IJBPAS, March, 2023, 12(3): 1076-1088

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

MICROWAVE ASSISTED SYNTHESIS OF CARBAZOLE MANNICH BASES AND NATURAL CARBAZOLE ALKALOIDS FROM *MURRAYA KOENIGII LEAVES:* AN *INSILICO* AND EXPERIMENTAL APPROACH

SINDHU.T.J*, SOUMYA P.V, ARATHI.K.N, ANUSHA.N.D, DIGI DAVIS.C, JASNA VARGHESE AND SREEJA.K

Sanjo College of Pharmaceutical Studies, Vellapara, Kuzhalmannam, Palakkad, Kerala-678702, India

***Corresponding Author: Sindhu.T.J: E Mail: sindhutj81@gmail.com**

Received 20th April 2022; Revised 15th May 2022; Accepted 13th Aug. 2022; Available online 1st March 2023

https://doi.org/10.31032/IJBPAS/2023/12.3.6925

ABSTRACT

The spread of drug resistant bacteria has badly affected the efficiency of many known antibacterial agents. Thus, the need of new antibacterial drugs is increased. Plant based drugs are gaining more importance because herbal drugs are considered safe and dependable. Comparison between antibacterial activity of synthetic and natural carbazole derivatives were carried out. Microwave assisted synthesis of synthetic carbazole derivatives and Soxhlet extraction of carbazole alkaloid from *Murraya koenigii* were carried out. Binding scores obtained from molecular docking studies, zone of inhibition from the experimental antibacterial study are used as the parameters for comparison.

Keywords: Docking, N-mannich base of carbazole, carbazole alkaloid, *Murraya koenigii, Zone of inhibition, Binding energy*

INTRODUCTION

Drugs are chemicals that prevent disease or assist in restoring health to diseased individual. In ancient time most of the drug used in the treatment of disease were derived from naturally occurring substance of the plant origin. Example: opium from poppy, quinine from cinchona, digitalis from foxgloves. Presently majority of new therapeutic agents are synthetic in nature. Antibiotic resistance is one of the leading public health concerns of this century **[1]**. This is particularly worrisome in the case of gram-negative bacteria for which treatment is already limited **[2]**. The spread of drug resistant bacteria has badly affected the efficiency of many known antibacterial agents **[3]**. This clearly highlights the urgent need for new and improved

antibacterial drugs with a novel target and new molecular structure agent to obviate resistance **[4]**. Interest in natural product as drug leads is being revitalized, particularly for tackling antimicrobial resistance **[5]**. Drug discovery can be described as the process of identifying chemical molecules that has the potential to become therapeutic agent. Drug discovery is a tedious process which is expensive and time consuming. Drug development is a process of bringing a new chemical molecule to the clinical practice **[6]**. Drugs have often been discovered starting from endogenous ligands or natural products followed by chemical modifications to improve in vivo efficacy **[7]**. Computer assisted drug design also called computer assisted molecular design represents more recent application of computer as a tool in the drug design process. It is important to emphasize that computer cannot substitute for a clear understanding of the system being studied. That is, computer is only an additional tool to gain better insight into the chemistry and biology of the problem at hand **[8]**. Molecular docking has become an important tool for drug discovery **[9]**. Docking enables the identification of novel compounds of therapeutic interest via predicting ligand-target interaction **[10]**. The organic compounds specifically N-heterocyclic compounds exhibit various pharmacological activities through effective binding to enzyme receptor site **[11]**. Carbazole skeleton is the key structural motif of many biologically active compounds including synthetic and natural product **[12]**. Carbazole and its derivatives are nitrogen containing aromatic heterocyclic compound that are found in nature or synthesized chemically **[13]**. Literature review revealed that tetrahydrocarbazole having the nitrogen atom and rigid aromatic moiety helps to the electronic transfer in the π-conjugated system possess the wide range of pharmacological activity **[14]**. Most carbazole alkaloids have been isolated from the taxonomically related higher plants of the genus Murraya, Glycomis, and Clausena from the family Rutaceae [15]. *Murraya koenigii* (Linn.) Spreng. (Family: Rutaceae) commonly called curry leaves occur throughout India **[16]**. *Murraya koenigii*is one the plant species with potential medicinal properties **[17]**. *Murraya koenigii* is a rich source of carbazole alkaloid **[18]**. The plant has been reported to exhibit antimicrobial, antitumor, antiepileptic, antioxidative, anti-inflammatory, anti-diarrheal, antihistaminic, analgesics properties **[19]**. Today there is a wide spread interest in drugs derived from plant **[20]**, herbal drugs symbolize safety, in contrast to the synthetic that are regarded as unsafe to

