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Comparative Antimicrobial Profiling of *Synedrella nodiflora* L. and *Spilanthes calva* DC. through Integrated *In Vitro*, *In Silico*, and Phytochemical Screening Approaches

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Abstract

The Asteraceae family includes numerous medicinal plants known for their broad-spectrum therapeutic properties, particularly antimicrobial activity. This study evaluates the phytochemical profiles and antimicrobial activity of methanol extracts from leaves of *Synedrella nodiflora* L. (MESN) and *Spilanthes calva* DC. (MESC), two Bangladeshi Asteraceae species, using in vitro disc diffusion assays and in silico docking, PASS prediction, and ADMET analysis. Phytochemical screening revealed glycosides, gums, flavonoids, and tannins in both (MESN also contained steroids); no alkaloids or reducing sugars were detected. Antimicrobial activity was evaluated via the disc diffusion method using 50 µg/disc extract concentrations. MESN demonstrated moderate antibacterial activity against *Staphylococcus aureus* (14.0 mm) and notable activity against *Shigella dysenteriae* (21.5 mm) and *Shigella sonnei* (23.5 mm). MESC exhibited broader and stronger antibacterial effects, with inhibition zones ranging from 13 mm to 30 mm across tested strains. In antifungal assays against seven pathogenic species, MESN showed significant activity against *Blastomyces dermatitidis* (22.0 mm) and *Microsporum* spp. (23.0 mm), while MESC displayed considerable inhibition against most fungi, except *Trichophyton* spp., with zones ranging from 7.5 mm to 24.0 mm. To complement *in vitro* findings, molecular docking, PASS prediction, and ADMET SAR analyses were conducted on commonly identified phytochemicals from these two plants to evaluate their binding affinities, predicted biological activities, and pharmacokinetic properties. *These in silico* analyses confirmed drug-likeness and binding affinities, supporting therapeutic potential against resistant pathogens. In conclusion, the study highlights the therapeutic promise of *S. nodiflora* and *S. calva* as sources of natural antibacterial and antifungal agents. The integration of phytochemical analysis, microbiological assays, and computational modeling underscores their potential for future drug development.

Key words: Antibacterial, antifungal, disc diffusion, *Synedrella nodiflora*, *Spilanthes calva*, molecular docking.

Introduction

For centuries, traditional remedies derived from medicinal plants have offered an accessible and effective way to treat various ailments, often with fewer side effects (Mallik and Akhter, 2012). These plants, particularly from families like Asteraceae, are rich in secondary metabolites natural compounds with potent antimicrobial and therapeutic properties

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(Bessada *et al.*, 2015). In fact, a significant portion of modern pharmaceuticals originate from plant sources, and according to the World Health Organization (WHO), over 80% of the global population still relies on traditional medicine for their healthcare needs (Laelago *et al.*, 2016). Today, scientific advancements, including computational (in silico) methods, are accelerating the discovery of these vital phytochemicals, reinforcing their value in preventing and treating diseases (Alam *et al.*, 2025a).

Diseases can arise from both external threats, like infections from microorganisms, and internal issues, such as autoimmune disorders (Alam *et al.*, 2020). Many common illnesses are caused by microbes, the earliest form of life on Earth. However, a growing challenge in medicine is the rise of antibiotic resistance, where many bacteria no longer respond to conventional treatments. This critical situation drives the search for new, safe, and effective antimicrobial agents. Nature, particularly the vast chemistry of plants, offers a promising frontier for discovering these urgently needed compounds (Alam *et al.*, 2025b; Richi *et al.*, 2025a).

Athlete's foot, dandruff, and thrush are among the most prevalent infections in humans that are linked to fungi. Although they are superficial and generally harmless, fungi can potentially result in much more serious illnesses such as systemic candidosis and invasive aspergillosis, which have extremely high related fatality rates (Sullivan *et al.*, 2005). Many strains of both gram-positive and gram-negative bacteria have developed high levels of resistance, which increases the urgency of identifying novel, safe, and effective antimicrobial agents from plant sources (Richi *et al.*, 2025b; Muschietti *et al.*, 2005).

Computer-simulated screening allows for a detailed understanding of the pharmacological mechanisms of phytochemicals (Parasuraman, 2011). Molecular docking plays a crucial role in identifying binding sites on three-dimensional protein structures and analyzing physicochemical interactions, making it an essential tool in computer-aided drug design and development. By simulating how natural ligands interact with target proteins, molecular docking aids in the rational design and preparation of new therapeutic compounds, enhancing the efficiency and accuracy of drug discovery processes (Hasib *et al.*, 2025; Emon *et al.*, 2021).

Plants belonging to the Asteraceae family have been found to contain around 1100 distinct acetylenes and biogenetically related compounds. The Asteraceae family is considered to be among the most evolved of all the Dicotyledonous based on its chemical composition and floral structure. Based on phytochemical screening, sesquiterpene, lactones are the main secondary metabolites that give this plant family its antibacterial qualities (Nino *et al.*, 2006). Compounds having two or more triple bonds in their structures are known as natural polyacetylenes. Asteraceae family polyacetylene compounds exhibit a variety of activities, including cytotoxic, antibacterial, anti-inflammatory, neurotoxic, and phototoxic effects (Mohammad *et al.*, 2025a; Konovalov, 2014).

