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In vitro antimicrobial activity of extracts from *Kydia calycina* and *in-silico* molecular docking studies of some phytochemicals

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A B S T R A C T

Drug-resistant microorganisms are a serious problem, particularly when more strains become immune to different antimicrobials. Antibiotic resistance has now developed in several microbes. Therefore, it is crucial to build new medications that are still efficient. The amount of funding that is often available for such progress is lower than what is necessary. *Kydia calycina* is a Malvaceae flowering plant used in traditional Indian medicine to cure several diseases, including infections. The goal of this study was to determine whether *K. Calycina* has antifungal and antibacterial properties. Infections are caused by the profusion of microbes in the environment; thus, plant products and active chemicals are employed to assess the antimicrobial property of the extracts and the inhibition zone of each extract on a range of bacterial and fungal strains. The results showed that when it was applied to the species that were studied, there was a considerable decrease in the growth of bacteria. The plant was subjected to a phytochemical analysis, which was completed. This plant may be employed in the quest for bioactive natural substances that might be used as leads in the creation of pharmaceuticals. The antimicrobial mechanism of action was investigated by molecular docking, and it was determined that Hibiscoquinone B and Hibiscone C showed both antibacterial and antifungal activity.

1. Introduction

Human ailments can be relieved with the help of medicinal plants. The World Health Organization (WHO) estimates that more than 80% of the global population, mostly in impoverished nations, relies on conventional plant-based medicines for their basic medical needs.[1] Scientific validation relates to the screening of plant bioactive chemicals and has helped develop novel medications with successful applications in the prevention and treatment of many ailments. Drug-resistant microorganisms are a serious problem, particularly when more strains become immune to different antimicrobials. Antibiotic resistance has now developed in several microbes. Therefore, it is crucial to build new medications that are still efficient.

Kydia calycina is a Malvaceae flowering plant used in traditional Indian medicine to cure several diseases, including infections, and is also known as Pula in Hindi. *Kydia calycina* is primarily found in mixed wet and deciduous woods and can be found in the tropical Himalayas from the Indus to Myanmar (Burma) and in peninsular India from Northern Maharastra to Madhya Pradesh.[2] In Rajasthan, it is prevalent in the Sariska Tiger Reserve Alwar.[3] It is a magnificent

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ornamental tree that bears clusters of white flowers in attractive arrangements. The tribes people in that area utilise this plant extensively to cure a variety of ailments, including rheumatoid arthritis, bodily pains, jaundice, ulcers, lumbago (pain in the muscles and joints if lower back) and Pavithra *et al.* in 2014 shows *Kydia calycina* against fungal pathogen against rhizome root of ginger[4] and Kumari Manikya *et al.* 2011 screened against bacterial strain results revealed that it shows significant antibacterial activity[5].This plant also has a number of additional therapeutic characteristics, including antioxidant,[6] anti-tumor,[7] anticariogenic,[8] analgesic and anti-inflammatory,[9] hepatoprotective,[10] and antihelmentic.[11] Aside from its medicinal properties, Due to the plant's superior bast fibre production compared to plants like jute and flax,[12] it is also utilised as a fibre in the textile industry.[13] Dried bark powder (5gm) and honey are combined to make a paste that should be swallowed in its whole early in the morning to lower blood glucose levels.[14] The molecular docking method offers considerable potential for computer-based drug discovery since it forecasts ligand conformation and orientation within the targeted binding site.[15]

Kydia calycina contains the following phytochemicals like lauric acid, myristic acid, palmitic acid, oleic acid, linoleic acid, cyclopropenoic acid, hibiscone C, hibiscoquinone B.[16] The goal of the current research is to ascertain the antimicrobial property of *Kydia calycina* floral extracts and to conduct *In silico* docking experiments on the phytochemicals listed above against the bacteria 1C14 and 1EA1. Figure 1 shows the 2D structures of the standard medications ciprofloxacin (for antibacterial action) and clotrimazole (for antifungal activity), and all the phytoconstituents discovered in *Kydia calycina*, as well as the 3D images of *E. coli* (PDB ID:1C14) and *Mycobacterium tuberculosis* (PDB ID:1EA1), respectively.

