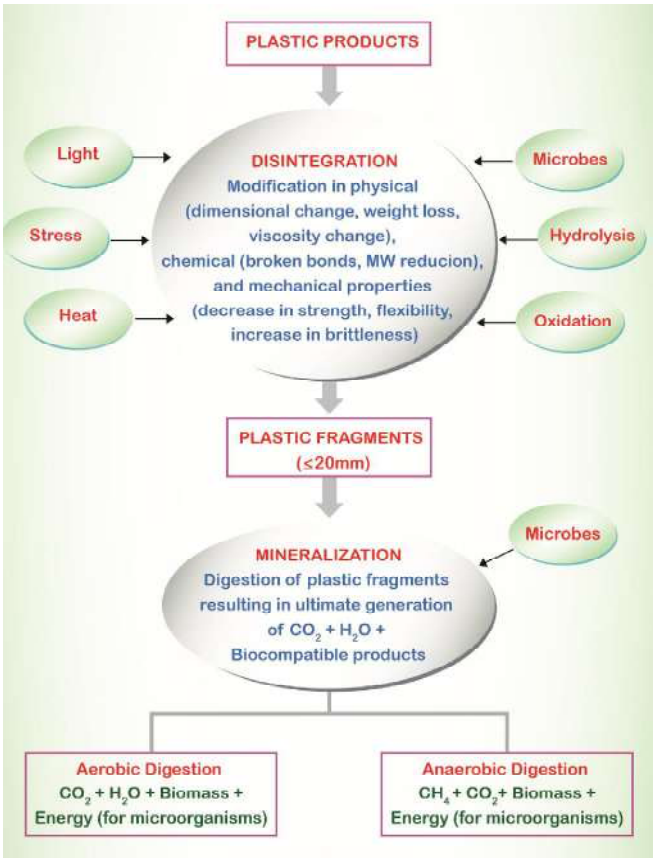

Figure 1: Schematic representation of biodegradation

(Adopted from a review entitled "Plastics degradation by microbes: A sustainable approach" by Elahi et al.,2021)

Conclusion

Plastics are used all over the world at large levels. The availability of plastics in aquatic environment has been increased many folds due to biodegradation, thermos-oxidative degradation, photo-degradation, thermal and hydrolysis processes in the ecosystem and poses serious threat to the aquatic life and human life through food web. There is a need to use adequate biodegradable methods to eradicate these polymers from the ecosystem. Due to the hydrophobic and inert nature, it is difficult to remove or degrade polymers. Besides physical and chemical methods, microbes have shown promising potential to degrade these polymers. The potential use of microbes for polymers removal needs to be further evaluated using original polymers contaminated wastewater. Long-term coordinated clean up operations are needed to evaluate the progressive ecosystem effects. A number of plastic-degrading bacteria have been sourced from the environment. But these results are from observation of individual strain of

organisms in in vitro condition. In nature various environmental factors (i.e., temperature, other micro organisms, water, availability of carbon source and oxygen) may influence the growth and biodegradable ability on above mentioned bacteria. Many chemical and physical methods are also considered to increase the rate of the degradation, which have shown significant results. The removal of microplastics/nanoplastics, their toxicity and the utilization of microbes remain to be addressed. The transfer of plastic polymers from the waste into the aquatic ecosystem including rivers and oceans through different processes and the strategy to shift these polymers from the wastewater to a suitable place for deposition/incineration should properly be encouraged.

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श्रद्धावान लभते ज्ञानम्

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A Review on Angiotensin Converting Enzyme gene polymorphism and its Implication in Pathophysiology

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Abstract:

The renin-angiotensin-aldosterone system (RAAS) plays a crucial role in blood pressure (BP) regulation and electrolyte balance. Angiotensin-converting enzyme (ACE) is a key regulatory enzyme of the RAAS and is responsible for converting angiotensin I to angiotensin II which is an active vasoconstrictor, thereby contributing to vasoconstriction and aldosterone secretion. It is produced mainly in the lungs, but also in other organs such as the kidneys and blood vessels. ACE is also involved in the breakdown of bradykinin, which is a substance that promotes vasodilation and increases blood flow. ACE inhibitors may increase the levels of bradykinin in the body, which may contribute to their beneficial effects on the cardiovascular system.

The ACE gene contains two insertion/deletion (I/D) polymorphic regions that are extremely variable. This polymorphism has been associated with numerous diseases, such as cardiovascular diseases, hypertension, diabetes, and renal diseases. The association between the ACE gene and hypertension has been widely studied, although the results have been inconsistent. Type 2 diabetes has been associated with increased insulin resistance, impaired glucose tolerance, and reduced insulin sensitivity.

Renal diseases are also associated with an increased risk of left ventricular hypertrophy (LVH), a common complication of hypertension and a strong predictor of cardiovascular mortality. The ACE gene polymorphism has been linked to various human diseases, including cardiovascular diseases, hypertension, diabetes, and renal diseases. The mechanism underlying these associations remains unclear, but alterations in RAAS activity, increased oxidative stress, and endothelial dysfunction have been associated with an increased risk of CVD. Further research is needed to explore the clinical utility of ACE genotype testing for risk stratification and personalized therapy. Hypertension or high blood pressure is a common condition that affects millions of individuals worldwide and is a major risk factor for cardiovascular diseases such as heart disease, stroke, and coronary artery disease. Various factors are involved in the development of hypertension, including genetic factors, environmental factors, lifestyle factors, and aging.

Introduction

Humans have two versions of the enzyme: the larger somatic form, which is present in many tissues, and the smaller germinal form, which is only present in the testes. Through transcription from alternative promoters, the same gene codes for both of these proteins. Both types are ectoenzymes (membrane-bound) that hydrolyze circulating peptides at the cell surface. It is monomeric, zinc and chloride-dependent peptidyl dipeptidase (Riordan JF, 2003).

The ACE gene is located on chromosome 17q23 and contains two highly variant polymorphic regions known as the insertion/deletion (I/D) polymorphism. The I/D polymorphism refers to the presence or absence of a 287 bp Alu repetitive sequence on intron 16, which results in the insertion (I) or deletion (D) of this sequence, respectively (Rigat et al, 1992). The I/D polymorphism has been extensively studied for its association with various diseases, including cardiovascular diseases (Butler R et al, 1997), hypertension (Ruiz et al, 1994), diabetes (Viswanathan et al, 2001), and renal diseases (Navis G et al, 1999).