humans and the environment **[21]**. In our study the potential of synthetic and natural carbazole derivatives to become antibacterial agent was studied through insilico approach and experimental studies. For this purpose, microwave assisted synthesis of N-mannich bases of 1,2,3,4-tetrahydrocarbazoles (synthetic carbazole derivatives) and chloroform extraction of carbazole alkaloid from *Murraya koenigii* were carried out. The antibacterial activity of synthetic and natural carbazole derivatives were evaluated and compared.

The aim of the present study includes

- Microwave assisted synthesis of mannich bases from 1,2,3,4-tetrahydro carbazole
- Soxhlet extraction of carbazole alkaloids from curry leaf
- Study of antibacterial activity through *in-silico* approach
- Evaluation of antibacterial activity through experimental method.

MATERIALS AND METHODS

PHARMACOGNOSTIC STUDY OF CURRY LEAF

Biological source: *Murraya koenigii* (Linn.) Spreng **[22]**

Family: Rutaceae **[22]**

Taxonomical status [23]

Kingdom: Plantae

Subkingdom: Tracheobionta

Super division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliospida

Sub class: Rosidae

Order: Sapindales

Family: Rutaceae

Genus: Murraya

Species: *Murraya koenigii*L. Spreng

Description

Murraya koenigii is semi deciduous, unarmed aromatic small spreading shrub or tree with strong woody stem but slender with the stem which is darker green to brownish in color. The tree is 4-8.7m tall, with a trunk up to 81 cm diameter. The diameter of main stem is about 16cm.Leaves are aromatic in nature. Leaves are pinnate, exstipulate, having reticulate venation and having ovate lanceolate with an oblique base, with 11-21 leaflets whose size description is each leaflet is 0.79-1.57 inch long and 0.39-0.79-inch broad **[24]**.

Habitat profile of curry leaf

Figure 1: Habitat profile of curry leaf, Location: Alappad, Kerala, India

Microscopy of curry leaf

Figure 2: Microscopy of curry leaf

Powder microscopy of curry leaf

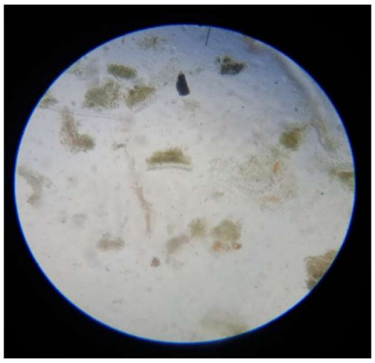


Figure 3: Powder microscopy of curry leaf

PREPARATION OF *Murraya koenigii* EXTRACT

Collection of curry leaf

Curry leaves were collected from the local houses of Alappad village in Kerala. These were washed thoroughly in order to remove the dirt and dust **[25]**. Then it was dried in the shade to protect leaves from losing the medicinal properties. The dried curry leaves were pulverized with mixer grinder. The powdered leaves were used for the study.

Soxhlet extraction

Requirements: RB flask, heating mantle, thimble, condenser, Soxhlet apparatus, dried curry leaf powder, chloroform.

Procedure

Curry leaves powder is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet apparatus is positioned into the flask containing the chloroform. The Soxhlet is then fitted with a condenser. The solvent is heated to reflux. The solvent vapor travels up a distillation tube and flood into the chamber housing the thimble holding the sample. The condenser ensures that solvent vapors condenses and drip back down into the chamber housing the sample. The chamber containing the sample slowly fills with warm solvent. When the Soxhlet sample is

almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. The cycle is allowed to repeat many times **[26]**. The content in flask is then concentrated to get the extract.

PREPARATION OF 1,2,3,4 –TETRA HYDRO CARBAZOLE

Take 4ml of cyclohexanone in RB flask and add 12ml of glacial acetic acid to it. Reflux with frequent shaking. Add 4g of phenyl hydrazine slowly to the RB flask. Heat for 5min under reflux. Cool with vigorous shaking. Filter the product and wash with cold water **[27]**.

