Comprehensive Liquid Chromatography-Mass spectrometry Profiling, Antioxidant and Antifungal Activity against Fish Fungi of Hexane Extract from *Artemisia vestita* Wall. Ex Besser

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ABSTRACT

The Himalayan medicinal herb *Artemisia vestita* Wall. Ex Besser is highly regarded for its therapeutic qualities; limited information is available on its non-polar extracts. The present study examined the phytochemical composition, antioxidant, and antifungal properties of the hexane extract of *A. vestita*. LC-MS analysis illustrated the presence of some major non-polar metabolites, predominantly terpenoids and fatty acids, including Gama-linolenic acid ethyl ester (1.34%), (+/-)-16-hydroxy-docosahexaenoic acid (1.38%), artemivestinolide D (1.82%), stigmasterol (6.12%), genkwanin (1.08%), etc. Only trace levels of flavonoids and phenolics were identified, such as Luteolin-7-methylether (0.02%), Cirsilineol (0.01%), etc. The agar well-diffusion experiment was used to assess antifungal activity against Aspergillus sp. and Fusarium sp., two significant fish-pathogenic fungi. The *in vitro* analysis demonstrated a concentration-dependent inhibitory effect against both fungal pathogens. Maximum inhibition was observed against *Aspergillus* sp. (14.46 ± 0.56 mm), followed by *Fusarium* sp. (13.2 ± 0.15 mm), although the activity was much lower than that of the positive control nystatin. Additionally, antioxidant activity was assessed using the DPPH assay revealed limited free radical scavenging potential due to the low phenolic content of the extract. Overall, the findings demonstrated moderate bioefficacy of the extract and the significant impact of solvent polarity on phytochemical output.

Keywords: *Artemisia vestita, LC–MS profiling, Hexane extract, Antifungal, Antioxidant activity*

INTRODUCTION

One of the largest and most chemically diverse genera in the plant world, Artemisia L. (family Asteraceae) comprises around 500 species, most of which are found in Asia, Europe, and North America. Many species of *Artemisia* have been widely used in traditional medicinal systems such as Ayurveda, Unani, and ancient Chinese medicine due to their multiple pharmacological benefits, including antibacterial, anti-inflammatory, antimalarial, antioxidant, and anticancer properties (Al-Sowayan *et al*., 2024). This medicinal potential is mainly due to the varied phytochemical composition, which contains terpenoids, flavonoids, coumarins, phenolic acids, and other pharmacologically active secondary metabolites.

The perennial shrub *Artemisia vestita* Wall. ex Besser, commonly referred to as "Russian Wormwood," is mostly found in the Himalayan areas of India, Nepal, and Tibet. This shrub has historically been used to cure fever, cough, infections, and digestive disorders (Dogra *et*

al., 2023). Previous phytochemical investigations of the essential oil of *Artemisia vestita* have revealed the presence of diverse classes of secondary metabolites, including monoterpene hydrocarbons (17.3%), oxygenated monoterpenes (73.1%), sesquiterpene hydrocarbons (1.7%), and oxygenated sesquiterpenes (2.1%) (Rather *et al*., 2017). Several studies have particularly focused on the composition of its essential oil.

Several studies have primarily focused on the composition of its essential oil. Chu et al., 2010 analysed the essential oil extracted from the aerial parts of *A. vestita* collected in China and identified grandisol (40.29%), 1,8-cineole (14.88%), and camphor (11.37%) as the major constituents. Similarly, Dogra et al., 2024 identified several major compounds including 1,8-Cineol (11.35), Artemisia alcohol (5.62), Grandisol (28.45), Borneol (11.12), Isofraxidin (2.12), Artemisia ketone (3.12%), Germacrene D (4.85), and β caryophyllene (5.67%) from its aqueous extract, highlighting the influence of regional variation on the volatile composition of *A. vestita*. In addition, they also reported its potential antibacterial, antioxidant, and cytotoxic activities, making it a viable option for pharmacological investigation and natural product research (Dogra *et al*., 2024). Nevertheless, there is still a lack of thorough phytochemical characterisation of *A. vestita* based on solvent-specific variations, despite its ethnomedical significance.

A promising screening technique for the thorough profiling of plant metabolites is liquid chromatography–mass spectrometry (LC–MS). The technique provides high sensitivity, selectivity, and resolution, which enables the simultaneous detection of a variety of chemical compounds, including highly polar glycosides and non-polar terpenes (Cannavacciuolo *et al*., 2023). In addition to making, it easier to identify bioactive components, LC-MS-based metabolomic investigations also help in understanding chemical composition, solvent extraction efficiency, and potential therapeutic implications.

The phytochemical composition of plant extracts is greatly influenced by the solvent selection. The class and yield of extracted chemicals can be determined by the polarity of the solvent; terpenoids, sterols, and fatty acids are mostly extracted by non-polar solvents like hexane, whereas flavonoids, phenolics, and coumarins are successfully extracted by moderately polar solvents like ethyl acetate (FOO, 2021). Thus, differential solvent extraction helps determine specific bioactive components for further biological analysis and provides insights into the distribution of phytochemicals within a plant species.

