IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Evaluation of Antimicrobial Efficacy of *Caralluma adscendens* var. *fimbriata* Extracts: A Molecular Docking Study.

[1]Pushpa Baban Ghogare, [2]R. K. Aher

[1,2]Department of Botany, New Arts, Commerce and Science College, Parner, Dist - Ahmednagar, Maharashtra - 414 302, INDIA.

Abstract: Caralluma adscendens var. fimbriata is a species used as traditional medicines in many parts of India as an anti-diabetic, anticancer, anti-oxidant, anti-inflammatory and etc. The aim of this study was to evaluate the anti-microbial properties of the aqueous extract of the plant and to determine the binding efficiency of the protein-ligand interactions. The study showed antibacterial activity against Gram positive and Gram negative bacteria making it a better chemotherapeutic agent. With reference to the computed study the ligand Sitosterol, Indole, Lupeol, Oleic acid, Palmitic acid, Tomentogenin, Triterpenoids with the proteins of anti- obesity, anti- oxidant, anti-cancer, anti-inflammatory and anti-diabetic shows good affinity making it a "lead compounds" for design and developing of new non-toxic effective drugs for the treatment of cancer, diabetes and obesity. The extracts and its compounds may be used as a safe nutraceutical for the treatment of diseases.

Index Terms **- Antimicrobial activity, Caralluma adscendens var. fimbriata, Protein-Ligands interaction.**

1. INTRODUCTION:

Plants have the healing properties since time immemorial. Around the globe the therapeutic usage of plant originated products is used from generation to generation and various medicines have been developed. The development of micro-organisms resistance against various drugs/antibiotics has resulted in major cause of infectious disease and clinical therapeutic problems. Hence, recent trend, requires understanding and awareness on traditional medicine and the presence of natural bioactive molecules have been drawing much attention inorderto develop alternate antibiotics from various plants. Synthetic antimicrobial drugs are costly, insufficient for the treatment and furthermore produce side impacts. This present circumstance gave the need to the pursuit for new antimicrobial drugs from medicinal plants[10]. The therapeutic properties of plants are due to the presence of phyto-chemicals and bioactive compounds like phenol, flavanoids, alkaloid and tannin that makes the plants acceptable for curing the infectious disease. *Caralluma adscendens var. fimbriata* known as Yugmaphallottatna in Sanskrit is an edible thick succulent cactus and is perennial herb growing abundantly in some villages of India[1] and in dry hill regions of Satara districts of Maharashtra. It belongs to asclepiadaceae family and its local name in Maharashtra is "Makadshenguli/Shengulmakad[9]. *Caralluma* species uses are becoming popular in various tribes of India as the species is used as an appetite suppressant and it has an ability to control weight[2] and lowers blood sugar level[3]. It has been found that the plant has some medicinal properties against cancer[4] rheumatism[1], gastric ulcer[5] and skin infections[6]. The *Caralluma* species found in India are the part of the medicine system of the country. The species are rich in triterpenes, alkaloids, Glycosides, Saponins and Tannins. The crucial phytochemical constituents of the species make them distinctive and diversified are pregenane glycoside, Flavone glycoside, Saponin glycoside, Megastamine glycoside, Sitosterol and Tomentoganin[7]. Extraction is the technique to recover the target compounds of interest by decreasing the co-extraction of undesirable compounds[8].

The traditional drug discovery and design methods are tedious, capital escalated processes and inseparable from a high disappointment rate. Computer based techniques are designed to evaluate the novel active compound having drug potential before laboratory preparation[11]. The computational process that identifies ligand which fits with the active site of the 3D structure of targeted protein to predict the binding interaction and binding energy is called Molecular Docking[12]. The drug hit identification and lead optimization is done by the process of molecular docking (Fig. 1).

However, to the knowledge a little is known about antibacterial activity and molecular docking study of*Caralluma adscendens var. fimbriata*. Hence, in the present study, an effort is made toidentify the antibacterial activities of ethanolic extracts of *Caralluma adscendens var. fimbriata* against *E. coli*, *Pseudomonas*, *Coliform* and *Staphylococcus aureus*.