Within the Asteraceae family, sesquiterpene lactones and polyacetylene compounds are important secondary metabolites that contribute to antibacterial and antifungal effects (Alam et al., 2024). These constituents make Asteraceae species attractive candidates for discovering new antimicrobial agents (Alam et al., 2024). The plant *S. nodiflora* L. belongs to the Asteraceae family. For hundreds of years, people have utilized this herb for therapeutic purposes. In Ghana, the leaves are used as a laxative to induce hiccups and to cure epilepsy and discomfort. A decoction of the entire plant is also taken. *S. nodiflora* has been the focus of numerous biological and chemical studies due to its applications in traditional medicine (Zheleva-Dimitrova *et al.*, 2020). The plant has been widely used in Nigeria for addressing cardiac issues, promoting wound healing, and halting bleeding (Idu and Onyibe, 2007) along with In Malaysia and Indonesia, the plant has been utilized for treating headaches, earaches, and stomachaches, as well as serving as a liniment for rheumatism (Dalizel, 1973). It has been observed that the whole plant extract possesses strong central

analgesic and anti-inflammatory properties (Forestieri *et al.*, 1996).

S. calva DC, a member of the Asteraceae family, is also referred to as paracress in English and marhatitiga in Bengali. It is a 60-centimeter-tall annual herb. Stimulant, sialogogue, and local anesthetic properties are present in the roots, flower heads, and entire aerial section. Flower tincture helps with throat infections, tongue paralysis, and toothaches (Ali *et al.*, 2011). This plant is widely recognized for its ability to boost immunity (Rai *et al.*, 2001), anti-aging abilities and treats several gum and dental conditions, including pyorrhea (Rai *et al.*, 2004), also used to treat liver diseases, rheumatism, scabies, scurvy, and gum disease (Jayaraj *et al.*, 2014).

Although *S. calva* DC and *S. nodiflora* (L.) are commonly utilized for the biological activity listed above, no comprehensive data on the plants' antibacterial and antifungal properties has been published yet. Our current study aims to assess the plants' antibacterial and antifungal properties and provide a scientific report on their traditional use in treating diseases, which could eventually lead to the creation of novel antibiotics.

Materials and Methods

Apparatus and reagents

Laminar air flow unit (Model: LCB-1202H, Korea), Hot plate (JHP-815/JHP-825), Micropipette (Diapette: 10-100), Water bath (China), Petri dish (70, 90 & 120mm in diameter), Screw-cap test tubes, Nutrient agar medium, Electric balance (AUY120; Japan), Incubator (XMT-78, China), Autoclave (YX-280A, China), Filter paper discs (5mm in diameter), Inoculating loop, Standard antibiotic discs (Amoxicillin), Griseofulvin.

Collection of samples

In March 2023, during the day, the chosen plant's part (Leaves) of *S. nodiflora* and *S. calva*, were collected from the University of Chittagong campus and identified by a certified taxonomist of the Department of Botany of the University of Chittagong. The herbarium kept voucher specimens of the samples that were gathered for future use.

Preparation of crude extracts

The plant pieces that were gathered were sorted from any unwanted materials. Aerial posts of the plant were then chopped into tiny bits and stored for 15 days in a dry, open area that was shaded. Then, using a grinder, the plant components were reduced to a coarse powder. The two samples' powders were kept dry, dark, and refrigerated until analysis started. Accurately weighed each sample's 300 grams of powdered material in a sterile, amber glass container with a flat bottom and soaked in 2500 milliliters of 99.9% methanol (MW: 46.07 g/mol, MERCK). The containers and their contents were sealed and stored for seven days while being shaken constantly. The whole combination was filtered through a piece of clean white cotton cloth. After using a rotary evaporator to concentrate the methanol extracts of the two plants under investigation, the samples were then allowed to dry at room temperature. It produced a dark and concentrated mass which was known as an methonolic crude extract (Mallik and Akhter, 2012; Alam *et al.*, 2024).

Phytochemical tests

The extract was qualitatively analyzed for the presence of several chemical groups, such as alkaloids, glycosides, steroids, carbohydrates, tannins, flavonoids, and saponins (Hossen *et al.*, 2022; Shandhi *et al*., 2024; Mallik and Akhter, 2012).

Screening for antibacterial activity

Disc diffusion method

The methanol extracts of *S. nodiflora* and *S. calva* were evaluated for antibacterial activity using the disc diffusion method. Amoxicillin (50 ug/disc) was used as reference standard. (Ali *et al.*, 2011) (Kondo *et al.*, 2010).Both Gm (+) and Gm (-) species were chosen to examine the antibacterial activity. The isolates *Salmonella typhi, Shigella dysenteriae, Vibrio cholerae, Shigella sonnei, Salmonella paratyphi, Escherichia coli, Staphylococcus aureus,*

Bacillus cereus, Bacillus subtilis, and *Salmonella megaterium* were selected for assessment based on their Gm (+) and Gm (-). The Faculty of Pharmacy at Southern University in Bangladesh provided all of the species.

Screening for antifungal activity

Fungal culture

The antifungal activity was examined by using seven fungus species. The species include *Pityrosporum ovale, Trichophyton spp., Microsporum spp., Aspergillus niger, Blastomyces dermattitidis, Candida albicans, and Cryptococcus neoformans.* The Southern University of Bangladesh's Faculty of Pharmacy provided all of the species. Every test strain was kept at 4ºC in culture media (Mallik and Akhter, 2012).

Preparation of test samples

The test samples were prepared by dissolving the extracts in methanol, with a concentration of 50 µg/disc. Using a micropipette, 10 µl of the methanol extract was applied to each disc. To ensure complete evaporation of the solvent, the discs were incubated under the conditions mentioned earlier (Ali *et al.,* 2011) (Mallik and Akhter, 2012).

Placement of disks, diffusion, and incubation

The discs (both the samples and the standards) were carefully placed at the center of the agar gels using sterile forceps to ensure complete contact with the surface of the previously inoculated medium. The plates were then inverted and stored in a refrigerator at 4°C for approximately 24 hours. During this period, the test materials diffused into a significant area of the medium. Finally, the plates were incubated at 37°C for 24 hours to assess the antibacterial and antifungal activity (Mallik and Akhter, 2012).