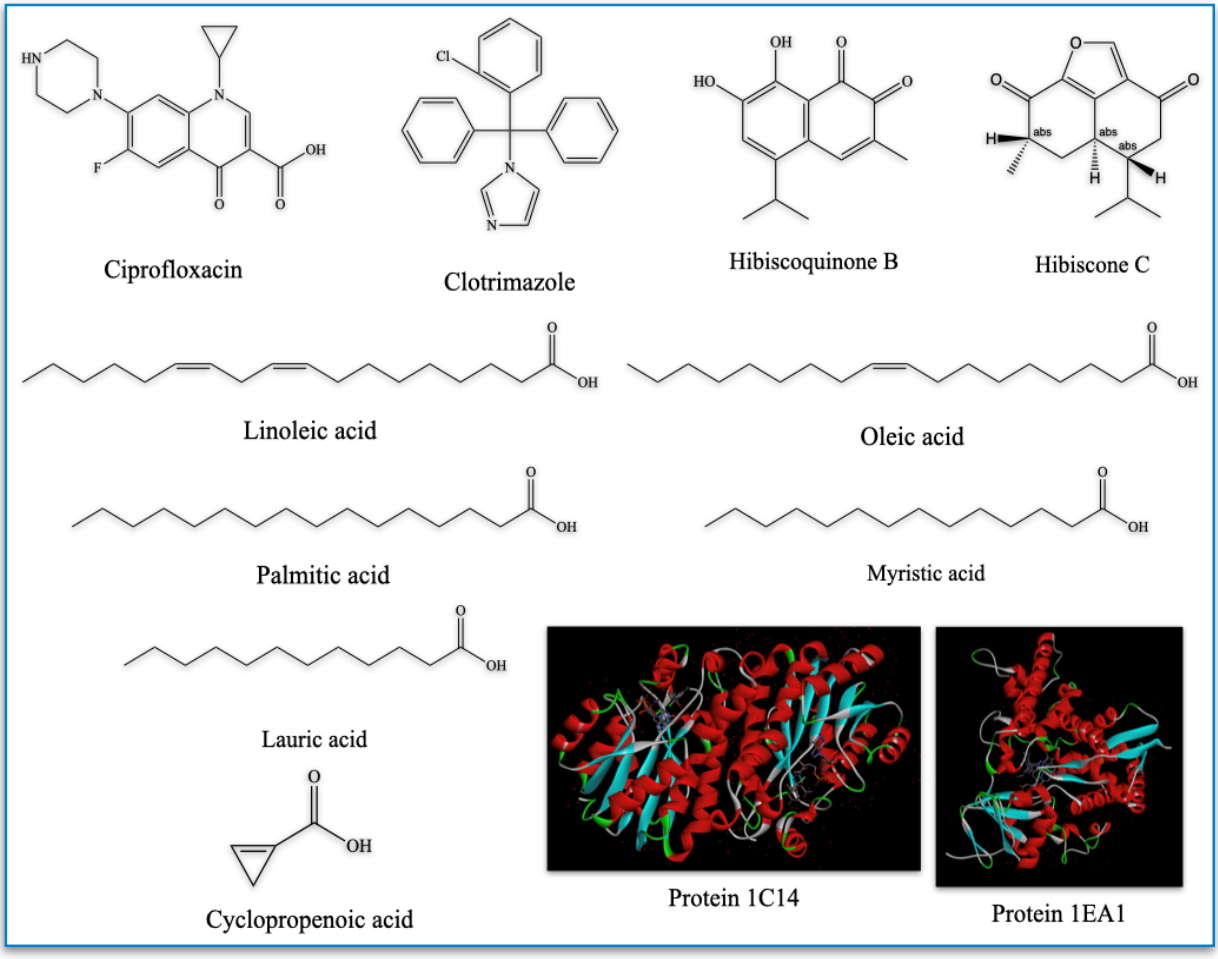

Figure 1. shows the 2D structures of the standard medications ciprofloxacin (for antibacterial action) and clotrimazole (for antifungal activity), and all the phytoconstituents (Hibiscoquinone B, Hibiscone C, Linoleic acid, Oleic acid, Palmitic acid, Myristic acid, Lauric acid, and Cyclopropenoic acid) discovered in *Kydia calycina*, as well as the 3D images of *E. coli* (PDB ID:1C14) and *Mycobacterium tuberculosis* (PDB ID:1EA1), respectively.

2. Results and Discussion

Phytochemical studies of extracts revealed the presence of various phytochemicals shown in Table 1.