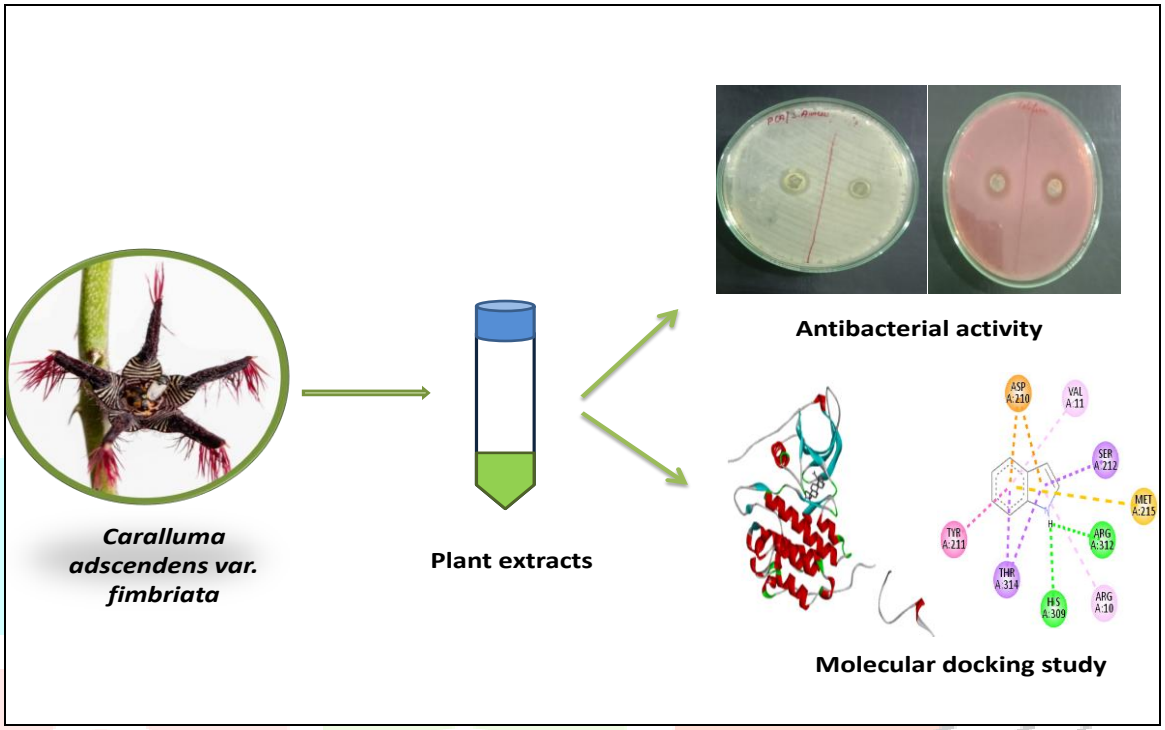


Figure 1: Pictorial representation of experimental work

2. MATERIAL AND METHODS:

2.1 Collection of Plant material

2.2 Preparation of Methanolic and Ethanolic extract:

The powdered plant material extracts were prepared using solvent of high polarity. A portion of 25g of powdered material was extracted using 250ml of Methanol and Ethanol in a Soxhlet apparatus for 24 hours. Consequently, the solvent extract was evaporated to dryness at 44°C in an incubator. The yield of each extract constituents was calculated and stored at 4°C for prior use. The extract was used for assessment of antimicrobial activity.

Figure 2: Ethanolic extraction of *Caralluma*

2.3 Preparation of Plant extracts standard concentration:

100mg/ml of stock was obtained as a standard concentration of ethanolic/methanolic extract (Fig. 2). The extract was sterilized by overnight incubation in an incubator at temperature ~37°C.

2.4 Well diffusion assay of plant extract:

Agar well diffusion method was used for screening the antibacterial activity of ethanolic extract of *Caralluma fimbriata*. Inoculum of each bacterial culture to be tested was spread on agar plates with a sterile swab. Subsequently, wells were punched into the agar medium and filled with 150µl (100mg/ml) of plant extract. The plates were then incubated in the upright position at 37° for 24 h. Two replicates were carried out for the extract against each of the test organism. After the incubation, the wells were observed and the zone of inhibition was noted with the help of measuring scale.

2.5 Disc diffusion assay of standard antibiotics disc:

Standard antibiotic discs of Penicillin, Vancomycin and Ampicillin were used as a control. The antibiotic discs were placed on the plates that were swabbed by particular bacterial culture on agar medium. After incubation for 24hrs, the diameters of the growth inhibition zones were observed and measured with the help of measuring scale in mm.

2.6 Molecular Docking study

The following software were used in this study: The ChemDraw® Ultra 13.0 (Cambridge soft, USA) software was used to draw the various chemical structures, Discovery Studio® 0.4software (Client, USA) was also used to visualize molecular docking, 3D crystal structure models of the targets (receptors) were downloaded from worldwide protein data bank (PDB) (http://www.rcsb.org), PyMOL software for molecular visualization, lastly the 3D structure of the ligands were taken from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