***In silico* study**

Validation of the ligands

At first, the common compounds identified through GC-MS protocol from *S. nodiflora* and *S. calva* plant extracts were taken from previous literature (Adesanwo *et al.,* 2019)(Begum *et al.,* 2008). The compounds include Octadecene (PID: 8217), Methyl hexadecanoate (PID: 8181), 1-docosene (PID: 74138), Methyl-9,12,15-octadecatrienoate (PID: 5367462) of MESN, and Caryophyllene Oxide (PID: 1742210), Caryophyllene (PID: 5281515), Limonene (PID: 22311), Myrcene (PID: 31253) of MESC**.** We retrieved the structures of the compounds from the PubChem database. These compounds are shown in Figure 3. The ligands were considered for in depth computer-aided molecular docking study. The compounds were then evaluated for drug potential using Lipinski's rule of five on the SwissADME web server (Hossain *et al.,* 2025).

Protein preparation

The RCSB protein data bank provided the crystal structures for the target proteins, which included bacterial enoyl-ACP reductase (FabI) inhibitors (PDB: 1LX6), benzamide FtsZ Inhibitor (PDB ID: 8HTB), 14-alpha-demethylase (PDB: 5FRB), and nucleoside diphosphokinase (PDB: 6K3H). Kurumbail *et al.* (Kurumbail *et al.,* 1996) supplied information that was utilized to determine the enzyme's active location. Swiss-PdbViewer and BIOVIA Discovery Studio 4.5 were used to remove water, cofactors, and heteroatoms throughout the preparation procedure. Following the addition of hydrogen atoms, protein reduction was carried out using PyRx and the MMFF94 force field. The target protein was stored in PDB format for docking studies (Mohammad *et al.,* 2025a).

Molecular docking and post-docking analysis

AutoDock 4.2 and PyRx 0.3 (http://pyrx.scripps.edu) were used for the docking operations (accessed July 31, 2021). (Kondo *et al.,* 2010). The docking findings were evaluated using PyMOL. These tools can assist in identifying if a particular kind of contact, such as a hydrogen bond, π-π interaction, or cation-π interaction, constitutes ligand binding. PyMOL was used to collect additional information on ligand-receptor interactions (Mohammad *et al.,* 2025b) (Mohammad *et al.,* 2025c).

PASS prediction

To ascertain the potential biological effects of the selected compounds, the PASS prediction was analyzed using the PASS online tools (http://www.pharmaexpert.ru/passonline/predict.php) . Between 0.000 and 1.000, the values of Pi and Pa varied. A chemical is considered to have biological potential when its Pa value is higher than its Pi value. On the other hand, Pa > 0.7 denotes high medicinal activities, 0.5 < Pa < 0.7 denotes intermediate therapeutic potentials, and Pa < 0.5 denotes poor pharmaceutical activity (Mohammad *et al.*, 2025d).

Results and Discussion

Phytochemical screening

This study aimed to confirm the presence or absence of preliminary phytochemicals. The results from various group tests performed on the selected plants, *S. nodiflora* L and *S. calva* DC, are presented in Table 3.

Table 3. Phytochemical analysis of the methanolic extract of *S. nodiflora* L and *S. calva* DC.

Phytochemicals	*Synedrella nodiflora* L	*Spilanthes calva* DC
Alkaloids	-	-
Glycosides	+	+
Steroids	+	-
Gums	+	+
Flavonoids	+	+
Reducing sugars	-	-
Tannins	+	+

+ = Presence, - = Absence.

Screening for antibacterial activity

Disc diffusion method

The antibacterial effectiveness of the test agents was assessed by their ability to inhibit bacterial growth, forming a clear zone of inhibition around the discs. After incubation, the diameter of these inhibition zones was measured in millimeters using a transparent scale, providing insights into the antimicrobial efficacy of the agents. The results are summarized in Table 4. Gram-negative bacteria were susceptible to the antibacterial activity of both MESN and MESC. With a 23.5 mm inhibitory zone against *S. sonnei*, MESN specifically shown moderate activity. With inhibition zones of 28 mm and 30 mm against *V. cholerae* and *S. paratyphi*, respectively, the extract MESC demonstrated more antibacterial activity than the common antibiotic amoxicillin. The results indicate that the efficacy of the common antibiotic amoxicillin and the plant extracts differed according on the type of bacterium that was tested.

***In Silico* study**

ADME/T analysis

The phytochemical structures of the MESN and MESC compounds were illustrated **in Figure 3** using ChemDraw Ultra 12.0. According to the study, all of the chemicals are orally bioavailable and comply with Lipinski's guidelines. The eight chemicals of MESN and MESC's toxicological characteristics are also predicted using the online admetSAR server (http://lmmd.ecust.edu.cn/admet sar2/) (Table 6).

Molecular Docking Analysis

Each activity's overall docking score is displayed in **table 7**. For each activity, the top compound of MESN and MESC's docking score and interaction analysis are displayed in **table 8** and **figurs. 4-7**, along with the reference medication for appropriate protein targets.

Table 4. ***In vitro*** **antibacterial activity of** ***S. nodiflora*** **and** ***S. calva*****.**

Bacterial strains		Zone of inhibition in mm		
		MESN	MESC	Amoxicillin (standard)
Gram-positive species	*B. subtilis*	10	16	32
	B.megaterium	8	14	31
	B. cereus	9	15	30
	S. aureus	14	17	28
Gram negative species	*P. aeruginosa*	14	24	30
	E. coli	15	25	32
	S. dysenteriae	21.5	28	31
	S. sonnei	23.5	13	25
	S. typhi	**R**	14	31
	V. cholerae	9	28	29
	S. paratyphi	**R**	30	32

R= Resistant / No growth.