Aspergillus sp. and *Fusarium* sp. are two fish pathogenic fungi causing gill necrosis, tissue damage, respiratory problems, considerable morbidity in farmed fish, and pose a serious danger to aquaculture worldwide. They cause significant financial losses and deteriorating fish quality through rapid proliferation in aquatic systems (Das *et al*., 2025). Even while chemical fungicides and antibiotics are frequently used to treat fungal diseases, their widespread use has led to environmental pollution, antimicrobial resistance, and detrimental effects on aquatic organisms that are not their intended targets. This situation

necessitates the search for safer and more environmentally friendly substitutes (Mohammad *et al*., 2025). Plant-derived extracts are increasingly popular as natural antimicrobials due to their chemical composition, low toxicity, and biodegradability. Due to their long history of potent antibacterial and antifungal properties, species of the genus *Artemisia* appear especially promising. Although the essential oil and polar extracts of *A. vestita* have been found to possess numerous bioactive terpenoids and flavonoids with antimicrobial activity (Dogra *et al*., 2024; Ding et al., 2010). However, lipophilic substances such as terpenoids, fatty acids, and sterols are preferentially extracted by nonpolar solvents and may exhibit mild but harmless antifungal effects; these nonpolar extracts have not received sufficient attention. In particular, solvent-specific LC-MS profiling has not been sufficiently documented, which limits our understanding of non-polar metabolites that may be involved in its biological activities. The present study represents the first comprehensive assessment of the LC-MS-based phytochemical composition of the hexane extract of A. vestita, along with an analysis of its antioxidant and antifungal activity. This solvent-focused analysis provides new viewpoints on phytochemical diversity and the therapeutic potential of the nonpolar extract of this medicinal plant.

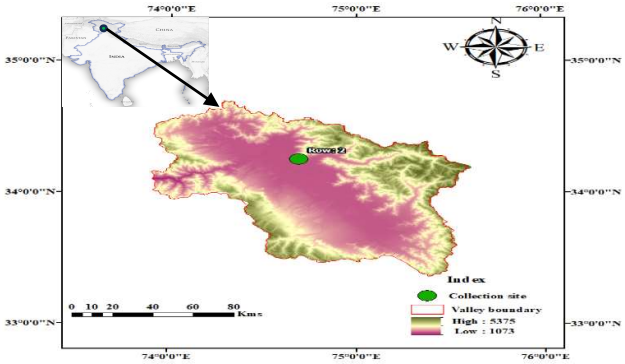

Fig. 1. Sampling site for collection of *Artemisia vestita*

MATERIALS AND METHODS

The flowchart showing the steps followed during the experiment is shown in Fig. **2.**