Cardiovascular Diseases: The I/D polymorphism has been implicated in the development of cardiovascular diseases, including coronary artery disease (CAD) and myocardial infarction (MI) (Niu et al, 2002). The DD genotype has been shown to be associated with an increased risk of CAD and MI in several studies. Similarly, the DD genotype has been found to be a significant risk factor for left ventricular hypertrophy (LVH), a common complication of hypertension and a strong predictor of cardiovascular mortality (Wuyts B et al, 1997; Uemura K et al, 2000; Saeed M et al, 2005; Falahati, A et al, 2019).

Hypertension: The association between the ACE gene and hypertension has been widely studied, and the results have been inconsistent. Some studies have found an association between the DD genotype and hypertension (Ruiz et al, 1994; Choudhury et al, 2012), while others have not (Gomez-Angelats E et al, 2000; Chiang FT et al, 1997). A meta-analysis of 36 studies involving over 11,000 subjects suggested that the DD genotype is associated with a 1.4-fold increased risk of hypertension compared to the II genotype. The mechanism underlying this association remains unclear but may involve increased systemic vascular resistance and salt sensitivity.

Diabetes: The I/D polymorphism has been associated with the development of type 2 diabetes in several populations. The DD genotype has been found to be associated with increased insulin resistance, impaired

glucose tolerance, and reduced insulin sensitivity (Panahloo A et al, 1995; Baroudi T et al, 2009; Feng Y et al, 2002). A meta-analysis of 14 studies involving over 4,000 subjects showed that the DD genotype is associated with a 1.3-fold increased risk of type 2 diabetes compared to the II genotype (Zhou JB et al, 2010).

Renal Diseases: The I/D polymorphism has been extensively studied for its association with renal diseases (Shanmuganathan R et al, 2015), including diabetic nephropathy (Ma H et al, 2015; Wang Y et al, 2016), IgA nephropathy (Syrjänen J et al, 2000), and renal transplantation (Yang CH et al, 2015). The DD genotype has been found to be associated with an increased risk of proteinuria and reduced glomerular filtration rate (GFR) in patients with diabetic nephropathy. Similarly, the DD genotype has been associated with an increased risk of graft failure and reduced GFR after renal transplantation.

ACE gene polymorphism and Cardiovascular disease

Cardiovascular diseases (CVDs) are a collection of heart and blood vessel disorders. They consist of: coronary heart disease – a disease of the blood vessels supplying the heart muscle; cerebrovascular disease – a disease of the blood vessels supplying the brain; peripheral arterial disease – a disease of blood vessels supplying the arms and legs; rheumatic heart disease – damage to the heart muscle and heart valves caused by streptococcal bacteria; congenital heart disease – birth defects that affect the normal development and functioning of the heart. Deep vein thrombosis and pulmonary embolism are blood blockages in the legs that can travel to the heart and lungs. Heart attacks and strokes are typically sudden occurrences caused by a blockage that prevents blood passage to the heart or brain. The most frequent cause is the accumulation of fatty deposits on the inner walls of the blood vessels that supply the heart or brain. Strokes can be induced by either haemorrhaging from a brain blood vessel or blood clots.

ACE gene polymorphism is a common genetic variation that influences the activity of the angiotensin converting enzyme (ACE) in the body. ACE plays a crucial role in the regulation of blood pressure and vascular function, and its activity is implicated in the development of cardiovascular disease (CVD) (Butler R et al, 1997).

There are two common ACE gene polymorphisms, known as the insertion (I) and deletion (D) alleles. Individuals with the DD genotype have been shown to have

higher levels of ACE activity than those with the II or ID genotypes (Rigat B et al, 1992). This increased ACE activity has been associated with an increased risk of CVD, including hypertension, stroke, and coronary artery disease.

Several studies have investigated the relationship between ACE gene polymorphism and CVD risk, with mixed results. Some studies have found that the DD genotype is associated with an increased risk of CVD (Ruiz et al, 1994; Uemura K et al, 2000; Settin A et al, 2009), while others have found no association or even a protective effect of the II genotype (Agerholm-Larsen B et al, 1997; Keavney B et al, 2000; Kumar et al, 2021).

Overall, the evidence suggests that ACE gene polymorphism may play a role in the development of CVD, but its contribution is likely to be complex and influenced by other genetic and environmental factors. Further research is needed to clarify the relationship between ACE gene polymorphism and CVD risk and to explore potential therapeutic interventions based on this genetic variation.

ACE gene polymorphism and hypertension

Hypertension or high blood pressure is a common condition that affects millions of individuals worldwide. It is a major risk factor for cardiovascular diseases such as heart disease, stroke, and renal failure. Various factors are involved in the development of hypertension, including genetic factors, environmental factors, lifestyle factors, and aging. The angiotensin-converting enzyme (ACE) is an important enzyme that plays a role in the regulation of blood pressure. ACE gene polymorphism is a common genetic variation that has been associated with hypertension.

The ACE I/D polymorphism has been extensively studied for its association with hypertension. The DD genotype has been found to be associated with higher levels of ACE activity and increased risk of hypertension. The II genotype, on the other hand, is associated with lower ACE activity and a lower risk of hypertension. The ID genotype is intermediate in terms of ACE activity and hypertension risk (Kario K et al, 1999; Rana G et al, 2018).

Several studies have shown a significant association between the ACE I/D polymorphism and hypertension. For example, a study conducted in Italy showed that the DD genotype was associated with a 2.4-fold increased risk of hypertension compared to the II genotype. Another study conducted in Turkey found that the DD genotype was associated with a 1.7-fold

increased risk of hypertension compared to the II genotype (Agachan B et al, 2003).

The mechanism by which the ACE I/D polymorphism increases the risk of hypertension is not fully understood. However, it is believed that the higher levels of ACE activity in individuals with the DD genotype lead to increased production of angiotensin II, which causes vasoconstriction and increased blood pressure. In addition, angiotensin II also stimulates the secretion of aldosterone, which promotes sodium retention and fluid retention, further contributing to hypertension.

The ACE I/D polymorphism has also been studied for its association with the response to antihypertensive therapy. Some studies have shown that individuals with the DD genotype have a poorer response to ACE inhibitor therapy, which is a common treatment for hypertension (Heran BS et al, 2008). This is because ACE inhibitors block the activity of ACE, and individuals with the DD genotype have higher levels of ACE activity, which may lead to reduced efficacy of ACE inhibitors.

On the other hand, other studies have shown that the DD genotype is associated with a better response to angiotensin receptor blocker (ARB) therapy, which is another type of antihypertensive medication that blocks the action of angiotensin II (Helmer A et al, 2018). This is because individuals with the DD genotype have higher levels of angiotensin II, and blocking its action through ARB therapy may be more effective in reducing blood pressure.