Screening for antifungal activity

Table 5. ***In vitro*** **antifungal activity of** ***S. nodiflora*** **and** ***S. calva*****.**

Fungal strains	Zone of inhibition in mm		
	MESN	MESC	Griseofulvin (standard)
Aspergillus niger	**R**	7.5	27
Blastomyces dermatidis	22	24	29
Candida albicans	**R**	16	26
Pityrosporum ovale	15	20	22.5
Trichophyton spp.	**R**	**R**	29
Microsporum spp.	23	24	28
Cryptococcus neoformans	15	21	26

R = Resistant / No growth.

Table 6. ADME/T Analysis of Reported phytochemicals of MESN and MESC. MW=Molecular Weight, HBA= Hydrogen Bond Acceptor, HBD= Hydrogen Bond Donor, nRB= Number of Rotational Bonds, TPSA= Topological Polar Surface Area, AOT= Acute Oral Toxicity, HIA= Human Intestinal Absorption, B.S.= Bioavailability Score, BBB= Blood Brain Barrier, NAT = Not Ames Toxic, and NC= Not Carcinogenic.

Plant extract	Compound name	Lipinski rules				Lipinski's Violation ≤ 1	Veber's rules		Toxicity parameters					
		MW (g/mol) <500	HBA <5	HBD ≤ 5	Log p ≤ 5		n RB ≤10	TPSA ≤ 140	Ames Toxicity	Carcino-gens	AOT	HIA	B.S.	BBB
S.nodiflora	Octadecene	252.486	0	0	7.0438	1	15	0.00Å²	NAT	C	III	90.834	0.55	0.987
	Methyl hexadecanoate	270.45	2	0	5.6407	1	14	26.30 Å²	NAT	C	III	92.335	0.55	0.749
	1-docosene	308.5	0	0	8.6042	1	19	0.00Å²	NAT	NC	III	89.46	0.55	1.06
	Methyl-9,12,15-octadecatrienoate	292.4	2	0	5.7489	1	13	26.3Å²	NAT	C	III	95.633	0.55	0.801
S.calva	Caryophyllene Oxide	220.356	1	0	3.9634	0	0	12.53 Å²	NAT	NC	III	95.421	0.55	0.654
	Caryophyllene	204.357	0	0	4.7252	1	0	0.00 Å²	NAT	NC	III	97.302	0.55	0.52
	Limonene	136.238	0	0	3.3089	0	1	0.00 Å²	NAT	NC	III	98.042	0.55	0.557
	Myrcene	136.238	0	0	3.475	0	4	0.00 Å²	NAT	C	III	97.594	0.55	0.68

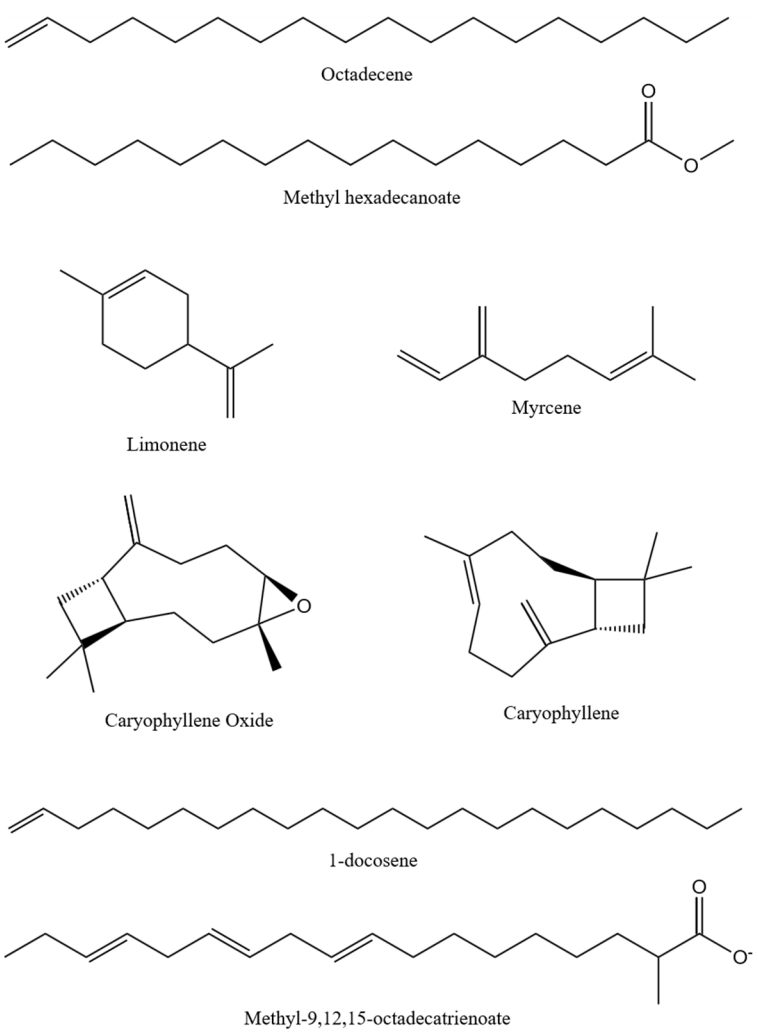

Figure 3. Phytochemical structures of MESN and MESC compounds.

Table 7. Binding scores of the reported compounds from the MESN and MESC for antibacterial and antifungal activity, respectively.