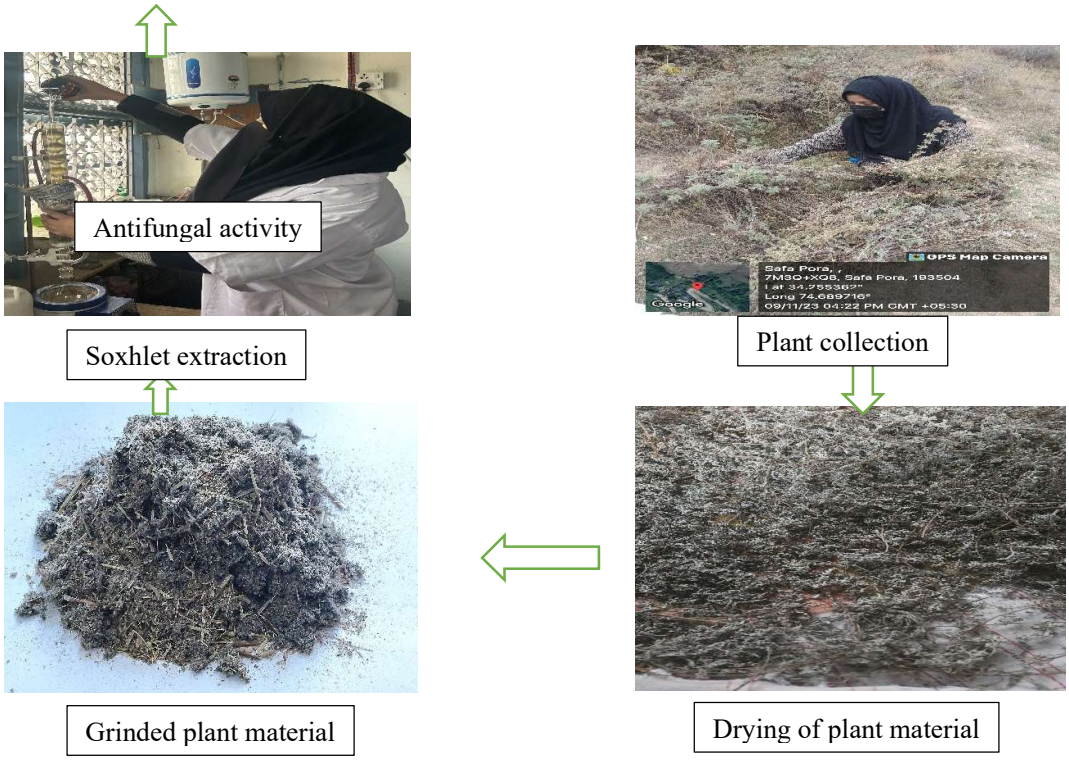

Fig. 2. Schematic diagram showing experimental design

Collection and identification of plant material

During the blossoming season (August to September), fresh aerial parts of *Artemisia vestita* Wall. ex Besser were collected from the Manasbal area of District Ganderbal, Jammu and Kashmir, India. A plant taxonomist, Akhtar H. Malik, Centre for Biodiversity and Taxonomy (CBT), Department of Botany, University of Kashmir, identified the experimental plant. For future reference, a voucher specimen (9081) was deposited in the departmental herbarium.

Preparation of plant extract

The plant powder and hexane solvent were used in a 1:5 ratio. Approximately 50 g of the

powder was extracted using a Soxhlet apparatus for 6-8 hours, until the solvent became clear (Mohammed *et al*., 2022). The resulting extract was filtered through Whatman No. 1 filter paper and concentrated to dryness under reduced pressure using a rotary evaporator maintained at 60 °C. The dried extract was stored in an Eppendorf tube at 4°C for future use.

Liquid chromatography and mass spectrometry (LC-MS) analysis

LC–MS analysis was carried out using a sophisticated analytical instrument facility (SAIF) at Punjab, Chandigarh, India. The instrument employed for LC-MS analysis was a model DBA064 equipped with a Waters Acquity autosampler. The analysis involved direct infusion mass spectrometry (MS, MS/MS) in positive electrospray ionisation (ESI). The mass range of 50 to 3200 amu was specified for the acquisition process, with a mass precision of less than 1 ppm and a scanning rate of 1 spectrum per second.

Identification of phytochemicals

Putative compound identification was performed by matching the mass-to-charge ratios (m/z) of mass spectra to those available in the online database PubChem and by reviewing the literature on *Artemisia* species (Blaženović *et al*., 2018). The respective structures of the identified compounds were sketched in ChemSketch Software 2025.

Microorganisms

The fungal pathogens used in the present study were obtained from the Advanced Research Laboratory at the University of Kashmir, India. These pathogenic fungal species were maintained on Potato Dextrose Agar (PDA) Slants at 4 °C for future use.

Antifungal activity

The agar well diffusion approach was implemented to determine the antifungal efficacy of hexane extract at various doses (1, 1.5 and 2 µg/mL). Before use, all glassware, media, and other materials were autoclaved for 15 minutes at 121°C and 15 psi. Sterile cotton swabs dipped in freshly grown fungal cultures were used to prepare and evenly inoculate Potato Dextrose Agar (PDA) plates. Sterile micropipette tips were used to aseptically punch 7 mm-diameter wells, which were then filled with 100 µL of each hexane extract concentration. Antifungal effectiveness was compared with nystatin (50 µg/disc) as the positive control (Jackalas and Mathew, 2023).

Antioxidant activity

The DPPH radical scavenging test was used to assess the hexane extract's free radical scavenging potential. The ascorbic acid (2–10 µg/mL) was used as a standard reference antioxidant. Different quantities of the plant extract (100-800 µg/mL) and ascorbic acid were combined in a 1:1 ratio with a 2 mM DPPH solution made in 80% methanol. The reaction mixtures were allowed to stand at room temperature in the dark for 40 minutes, and the absorbance was measured at 520 nm using a UV-Vis spectrophotometer (Gulcin and Alwasel, 2023). The percentage of DPPH radical scavenging activity was determined by employing the formula:

$$\% \text{ antioxidant activity} = \frac{Ac-As}{Ac} \times 100$$

Where *As* is the absorbance of the sample mixture and Ac is the absorbance of the control.

RESULTS AND DISCUSSION

The LC–MS profiling of the hexane extract of Artemisia vestita offered a thorough metabolite fingerprint and demonstrated a varied diversity of secondary metabolites. A discrete LC-MS profile was obtained for the hexane extract, demonstrating the impact of solvent polarity on chemical composition.