3. RESULTS AND DISCUSSIONS:

3.1 Extract yield

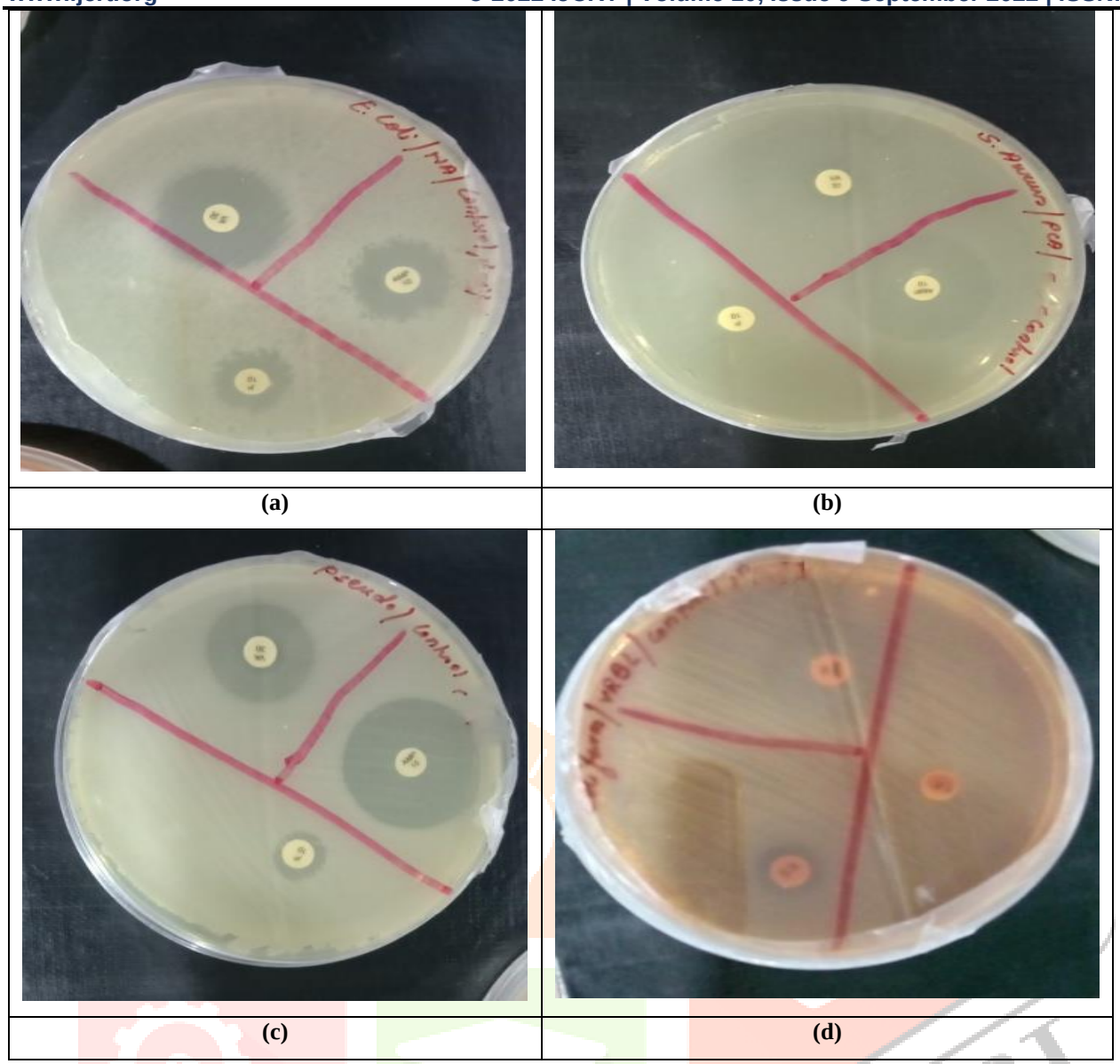
We used ethanol solvent for extraction of compounds from *Caralluma adscendens var. fimbriata* to see the antibacterial activity against various strain of bacteria. The yield percentage of the ethanolic extracts was 32.3%.

3.2 Evaluation of antibacterial activity

By disc method the effectiveness of three antibiotics was determined against the bacterial strains (Fig. 3). Ampicillin have shown highest zone of inhibition (20mm) against *Staphylococcus auereus* and Penicillin showed minimum zone of inhibition against *Coliform*.

Table 1: Showing results for Zone of Inhibition of market available antibiotic disc

S. No.	Bacterial Strains	Zone of inhibition in diameter(mm) of Control		
		Penicillin (10mg)	**Ampicillin** (10mg)	**Vancomycin** (30mg)
1	*Staphylococcus aureus*	6mm	20mm	5mm
2	*Pseudomonas aeruginosa*	6mm	16mm	15mm
3	*Escherichia coli*	8mm	13mm	18mm
4	*Coliform*	1mm	10mm	4mm



(a) **(b)**

(c) **(d)**

a

Figure 3: Showing the result of Inhibition zone of marketed antibiotic disk: a) Inhibition zone of antibiotics against *Escherichia coli.* b) Inhibition zone of antibiotics against *Staphylococcus aureus* c) Inhibition zone of antibiotics against *Pseudomonas aeruginosa.* d) Inhibition zone of antibiotics against *Coliform.*

3.3 Evaluation of plant extracts bioactivity-

In present study the antibacterial activity of the ethanolic extract of *Caralluma fimbriata* was assayed *in- vitro* by agar well diffusion method against 4 bacterial strains (Fig. 4). Among the four bacterial strains*Escherichia coli* have shown highest zone of inhibition in ethanolic extract while *Staphylococcus aureus* and *Pseudomonas aeruginosa* have shown lower zone of inhibition in ethanolic extract compared to *E. coli*. The *coliform* bacterial have shown intermediate result between the above bacterial strains.

Table2: Showing results for zone of inhibition of ethanolicplant extract

S.No.	Sample	Bacterial strains	Zone of inhibition in diameter(mm)
			Ethanolic extract
1	*Caralluma fimbriata*	*Staphylococcus aureus*	13.5mm
2		*Pseudomonas aeruginosa*	13.5mm
3		*Escherichia coli*	18mm
4		*Coliform*	16mm