Sample	Compounds	PubChem CID	Docking Score (Kcal/mol)			
			Antibacterial		Antifungal	
			1lx6	8htb	5frb	6k3h
MESN	Octadecene	8217	-5.2	-5.7	-5.5	-4.3
	Methyl hexadecanoate	8181	-4.8	-5.7	-5.5	-4.7
	1-docosene	74138	-5	-6.4	-6.3	-4.7
	Methyl-9,12,15-octadecatrienoate	5367462	-5.4	-6.5	-6.4	-5.4
MESC	Caryophyllene oxide	1742210	-6.3	-6.5	-8.5	-5.4
	Caryophyllene	5281515	-6.3	-6.4	-8.6	-5.4
	Limonene	22311	-5.7	-6	-6.3	-4.6
	Myrcene	31253	-5.1	-6	-5.5	-4.4
Standards	Amoxicillin	33613	-8.2	-7.7	---	---
	Griseofulvin	441140	---	---	-7.8	-5.6

Table 8. MESN and MESC's reported compounds *in silico* binding affinity and non-bonding interaction for antibacterial and antifungal properties, respectively.

Section Number	Receptor	Compound	Binding affinity (kcal/mol)	Bond type	Amino acids
1	1lx6	Methyl-9,12,15-octadecatrienoate (MESN)	-4.8	Carbon hydrogen bond	ILE92, LEU144
				Pi-Sigma	TYR146
				Alkyl	ALA189 (2), ILE192, ILE20, LEU144 (2)
				Pi-Alkyl	TYR146 (2), TYR156 (2)
		Caryophyllene (MESC)	-6.3	Pi-Sigma	TYR146
				Alkyl	ALA189, ILE20, ILE192
				Pi-Alkyl	TYR146 (2), TYR156
		Amoxicillin **(Standard)**	-8.2	Conventional hydrogen bond	ALA21, LYS163 (2), GLY13,
				Hydrogen bond	SER19, SER91
				Pi-Pi Stacked	TYR146
				Alkyl	ALA189
				Alkyl	ILE20
2	8htb	Methyl-9,12,15-octadecatrienoate (MESN)	-6.5	Alkyl	VAL203, VAL297, VAL307, LEU200, MET226, ILE311
		Caryophyllene Oxide (MESC)	-6.5	Alkyl	VAL189 (2), PRO248 (4), LEU249 (3), LEU250 (2), ILE172
		Amoxicillin **(Standard)**	-7.7	Conventional Hydrogen Bond	ASN263, THR265, ASN299 (2), THR309, VAL203
				Amide Pi-Stacked	ASP199, LEU200
				Pi-Alkyl	LEU200, VAL203, VAL297
3	5frb	Methyl-9,12,15-octadecatrienoate (MESN)	-6.4	Alkyl	ILE373 (2), LEU503 (2), LEU125, ILE376
				Pi-Alkyl	TYR122 (3), PHE130, PHE229, PHE234, PHE504 (2)
		Caryophyllene (MESC)	-8.6	Alkyl	LEU503 (5), ILE377, ILE373
				Pi-Alkyl	TYR122, PHE229, PHE234, PHE504, HIS374
		Griseofulvin (**Standard**)	-7.8	Alkyl	ALA307, ILE373, LEU503
				Pi-Alkyl	PHE504, ILE373
5	6k3h	Methyl-9,12,15-octadecatrienoate (MESN)	-5.4	Conventional hydrogen bond	LYS11 (2), ARG104 (2), ASN114, GLY118
				Carbon hydrogen bond	GLY118, THR93
				Alkyl	VAL111, LEU63, LEU54 (3)
				Pi-Alkyl	TYR51, PHE59
		Caryophyllene (MESC)	-5.4	Alkyl	LEU54, VAL111 (2)
				Pi-Alkyl	TYR51, PHE59, HIS117
		Griseofulvin **(Standard)**	-5.6	Conventional hydrogen Bond	LYS11, ASN114 (2)
				Carbon hydrogen bond	CYS116
				Alkyl	LEU54, LEU63
				Pi-Alkyl	PHE59, HIS117 (2)

Pass prediction

Eight carefully selected compounds from MESN and MESC were assessed for their antibacterial and antifungal potential using the PASS online tool. The results showed that compounds with notable molecular activity had Pa (probability of activity) values higher than Pi (probability of inactivity), indicating a strong likelihood of biological activity (Table 9).