LC–MS analysis of hexane extract of *Artemisia vestita*

The hexane extract was markedly comprised of some major non-polar compounds, including ɣ linolenic acid ethyl ester (1.34%), 16-Hydroxy-4Z,7Z,10Z,13Z,17E,19Z-docosahexaenoic acid (1.38%), himachalol (0.28%), artemivestinolide D (1.82%), stigmasterol (6.12%), and urosolic acid (2.34%). Furthermore, some flavonoids, alkaloids, and phenols were also reported, but in very small quantities, including caffeic acid (0.51%), luteolin-7-methylether (0.02%), and methoxytricin (0.03%). These compounds are known for their antioxidant, antimicrobial, and anti-inflammatory potential. The chromatogram of the hexane extract is shown in Fig. 3, and **Table 1** provides a comprehensive list of tentatively identified compounds, including their molecular masses, molecular formulas, retention times, and m/z ratios. The chemical structures and mass spectra of these compounds are shown in Figs. 4 and 5, respectively**.**

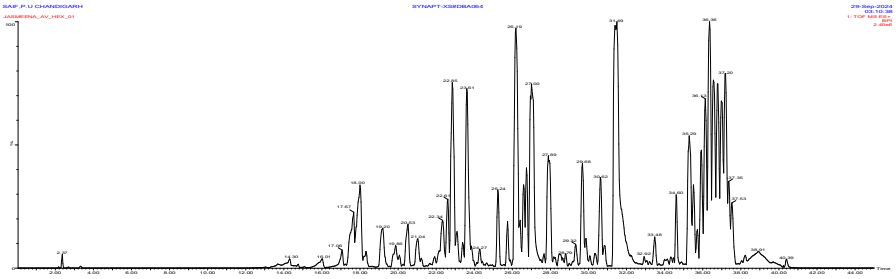

Fig.3. LC-MS chromatogram of hexane extract of *Artemisia vestita*

Table 1. LC-MS analysis of hexane extract of *Artemisia vestita.*

S. No.	Compound Name	Compound Nature	RT	m/z	Adducts	Molecular formula	Peak %	Match score
1	Gama linolenic acid ethyl ester	Fatty acid	25.168	345.09	M+K	$C_{20}H_{34}O_2$	1.34	94.7
2	16-Hydroxy-docosahexaenoic acid	Fatty acid	26.743	345.09	M+H	$C_{22}H_{32}O_3$	1.38	92
3	Daidzin	Flavonoid	23.38	383.14	M+H-2H2O	$C_{21}H_{20}O_9$	0.28	85.9
4	Caffeic acid	Phenol	33.4	219.18	M+K	$C_9H_8O_4$	0.51	92.9
5	Dihydrosantamarin	Alkaloid	28.78	273.19	M+Na	$C_{15}H_{22}O_3$	0.17	89.2
6	Luteolin-7-methylether	Flavonoid	15.761	301.10	M+H	$C_{16}H_{12}O_6$	0.02	93.6
7	Stigmasterol	Terpenoid	37.12	451.36	M+K	$C_{29}H_{48}O$	6.12	94.5
8	Urosolic acid	Terpenoid	36.97	495.39	M+K	$C_{30}H_{48}O_3$	2.34	95.7
9	Alpha pinene	Terpenoid	29.32	159.12	M+Na	$C_{10}H_{16}$	0.44	93.5
10	Cirsilineol	Flavonoid	27.94	345.09	M+H	$C_{18}H_{16}O_7$	0.01	93.8
11	Methoxytricin	Flavonoid	22.64	361.09	M+H	$C_{18}H_{16}O_8$	0.03	96.6
12	Genkwanin	Flavonoid	26.59	285.08	M+H	$C_{16}H_{12}O_5$	1.08	94.8
13	Artemivestinolide D	Terpenoid	30.65	359.11	M+K	$C_{19}H_{28}O_4$	1.82	96.9
14	Himachalol	Terpenoid	22.33	221.04	M	$C_{15}H_{26}O$	0.28	94.2