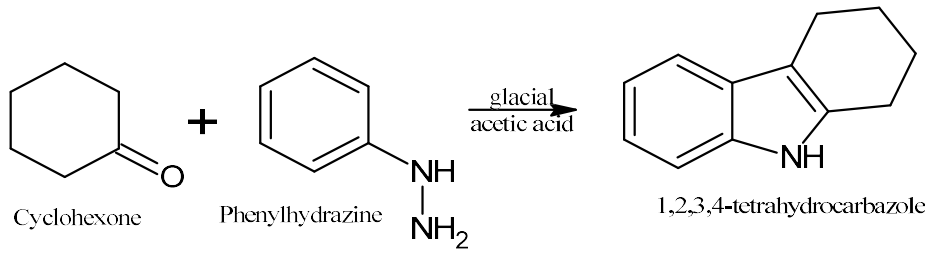


Figure 4: preparation of 1,2,3,4-tetrahydrocarbazole

MICROWAVE ASSISTED SYNTHESIS OF MANNICH BASES FROM 1,2,3,4 – TETRAHYDROCARBAZOLE

1.7 g of 1,2,3,4 - tetrahydro carbazole was taken in a beaker and 6ml of ethanol and 1ml of formaldehyde were added. Appropriate primary or secondary amine was also added to itshown in table 1. Mixture was heated in a microwave at power of 300 watt for 10 seconds. the product is kept overnight in refrigerator and is filtered **[28]**.

Table 1: amines used for preparation and their weight

Amine	Weight taken or volume taken
Primary amine	
Aniline	**0.9ml**
Secondary amines	
Piperazine	**0.86g**
N-methyl piperazine	**1ml**
Diphenylamine	**1ml**

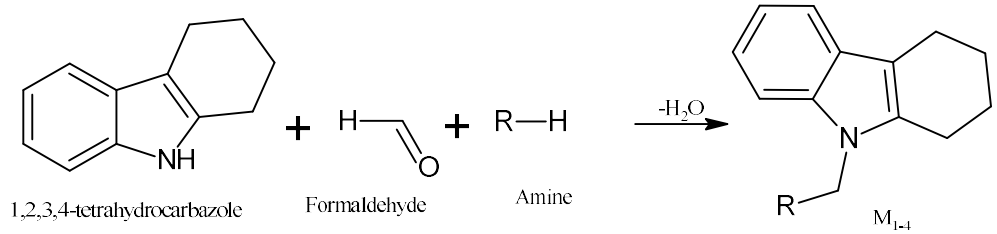
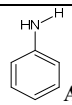
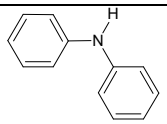
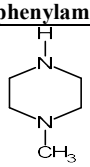
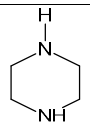


Figure 5: preparation of mannich bases from 1,2,3,4-tetrahydrocarbazole

Table 2: structure of amines used in the preparation

	Amine	Product
R—H	 Aniline	M_1
	 Diphenylamine	M2
	 N-methyl piperazine	M_3
	 Piperazine	M_4

M_1 -N-Mannich base of aniline
M_2 -N-Mannich base of diphenylamine
M_3 -N-Mannich base of N-methyl piperazine
M_4 -N-Mannich base of piperazine

IN – SILICO STUDIES

Software and databases used

- ❖Auto Dock 4.2 combines
- ▪ AutodockTools1.5.4
- ▪ Python Molecule viewer1.5.4
- ▪ Vision 1.5.4
- ❖ Python 2.5
- ❖ Accelrys discovery studio viewer
- ❖ Pre ADMET software
- ❖ Molinspiration server
- ❖ RCSB protein data bank
- ❖ Online SMILES translator

All *in-silico* studies were carried out at Sanjo college of pharmaceutical studies, Vellapara, Palakkad.

DOCKING STUDIES OF MANNICH BASES FROM 1,2,3,4-TETRAHYDROCARBAZOLEAND CARBAZOLE ALKALOID FROM CURRY LEAF

Enzyme preparation

Enzyme: Glutamate racemase of *Escherichia coli*

PDB ID: 2JFN

Resolution :1.90 A^0

Sequence length:285

Chain A

X -ray crystalline structure of protein was downloaded from protein data bank (www.rcsb.pdb.org) **[29]** and refined using Biovia discovery studio.