Table 1. Phytochemical studies of extracts revealed the presence of various phytoconstituents in *Kydia calycina.*

Phytochemical Tests	Methanolic extract	Ethanolic extract	Aqueous extract
Alkaloids	+	+	
Carbohydrates	+	+	
Glycosides	+	+	+
Phytosterols		+	
Phenolic compounds and tannins	+	+	+
Steroids			
Flavonoids	+	+	+
Saponins			+
Terpenoids	+	+	

2.1. Antimicrobial study

2.1.1 Antibacterial

By using a two-fold serial dilution method, the test compounds 1 to 25 were examined for their *in vitro* antibacterial activity in contrast to the Gram positive bacteria *Staphylococcus aureus* (MTCC 3160), *Bacillus cereus* (MTCC 9786), and the Gram negative bacteria *Escherichia coli* (MTCC 118), and *Pseudomonas aeruginosa* (MTCC 4673). The standard drug used was ciprofloxacin. Compounds have demonstrated good to moderate activity in comparison to the reference drug ciprofloxacin, as measured by the Minimum Inhibitory Concentration (MIC) (µg/ml). Results revealed that amongst the all extracts ethanolic extract shows highly active against all bacterial species.

2.1.2 Antifungal

By using a two fold serial dilution approach, the test compounds 1 to 25 were evaluated for their *in vitro* antifungal activity in contrast to *Candida albicans* (MTCC 1637) and *Aspergillus niger* (MTCC 282). The standard drug used was clotrimazole. Compounds have demonstrated good to moderate antifungal activity in comparison to the reference drug clotrimazole, as measured by the Minimum Inhibitory Concentration (MIC) (µg/ml) of antifungal activity. Results revealed that amongst the all extracts ethanolic extract shows highly effective both antifungal species.

2.2. Computational Study

In silico studies of compounds present in *Kydia calycina* using pyrx showed the following results. To ascertain the manner of binding and interactions amongst the most potent phytoconstituents (lauric acid, myristic acid, palmitic acid, oleic acid, linoleic acid, cyclopropenoic acid, and hibiscone C and hibiscoquinone B) found in *Kydia calycina*, a molecular docking research was carried out against bacterial activity and fungal activity, the proteins 1C14, 1ea1 by means of Pyrx software. The outcomes were contrasted with the reference drugs' molecular docking models, specifically Ciprofloxacin, Clotrimazole the Complex of GS-Alpha with the Catalytic Domains of Mammalian Adenylyl Cyclase: Complex with Pyrophosphate and Ca crystal structure of (PDB ID:1C14), and Cytochrome P450 14 alpha-sterol demethylase from *Mycobacterium tuberculosis* in complex through fluconazole bound to DNA (PDB id: 1ea1) were downloaded from the Protein Data Bank, and used for antibacterial, antifungal, and respectively. The 3D structures of ligands and reference drugs were prepared by using ChemDraw Ultra 16.0 software. The search grid of the 1C14 protein was identified as center_x = -13.805, center_y = 42.0267, and center_z = 148.9841 with the dimensions of size_x = 54.9228, size_y = 44.7239, and size_z = 41.4330 at a spacing of 0.375 Å. The search grid of the 1ea1 protein was identified as center_x = -12.056, center_y = -2.8535, and center_z = 62.4432 with the dimensions of size_x = 64.1390, size_y = 56.4764, and size_z = 69.4023 at a spacing of 0.375 Å. The Vina docking program's default values were used for all other parameters, which are not listed here. The substance that scored the highest had the lowest binding affinity value. All data were visually analysed using Biovia Discovery studio visualizer software. The structures of compounds from *Kydia calycina* are shown in **Fig. 1**. The binding energy for each chosen compound with the *E. coli* (1C14) and *Mycobacterium tuberculosis* (1EA1) protein are given in Table 2 & 3. Results revealed that in case of interaction with 1C14 protein Hibiscoquinone B having same docking score like ciprofloxacin and shows three hydrogen bond interaction and less hydrophobic interaction or in case of protein 1EA1 both Hibiscoquinone B and Hibiscone C shows good docking score and strong hydrogen bonding and week hydrophobic interaction.

Table 2. Binding affinities of isolated compounds at the active site of 1C14.

Sr. No.	Ligands	Dock score
1	Ciprofloxacin	-8.1
2	Hibiscoquinone B	-8.3
3	Hibiscone C	-8
4	Linoleic acid	-6.3
5	Oleic acid	-6.3
6	Palmitic acid	-6.1
7	Myristic acid	-5.8
8	Lauric acid	-5.6
9	Cyclopropenoic acid	-4.4

Table 3. Binding affinities of isolated compounds at the active site of 1EA1.