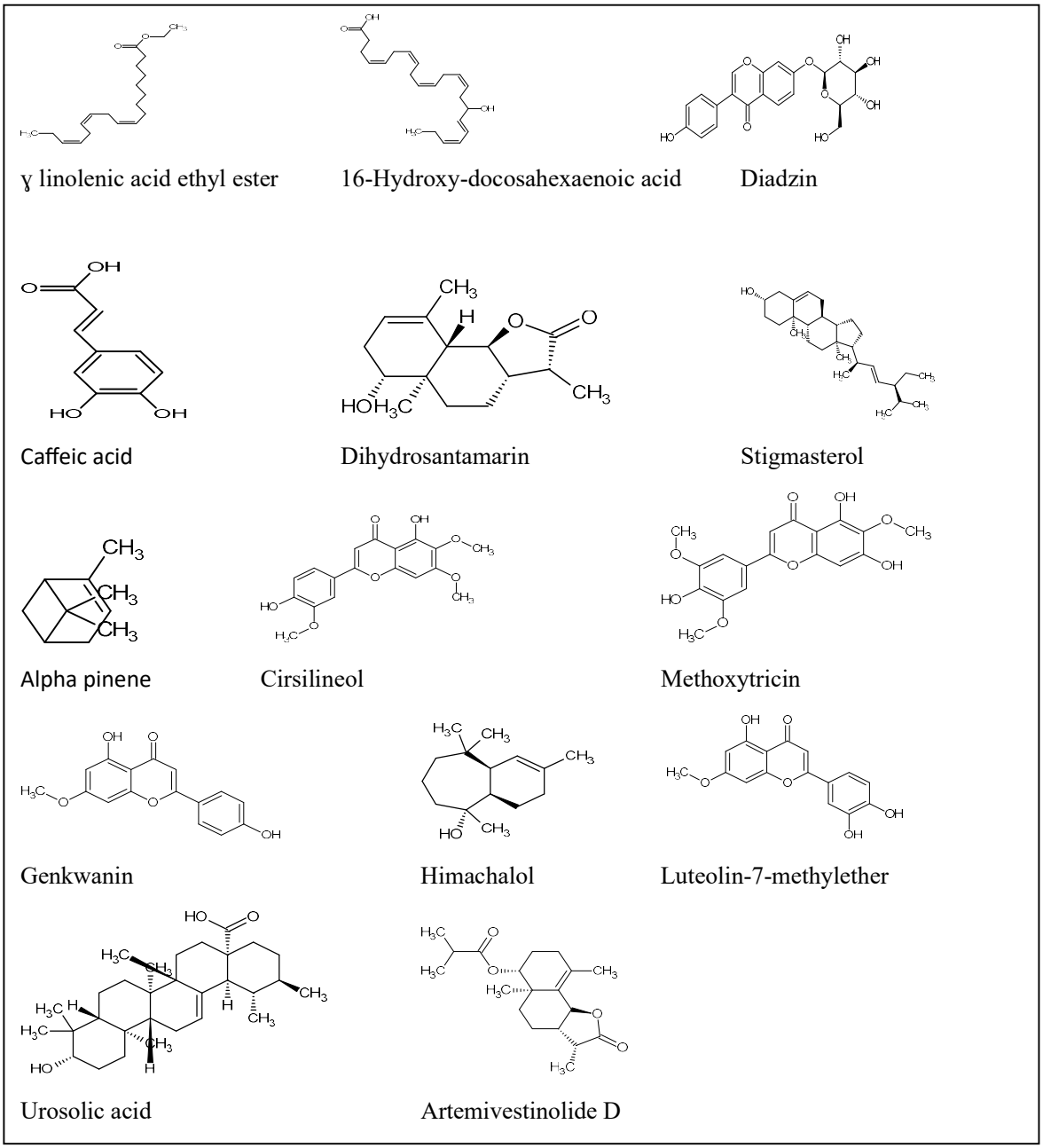

Fig.4. Chemical structures of phytocompounds tentatively identified from hexane extract of *Artemisia vestita*.