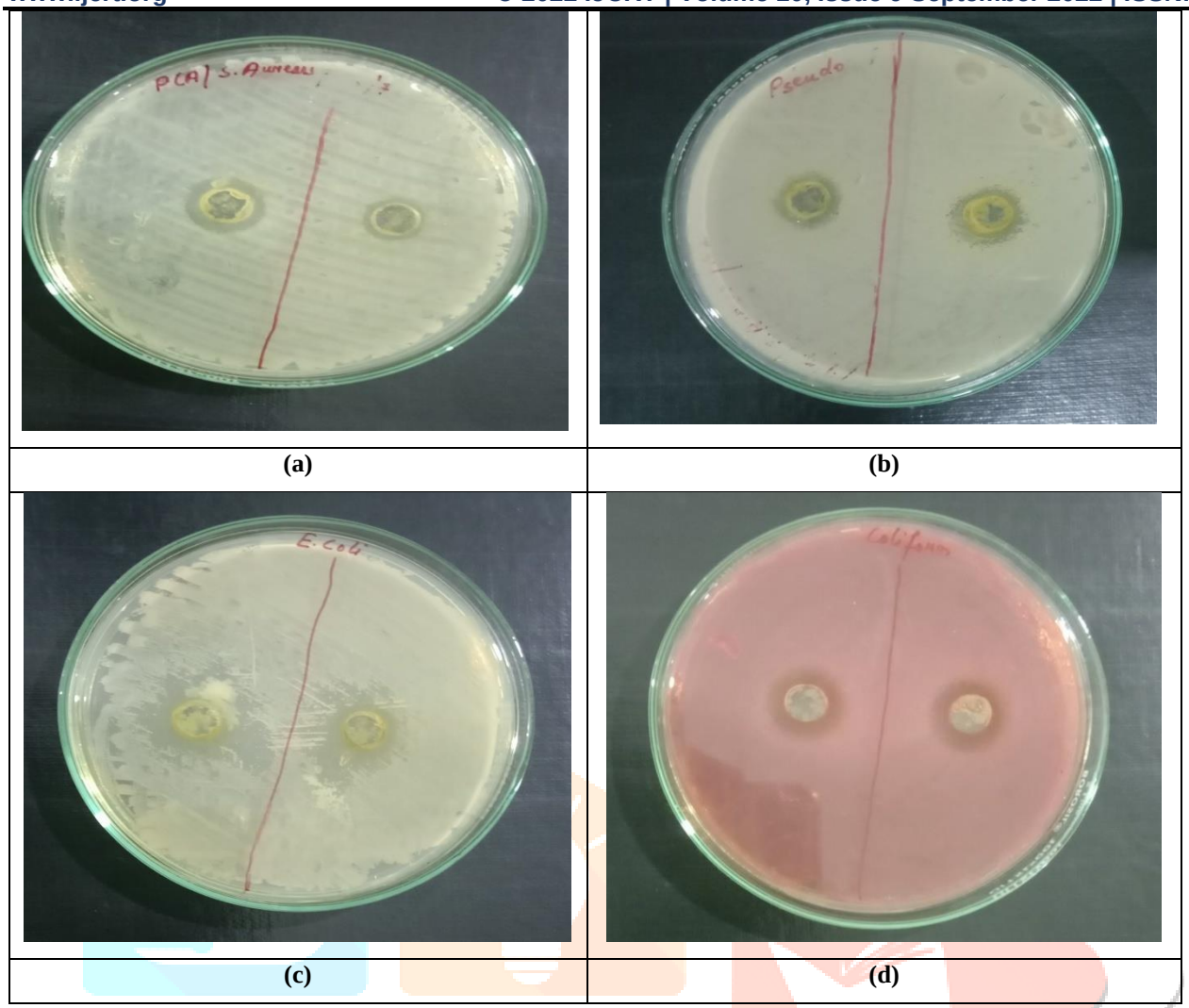


Figure 4: Showing the inhibition zone of Plant extract: a) Zone of inhibition shown by Ethanolic plant extract against *Staphylococcus aureus*, b) Zone of inhibition shown by Ethanolic plant extract against *Pseudomonas aeruginosa*, c) Zone of inhibition shown by Ethanolic plant extract against *Escherichia coli*, d) Zone of inhibition shown by Ethanolic plant extract against *Coliform.*

3.4 Docking result by receptor and ligand complex:

In the present study, selected ligands and macromolecules were evaluated through molecular docking studies using Auto-dock tool. Initially, the structures of these molecules were generated and energy was minimized. The molecular docking interaction was identified based on binding orientation of Placental Lactogen (1F6F), Epidermal Growth Factor Receptor (1M17), Casein Kinase II Subunit Alpha (2OXX), Prostaglandin E Synthase (4YK5), Peroxisome Proliferator Activated Receptor Gamma (2PRG) protein with its binding affinity. In the present study, Molecular docking study has been performed and higher binding energy was observed, which is being reported for the first time.

The Placental lactogen (PDB ID: 1F6F) plays a crucial role in secretion of insulin in β-cells of pancrease and stimulating the promotion of anti-apoptotic proteins expressions. Obesity and diabetes of a person is related to lower and higher level of placental lactogen in serum[13]. Epidermal growth factor receptor tyrosine kinase (EGFRK) (PDB ID: 1M17) is used to study the antitumor activity. EGF may act as an antioxidant by scavenging toxic oxidation products in wound tissue by contributing in the process of healing of wound tissue[14]. CK2 (casein kinase II) (PDB ID: 2OXX) is regulating the various processes in the cell, such as cancer development, cell cycle, apoptosis and transcriptional regulation (Fig. 6). It became the significant target for cancer therapy due to its proliferating and anti-apoptotic properties[15]. The protein Prostaglandin E synthase (PDB ID: 4YK5) have anti- inflammatory properties making it a key mediator of inflammatory pain and fever. The increased expression of microsomal prostaglandin E2 synthase-1 (mPGES-1) indicates increased PGE2 formation[16]. Protein Peroxisome Proliferator Activated Receptor Gamma (PDB ID: 2PRG) shows anti- diabetic property and they are closely involved in the regulation of dietary fat storage and catabolism[17].

The compounds Sitosterol, Indole, Lupeol, Oleic acid, Palmitic acid, Tomentogenin, Triterpenoids were isolated from PubChem database (Fig. 5). The structures of the ligands prepared and used for this study are presented in Table.