Sr. No.	Ligands	Dock score
1	Clotrimazole	-8.1
2	Hibiscone C	-7.6
3	Hibiscoquinone B	-7.2
4	Linoleic acid	-6
5	Oleic acid	-5.5
6	Myristic acid	-5.2
7	Lauric acid	-5
8	Palmitic acid	-4.5
9	Cyclopropenoic acid	-4.4

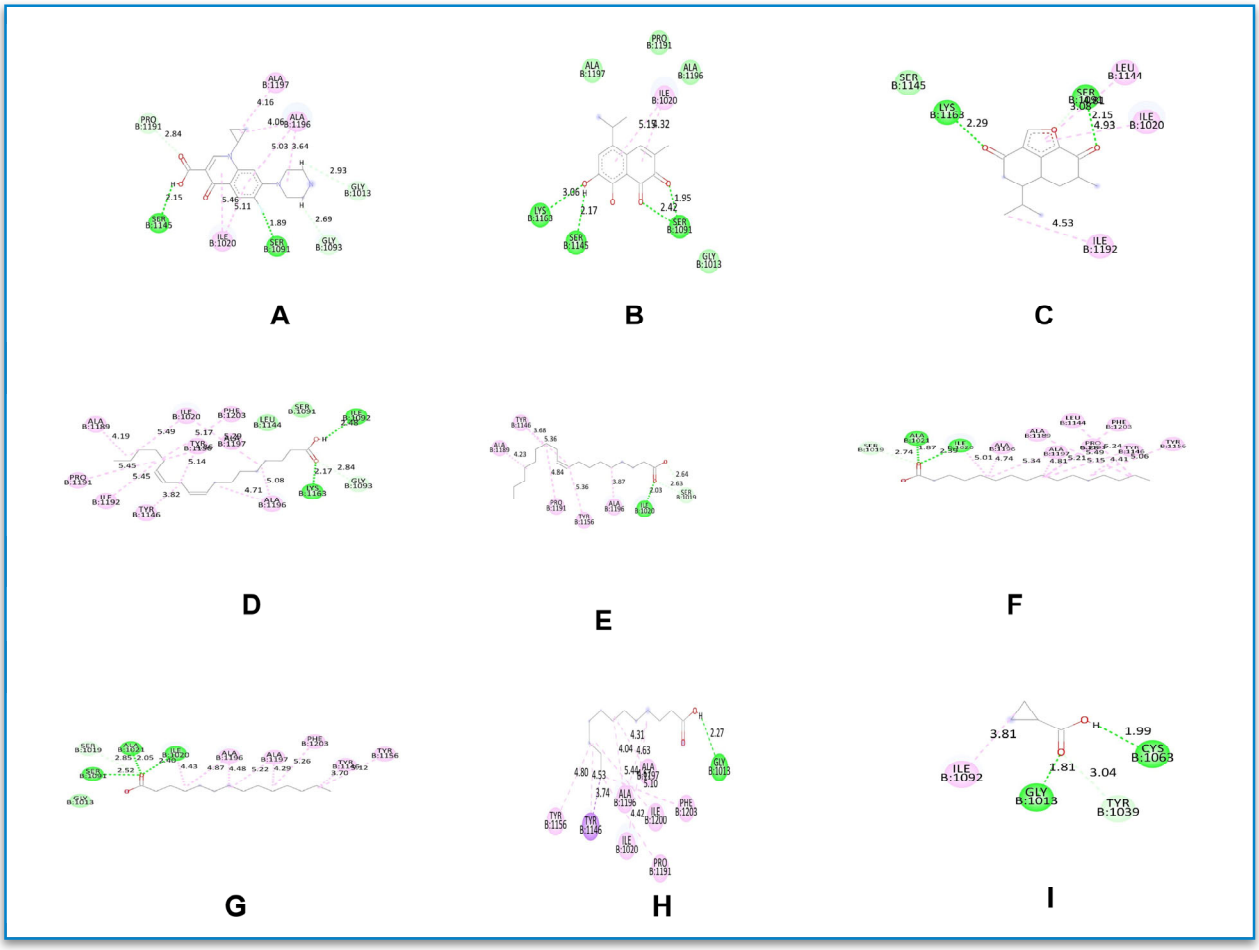

Fig. 2. 2D Interactions of Standard drug (a) ciprofloxacin and ligands (b) Hibiscoquinone B,(c) Hibiscone C, (d) Linoleic acid, (e) Oleic acid, (f) Palmitic acid, (g) Myristic acid, (h) Lauric acid, and (i) Cyclopropenoic acid with *E. coli* (PDB ID:1C14) respectively.

Table 4. Interactions of 1C14 amino acid residues with ligands at receptor site.

