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EVALUATION OF ANTIMICROBIAL PROPERTIES OF *Adiantum latifolium* Lam.: A SOURCE OF TRITERPENOID LEADS AGAINST PATHOGENIC BACTERIA

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ABSTRACT

Secondary bacterial infections and the emergence of drug resistant microbial strains are two consequences concomitant with COVID-19 pandemic. It demands novel antibacterial drugs to treat secondary bacterial diseases. The present study reveals that the ethyl acetate extract of the leaves of *A. latifolium* has antibacterial efficacy against six pathogenic bacteria, *viz*., *Bacillus cereus, Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia,* reported to be infecting COVID-19 patients. Molecular docking and FTIR studies have been used to find out the lead molecules of the extract. The triterpenoids 22-hydroxyhopane and 3β-acetoxy-21α-H-hop-22(29)ene have shown high binding affinity (binding energy from -6.82 to -9.28 kcal mol^{-1}) with DNA gyrase and penicillin binding proteins provided as targets. The study indicates that the phytocompounds present in *A. latifolium* may serve to combat COVID-19 associated ailments.

Keywords: *Adiantum latifolium*, 3β-acetoxy-21α-H-hop-22(29)ene, Covid-19, FTIR, 22-hydroxyhopane, molecular docking

INTRODUCTION

Infectious diseases are always a recurring threat to human health and welfare. The Covid-19 pandemic, caused by the retrovirus SARS-CoV-2, resulted in huge human and economic losses. The virus deranges immune response and causes cytopathological changes and tissue destruction facilitating bacterial infections. Such secondary bacterial infections are reported to be the primary cause of Covid-19 deaths (Shafran *et al.,* 2021). Similar effects also occur during rhinovirus and influenza virus infections (Manohar *et al*., 2020).

Several multidrug resistant bacteria, including *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus,* and *Bacillus cereus* have been cultured from Covid-19 patients (Du *et al.,* 2020; Lansbury *et al.,* 2020; Osakwe, 2020; Sepulveda *et al.,* 2020). Hospitalized Covid-19 patients with bacterial coinfections may be resistant bacteria super-spreaders. In such cases, treatment with the existing antibacterial drugs becomes less effective or even ineffective. These conditions demand the development of new antibacterial drugs with little to no side effects. Natural products derived from plants are considered an alternative choice for discovering new agents or active templates against pathogenic bacteria. The co-evolution of plants with microbes and their metabolic diversity made medicinal plants rich in medicinally active biomolecules. Dehydroabietic acid from *Pinus elliottii*, ellagic acid from *Rosa rugosa* and triterpenes from *Planchonia careya* are some plant derived compounds revealed to have antimicrobial activity against

multidrug resistant bacteria (Subramani *et al.*, 2017). Such phytochemicals besides possessing antibacterial activity, help to prevent the emergence of drug resistant strains of bacteria, whether used alone or in conjunction with conventional antibiotics. As a result, a new area of intense interest in medical research is synergistic therapy regimens, including phytocompounds or their derivatives.

The plant genus *Adiantum* is medicinally significant and has long been used in Ayurvedic medicine systems in India. *Adiantum latifolium* Lam., commonly known as 'Broadleaf Maidenhair fern', has been used in Chinese medicine to cure fever, diarrhoea, and bleeding and also as diuretics (Pan *et al.*, 2011). The presence of phytocompounds with antibacterial characteristics is primarily responsible for the plant's resistance to infections (Archer and Cole, 1986). Ferns differ from flowering plants in a number of ways, one of which is their triterpene metabolism. The triterpenoids ursolic acid and oleanolic acid are reported to have antibacterial activity against gram positive and gram negative bacteria (Wrońska *et al*., 2022). Though these plants have been used in folk medicine and are rich in bioactive compounds, their biological effects have not been thoroughly studied, probably due to little economic interest.

The initial stages of drug research involve screening of plant extracts and identification of bioactive compounds. Lead molecules present in plant extracts and their targets can be identified by molecular docking methods. Penicillin-binding proteins (PBP) and DNA gyrase are promising targets for design and discovery of antibacterial drugs. For instance, coumarins and cyclothialidines are natural antibiotics isolated from *Streptomyces* species that inhibit DNA gyrase activity by blocking the binding of ATP to the enzyme (Khan *et al.*, 2018). The present study was focused to assess the *in vitro* antibacterial efficacy of *A. latifolium* against pathogenic bacteria causing secondary infections in Covid-19 patients. *In silico* method was used to predict the bioactive compounds of the plant against the bacteria.