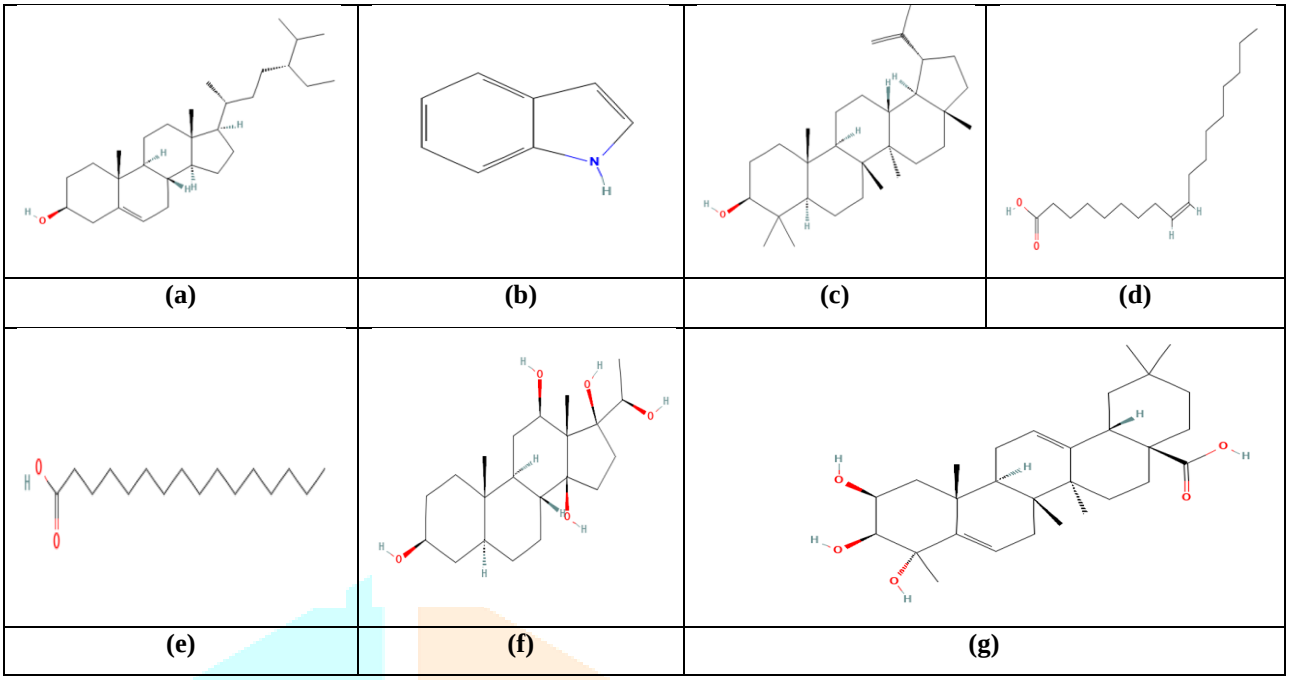


Figure 5: Structures of seven compounds: a) Sitosterol, b) Indole, c) Lupeol, d) Oleic Acid, e) Palmitic Acid, f) Tomentogenin and g) Triterpenoid.