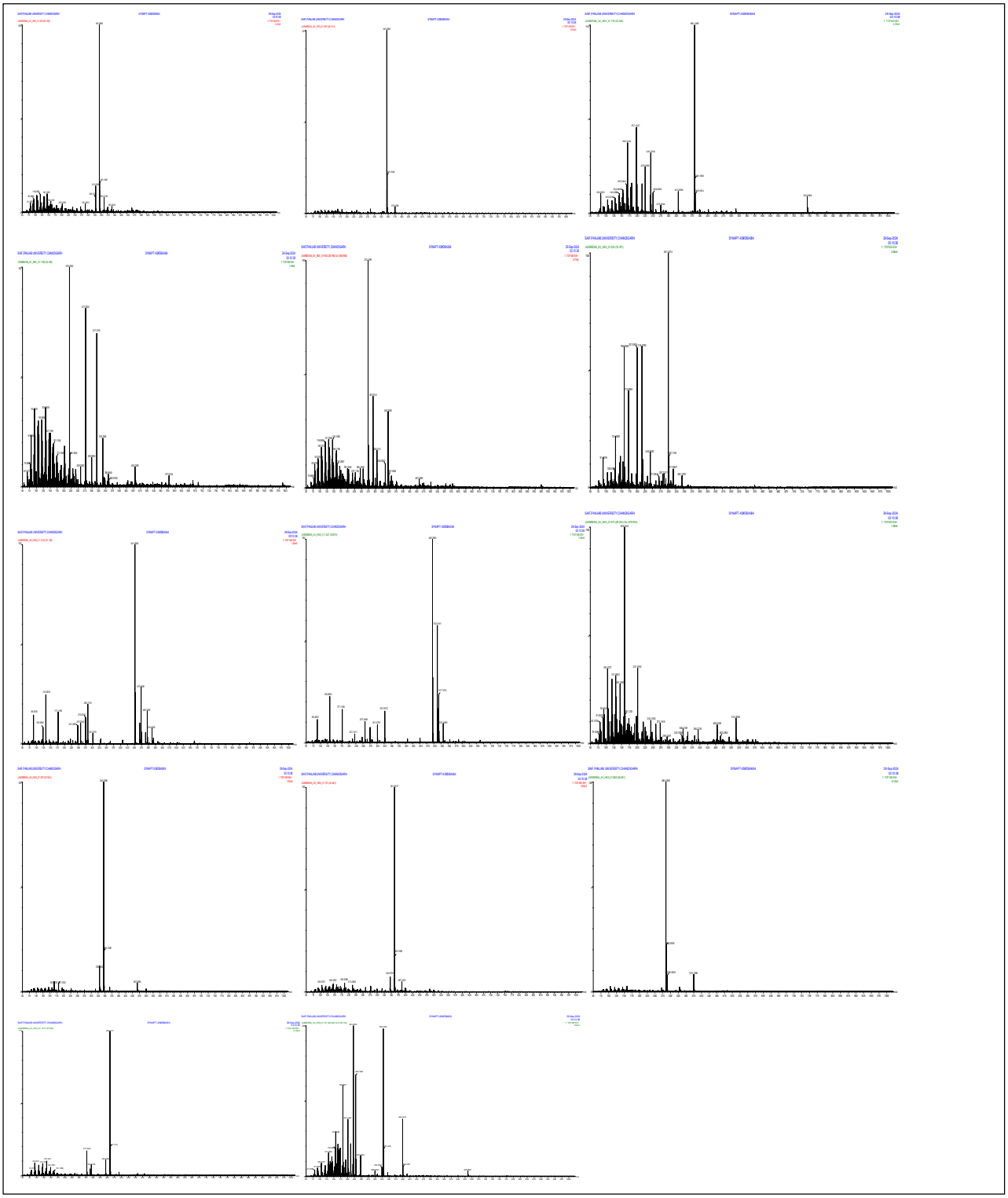

Fig. 5. Mass spectra of tentatively identified compounds (Left to right row wise: Gama linolenic acid, Hydroxy-docosahexaenoic acid, Daidzin, Caffeic acid, Dihydrosantamarin, Stigmasterol, Alpha pinene, Cirsilineol, Methoxytricin, Genkwanin, Himachalol, Luteolin-7-methylether, Urosolic acid and Artemivestinolide D) of the hexane extract of *Artemisia vestita.*

Antifungal activity of hexane extract

The agar well diffusion assay was used to determine the antifungal activity of the crude hexane extract of the experimental plant. Both fungal pathogens were inhibited by the extract in a concentration-dependent manner. *Aspergillus* sp. exhibited greater sensitivity with larger zones of inhibition as compared to *Fusarium* sp. As shown in **Table 2** and Fig. 6, the inhibitory diameter increased gradually with increasing extract concentration, confirming the dose-dependent antifungal activity of the hexane extract.

Table 2: Antifungal activity of crude *A. vestita* hexane extract against fish pathogenic fungal species.

S. No.	Fungi	Zone of Inhibition (mm)			PC (Nystatin)	NC (DMSO)
		1 mg/mL	1.5 mg/mL	2 mg/mL	50 µg/mL	2%
1	*Aspergillus* sp.	9.56 ± 0.2	12.26 ± 0.2	14.46 ± 0.5	20.2 ± 0.1	7
2	*Fusarium* sp.	9.4 ± 0.2	11.3 ± 0.1	13.2 ± 0.15	19.5 ± 0.1	7
The experiment was carried out in triplicate, and the data are presented as mean ± standard deviation. Where PC=Positive control and NC=Negative control.						

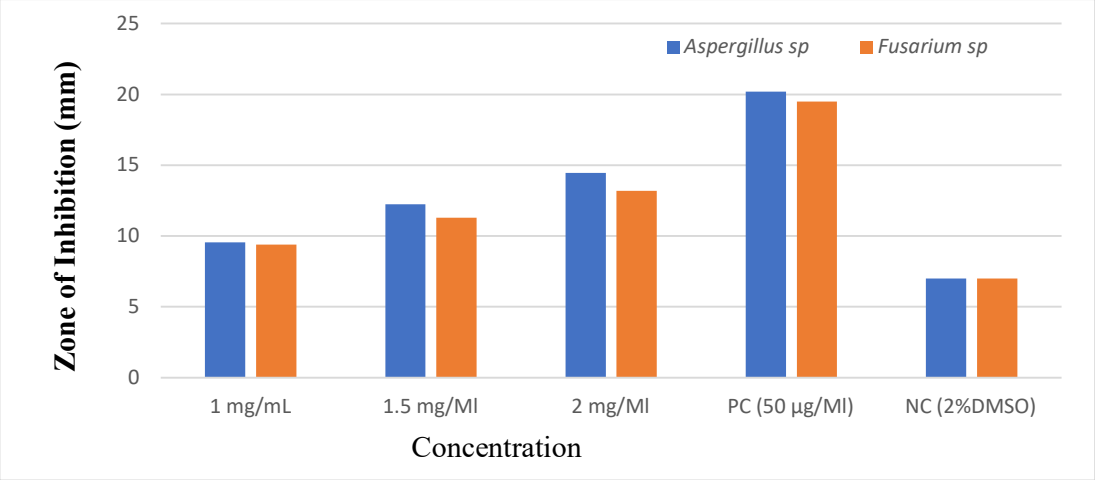

Fig. 6. Graph depicting the zone of inhibition by the hexane extract against fish pathogenic fungi.