MATERIALS AND METHODS

Preparation of plant extract

Adiantum latifolium leaves were collected from several places in Thiruvananthapuram district, Kerala (India). The leaves were dried in shade, finely ground to powder using a flour mill. Ethyl acetate extract was prepared in a conical flask at a concentration of 200 g sample L^1, kept in a rotary shaker for 12 h, then filtered and concentrated to dryness under vacuum.

In vitro study of antibacterial activity

Agar well diffusion method was used to study antibacterial activity (Pradeepkumar *et al.*, 2018). The strains used were *Staphylococcus aureus* (ATCC 29213), *Bacillus cereus* (ATCC 10876), *Proteus mirabilis* (GC 10), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 700603). The bacterial inocula were procured from the National Collection of Industrial Microorganisms (NCIM), Pune. Plates were prepared by pouring 20 mL of molten nutrient agar medium into the sterile petridishes (95 mm diameter). The agar was left to solidify for 5 min, and then the medium surface was impregnated with 24 h grown strains. Wells (8 mm diameter, 2 cm apart) were made on each of these plates using a sterile cork borer. Then 100 µL extracts were added to the wells and the plates were incubated at 37◦C for 24 h. The extent of the clear zone indicated by diameter was measured and antibacterial activity was evaluated based on comparison with controls. Experiments were repeated in triplicates, and the results were documented.

Identification of bioactive compounds

The bioactive compounds in the extract were identified based on *in silico* and FTIR analysis (Ali *et al*., 2023) and information from previous studies (Pradeepkumar *et al.*, 2018, 2019a, b). For FTIR analysis (IRPrestige-21, Shimadzu) the sample was prepared using KBr. Bacterial target proteins

selected were DNA gyrase subunit A (PDB Id: 6RKS), Penicillin binding protein 1b (PDB Id: 2Y2H) and Penicillin binding protein 5 (PDB Id: 3MZF). The three dimensional structures of the selected therapeutic targets were retrieved from PDB (*https://www.rcsb.org/search*). Auto-Dock version 4.2 software and BIOVIA Discovery Studio 2020 were used for docking studies and visualization of molecular structures, respectively (Morris *et al*., 2009). The grid box parameters were selected for each protein in such a way as to cover the entire three-dimensional structure of the protein and saved in grid parameter file (GPF) format. Grid generation was done using Autogrid software. The optimization conditions for docking were 50 genetic algorithm runs, a population size of 300, and a maximum number of evaluations of 2.5 x 10^6. CASTp webserver is used to predict the active sites of the targets.

Statistical analysis

The statistical analysis of antibacterial activity was done using SPSS software. Statistical significance was accepted when $P \leq 0.05$.

RESULTS AND DISCUSSION

Antibacterial activity

The ethyl acetate extract of leaves of *A. latifolium* showed significant antibacterial activity against the six strains of pathogenic bacteria in a dose dependent manner (Table 1, Fig. 1).

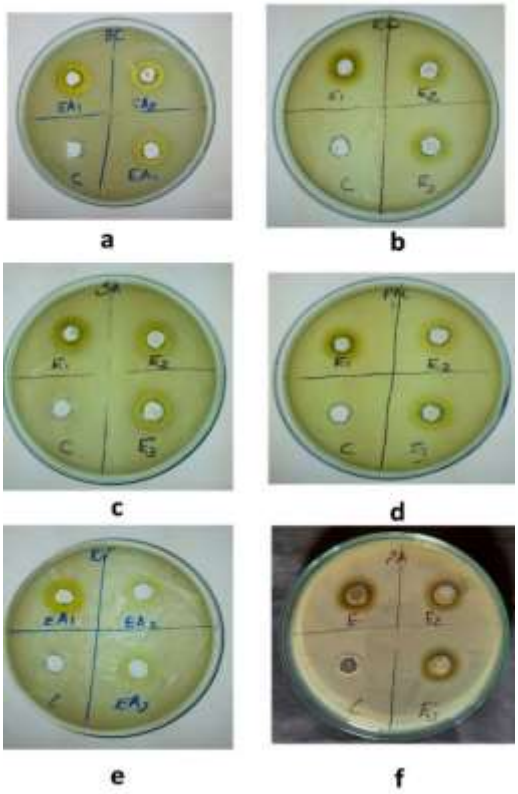

Fig. 1: Antibacterial activity of ethyl acetate extract of *A. latifolium* leaves; a) *B. cereus,* b) *E. coli, c*) *S. aureus,* d) *P. mirabilis,* e) *K. pneumonia,* f) *P. aeruginosa;* (concentration: EA1/E1 - 100 mg mL^{-1}, EA2/E2 - 50 mg mL^{-1}, EA3/E3 - 25 mg mL^{-1}, C - control)

Identification of bioactive molecules

Molecular docking study revealed the binding interactions and affinity of the triterpenoids 22-hydroxyhopane (Hh, $C_{31}H_{54}O$, 442.76 g mol^{-1}) and 3β-acetoxy-21α-H-hop-22(29)ene (Ah, $C_{32}H_{52}O_2$, 468.75 g mol^{-1}) present in the extract (Table 2, Fig. 2 & 3).