Ligand preparation

Calculation of pharmacokinetic parameters through Molinspiration cheminformatics software

Lipinski's rule of five is commonly used in drug design to predict oral bioavailability of drug candidates **[30]**. Smile notations generated by ACD labs chemsketch software were fed in the molinspiration software for calculation of molecular properties **[31]** (www.molinspiration.com) **[32]** of carbazole derivatives and carbazole alkaloids. Online smile translator was used for preparing ligand in the pdb format.

♦After optimization of lead docking studies were carried out using Autodock4.

ANTIBACTERIAL STUDY OF MANNICH BASES AND PLANT EXTRACT

Chemicals and apparatus required

Nutrient media: Muller-Hinton agar

Sterile swab: non adsorbent cotton

Conical flask, test tubes, petri dishes, micropipettes, Autoclave, Laminar air flow unit, Micro tips.

Antibacterial study was performed in pharmaceutical microbiology laboratory, Sanjo college of pharmaceutical studies, Vellapara, Palakkad

Procedure

Anti-bacterial activity was studied using well agar disc diffusion method. Bacterial growth inhibition was investigated against gram negative bacteria *Escherichia coli*.50mg/ml of samples (M_{1-4}) was prepared using DMF (dimethylformamide). Inoculate the Muller- Hinton agar plate by streaking the swab over the surface. Allow it to dry.Wells are created and samples are applied using micropipette. Plates are incubated at 37^0C for 12- 18hr. DMF is used as negative control and ciprofloxacin is used as positive control. Zone of inhibition was measured and compared with that of standard. Similarly antibacterial activity of plant extract was also performed. The zone of inhibition measured was tabulated.

RESULT AND DISCUSSION

Microwave assisted synthesis of N-mannich base of tetrahydrocarbazole

Microwave assisted synthesis of N-mannich base of tetrahydrocarbazole were carried out and Characterization of synthesized carbazole derivatives is given in **Table 3**.

RESULT OF ANTIBACTERIAL STUDY

The zone of inhibition of synthesized carbazole derivatives and that of carbazole alkaloid from curry leaf are shown in **Figure 6** and the diameters are entered in **Table 4**. The results are compared with standard.

In experimental study of antibacterial activity, all carbazole derivatives (M_{1-4}) and plant extract (carbazole alkaloid) have zone of inhibition lesser than that of the standard ciprofloxacin. Among the synthetic carbazole derivatives N-mannich base of N- methyl piperazine has greater zone of

inhibition. The anti-bacterial activity of plant extract is lesser than that of the synthetic carbazole derivatives. Still, it has the antibacterial activity (zone of inhibition =10mm)

RESULT OF DOCKING STUDIES

Drug likeness score of ligands were computed using Molinspiration software to predict the oral activity and are shown in **Table 5 & 6**.

Docking results of glutamate racemase of E. coli (2JFN) with mannich bases of carbazoles (M_{1-4}) and plant extract are shown in the **Table 7**. The best docked structure should have binding energy lesser than that of standard ciprofloxacin. The binding site of enzyme and ligands are shown in the snap shots given in **Figure 7-12**. The binding energy was compared with that of the standard ligand.

N- mannich base of carbazoles (M_{1-4})and carbazole alkaloids of curryleaf shows good binding interaction with glutamate racemase. N-mannich base of diphenylamine showsleast binding energy (-7.04) than ciprofloxacin (-5.67). All other mannich bases also have least binding energy(M_1=-6.77, M_3=-6.59, M_4=-6.54)) than ciprofloxacin(-5.67). Binding energy of plant extract(-6.06) is also lesser than the ciprofloxacin. Carbazole derivatives and carbazole alkaloids have greater binding interaction with glutamate racemase than standardciprofloxacin. Among them binding interaction of N-mannich base of diphenylamine predominates. Binding energy of plant extract is higher than that of synthetic carbazole derivatives. However it also have antibacterial activity since the binding energy is lesser than that of standard.