ACE gene polymorphism is a common genetic variation that has been associated with hypertension. The DD genotype is associated with higher levels of ACE activity and increased risk of hypertension, while the II genotype is associated with lower ACE activity and a lower risk of hypertension. The ID genotype is intermediate in terms of ACE activity and hypertension risk. The mechanism by which the ACE I/D polymorphism increases the risk of hypertension is not fully understood, but it is believed to involve increased production of angiotensin II, which causes vasoconstriction and increased blood pressure. The ACE I/D polymorphism has also been studied for its association with the response to antihypertensive therapy, with some studies showing that the DD genotype has a poorer response to ACE inhibitor therapy but a better response to ARB therapy.

ACE gene polymorphism and Diabetes

Diabetes is a complex metabolic disorder characterized by high blood glucose levels. It occurs when the body is either unable to

produce enough insulin, the hormone that regulates glucose metabolism, or when the cells become less sensitive to it. The prevalence of diabetes is increasing worldwide, and it is estimated that over 400 million people are affected by this condition. Genetic factors have been shown to play an important role in the development of diabetes, and it is likely that multiple genes are involved. One such gene that has been associated with diabetes is the ACE gene.

The ACE gene encodes for angiotensin-converting enzyme, an enzyme that plays a key role in the regulation of blood pressure and fluid balance. The ACE gene has a common polymorphism known as the insertion/deletion (I/D) polymorphism, which refers to the presence or absence of a DNA sequence in a specific location of the gene. The I/D polymorphism has been extensively studied in relation to its association with various diseases, including diabetes.

Several studies have investigated the association between the ACE I/D polymorphism and diabetes. In a study of 777 patients with type 2 diabetes and 661 healthy controls, a significant association was found between the ACE I/D polymorphism and diabetes (Feng Y et al, 2002; Habibullah M et al, 2021). The frequency of the DD genotype (where there is a deletion of the DNA sequence) was significantly higher in patients with type 2 diabetes compared to healthy controls (Yang M et al, 2006). The DD genotype was also associated with a higher risk of diabetic complications such as retinopathy and nephropathy.

Another study of 186 patients with type 1 diabetes and 95 healthy controls found that the frequency of the DD genotype was significantly higher in patients with type 1 diabetes compared to controls (Alsaeid M et al, 2004). Furthermore, the DD genotype was associated with higher levels of glycated hemoglobin (HbA1c), a marker of long-term glucose control, and a higher risk of diabetic nephropathy.

The mechanism by which the ACE I/D polymorphism may be involved in the development of diabetes is not fully understood, but several hypotheses have been proposed. One hypothesis suggests that the ACE I/D polymorphism may affect the activity of the renin-angiotensin-aldosterone system (RAAS), a hormonal system that regulates blood pressure and fluid balance. The activity of the RAAS is known to be increased in diabetes, and it has been shown to contribute to the development of diabetic complications. The DD genotype has been shown to be associated with higher levels of

ACE activity compared to the II genotype, and this may lead to increased activation of the RAAS and a higher risk of diabetic complications.

Another hypothesis suggests that the ACE I/D polymorphism may be involved in the development of insulin resistance, a key feature of type 2 diabetes (Ruiz J et al, 1994). Insulin resistance refers to the decreased ability of cells to respond to insulin, leading to high blood glucose levels. It has been shown that the DD genotype is associated with higher levels of insulin resistance compared to the II genotype (Panahloo A et al, 1995). This may be due to the fact that ACE is involved in the breakdown of bradykinin, a peptide that has insulin-sensitizing properties. Thus, higher levels of ACE activity may lead to decreased levels of bradykinin and increased insulin resistance.

In conclusion, the ACE I/D polymorphism has been shown to be associated with diabetes, particularly in relation to the risk of diabetic complications and insulin resistance. However, further studies are needed to fully understand the mechanism by which this polymorphism may contribute to the development of diabetes. The identification of genetic factors involved in diabetes may lead to the development of new strategies for the prevention and treatment of this complex disease.

ACE gene polymorphism and Renal disease

Renal disease is a broad term that encompasses a variety of conditions affecting the kidneys. One of the most common types of renal disease is chronic kidney disease (CKD), which is characterized by a gradual loss of kidney function over time. CKD can eventually progress to end-stage renal disease (ESRD), which requires dialysis or a kidney transplant for survival. Other types of renal disease include glomerulonephritis, pyelonephritis, and nephrotic syndrome.

There is substantial evidence to suggest that the ACE I/D polymorphism is associated with renal disease. Several studies have shown that individuals with the DD genotype (i.e. homozygous for the ACE deletion allele) have an increased risk of developing CKD and ESRD, as well as a faster progression of disease (Shanmuganathan R et al, 2015; Solini A et al, 2002; Fawwaz S et al, 2017). For example, a meta-analysis of 68 studies involving 40,908 patients found that individuals with the DD genotype had a 50% increased risk of developing ESRD compared to those with the II genotype (i.e.

homozygous for the ACE insertion allele) (Kato et al., 2008).

The mechanism by which the ACE I/D polymorphism affects renal disease is not entirely clear, but it is thought to involve alterations in the RAAS system. The RAAS system is a complex network of hormones and enzymes that regulate blood pressure and sodium balance. The ACE enzyme is responsible for converting angiotensin I to angiotensin II, which is a potent vasoconstrictor that increases blood pressure. Angiotensin II also promotes the release of aldosterone, which increases sodium reabsorption in the kidneys and further raises blood pressure.

Several studies have shown that individuals with the DD genotype have higher levels of ACE activity and angiotensin II production compared to those with the II genotype (Kim DK et al, 1997). This may lead to increased vasoconstriction and sodium retention, which can contribute to the development and progression of renal disease. In addition, angiotensin II has been shown to promote inflammation and fibrosis in the kidneys, which can further damage renal function.

Overall, the association between the ACE I/D polymorphism and renal disease is complex and multifactorial. Other genetic and environmental factors may also play a role in the development and progression of renal disease. However, the evidence suggests that individuals with the DD genotype have an increased risk of developing renal disease and may benefit from closer monitoring or earlier intervention to prevent disease progression.

In conclusion, the ACE I/D polymorphism is a common genetic variant that is associated with a variety of diseases, including renal disease. The DD genotype is associated with an increased risk of developing CKD and ESRD, as well as a faster progression of disease. The mechanism by which this occurs is thought to involve alterations in the RAAS system, leading to increased vasoconstriction, sodium retention, and inflammation/fibrosis in the kidneys. Further research is needed to better understand the molecular mechanisms and clinical implications of this association.