The study on binding with DNA gyrase revealed that Hh showed strong binding affinity with DNA gyrase with a binding energy of -8.03 kcal mol^{-1}, Ki of 1.30 μM, 14 interactions with 12 amino acids including 8 from active site. Ah-DNA gyrase binding revealed a binding energy of -6.82 kcal mol^{-1}, Ki of 10.1 μM, and indicated 17 interactions with 14 amino acids. All interacted amino acids fall within the active site.

The study on binding with PBP1b showed that Hh docked with PBP1b with a high binding energy of -9.28 kcal mol^{-1}, Ki of 0.61 μM, revealing 19 interactions with 13 amino acids, of which 7 belong to the active site. Ah binds with PBP 1b with a binding energy of -7.36 kcal mol^{-1}, Ki of 4 μM and had 15 interactions with 14 amino acids of which 9 are from the active site.

The study on binding with PBP5 revealed that the binding energy of Hh with PBP5 is -8.14 kcal mol^{-1}, Ki 0.96 μM, revealing 14

Table 1: Antibacterial activity of ethyl acetate extract of *A. latifolium* leaves against pathogenic bacteria

Bacterial species		Inhibition zone (mm)*		
		100 mg mL^{-1}	50 mg mL^{-1}	25 mg mL^{-1}
Gram + ve	*Bacillus cereus*	15.00 ± 0.70	13.51 ± 0.01	13.26 ± 0.01
	Staphylococcus aureus	16.18 ± 0.41	14.83 ± 0.54	14.17 ± 0.11
Gram – ve	*Proteus mirabilis*	17.30 ± 0.43	16.00 ± 0.35	15.00 ± 0.14
	Pseudomonas aeruginosa	11.00 ± 1.41	10.50 ± 0.35	10.00 ± 1.41
	Escherichia coli	15.83 ± 0.20	15.00 ± 1.41	13.76 ± 0.17
	Klebsiella pneumonia	12.33 ± 1.08	11.35 ± 0.21	10.49 ± 0.01

Control showed no activity, *significance at 0.001 level as compared to control, values are mean ± SE of 3

Table 2: Ligand binding interactions of DNA gyrase, PBP1b and PBP5

Target	Ligand	Binding energy (kcal mol^{-1})	Ki (μM)	H bond length (Å)	Interacted amino acids of active site
DNA gyrase A	Hh	-8.03	1.30	-	GLN434 GLU435 ARG436 ASN437 ARG438 PRO747 GLU748 TRP751
	AHh	-6.82	10.10	2.17 3.31 3.75	PRO95 PHE96 PHE109 GLY110 SER111 ILE112 MET201 LYS217 SER296 ASP297 GLN434 ARG438 PRO747 GLU748
Penicillin binding protein1b	Hh	-9.28	0.16	2.01	TYR515 SER626 ARG627 VAL628 THR629 GLN687 TYR690
	AHh	-7.36	4	3.66	TYR498 ALA499 TYR515 SER626 ARG627 VAL628 THR629 GLN687 TYR690
Penicillin binding protein 5	Hh	-8.14	0.96	1.94 2.84 3.07	ARG40 HIS216 ASP218 LYS219 GLY221 TYR222 GLY241 GLY242 ARG243 THR244 PHE245
	AHh	-7.28	4.58	-	THR7 ILE9 LYS29 PHE263 THR299 ILE300 PRO301 ARG304 LEU336 ASP337 LYS339 ILE341

Hh- 22 Hydroxyhopane, AHh- 3β-Acetoxy-21α-H-hop-22(29)ene
replicates.

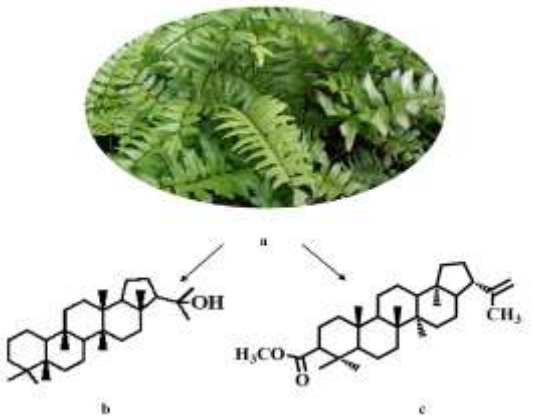

Fig. 2: a) *Adiantum latifolium*, b) 22 hydroxy-hopane, c) 3β-acetoxy-21α-H-hop-22 (29)ene

interactions with 12 amino acids, of which 11 belong to the active site. Ah binds with PBPwith binding energy of -7.28 kcal mol^{-1}, Ki of 4.58 μM and 17 interactions with 12 amino acids. All amino acids with which Ah interacted were active site amino acids.