Ligands	Binding Affinity, ΔG (Kcal/mol)	Amino acids involved and Distance (A°)	
		Hydrogen Binding Interactions	Hydrophobic Interactions
Ciprofloxacin	-8.1	SER B:1145 (2.15), SER B:1091 (1.89), GLY B:1013 (2.93), GLY B:1093 (2.69), PRO B:1191 (2.84)	ALA B:1197 (4.16), ALA B:1196 (4.06, 5.03, 3.64), ILE B:1020 (5.46, 5.11)
Hibiscoquinone B	-8.3	LYS B:1163 (3.06), SER B:1145 (2.17), SER B:1091 (2.42, 1.95)	ILE B:1020 (5.15, 4.32)
Hibiscone C	-8	LYS B:1163 (2.29), SER B:1091 (2.15, 3.08)	LEU B:1144 (4.81), ILE B:1020 (4.93), ILE B:1192 (4.53)
Linoleic acid	-6.3	LYS B:1163 (2.17), GLY B:1093 (2.84), ILE B:1092 (2.48)	ILE B:1020 (5.49, 5.29), ILE B:1192 (5.45), ALA B:1189 (4.19), PRO B:1191 (5.45), TYR B:1146 (3.82), ALA B:1196 (4.71, 5.08), ALA B:1197 (4.36), TYR B:1156 (5.14), PHE B:1203 (5.17)
Oleic acid	-6.3	SER B:1019 (2.64, 2.63), ILE B:1020 (2.03)	ALA B:1189 (4.23), TYR B:1146 (3.68, 5.36), PRO B:1191 (4.84), TYR B:1156 (5.36), ALA B:1196 (3.87)
Palmitic acid	-6.1	ALA B:1021 (1.87), ILE B:1020 (2.39), SER B:1019 (2.74)	ALA B:1196 (4.74), ILE B:1020 (5.01), ALA B:1189 (5.23), ALA B:1197 (5.34, 4.81), PRO B:1191 (5.21), LEU B:1144 (5.24), PHE B:1203 (5.49), TYR B:1146 (5.15, 4.41), TYR B:1156 (5.06)
Myristic acid	-5.8	SER B:1091 (2.52), ILE B:1020 (2.40), ALA B:1021 (2.05), SER B:1019 (2.85)	ILE B:1020 (4.43), ALA B:1196 (4.87, 4.48), ALA B:1197 (5.22, 4.29), PHE B:1203 (5.26), TYR B:1146 (3.70), TYR B:1156 (5.12)
Lauric acid	-5.6	GLY B:1013 (2.27)	TYR B:1156 (4.80), TYR B:1146 (4.53, 3.74), ALA B:1196 (4.04, 4.63), PRO B:1191 (4.42), ILE B:1020 (4.67), ILE B:1200 (5.44), PHE B:1203 (5.10), ALA B:1197 (4.31)
Cyclopropenoic acid	-4.4	GLY B:1013 (1.81), CYS B:1063 (1.99), TYR B:1039 (3.04)	ILE B:1092 (3.81)