Conclusion

In conclusion, the ACE gene polymorphism has been extensively studied for its association with various human diseases, including cardiovascular diseases, hypertension, diabetes, and renal diseases. The DD genotype has been consistently associated with an increased risk of several diseases, including CAD, MI, LVH, hypertension, type 2 diabetes, and renal diseases. The mechanism underlying these

associations remains unclear and may involve altered RAAS activity, increased oxidative stress, and endothelial dysfunction. Further studies are needed to explore the clinical utility of ACE genotype testing for risk stratification and personalized therapy.

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श्रद्धावान लभते ज्ञानम्

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A review on solvent promoted preparation of mononuclear and dinuclear vanadium complexes having hydrazone moiety

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Abstract: A quick review on the chemistry of vanadium complexes with hydrazone functionalised moiety is reported herein inspired by our recent findings of the mononuclear oxidovanadium(V) complexes of type $[V^{V}O(L^{1-2})(OMe)(MeOH)]$ and dinuclear μ-oxidodioxidodivanadium(V) complexes of type $(L^{1-2})(O)V^{V}-O-V^{V}(O)(L^{1-2})$ where L^1 and L^2 are the dianionic forms of the conjugated keto-imine functionalized substituted hydrazone ligands. Relevant researches on various other mono and dinuclear oxovanadium complexes have been discussed briefly with respect to structure and coordination environment. A general solvent dependent synthetic strategy is established thereof for facile preparation of the corresponding mononuclear and dinuclear bridge complexes.

Keywords: Vanadium, Aroylhydrazone, Solvent dependence, X-ray Structure

Introduction

Vanadium was first discovered by Andres Manuel del Rio in 1801 by analyzing a new lead-bearing mineral called ‘brown lead’ and he named the new element erythronium (Greek for ‘red’) as most of

Its salts turned red upon heating. Nils Gabriel Sefstromre discovered the element in 1831 and he named it vanadium after the Scandinavian goddess of beauty and fertility Vanadis. It is a soft, ductile and greyish silvery metal and found naturally in soil and sea water as trace metal. Vanadium has multiple biological roles, its coordination chemistry has provided an impetus in a variety of catalytic processes, [1-4] biochemical processes such as peroxidase mimicking activity [5], insulin mimicking activities, [6] cytotoxic activities, [7] nitrogen fixation, [8]haloperoxidation, [9], epoxidation[10], inhibition of phosphate-metabolizing enzymes [11], alleviation of diabetes mellitus symptoms [12] and so on.

On the other hand, nitrogen-oxygen donor ligands, aroylhydrazones are a class of azomethines having the group –C=N−N– and are widely employed as ligands in coordination chemistry. These ligands are readily available and depending on the nature of the starting materials employed for their preparation, they can exhibit versatile denticities and functionalities. These types of ligands play crucial part in determining the range of applications due to easily tunable electronic properties, conformational diversity [13], widespread biological applicability [14], in determining the overall charge of the metal complexes and therefore their stability depending on factors like oxidation state of the metal ions, reaction conditions, nature of the substituents on hydrazone skeleton and tautomerisation. [15, 16]

The objectives of present review includes

- Syntheses of vanadium metal chelates using the aroylhydrazones as principal ligands.
- To study the coordination modes of different aroylhydrazones in vanadium metal chelates by using different solvent system.
- Establish the structural diversity and coordination environment of the compounds

Coordination diversity of hydrazones

A quick survey of the general structure of a substituted hydrazone reveals that it has (i) nucleophilic imine and amino-type nitrogens, (ii) an imine carbon that has both electrophilic and nucleophilic character, (iii) configurationally isomerism arising out of the intrinsic nature of the C=N bond and (iv) an acidic N–H proton for most of the cases (**Figure 1**). The coordination mode adopted by a hydrazone depends on different factors like amido-iminol tautomerism, reaction conditions, stability of the complex formed, number and nature of the substituents on hydrazone skeleton. Therefore, they can coordinate in neutral, mono-anionic, di-anionic or tetra-anionic form bearing

unusual coordination numbers such as six and seven in some mononuclear or binuclear species. The amido-iminol equilibrium (**Figure 2**) depends on the nature of the substituents present in the hydrazide moiety, pH of the medium used, solvent system, and the metal salts employed.

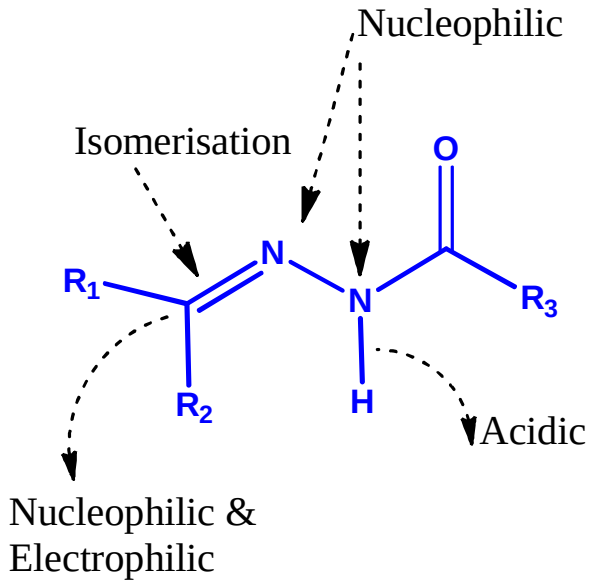


Figure 1. Structural and functional diversity of the hydrazone entity

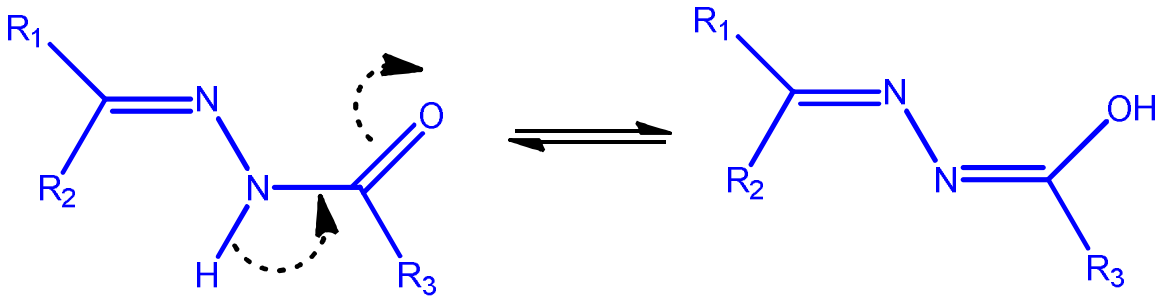


Figure 2. Tautomerism in aroylhydrazones.