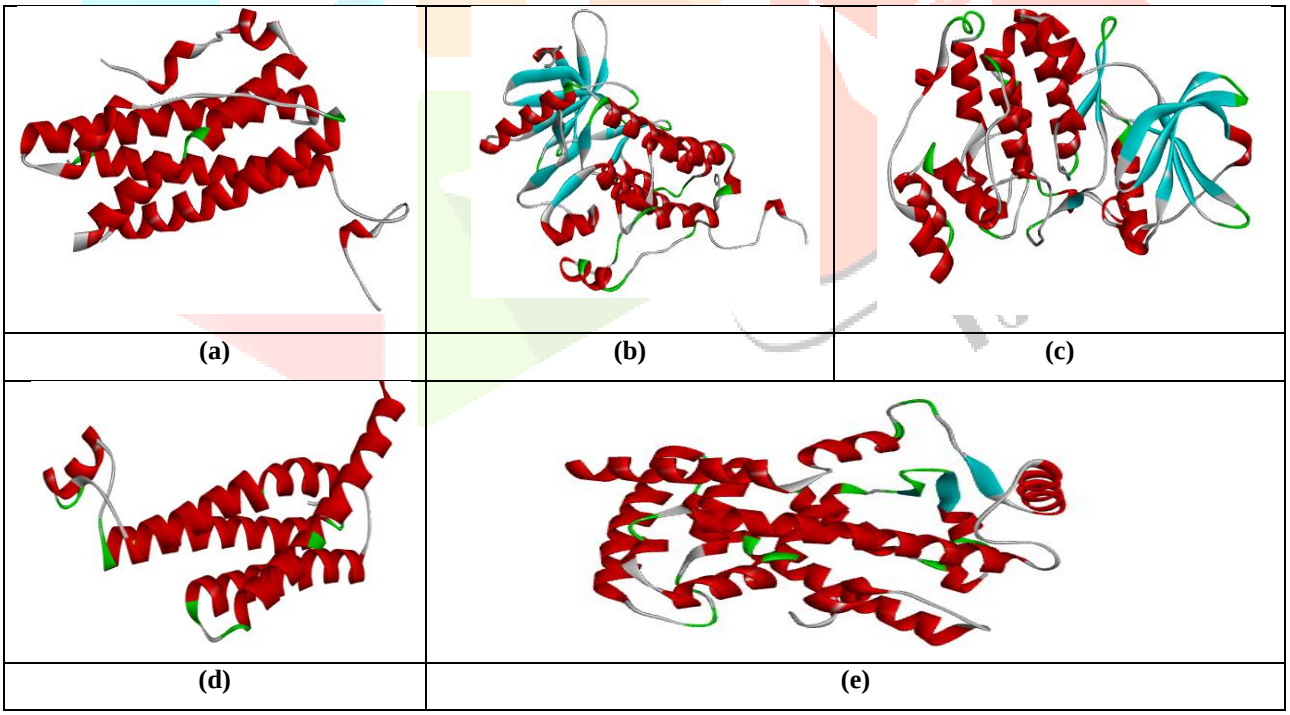


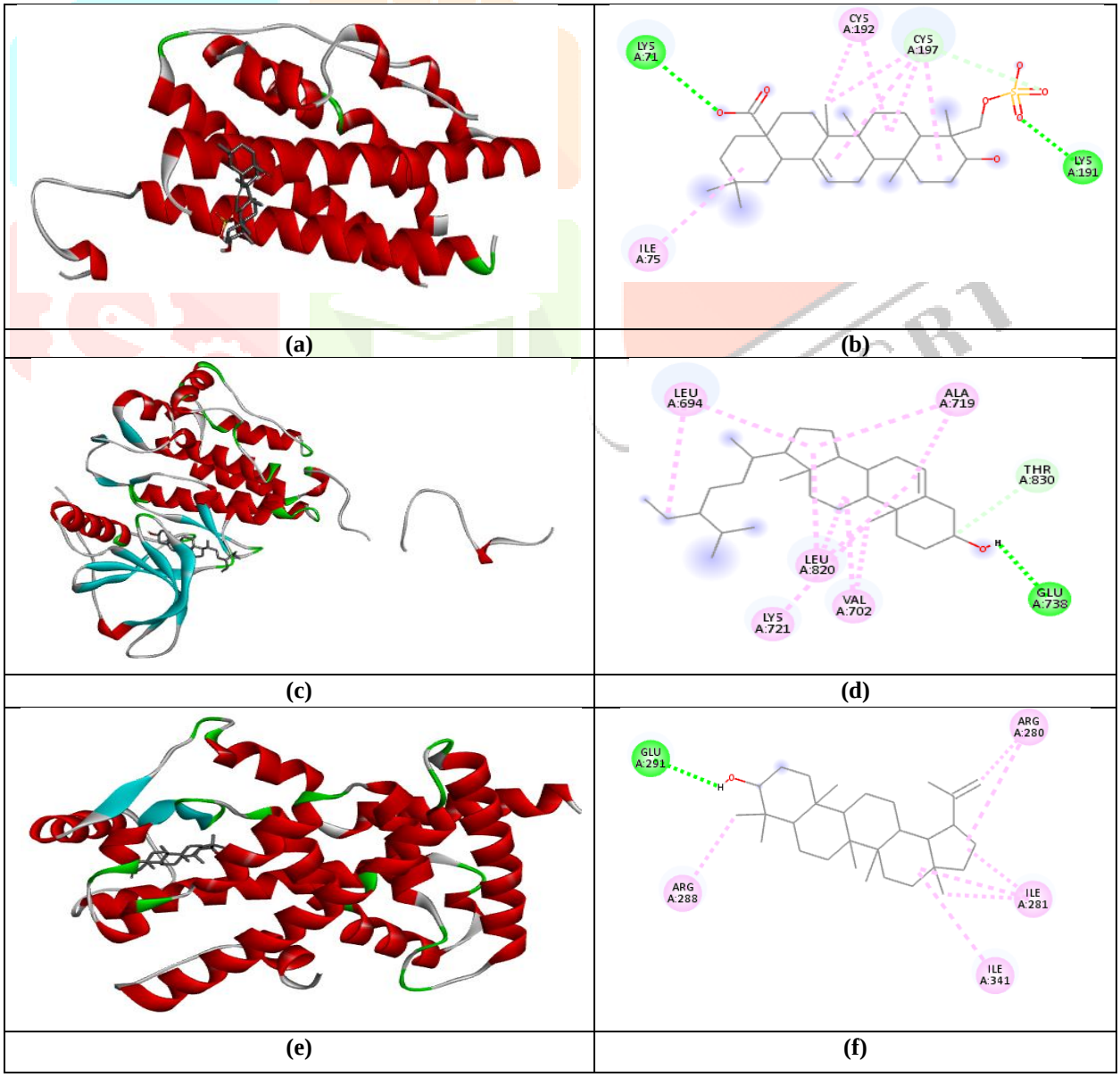
Figure 6: 3D structures of receptors: a) Placental Lactogen (1F6F), b) Epidermal Growth Factor Receptor (1M17), c) Casein Kinase II Subunit Alpha (2OXX), d) Prostaglandin E Synthase (4YK5) and e) Peroxisome Proliferator Activated Receptor Gamma (2PRG).

Docking simulations of candidate ligands Sitosterol, Indole, Lupeol, Oleic acid, Palmitic acid, Tomentogenin, Triterpenoids with Placental Lactogen (1F6F), Epidermal Growth Factor Receptor (1M17), Prostaglandin E Synthase (4YK5), Casein Kinase II Subunit Alpha (2OXX) and Prostaglandin E Synthase (4YK5) shows that ΔG score of Triterpenoids of 1F6F, Sitosterol of 1M17, Lupeol of 4YK5, Sitosterol of 2OXX and Lupeol of Peroxisome Proliferator Activated Receptor Gamma (2PRG) are -6.56 kcal/mol,

-8.0 kcal/mol, -7.22 kcal/mol, -6.63 kcal/mol and -8.33 kcal/mol respectively at the lowest (1st) conformation. These results indicate that, of the six ligands, the one with lowest conformation possesses the greatest binding affinity (Fig 7).