The hexane extract showed noticeable antifungal activity; however, it had a much less inhibitory impact than the positive control **(**Fig. 7A-B). The low number of phytoconstituents extracted by hexane, which mainly extracts non-polar molecules, may be the cause of this decreased effectiveness. Fatty acids and a few non-polar terpenoids, which often have lesser antibacterial qualities than the polar flavonoids, phenolics, and oxygenated terpenoids frequently found in more polar solvent extracts, dominated the chemical composition of this extract. As a result, the relatively weak antifungal effect of the hexane extract was probably caused by the low concentration of strong bioactive metabolites.

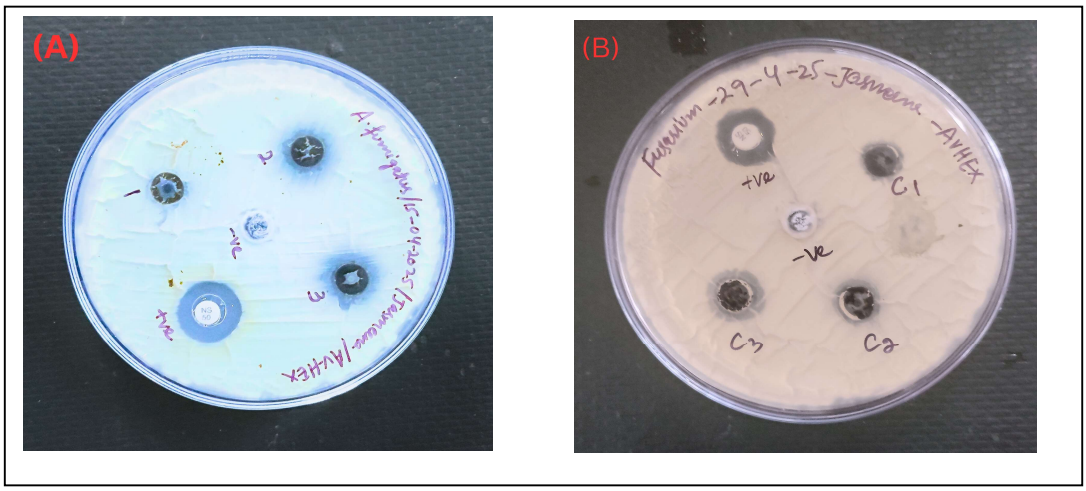

Fig. 7. Zone of inhibition shown by (A) *Aspergillus* sp. and (B) *Fusarium* sp.

Antioxidant activity

According to the DPPH radical scavenging assay, the hexane extract showed lower antioxidant activity than the standard, as shown in Table 3 and illustrated in Fig**. 8 and 9**. This can be due to the reason that the hexane solvent more frequently separates lipophilic elements like fatty acids and non-polar terpenoids, which show limited hydrogen-donating ability in contrast to polyphenolic compounds (flavonoids, phenolic acids) that are abundant in more polar extracts. Thus, the inadequate DPPH scavenging would be accounted for by the low phenolic and flavonoid constituents of the hexane extracts.

Table 3. Antioxidant activity of crude hexane extract of *Artemisia vestita.*

Sample	Concentration (µg/mL)	% Inhibition
Ascorbic acid	1	59.26± 0.03
	2	65.93±0.02
	4	74.59±4.77
	6	84.99±2.93
	8	94.44±1.29
Hexane extract	100	14.73±2.57
	200	26.5±1.39
	400	33.04±1.96
	600	57.66±0.37
	800	58.00±0.29
The experiment was carried out in triplicates and the data is presented as Mean±SD		