Generally, in basic solution amide oxygen gets deprotonated and coordinates to the metal centre in the iminolate form where as strongly acidic condition favors compounds formulated with neutral mode. Here are some examples[16] depicted in **Chart I**in which hydrazones have ligated in different ways. In Structure **I**, bi dentate mode with the carbonyl oxygen and the azomethine **N** as possible donor sites. When the heteroatom in the substituent hydrazones coordinate to the metal centre, they can behave as a tridentate ligand.

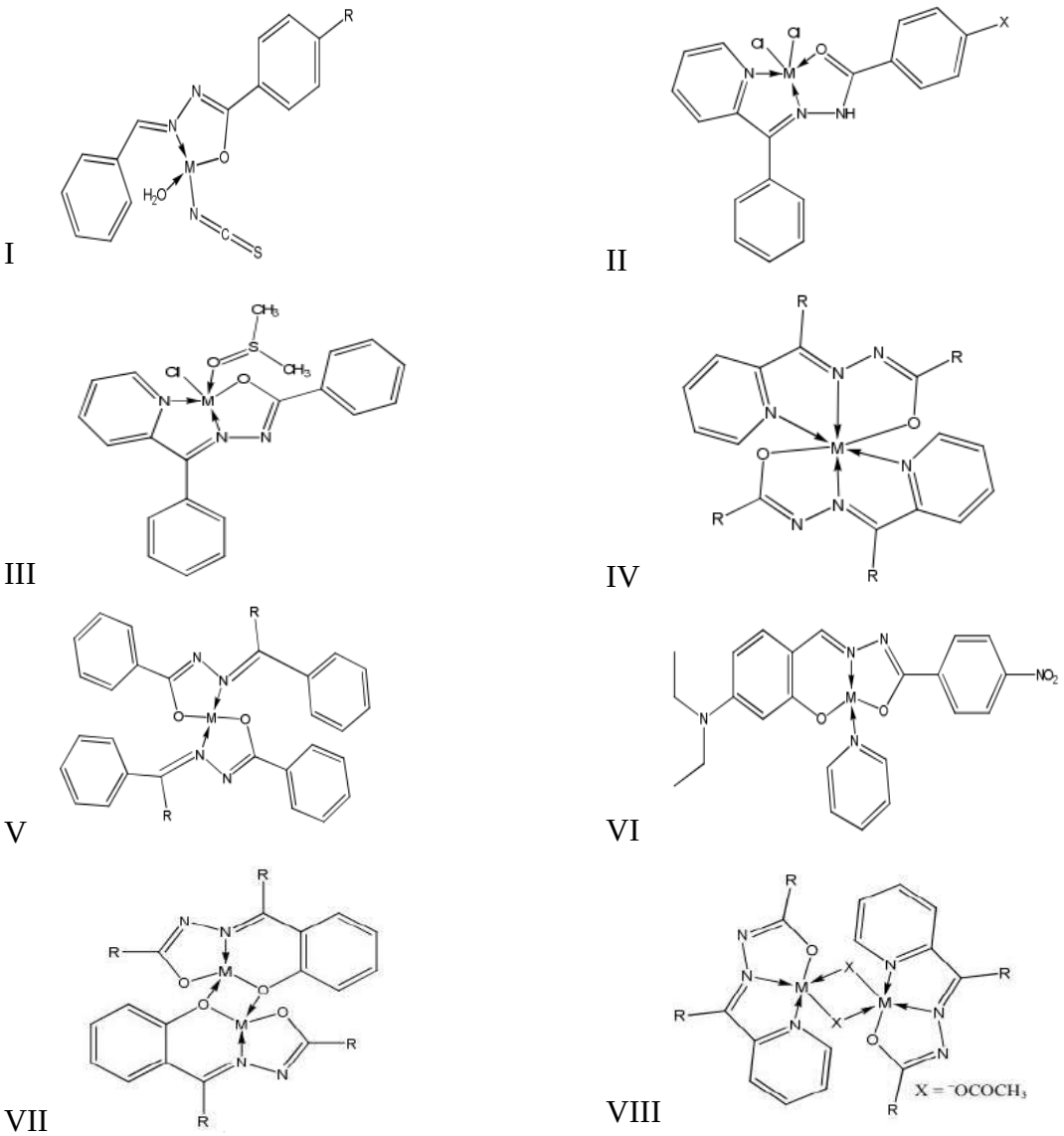


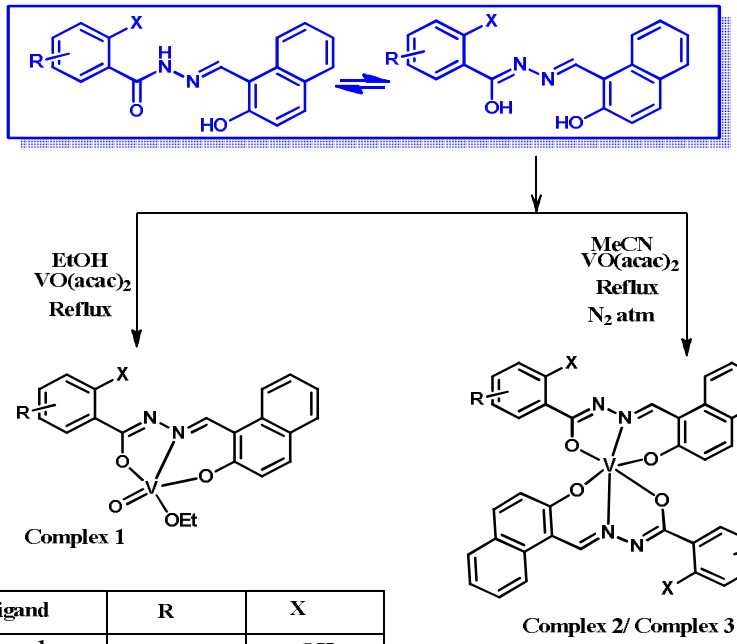
Chart I Possible binding modes of some aroyl hydrazones ligands.