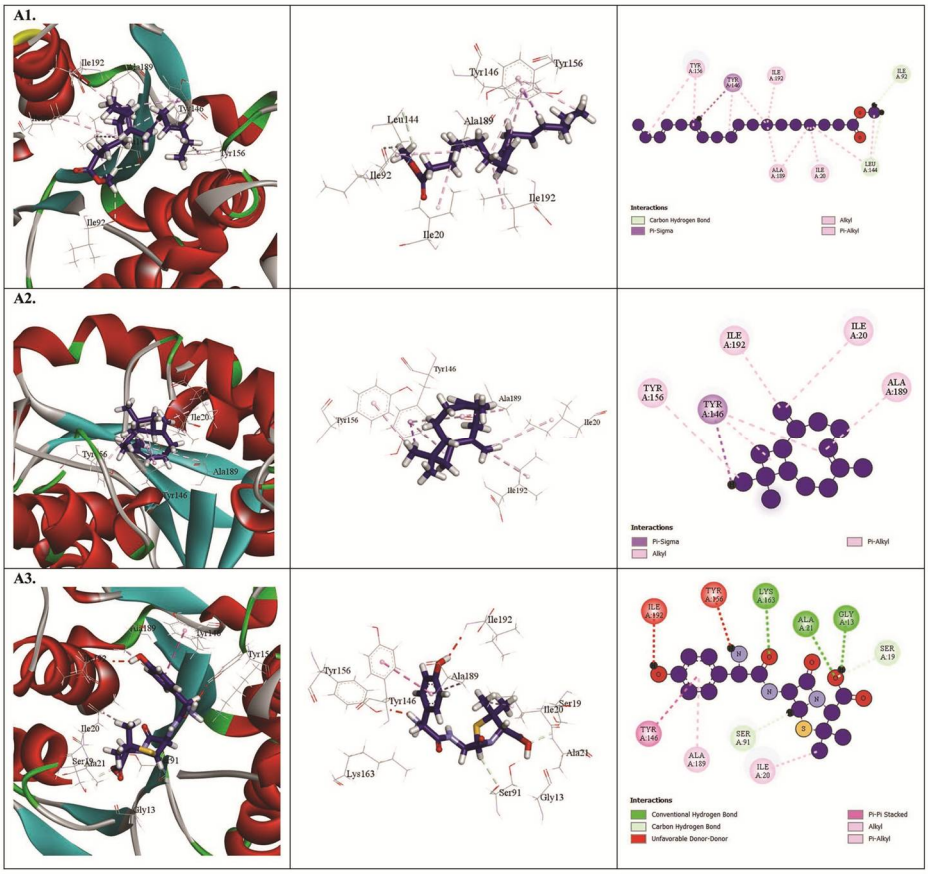

Figure 4. Molecular Docking Interactions of Compounds against bacterial enoyl-ACP reductase (FabI) inhibitors (PDB: 1lx6): A1. Methyl-9,12,15-octadecatrienoate (MESN) A2. Caryophyllene (MESC) A3. Amoxicillin (Standard).

Table 9. Pass Prediction analysis of biologically active compounds of MESN and MESC.

Sample	Compound name	Biological activity			
		Antibacterial		Antifungal	
		Pa	Pi	Pa	Pi
MESN	Octadecene	0.353	0.042	0.535	0.025
	Methyl hexadecanoate	0.263	0.076	0.424	0.045
	1-docosene	0.353	0.042	0.535	0.025
	Methyl-9,12,15-octadecatrienoate	0.315	0.055	0.517	0.028
MESC	Caryophyllene oxide	0.532	0.014	0.647	0.014
	Caryophyllene	0.437	0.023	0.582	0.020
	Limonene	0.405	0.029	0.582	0.020
	Myrcene	0.398	0.030	0.584	0.020

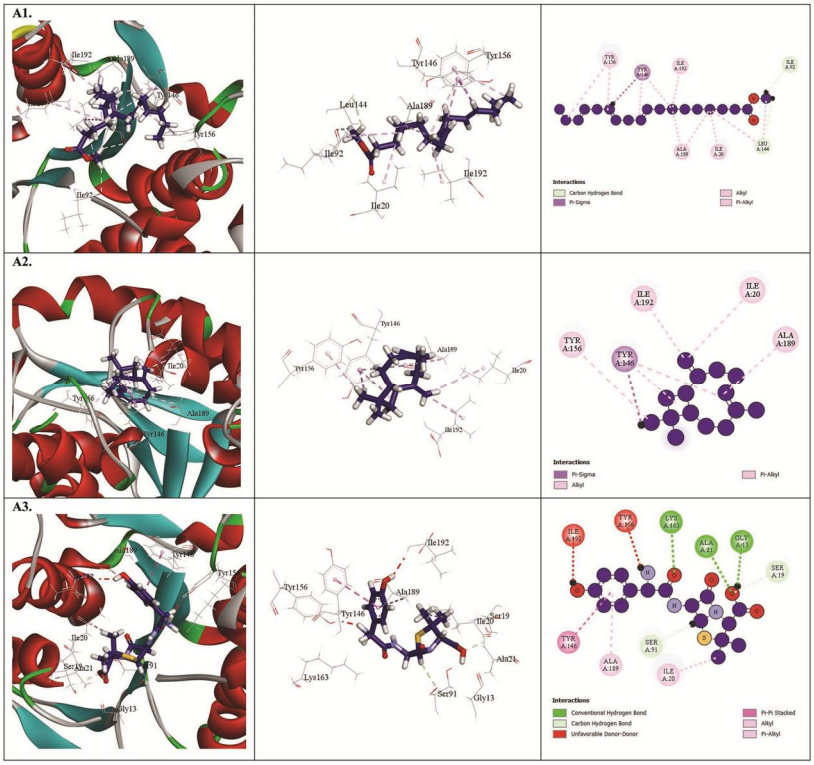

Figure 4. Molecular Docking interactions of compounds against bacterial enoyl-ACP reductase (FabI) inhibitors (PDB: 1lx6): A1. Methyl-9,12,15-octadecatrienoate (MESN) A2. Caryophyllene (MESC) A3. Amoxicillin (Standard).