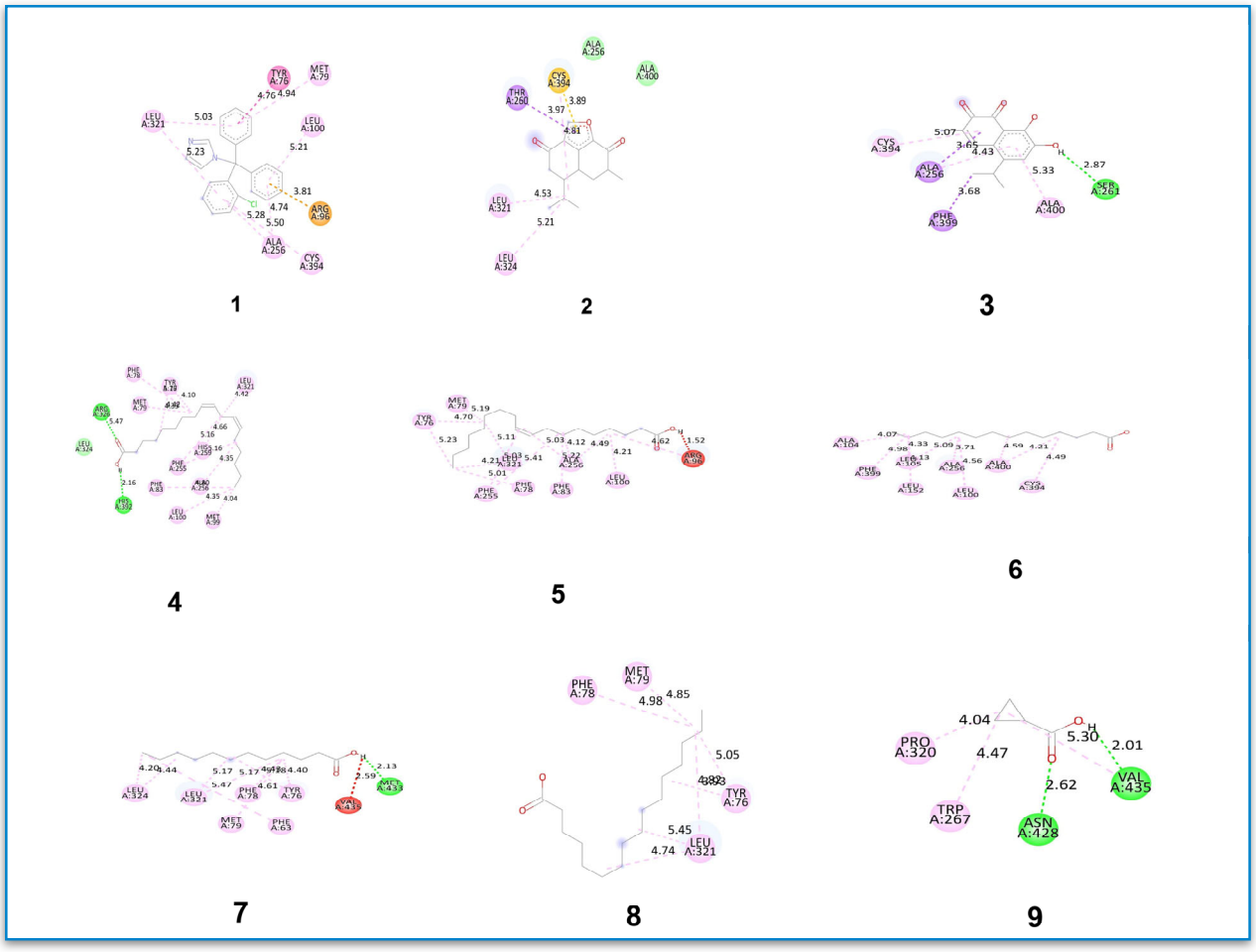

Fig. 3. 2D Interactions of Standard drug (1) Clotrimazole and ligands (2) Hibiscone C, (3) Hibiscoquinone B, (4) Linoleic acid, (5) Oleic acid, (6) Myristic acid, (7) Lauric acid, (8) Palmitic acid, and (9) Cyclopropenoic acid with *Mycobacterium tuberculosis* (PDB ID:1EA1).

Table 5. Interactions of 1EA1 amino acid residues with ligands at receptor site

Ligands	Binding Affinity, ΔG (Kcal/mol)	Amino acids involved and Distance (A°)	
		Hydrogen Binding Interactions	Hydrophobic Interactions
Clotrimazole	-8.1		LEU A:321 (5.03, 5.23), TYR A:76 (4.76), MET A:79 (4.94), LEU A:100 (5.21), ALA A:256 (5.28, 4.74), CYS A:394 (5.50), ARG A:96 (3.81)
Hibiscone C	-7.6		THR A:260 (3.97), LEU A:321 (4.53), LEU A:324 (5.21), CYS A:394 (3.89, 4.81)
Hibiscoquinone B	-7.2	SER A:261 (2.87)	CYS A:394 (5.07), ALA A:256 (3.65, 4.43), PHE A:399 (3.68), ALA A:400 (5.33)
Linoleic acid	-6	ARG A:326 (5.47), HIS A:392 (2.16)	PHE A:78 (5.16), MET A:79 (4.33), TYR A:76 (4.10, 4.42), LEU A:321 (4.42), HIS A:259 (4.66), PHE A:255 (5.16, 5.16), ALA A:256 (4.35), PHE A:83 (4.80), LEU A:100 (4.35), MET A:99 (4.04)
Oleic acid	-5.5	ARG A:96 (1.52)	ARG A:96 (4.62), MET A:79 (5.19), TYR A:76 (4.70, 5.23), LEU A:321 (5.11, 4.21), PHE A:255 (5.03, 5.41), ALA A:256 (5.03, 4.12, 4.49), PHE A:83 (5.22), LEU A:100 (4.21), PHE A:78 (5.01)
Myristic acid	-5.2		CYS A:394 (4.49), ALA A:400 (4.59, 4.21), LEU A:100 (4.56), ALA A:256 (3.71), LEU A:152 (4.13), LEU A:105 (4.33, 5.09), PHE A:399
Lauric acid	-5	MET A:433 (2.13), VAL A:435 (2.59)	LEU A:324 (4.20, 4.44), LEU A:321 (5.17, 5.17), PHE A:78 (5.18), TYR A:76 (4.43, 4.40), PHE A:63 (5.47), MET A:79 (4.61)
Palmitic acid	-4.5		LEU A:321 (4.74, 5.45, 4.82), TYR A:76 (3.93, 5.05), PHE A:78 (4.98), MET A:79 (4.85)
Cyclopropenoic acid	-4.4	ASN A:428 (2.62), VAL A:435 (2.01)	PRO A:320 (4.04), TRP A:267 (4.47), VAL A:435 (5.30)