If we take a hydrazone having a pyridine group on the carbonyl part, it can coordinate to the central metal by adopting an NNO coordination mode, either through neutral amido form (Structure **II**) or through deprotonated iminolate form (Structure **III**). The lengths of the N–C and C=O bonds are very important indicators of the mode of coordination. Structure **IV-VI** exhibit interesting coordination modes with transition metal ions to form octahedral, square planar and tetrahedral complexes. The presence of phenolate oxygen in the hydrazine group can form a bridge between the metal centers and thus can form dimeric complexes (Structure **VII**). Bridging ligands like acetate ions, dicyanamide, azide, etc. can form a bridge between

the metal centers and thus can form dimeric complexes (Structure **VIII**).

Some preview of solvent dependent syntheses of vanadium-hydrazone complexes

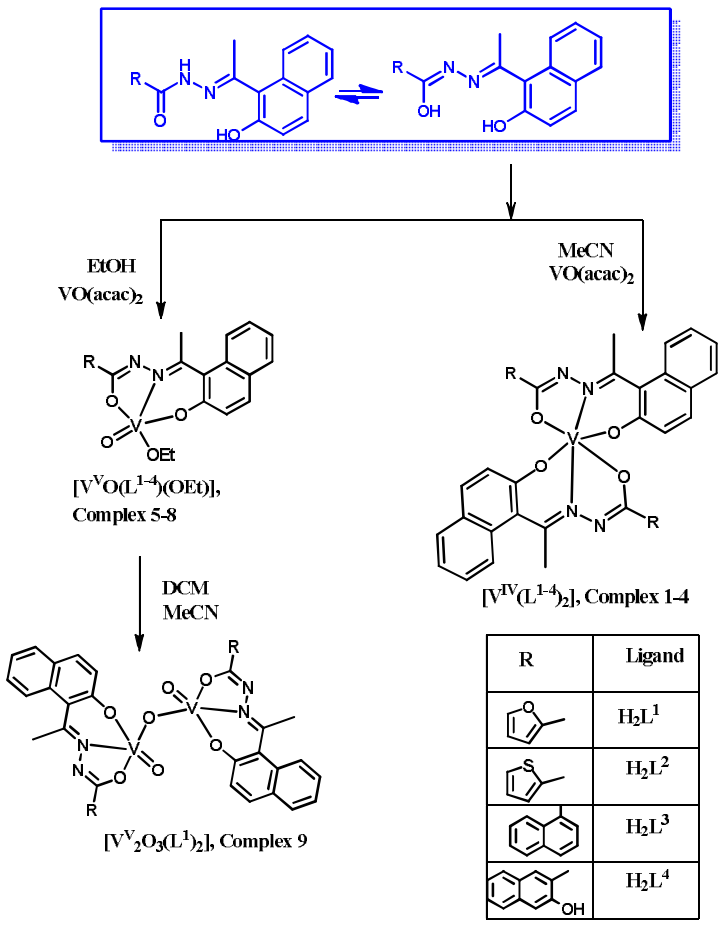
Case I-Three aroylhydrazones H_2L^{1-3} and the corresponding vanadium complexes, one oxido$[V^{V}O(L^{1})(OEt)]$ (**1**) with ligand $H_2L^{1}$and two monoxido compounds, $[V^{IV}(L^{2-3})_2]$ (**2** and **3**) with ligands H_2L^{2-3} were prepared. The aroylhydrazone Schiff base compounds, were synthesized by the condensation of 2-hydroxy-1-naphthaldehyde with their respective acid hydrazides (where H_2L^{1} = 3-hydroxy-2-naphthoichydrazide, H_2L^{2} = salicyloylhydrazide, and H_2L^{3} = anthranilic acid hydrazide) in equimolar proportions in ethanol [17]. To Synthesis complex **1**, the metal precursor $[V^{IV}O(acac)_2]$ was added to a hot ethanolic solution of H_2L^{1} in equal stoichiometric ratio and refluxed for 3 h. For complex **2** and **3**, ligand precursors (H_2L^{2} or H_2L^{3}) were added to a hot acetonitrile solution of $[V^{IV}O(acac)_2]$ in 2:1 ratio under nitrogen atmosphere. Noteworthy point is that the metal binds to the tautomeric iminolate forms of the hydrazone moiety. Actually, we can safely say that here tautomerisation precedes chelation. Moreover, the nature of solvent as well as the molar ratio of the reacting substances attributes a significantly in final structure. The corresponding synthetic route is given in Scheme **1.**



Ligand	R	X
H_2L^{1}	Ph	OH
H_2L^{2}	------	OH
H_2L^{3}	------	NH_2

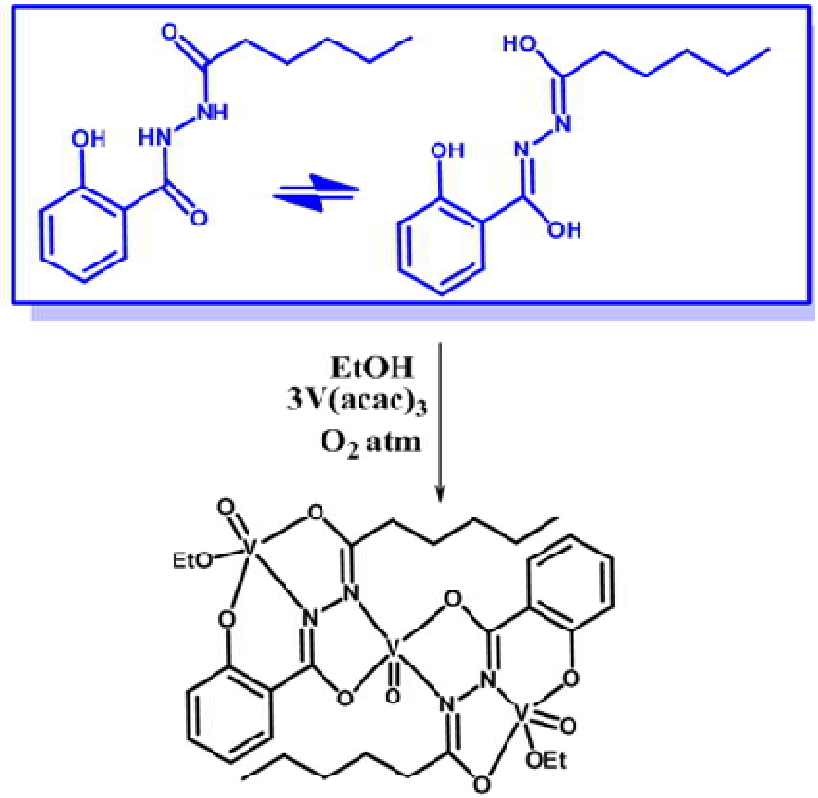
Scheme 1. Schematic Representation of the Synthesis of $[V^{V}O(L^{1})(OEt)]$ (**1**) and $[V^{IV}(L^{2-3})_2]$ (**2**,**3**)