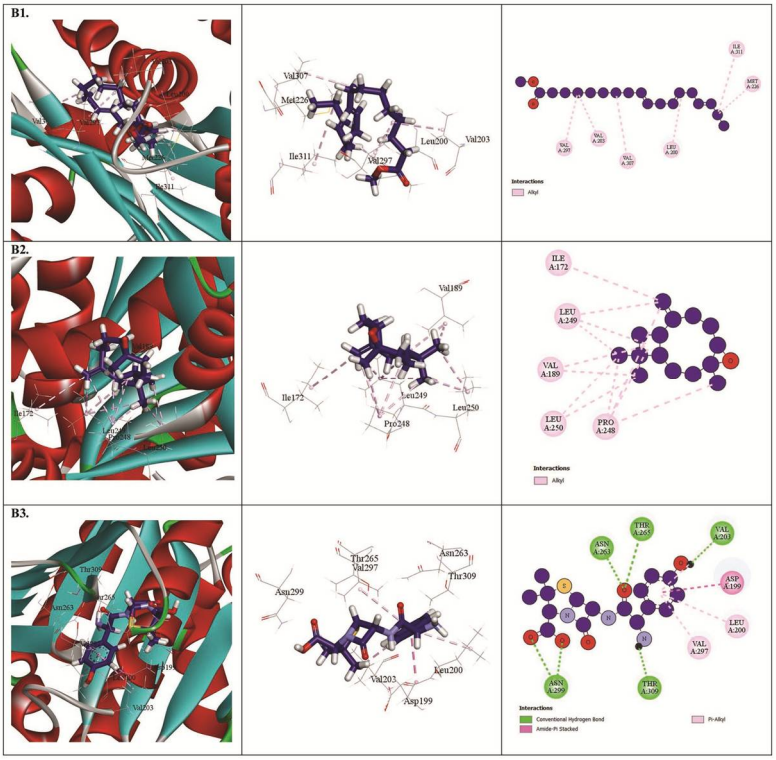

Figure 5: Molecular Docking interactions of compounds against benzamide FtsZ inhibitor (PDB: 8htb): B1. methyl-9,12,15-octadecatrienoate (MESN) B2. Caryophyllene Oxide (MESC) B3. Amoxicillin (Standard).

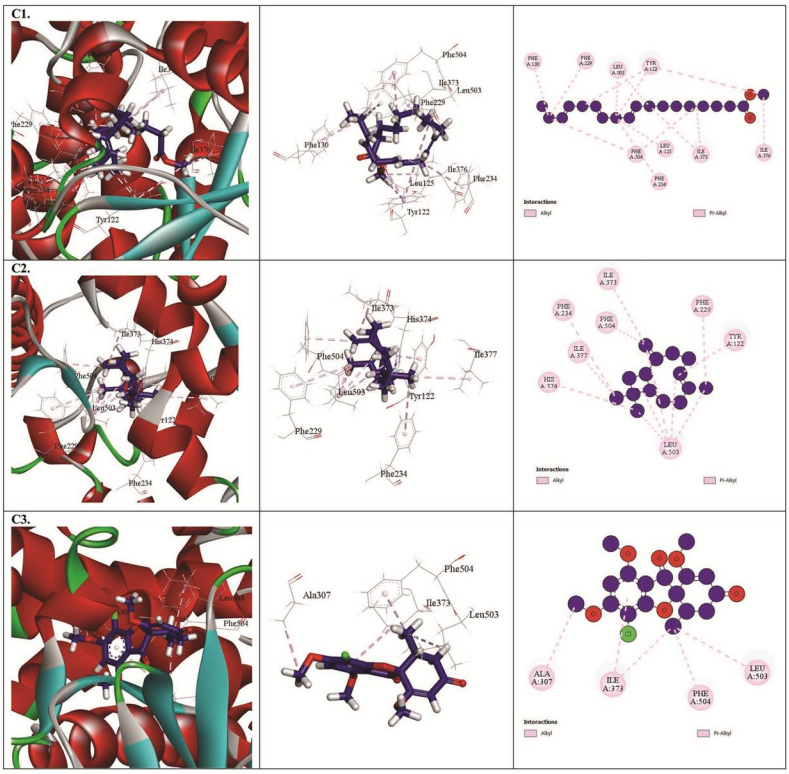

Figure 6: Molecular Docking Interactions of Compounds against 14-alpha-demethylase (PDB: 5frb): C1. Methyl-9,12,15-octadecatrienoate (MESN) C2. Caryophyllene (MESC C3. Griseofulvin (Standard).

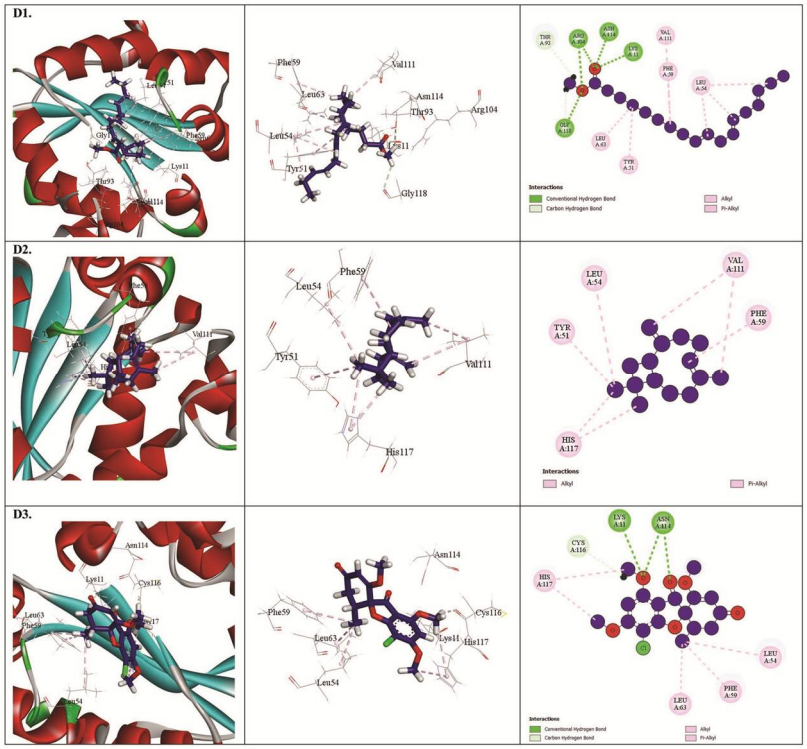

Figure 7: Molecular Docking Interactions of Compounds against Nucleoside Diphosphokinase (PDB: 6k3h): D1. Methyl-9,12,15-octadecatrienoate (MESN) D2. Caryophyllene (MESC) D3. Griseofulvin (Standard).