Table 3: Binding Energy of complex

Ligands / Proteins	Sitosterol	Indole	Lupeol	Oleic acid	Palmitic acid	Tomentogenin	Triterpenoids
Anti-obesity (1F6F)	-6.52	-3.78	-6.32	-2.16	-2.51	-5.58	-6.56
Anti-oxidant (1M17)	-8.0	-4.63	-7.55	-3.1	-3.06	-7.23	-6.01
Anti-diabetic (2PRG)	-7.69	-4.30	-8.33	-4.06	-4.17	-7.6	-6.53
Anti-cancer (2OXX)	-6.63	-4.56	-5.7	-3.84	-4.02	-6.44	-6.37
Anti-inflammatory (4YK5)	-6.54	-3.68	-6.81	-2.08	-2.28	-6.43	-7.22



(a) (b)

(c) (d)

(e) (f)

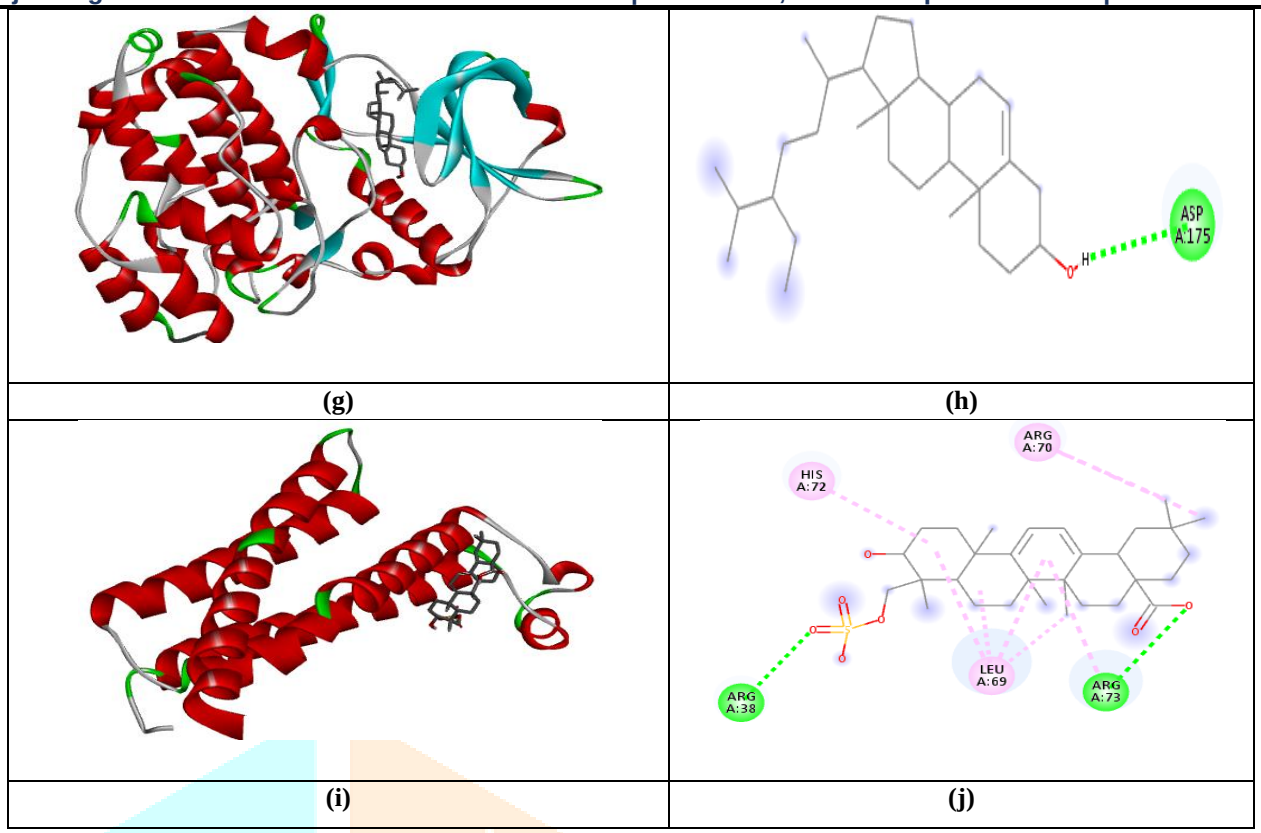


Figure 7: The binding mode (interactions) of compounds with ligands a) Interaction between the molecule and the receptor amino acids residues of 1F6F with triterpenoids, b) Interaction diagram of complex (PDBID: 1F6F) with the alkyl group residues and two hydrogen bond (green), c) Interaction between the molecule and the receptor amino acids residues of 1M71 with sitosterol, d) Interaction diagram of complex (PDBID:1M17) with the alkyl group residues and one hydrogen bond (green), e) Interaction between the molecule and the receptor amino acids residues of 2PRG with lupeol, f) Interaction diagram of complex (PDBID: 2PRG) with the alkyl group residues and one hydrogen bond(green), g) Interaction between the molecule and the receptor amino acids residues of 2OXX with sitosterol, h) Interaction diagram of complex (PDBID:2OXX) with one hydrogen bond (green), i) Interaction between the molecule and the receptor amino acids residues of 4YK5 with triterpenoids and j) Interaction diagram of complex (PDBID:4YK5) with the alkyl group residues with two hydrogen bonds (green)