Case II- In this case four Schiff bases, 2-furoylazine of 2-hydroxy-1-acetonaphthone (H_2L^1), 2-thiophenoylazine of 2-hydroxy-1-acetonaphthone (H_2L^2), 1-naphthoylazine of 2- hydroxy-1-acetonaphthone (H_2L^3), and 3-hydroxy-2-naphthoylazine of 2-hydroxy-1-acetonaphthone (H_2L^4) were synthesized by the condensation of 2-hydroxy-1-acetonapthone and the respective acid hydrazide in equimolar ratio in ethanol [18]. The resulting ligands were used to synthesis a series of mononuclear non-oxidovanadium(IV) $[V^{IV}(L^{1-4})_2]$ (**1-4**), oxidoethoxido vanadium(V) $[V^{V}O(L^{1-4})(OEt)]$ (**5-8**),and dinuclear μ oxidodioxidodivanadium(V) $[V^{V}_2O_3(L^1)_2]$ (**9**) complexes.$[VO(acac)_2]$ was added to a hot acetonitrile solution of the ligands H_2L^{1-4} in 1:2 ratio to prepare complexes **1-4**. For complexes **5-8**, it was added in 1:1 ratio to hot ethanolic solution of the ligands. The μ-oxidodioxidodivanadium(V) (**9**) was synthesized by dissolving complex **5** in dichloromethane and layered with acetonitrile solution. Therefore, here three different types of solvents resulted in three different coordination modes around vanadium centre. The corresponding synthetic route is given in Scheme **2.**



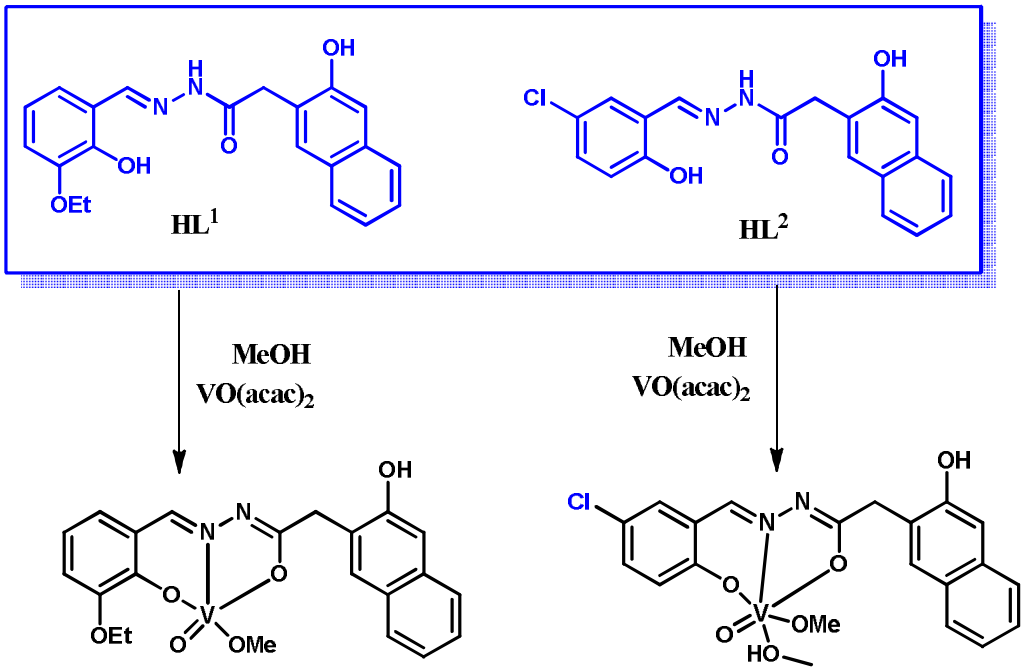
Scheme 2. Schematic Representation of the Synthesis of complexes **1-9**

Case III- An interesting N-hexanoylsalicylhydrazide (H_3hshz) ligand that functioned as a potential trianionic pentadentate chelating ligand ($hshz^{3-}$) yields a linear trinuclear mixed valence vanadium(V/IV/V) complex when reacted with vanadium(III) acetylacetonate in ethanol [19]. Onevanadium(IV) atom is at the center of complex **1**, and is spanned by two terminal vanadium(V) ions bridged via hydrazido ligands. Interestingly here the ligand acts both as a pentadentate chelating ligand as well as bridging ligand at the same time. The corresponding synthetic route is given in Scheme **3**



Scheme 3. Schematic Representation of the Synthesis of $[V_3O_3(hshz)_2(OEt)_2]$, Complex **1**

Case IV- Two ligands HL1 = N'-(2-hydroxy-3-ethoxybenzylidene)-3-hydroxy-2-naphthohydrazide, and HL2 = N'-(2-hydroxy-5- chlorobenzylidene)-3-hydroxy-2-naphthohydrazide) have been used in this case 20 for the synthesis of $[VO(OCH_3)L_1]$ and $[VO(OCH_3)(HOCH_3)L_2]$. Herea methanolic solution of the Schiff base was added with stirring to a methanolic solution of $VO(acac)_2$ in equal molar ratio. Here the halo substituted ligand gives free solvent coordinated product. whereas ethoxy substituted ligand gives only mono oxo mono methoxy coordinated product. The corresponding synthetic route is given in Scheme 4



Scheme 4. Schematic Representation of the Synthesis of $[VO(OCH_3)L^1]$ and $[VO(OCH_3)(HOCH_3)L^2]$.

Case IV (Our Recent study):

Inspired by the above fact, we have synthesized two mononuclear oxidovanadium(V) complexes type of $[V^VO(L^1)(OMe)(MeOH)]$ (**1**), $[V^VO(L^2)(OMe)(MeOH)]$ (**2**) and two $[V_2O^3]^{4+}$ core of μ-oxidodioxidodivanadium(V) complexes $(L^1)(O)V^V–O–V^V(O)(L^1)$ (**3**) and $(L^2)(O)V^V–O–V^V(O)(L^2)$ (**4**) have been reported where L^1 and L^2 are the dianionic forms of the conjugated keto-imine functionalized substituted hydrazone ligands HL^1 [(E)-N'-(2-hydroxybenzylidene)cinnamohydrazide)] and HL^2 [(2E,N'E)-N'-(2-hydroxybenzylidene)-3-(naphthalen-1-yl)acrylohydrazide].