Due to the depletion of natural resources and related issues, certain nations have recently required the use of natural resources for a variety of reasons, despite notable advances in technology and medicine (Ertürk, 2006). It is estimated that approximately two-thirds of medications are derived from plants. This has led to the emergence of a new field of study known as phytopharmacology, which focuses on exploring plant-derived compounds that are effective in treating specific chronic diseases and has sparked growing interest in herbal medicines (Zishan *et al.*, 2023). Similar to many other countries, people in Bangladesh collect and utilize plants with known health benefits to treat various ailments. This study examined the phytochemical properties as well as the antimicrobial efficacy of methanolic extracts from *S. nodiflora* and *S. calva* against bacteria and fungi.

To determine whether phytoconstituents are present in plants and to forecast their possible therapeutic advantages, phytochemical screening is a useful technique (Pant *et al.,* 2017). Numerous of these phytoconstituents, including terpenoids, flavonoids, and alkaloids, are secondary metabolites recognized to have therapeutic uses (Batiha *et al.,* 2020). This investigation identified the presence of numerous secondary metabolites, which significantly enhanced the bioactivity of methanolic leaf extracts from *S. nodiflora* and *S. calva* using phytochemical analysis. For example, tannins, which have astringent effects that cause tissue to tighten, are used in a variety of medicinal applications, including anti-cancer, hypoglycemic, and antioxidant therapies (Rajasekaran *et al.,* 2021). Another class of chemicals, triterpenes, are known to have anti-inflammatory and anti-tumor properties (Renda *et al.*, 2022). Due to their diverse actions, alkaloids serve as a broad range of pharmacological tools in the therapeutic field. They exhibit various effects, including antihypertensive (e.g., reserpine), antipyretic (e.g., quinine), anticancer (e.g., vincristine), antiasthmatic (e.g., ephedrine), analgesic (e.g., morphine), and antihyperglycemic (e.g., piperine) properties (Ashrafi *et al.*, 2023). Furthermore, heart-related disorders can be effectively treated with glycosides, especially cardiac glycosides (Fatema *et al.,* 2024).

Eleven bacterial strains were evaluated using the disc diffusion method, and the methanolic extracts of S. nodiflora and S. calva showed moderate antibacterial activity. Direct comparison of the two extracts indicates that MESC generally produced larger inhibition zones and affected a broader spectrum of bacteria than MESN, suggesting that S. calva may be more suitable as a source of broad-spectrum antibacterial leads, whereas S. nodiflora shows more selective activity against certain gram-negative pathogens such as *Shigella* spp.

Meanwhile, the antibacterial activity of these extracts may be attributed to the presence of bioactive compounds such as phenols, flavonoids, alkaloids, glycosides, and tannins, which are known for their antimicrobial properties. However, these findings are based on crude methanolic extracts at a single disc concentration, and minimum inhibitory or fungicidal concentrations were not determined, which limits direct comparison with standard antibiotics and clinical relevance (Hossain *et al.,* 2023).

The antifungal properties of two extracts were evaluated against various fungal species. Flavonoids have been reported to have antifungal properties (Al Aboody and Mickymaray, 2020). Both extracts have flavonoids, which may be the cause of their antifungal attributes, according to phytochemical screening. However, further studies are necessary to isolate and purify the active compounds and confirm their antifungal efficacy. The different response patterns, with MESN showing stronger effects against *Blastomyces dermatitidis* and *Microsporum* spp. and MESC inhibiting a wider range of fungi but not Trichophyton spp., indicate that these extracts may be useful for different types of fungal infections rather than as universal antifungal agents.

Molecular docking studies are widely used to predict ligand-target interactions and enhance the understanding of the bioactivities of natural products.

These studies also provide valuable insights into potential binding mechanisms within protein active sites. In this research, molecular docking was employed to clarify and support the observed biological activities, helping to interpret their underlying pharmacological effects (Mohammad *et al.,* 2025c). A total of 8 compounds from *S. nodiflora* (MESN) and *S. calva* (MESC) were selected for a more detailed analysis. These compounds were docked against four target proteins: bacterial enoyl-ACP reductase (FabI) inhibitors (PDB: 1LX6) and benzamide FtsZ inhibitor (PDB ID: 8HTB) for antibacterial activity, as well as 14-alpha-demethylase (PDB: 5FRB) and nucleoside diphosphokinase (PDB: 6K3H) for antifungal activity. The compounds from the extracts MESN and MESC showed notable binding toward FabI. These findings suggest that these compounds have promising antibacterial potential through their ability to inhibit essential bacterial enzymes and proteins involved in cell wall synthesis and fatty acid biosynthesis. Nonetheless, docking and PASS prediction provide only theoretical support; experimental confirmation with purified compounds and in vivo models is required before any therapeutic application can be proposed. This study investigated potential antifungal compounds by screening them against two key target proteins: 14-alpha-demethylase (PDB: 5FRB) and nucleoside diphosphokinase (PDB: 6K3H). These results suggest that these compounds have considerable antifungal activity by targeting essential enzymes involved in fungal cell function and survival. This approach enables the identification of promising bioactive molecules with potential therapeutic applications in combating bacterial and fungal infections.

Conclusion

In the present study, methanolic extracts of *S. nodiflora* (MESN) and *S. calva* (MESC) leaves were found to contain a variety of phytochemicals that likely contribute to their therapeutic potential. MESN exhibited mild antibacterial activity against Gram-positive bacteria, while showing significant activity against Gram-negative bacteria. In contrast, MESC demonstrated notable antibacterial effects against all tested bacterial strains. Both MESN and MESC displayed significant antifungal activity against most of the fungal species evaluated. Additionally, Methyl-9,12,15-octadecatrienoate of *S. nodiflora* and Caryophyllene of *S. calva* showed significant binding affinity in the molecular docking study. Further research is necessary to identify the active compounds and assess the in vivo antibacterial and antifungal activities of these plant extracts.

Conflict of interest

The authors declare that they have no competing interests.

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None

Data availability statement

Data will be available upon request.

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