4. CONCLUSION

This study presents an overview of the *Caralluma adscendens var. fimbriata* that can be used to treat against obesity, diabetes, hypertension, ulcers, and cancer. The *Caralluma adscendens var. fimbriata*genus is a promising source for phyto-chemicals with therapeutic usage and suggests possible leads for researchers to discover herbal effects but most of the evidence remains suggestive but not conclusive so further phytochemical and pharmacological research is needed. The demonstration of antibacterial activity of *Caralluma adscendens var. fimbriata* against Gram positive (*Staphylococcus auereus*) and Gram negative bacteria (*Coliform, Pseudomonas, E. coli*) provides the scientific basis for its use in the traditional treatment. Further studies are required to establish the exact mechanism of antibacterial activity of phytochemicals extracted from so *Caralluma adscendens var. fimbriata* that better and safer chemotherapeutic agents can be developed from this plant. With reference to the computated study the ligand Sitosterol, Indole, Lupeol, Oleic acid, Palmitic acid, Tomentogenin, Triterpenoids with the proteins of anti- obesity (Placental Lactogen 1F6F), anti- oxidant (Epidermal Growth Factor Receptor1M71), anti-cancer Casein kinase II subunit alpha 2OXX), anti-inflammatory (Prostaglandin E Synthase 4YK5) and anti-diabetic (Peroxisome proliferator activated receptor gamma 2PRG) shows good affinity with ligands. From the *in-silico* studies it can be concluded that all the receptor -ligand complex isolated from the plant could be the "lead compounds" for design and developing new nontoxic and effective drugs for the treatment of cancer, diabetes, inflammatory, obesity and also as an anti- oxidant property. Further phytochemical and pharmacological research with more work should be done to address critical health concerns prevailing in developed as well as developing countries for designing a safer nutraceuticals approach for these diseases.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGEMENTS

The authors thank the SARATHI of Maharashtra for the funding provided through CSMNRF-2021/2021-22/546. Further authors are thankful to the Principal and Management of the New Arts Commerce and Science College Parner for the laboratory facilities.

REFERENCES

1. Packialakshmi, N., & Naziya, S. (2014). Screening of antibacterial and phytochemical analysis of Caralluma fimbriata. The Pharma Innovation, 3(6, Part B), 65.
2. Vitalone, A., Di Sotto, A., Mammola, C. L., Heyn, R., Miglietta, S., Mariani, P., ... & Mazzanti, G. (2017). Phytochemical analysis and effects on ingestivebehaviour of a Caralluma fimbriata extract. Food and Chemical Toxicology, 108, 63-73.
3. Qayyum, N., Rani, H., Mir, K. B., & Khan, A. Q. (2018). Caralluma Pharmacological Attributes. Journal of Food, 2(2), 13.
4. Anwar, R., Rabail, R., Rakha, A., Bryla, M., Roszko, M., Aadil, R. M., &Kieliszek, M. (2022). Delving the Role of Caralluma fimbriata: An Edible Wild Plant to Mitigate the Biomarkers of Metabolic Syndrome. Oxidative Medicine and Cellular Longevity, 2022.
5. Abdel-Sattar, E., & Ali, D. E. (2022). Russelioside B: a Pregnane Glycoside with Pharmacological Potential. Revista Brasileira de Farmacognosia, 1-13.
6. Adnan, M., Jan, S., Mussarat, S., Tariq, A., Begum, S., Afroz, A., & Shinwari, Z. K. (2014). A review on ethnobotany, phytochemistry and pharmacology of plant genus C aralluma R. Br. Journal of Pharmacy and Pharmacology, 66(10), 1351-1368.
7. Tambe, D. A., Chaudhari, T. B., & Chaudhari, S. R. (2010). Phyto-pharmacology of Caralluma adscendens roxb: a review. Pharmacognosy Journal, 2(14), 33-38.
8. Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. Trends in Food Science & Technology, 17(6), 300-312.
9. Mali, K. K., Dias, R. J., Hawaldar, V. D., & Mahajan, N. S. (2009). Hypoglycemic activity of Caralluma adscendens in alloxan induced diabetic rats. Int J Chem Sci, 7, 517-522.
10. Cordell, G. A. (2000). Biodiversity and drug discovery—a symbiotic relationship. Phytochemistry, 55(6), 463-480.
11. Zonta, N., Coluccia, A., & Brancale, A. (2010). Advanced in silico approaches in antiviral research. Antiviral Chemistry and Chemotherapy, 20(4), 147-151.
12. Teodoro, M. L., Phillips, G. N., &Kavraki, L. E. (2001, May). Molecular docking: A problem with thousands of degrees of freedom. In Proceedings 2001 ICRA. IEEE International Conference on Robotics and Automation (Cat. No. 01CH37164) (Vol. 1, pp. 960-965). IEEE.
13. Miller, W. L., & Eberhardt, N. L. (1983). Structure and evolution of the growth hormone gene family. Endocrine Reviews, 4(2), 97-130.
14. Mohapatra, R. K., El-ajaily, M. M., Alassbaly, F. S., Sarangi, A. K., Das, D., Maihub, A. A., ... & Al-Noor, T. H. (2021). DFT, anticancer, antioxidant and molecular docking investigations of some ternary Ni (II) complexes with 2-[(E)-[4-(dimethylamino) phenyl] methyleneamino] phenol. Chemical Papers, 75(3), 1005-1019.
15. Cozza, G. and Moro, S., 2013. Casein Kinase 2 (CK2) Inhibitors: Emerging Anticancer Therapeutic Agents? Advances in Anticancer Agents in Medicinal Chemistry, 1, pp.3-42.
16. Scher, J. U., & Pillinger, M. H. (2009). The anti-inflammatory effects of prostaglandins. Journal of Investigative Medicine, 57(6), 703-708.
17. Vazquez, M., Silvestre, J. S., &Prous, J. R. (2002). Experimental approaches to study PPAR gamma agonists as antidiabetic drugs. Methods Find Exp Clin Pharmacol, 24(8), 515-523.