Table 3: Characterization of synthesized N-mannich bases from 1,2,3,4-tetrahydrocarbazole

Compound code	Molecular weight	Molecular formula	Practical yield (grams)	Solubility	
				Water	DMF
M_1	276.38	$C_{19}H_{20}N_2$	5.50	insoluble	soluble
M_2	352.48	$C_{25}H_{24}N_2$	1.02	insoluble	Soluble
M_3	283.42	$C_{18}H_{25}N_3$	3.62	insoluble	Soluble
M_4	269.39	$C_{17}H_{23}N_3$	1.96	insoluble	soluble

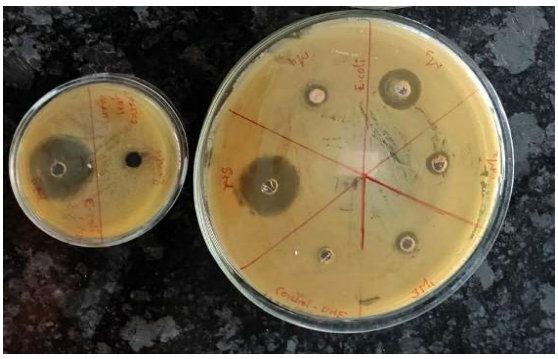


Figure 6: Antibacterial activity of M_{1-4} &C_1 in *Escherichia coli*

Table 4: Zone of inhibition of synthetic carbazole derivatives and plant extract

Compound code	Zone of inhibition diameter (mm)
M_1	11
M_2	12
M_3	21
M_4	11
Plant extract	10
Ciprofloxacin	30
DMF	8

Table 5: Drug likeness score of M_{1-4} using molinspiration server

S. No.	Compound code	mi Log P	Molecular weight	No. of H bond acceptor	No. of H bond donor	No. of violations
1	M_1	4.87	276.38	2	1	0
2	M_2	6.82	352.48	2	0	1
3	M_3	3.31	283.42	3	0	0
4	M_4	2.72	269.39	3	1	0

Table 6: Drug likeness score of carbazole alkaloid in Murraya koenigii

S. No.	Compound code	mi Log p	Molecular weight	No. of H bond acceptor	No. of H bond donor	No. of violations
1	C_1	2.40	241.25	4	1	0

C_1 -Carbazole alkaloid 1 - Koenigine Quinone A - 7-methoxy-3-methylcarbazole-1,4-quinone

Table 7: Binding energy of M_{1-4} and C_1 with 2JFN

Compound code	Binding energy (Kcal/mol)	Hydrogen bond
M_1	-6.77	0
M_2	-7.04	0
M_3	-6.59	1
M_4	-6.54	1
Plant extract	-6.06	1
Ciprofloxacin	-5.67	0