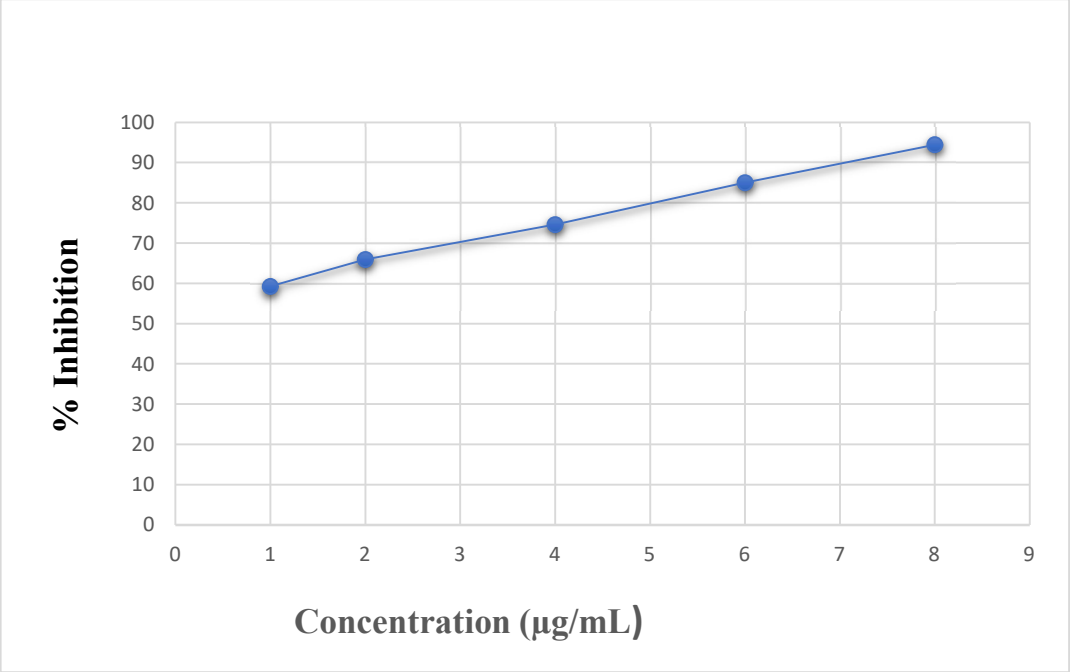

Fig. 8. Graph showing % inhibition of ascorbic acid used as a reference in the experiment.

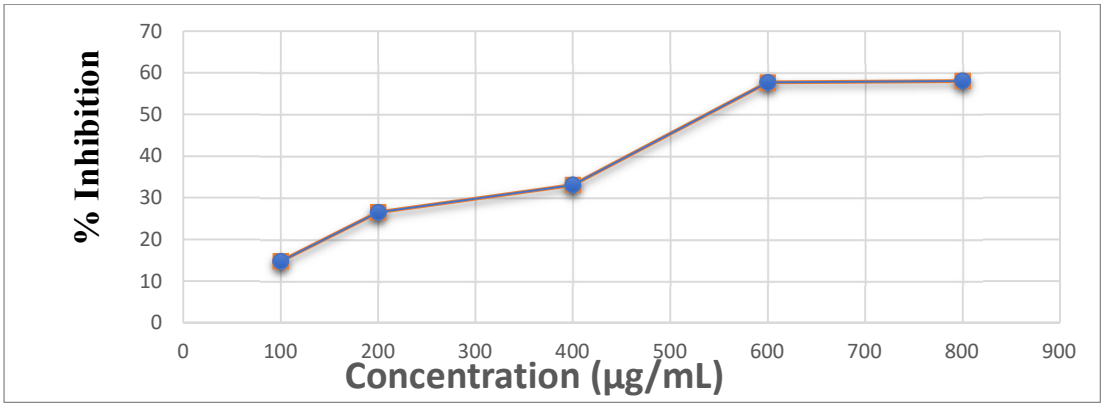

Fig. 9. Graph depicting %inhibition of hexane extract of *Artemisia vestita*.

The current work offers a comprehensive phytochemical and biological assessment of the hexane extract of *A. vestita*, providing fresh perspectives on its non-polar metabolite composition and bioactivity. Terpenoids, fatty acids, and other lipophilic components make up the majority of the hexane extract, according to LC–MS profiling, which is consistent with the anticipated extraction capabilities of non-polar solvents. Among the main metabolites detected were substances like γ-linolenic acid ethyl ester, 16-Hydroxy-docosahexaenoic acid, and himachalol. The results are comparable with some previous reports (Chu *et al*., 2010; Rather *et al*., 2017).

The antifungal findings showed a concentration-dependent activity against Aspergillus sp. and Fusarium sp., which are associated with high mortality in aquaculture species. These findings are consistent with earlier studies, which demonstrated solvent-dependent antimicrobial activity of *A. judaica* and reported least activity of hexane extract compared to highly polar and intermediate-polar solvent extracts (Khan *et al*., 2022). Furthermore, our results are also supported by Dogra *et al*. (2024) who demonstrated that methanol and aqueous solvents are the best for extracting the highly compatible antimicrobials from *Artemisia vestita leaf* extract as compared to nonpolar and intermediate polar solvents.

The antioxidant activity further confirmed the limited bioactivity of the hexane extract. These results imply that polar components that are better recovered by ethyl acetate or methanol than by hexane are mostly linked to antioxidant action in *A. vestita* and are in line with Dogra *et al*., (2024).

The findings showed that, contrary to the predicted behaviour of polar extracts, the hexane extract of *A. vestita* is chemically rich in non-polar metabolites but physiologically less potent in antioxidant and antifungal properties. Its mild inhibitory activity against fish fungal infections, however, suggests its potential use as a safer, plant-based supplemental drug for the treatment of aquaculture diseases.

CONCLUSION

The study provides a thorough LC–MS fingerprint of the hexane extract of *Artemisia vestita*, an essential feature that has remained largely unexplored and demonstrated fresh insights into the solvent-specific phytochemical composition of the plant. The extract exhibited limited antioxidant and moderate antifungal activity, likely due to its low phenolic content. The findings emphasise the significance of solvent selection in natural product research by establishing a correlation between solvent polarity and phytochemical output and bioefficacy. All things considered, the study broadens our understanding of *A. vestita* and lays the groundwork for further research focusing on non-polar bioactive components for pharmaceutical uses.

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