3. Conclusions

A series of phytoconstituents were discovered and evaluated antimicrobial activity by serial broth dilution method some compounds found to be active. These compounds showed the good antibacterial activity against *S. aureous* and *E. coli* and in antifungal study compounds showed the good antifungal activity against *C albicans*. In docking studies antibacterial ligands (b) Hibiscoquinone B, (c) Hibiscone C, (d) Linoleic acid, (e) Oleic acid, has been found significantly bound with the target protein 1C14, crystal structure of *E. coli* enoyl reductase-nad+-triclosan complex and in antifungal study against protein 1ea1 o 14 alpha-sterol demethylase enzyme active sites. The crystal structure of enzyme (PDB ID: 1EA1) molecule demonstrated antifungal (2) Hibiscone C, (3) Hibiscoquinone B, (4) Linoleic acid, (5) Oleic acid, activity, among other ligands, molecule possessed profound antifungal activity. Phytoconstituents Hibiscoquinone B, and Hibiscone C showed both antibacterial and antifungal activity.

4. Experimental

4.1. Materials and methods

4.1.1. Plant material

In the Sariska Tiger Reserve in Alwar, *Kydia calycina* flowers were harvested. It was verified by Dr. Laxmikant Sharma, a plant taxonomist from the Department of Botany at Raj Rishi College in Alwar.

4.1.2. Extraction and isolation

Flowers were picked, air dried in the shade to room temperature, and then crushed. The powder was extracted in a round bottom flask for 7 days separately using a maceration process with ethanol, methanol, and distilled water. The content of the flasks was occasionally agitated to ensure the efficiency of the extraction. After a week, Whatman number 1 filter paper was used to filter the extracts via a Buchner funnel and concentrated under reduced pressure to yield corresponding extracts, and the extracts were kept in a desiccator to remove moisture and stored properly until used for preparing dilutions and performing antimicrobial activity.

4.1.3. Microorganisms

Gram positive bacterial strains like *Staphylococcus aureus* (MTCC No 3160), Bacillus cereus (MTCC No 9786), and gram-negative bacteria like *Escherichia coli* (MTCC No 118), and *Pseudomonas aeruginosa* (MTCC No 4673) were used to assess the antibacterial activity, respectively. In the experiment *Candida albicans* (MTCC No 1637) and *Aspergillus niger* (MTCC No 282) were used to determine the antifungal activity of different extracts of *Kydia calycina*.

4.1.4. Antimicrobial assay

Antimicrobial activity was carried out using disc-diffusion method. Mueller Hinton Agar (MHA) (Hi-media, Mumbai) in a volume of 20 ml and SDA in a volume of 20 ml were used to produce petri plates for bacteria and fungi, respectively. After being swabbed on top of the hardened media, the test cultures were left to dry for 10 minutes. Three replicates of the experiments were carried out with three different concentrations of the crude extract (5, 2.5, and 1.25 mg per disc). In order to allow for compound diffusion, the loaded discs were placed on the medium's surface and kept there for 30 minutes at room temperature. Ethanol was used to prepare the negative control. As a positive control, ciprofloxacin (10 mg/disc) was used. For 24 hours at 35–37 °C for bacteria and 48 hours at 25–27 °C for fungus, the plates were incubated. The experiment was conducted twice, with the zone of inhibition measured in millimeters.

4.2. Biological activity

4.2.1. Antibacterial activity

By using the serial broth dilution method, different *Kydia calycina* flower extracts (ethanol, methanol, and aqueous) were tested for their antibacterial activity against the bacterial strains *Staphylococcus aureus* (MTCC No 3160), Bacillus cereus (MTCC No 9786), *Escherichia coli* (MTCC No 118), and *Pseudomonas aeruginosa* (MTCC No 4673).[17-18] Each substance's effectiveness was evaluated using ciprofloxacin as the benchmark. The Institute of Microbial Technology in Chandigarh provided the necessary bacterial strains.