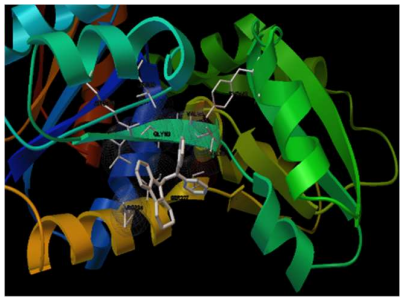


Figure 7: Binding interaction of 2JFN with M_1

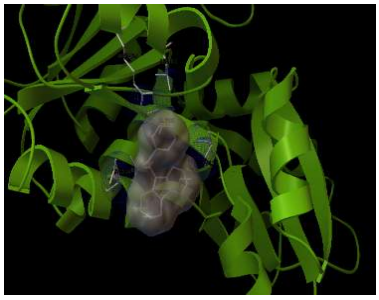


Figure 8: Binding interaction of 2JFN with M_2

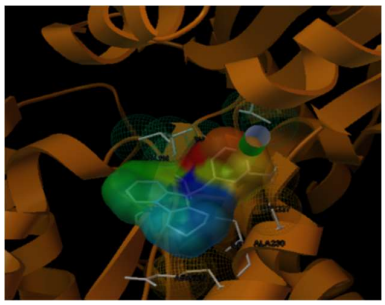


Figure 9: Binding interaction of 2JFN with M_3

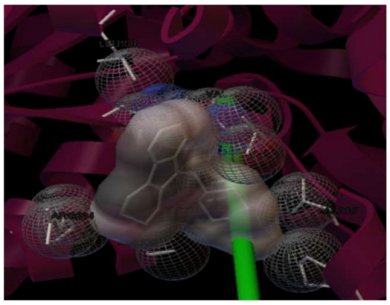


Figure 10: Binding interaction of 2JFN with M_4

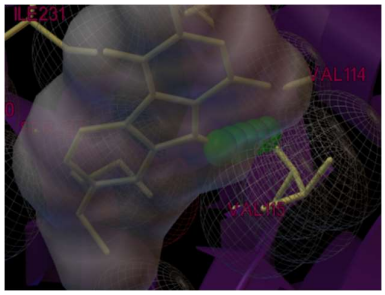


Figure 11: Binding interaction of 2JFN with C_1

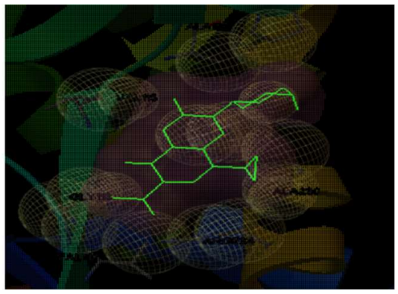


Figure 12: Binding interaction of 2JFN with ciprofloxacin

CONCLUSION

Computer aided drug design helped to minimize the complications accociated with traditional drug design process. Drug likeness score of ligand observed through molinspiration sofware helped to predict the pharmacokinetic activity of the drug. Microwave assisted synthesis were carried out for the preparation of carbazole derivatives and it make the preparation easier than the reflux method. Antibacterial activity of synthetic carbazole derivatives and that of carbazole alkaloid from the plant extract was confirmed by insilico (docking studies) and experimental met(agar disc diffusion method). Comparison of anti bacterial activity between natural and synthetic derivatives were carried out using parameters such as binding scores and zone of inhibition obtained from present study. In silico study proved that the both the synthetic and natural derivatives have the potential to become antibacterial agent. Synthetic derivatives shows more antibacterial activity than natural ones. In future, isolation of natural carbazole alkloid from ***Murraya koenigii*** will become more effective and we can avoid adverse reactions.

REFERENCES

[1] Maria Barbara Pisano, Amit Kumar, Rosaria Medda, Gianluca Gatto, Rajesh Pal, Antonella Fais, Benedetta Era, Sofia Cosentino, Eugenio Uriarte, Lourdes Santana, Francesca Pintus and Maria Joao Matos, Antibacterial activity and molecular docking studies of a selected series of Hydroxy-3-arylcoumarins, molecules 2019,24,2815

[2] Nicole Jackson, Lloyd Czaplewski, Laura J V Piddock, Discovery and development of new antibacterial drugs: learning from experience, Journal of antimicrobial chemotherapy, volume73, issue 6, June2018, pages 1452-1459

[3] Maryam Bashir, Afifa Bano, Abdul Subhan Ijaz and Bashir Ahmad Chaudhary, Recent developments and biological activities of N-substituted carbazole derivatives: A

review, Molecules 2015, 20,13496-13517

[4] Tafere Mulaw Belete , Novel target to develop new antibacterial agent and novel alternatives to antibacterial agent, Human Microbiome journal, volume 11, March 2019, 100052

[5] Atanas G. Atanasov, Sergey B. Zotchev, Verena M. Dirsch and Claudiu T. Supuran, Natural product in drug discovery:advances and opportunities, Nature review drug discovery, march 2021, volume 20, pages 200-216.

[6] Edward A. Sausville, Drug discovery, principles of clinical pharmacology, 3rd edition, 2012

[7] Tobias Wunberg, Martin Hendrix, Alexander Hillisch, Mario Lobell, Heinrich Meier, Carsten Schmeck, Hanno Wild and Berthold Hinzen, Improving the hit -to-lead process: data-driven assessment of drug-like and lead-like screening hits, Drug discovery today, volume 11, number 3/4, February 2006

[8] V K Ahluwalia, Madhu Chopra, Textbook of medicinal chemistry, 2nd edition, page 151.

[9] Xuan-Yu Meng, Hong-Xing Zhang, Mihaly Mezei, Meng Cui, Molecular docking: A powerful approach for structure-based drug discovery, curr comput aided drug des. 2011 June 1;7(2), pages 146-157

[10] Luca Pinzi and Giulio Rastelli, Molecular docking: shifting paradigms in drug discovery, international journal of molecular sciences, 2019, 20, 4331

[11] Kumaraswamy Gullapelli, G. Brahmeshwari, M. Ravichander and Uma Kusuma, Synthesis, antibacterial and molecular docking studies of new benzimidazole derivatives, Egyptian journal of basic and applied sciences 4, 2017, pages 303-309

[12] Agata Gluszynska, Biological potential of carbazole derivatives, European journal of medicinal chemistry 94, 2015, pages 405-426

[13] Kestutis Dabrovolskas, Ilona Jonuskiene, Simona Sutkuviene, Dalius Gudeika, Synthesis and evaluation of antibacterial and antioxidative activities of carbazole derivatives, CHEMIJA, 2020, volume 31, No.1, pages 42-51