FTIR study

A sharp peak at 1728 in ethyl acetate extract indicated the presence of C=O groups. Peaks at 3500-3600 indicated the presence of the compound containing –OH group (Fig. 4). The peak at 3005 showed the presence of alkene C-H stretching (=C-H) and peaks in between 1630 and 1700 C=C (double bonds). The common peaks corresponding to –OH and

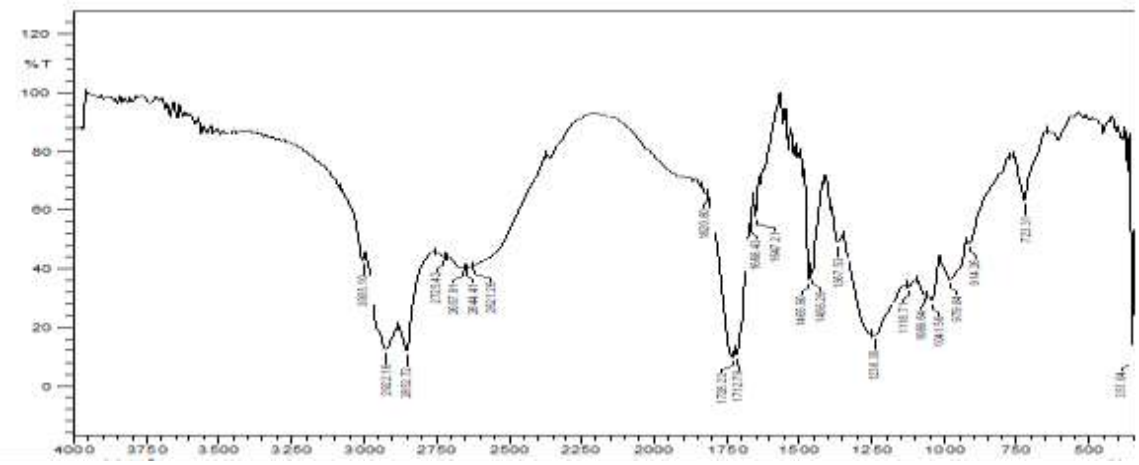

Fig. 4: FTIR spectrum of ethyl acetate extract of *A. latifolium* leaves

Fig. 3: Docked poses of bacterial targets with ligands, a,d,g-3D structure of DNA gyrase, PBP1b and PBP5 respectively. b, e, h- binding interactions of 22-hydroxyhopane, c, f, i -binding interactions of 3β-Acetoxy-21α-H-hop-22(29)ene

C=O groups represent the presence of active compounds Hh and Ah, respectively.

The present study revealed the antibacterial efficacy of *A. latifolium* leaves against the pathogenic bacteria including two strains of Gram positive and four strains of Gram negative bacteria. Through molecular docking studies, target specific bioactive triterpenoids Hh and Ah have been identified from the plant extract. The compounds attach to each other strongly and spontaneously without expending any energy. The ligand-target complex is more stable when the binding energy is negative. The ability of the ligand to prevent the target protein from performing its function is measured by the inhibition constant (Ki). A lower Ki value (0.16 to 10.10 kcal mol^{-1}) indicates higher binding affinity because it signifies that less ligand is required to inhibit the target activity. Potent inhibitors can have Ki values less than 100 μM (Zheng and Polli, 2010). Hh shows higher binding affinity towards all the target proteins and lower Ki values. This can be attributed to the presence of reactive -OH group attached to the pentacyclic ring of the compound.

The extract is a compound composition of various phytochemicals, predominantly triterpenoids. The bioactive compounds have been shown to have multi-targeted activity against the bacteria under investigation. It is worth mentioning that drugs with several targets are safer and more efficient than those with a single target (Zhang *et al.*, 2017). Such compounds can also overcome drug resistance by pathogens and may have a different mode of action compared with the available drugs (Watanabe and Kawaoka, 2015). The identified compounds were also reported for their antiviral potency against SARS- COV-2 (Kumar and Siddique, 2021; Siddique and Kumar, 2022).

The study reveals that the phytocompounds present in *A. latifolium* can be considered natural lead molecules in drug design against Covid-19 and associated bacterial infections since they possess both antibacterial and antiviral properties. Further studies involving chemical modifications of the compounds for improving bioactivity and pharmacological evaluations in *in vivo* models are required for realizing their potential in medicinal chemistry.

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