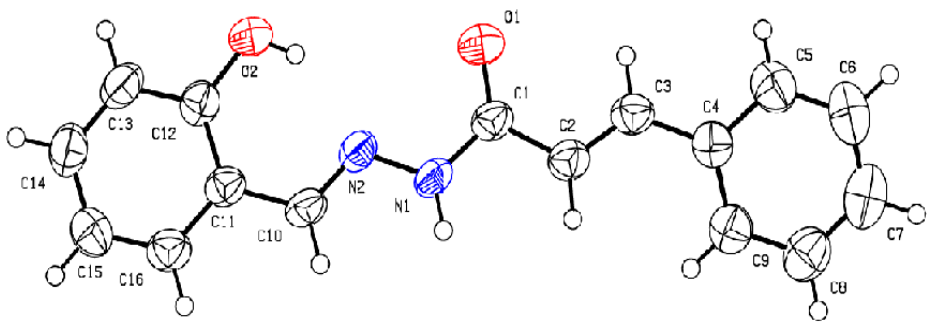


Figure 3. Ortep view of HL^1

The μ-oxidodioxidodivanadium complexes are generated upon switching the solvent from methanol to acetonitrile. The X-ray analysis showed octahedral geometry for the mononuclear complexes **1**, **2** but oxido-bridged dinuclear complexes **3** and **4** formed penta-coordinated square-pyramidal geometry about metal atoms. Solid-state crystal structure of the ligand[21] HL^1 is shown in **Figure 3** and that of the complexes[22] in **Figure 4.** The corresponding synthetic route is given in Scheme **5.**

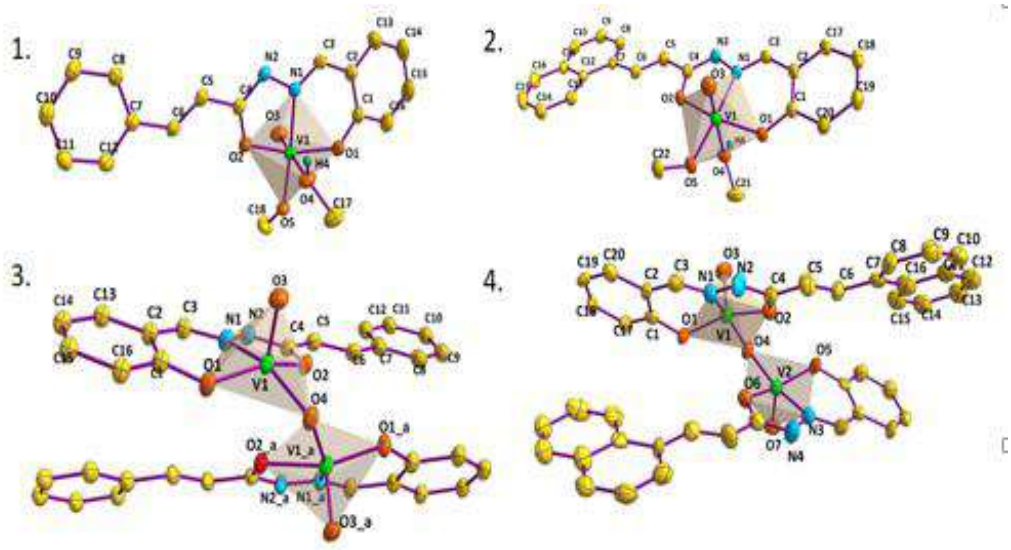
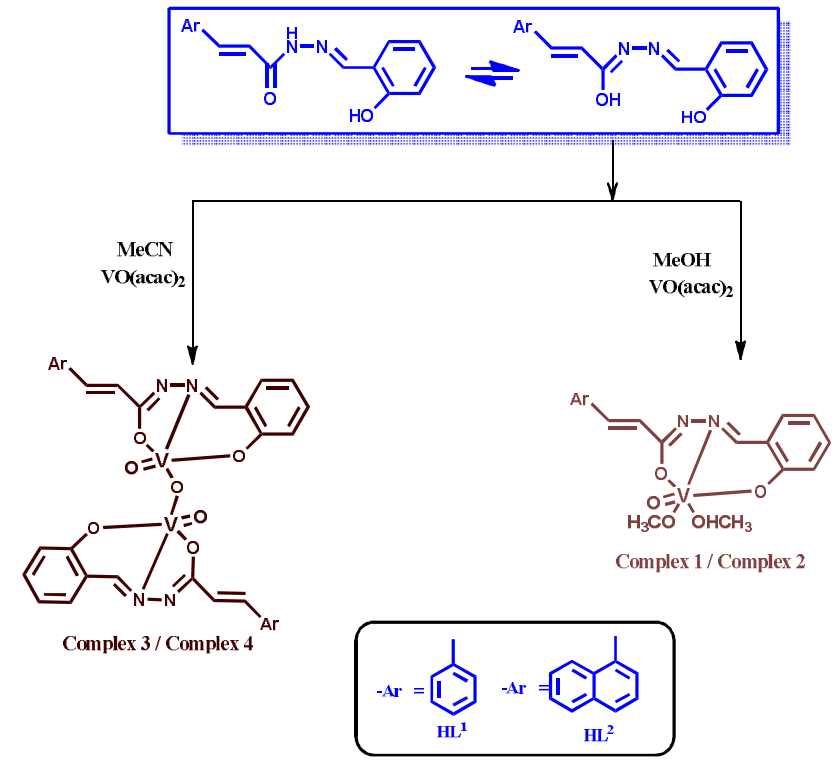


Figure 4. Ortep view of the complexes **1**, **2**, **3** and **4**



Scheme 5. Schematic Representation of the synthesis of complexes **1-4**

Conclusion:

In conclusion, it can be well said that, various factors like stoichiometric ratio of the reactants, reaction conditions and medium of reaction attributed the coordination environment of the vanadium centre. Most importantly, the appropiate choice of solvent plays a pivotal role in determining the nature of the complexes. Consequently it effects the oxidation state of the metal centre. Through out the review we have experienced that polar protic solvent like methanol or ethanol always gives the solvent coordinated product whereas aprotic solvent like acetonitrile ordichloromethane give mainly non-oxido VL_2 type or dinuclear oxo bridge product with hydrazone ligand. The later types of products sometimes generate valence tautomerism depending on the elctronic structure and environment of ligand moiety.

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Conflicts of Interest

There are no conflicts to declare.

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