[14] Sakinala Padmavathi and Madhukar Rajaram Tajne, Design, synthesis, molecular docking studies and antimicrobial activity of 1,2,3,4-tetrahydrocarbazole

derivatives, International current pharmaceutical journal, August 2016, 5(9), pages 73-78

[15] Hans-Joachim Knolker, Kethiri R Reddy, Chemical reviews, 2002, 102 (11), pages 4303-4428

[16] Manisha Vats, Harneet Singh, Satish Sardana, phytochemical screening and antimicrobial activity of roots of *Murraya koenigii* (Linn.) Spreng. (Rutaceae),Brazilian journal of microbiology, 2011,42, pages 1569-1573

[17] Aju B Y, Rajalekshmi R and Mini S, Pharmacognostical evaluation of curry leaf (Murraya *koenigii)*, Journal of pharmacognosy and phytochemistry,2018,7(6):779-784

[18] Surva Mandal, Anupam Nayak, Samir K. Banerjee, Julie Banerji and Avijit Banerji, A new carbazole alkaloid from *Murraya koenigii* Spreng (Rutaceae), Natural product communication ,2008, volume3, No.10, 1679-1682

[19] Shashank Tiwari, Sherya Talreja, A pharmaceutical importance of *Murraya koenigii*- A complete study, Indian journal of public health research and development, November 2020, volume11, No.11 pages 226-284

[20] R Nair, S Chanda, Activity of some medicinal plants against certain pathogenic bacterial strain, Indian journal of pharmacology, 2006, volume38, issue2, pages 142-144

[21] Gunjan Kumar, M D Jalaluddin, Purnendu Rout, Rajat Mohanty, C L Dileep, Emerging trends of herbal care in dentistry, Journal of clinical and diagnostic research 2013, 7(8), 1827-1829

[22] Satish Chand Saini, Dr. GBS Reddy, Murraya koenigii, IOSR journal of pharmacy and biological sciences,2013, volume 7, issue 6, September-October pages 15-18

[23] Suman Singh, P K Omre and Sandhya Madanmohan, Curry leaves (*Murraya koenigii* L. Sprengal)-A miracle plant, Indian journal of scientific research,2014, 4(1), pages 46-52

[24] Manshu Jain, Ritu Gilhotra, Ravindra Pal Singh, Jitendra Mittal, Curry leaf (*Murraya koenigii*): a spice with medicinal property, MOJ biology and medicine, 2017,2(3), pages 236-256

[25] Dr. Rupali Rajvanshi, Dr. Kusum Mittal, Phytochemical analysis of curry leaves, international journal of science and research,

September 2019, volume8, issue9, pages 366-367

[26] Radheshyam Sharma, Umesh Kumar, Exploration and phytochemical estimation of Murraya Koenigii leaves for pharmaceutical application, Asian journal of pharmaceutical research, 2019, volume9, issue3, 159-168

[27] Sindhu T J, Arathi K N, Akhila Devi, Aswathi T A, Noushida M, Midhun M, Sajil kuttiyil, Synthesis, Molecular docking and antibacterial studies of novel azole derivatives as enoyl ACP reductase inhibitor in *Escherichia coli*, Asian journal of research in pharmaceutical sciences, July-September 2019, 9(3)

[28] Ruddarraju RR , Murugulla AC , Kotla R , Tirumalasetty MB , Wudayagiri R , Donthabakthuni S , et al. Design, synthesis, anticancer activity and docking studies of theophylline containing 1,2,3-triazoles with variant amide derivatives. Med Chem Comm. 2017;8:176–83.

[29] www.rcsb.pdb.org

[30] Asif Husain, Aftab Ahmad, Shah Alam khan, Mohd Ali, Rubina Bhutani, Fahad A Al- Abbasi, synthesis molecular properties, toxicity and biological evaluation of some new substituted imidazolidine derivatives in search of potent anti-inflammatory agents, Saudi pharmaceutical journal 2016, 24, 104-114

[31] Lakshmi Sree Nath, Shah Alam khan, Ayaz Ahmad, Computer aided screening of natural products of lead molecules for design and development of potent anti-inflammatory agents, Scholars academic journal of pharmacy,2014, 3(6), pages 496-503

[32] www.molinspiration.com