Determination of Minimum Inhibitory Concentration (MIC):

Numbers 1 through 9 were written on sterilized test tubes, and from this, 0.1 ml was extracted and diluted with sterile water to make 10 ml. It was then used as the working inoculum for testing the freshly prepared analogues for their

antibacterial activity. The following procedures were all completed with aseptic technique. In order to get at 100µ g/ml as the initial dose, in a first sterile test tube with 3.8 ml of nutritional broth, 0.2 ml of a 2000 µg/ml test stock solution in DMSO was added. Thereafter, 2 cc of nutritional broth was added to test tubes 2 through 9. At a 10% concentration, DMSO had no antibacterial effect when used as a control. The concentrations in these test tubes were diluted in series to yield 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 µg/ml. The vehicle control was one test tube with the same volume of solvent DMSO (10%) but no test chemical. As a positive control, one test tube that contained only nutritional media and no test substance or vehicle was used to confirm the growth property of the medium. Each test tube received 0.1 ml of the bacteria suspension (working inoculate), and each test tube was then incubated at 35–37°C for 24 hours. The test drug's MIC value, given in µg/ml, was calculated as the maximum dilution at which the test compound totally prevented the development of the test organism. The outcomes are compiled in Table 6.

Table 6. Minimum inhibitory concentration (MIC) (µg/ml) of the Plant extract against Gram positive and Gram-negative bacteria

Plant extract	*S. aureus* (MTCC 3160)	*B. cereus* (MTCC 9786)	*E.coli* (MTCC 118)	*P. aeruginosa* (MTCC 4673)
Ethanolic extract	3.5	3.5	2.5	3
Methanolic extract	18	22	23	22
Aqueous extract	25	18	12	17

4.2.2. Antifungal activity

By using the serial broth dilution method, several *Kydia calycina* flower extracts (ethanol, methanol, and aqueous) were tested for their antifungal activity against *Candida albicans* (MTCC No 1637) and *Aspergillus niger* (MTCC No 282).[17-19] Clotrimazole was used as the benchmark to compare each compound's activity. The Institute of Microbial Technology in Chandigarh supplied the necessary bacterial strains.

Determination of Minimum Inhibitory Concentration (MIC):

Numbers 1 through 9 were written on sterilized test tubes and were sterilised. The following procedures were all completed with aseptic technique. In order to get at 100 µg/ml as the starting dose, 3.8 ml of Sabouraud dextrose broth and 0.2 ml of a 2000 µg/ml test stock solution in DMSO were placed in a first sterile test tube. Following that, 2 cc of Sabouraud dextrose broth was added to the remaining test tubes 2 through 9. At a 10% concentration, DMSO had no antibacterial effect when used as a control. The concentrations in these test tubes were diluted in series to yield 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 µg/ml. The vehicle control was one test tube with the same volume of solvent DMSO (10%) but no test chemical. The positive control was one test tube that just contained Sabouraud dextrose media, with no test substance or vehicle, in order to guarantee the media's ability to promote growth. All of the test tubes received 0.1 ml of fungal suspension (the working inoculum) for 48 hours at a temperature of 25 to 27 °C. The results are reported in Table 7 and the MIC value of the test substance was determined to be the highest dilution that totally prevented the growth of the test organism.

Table 7. Minimum inhibitory concentration (MIC) (µg/ml) of the synthesized compounds Plant extracts against fungal strains

Plant extract	*C. albicans* (MTCC 1637)	*A. niger* (MTCC 282)
Ethanolic extract	3.125	6.25
Methanolic extract	25	25
Aqueous extract	12.5	12.5

4.3. Molecular docking studies

The most effective phytoconstituents found in *Kydia calycina* were studied using molecular docking to establish their interactions and method of binding with bacterial and fungal proteins. The subsequent Docking experiments were performed on phytochemicals such as lauric acid, myristic acid, palmitic acid, oleic acid, linoleic acid, cyclopropenoic acid, hibiscone C and hibiscoquinone B.[16] The PubChem database (www.ncbi.nlm.nih.gov/pubchem) was used to get the chemical structures and physiochemical characteristics of these substances.[20]

E. coli and *Mycobacterium tuberculosis* protein structure: The Protein Data Bank (PDB) data- base (www.rcsb.pdb) was used to obtain the three-dimensional (3D) structures of *Mycobacterium tuberculosis* and *E. coli.* Large biological macromolecules like proteins and nucleic acids have 3D structural data that is stored in the PDB. *Mycobacterium tuberculosis* and *E. coli* both have PDB IDs of 1EA1 and 1C14, respectively.

Docking studies: Pyrx and Discovery studio Biovia 2017 software were used to conduct compound docking studies to determine how ligands interacted with the target protein.[21]

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