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UNVEILING THE PHYTOCHEMICAL PROFILE AND BIOACTIVE POTENTIAL OF SILENE INDICA VAR. INDICA

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Abstract

A great deal of interest in studying plants has been sparked by scientific research due to their natural compounds that have been employed for medicinal purposes. *Silene indica*, a perennial herb that belongs to the Caryophyllaceae family, is commonly used in traditional Chinese medicine. This research explored the phytochemical composition, antioxidant capacity, and antibacterial characteristics of *S. indica* var. *indica* to fully understand its wide range of therapeutic uses. The methanolic extract of *S. indica* var. *indica* was used for preliminary phytochemical investigation of reducing sugars, glycosides, proteins, flavonoids, steroids, saponins, alkaloids, tannins and phenols. Qualitative results revealed that *S. indica* var. *indica* contained significant primary (carbohydrate, proteins, essential oil, steroids) and secondary bioactive compounds (alkaloids, flavonoids, terpenoids, saponins, and phenols) which validate its traditional medicinal uses. Moreover, the DPPH free radical scavenging assay was used to examine the antioxidant property, and the agar well diffusion method was used to investigate the antibacterial capability against *Escherichia coli* and *Enterococci.* Results of antioxidant bioassay showed that n-Hexane fraction exhibited the highest inhibition (71.24%), followed by the aqueous fraction (60.7%).Antibacterial research exposed distinct inhibition zones at concentration ranging from 300-500 µg /mL. The higher inhibition percentage (80.1% and 89.47%) against *Enterococci* and *E. coli* was recorded in MeOH fraction. These findings suggested that *Silene indica* var. *indica* has a promising source of natural antioxidants and antibacterial agents that supports its traditional use in treating various ailments. Advance research is needed to explore its mechanisms of action and potential clinical applications.

Key words: *Sileneindica*; Phytochemical; Antioxidant; Antibacterial; *Enterococci; E. coli*

Introduction

Investigating the pharmacological and nutritional potential of phytochemicals found in native and naturalized plants has attracted botanists and provided a window for studying and increasing the interest of plant researchers in recent years (Jayaprakash, 2017). Consequently, there has been a surge in interest in using traditional treatments among patients and the scientific community (Smillie & Khan, 2010). This therapeutic revolution has opened new avenues for treatment and cure worldwide, not only in underdeveloped countries but also in those where traditional medicine is the norm (Harrison, 2015). This kind of re-birth has prompted the development and application of numerous novel instruments and techniques to detect and separate antioxidants from plant sources and protocols to examine their effectiveness in assumed purpose (BaleirasCouto *et al*., 1996).

Silene indica var. *indica* is a member of the Caryophyllaceae family, which has important applications in many different industries, such as horticulture, medicine, and cosmetics (Haq *et al*., 2023). Because of the abundance of their bioactive compounds including flavonoids and saponins, that may help to cure inflammatory, digestive, and respiratory disorders. Numerous species of this family have long been prized for their medicinal qualities (Abbas *et al*., 2023). The Caryophyllaceae family is economically significant in the landscaping and decorative plant sectors because it has many attractive species that are commonly utilized in horticulture (Yang *et al.,* 2024). Additionally, this family has many valuable species that are used to manufacture organic cosmetics and cleaning products. According to Boğa (2017), this is the integration of natural plant resources in the cosmetics sector. According to Arif *et al*., (2022) and Yeshi *et al*., (2022), many pharmacological and nutritional traits are known to be present in *Silene* species. Its secondary metabolites are crucial for defining defence systems against microorganisms and herbivores. In a number of *Silene* species, almost 400 bioactive compounds have been identified, including lipids, terpenes, and saponins (Makhmudova *et al.,* 2022). The vitality and importance of genus historically and in traditional medicine is boomed by these compounds (Khan *et al.,* 2014). Investigations on ethnobotany have explored their strong antioxidant salient features which made possible

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to treat diseases like fever, bronchitis, asthma, and the common cold. For that reason, *Silene* species is often considered as conventional medicine to treat bronchitis, fever and magnified their importance in ethnomedicine. These species are utilized as food purpose in Morocco, Austria, Spain, and Italy, among other places. Its fresh aerial portions are eaten as food in Turkey, underlining its nutritious benefits (Hussain *et al.,* 2023; Gürbüz *et al.,* 2019). *Silene* species have been used in different areas. In ancient time people used as soap and recently bring it use in organic cleaning products, cosmetics because of their saponin concentration (Mamadalieva *et al.,* 2023). For that reason, *Silene* species become most attractive field for additional research due to their antiviral, antibacterial, and antioxidant properties (Roya & Fatemeh, 2013) andsignificance of *Silene indica* var. *indica* become most vital thatin the production of natural medications is illuminated by these bioactive compounds, which also stresses upon the importance of further exploration and study of the chemical makeup and therapeutic richness of this plant (Wali *et al*., 2022). Modern pharmacological tool and methodology aligned with conventional study of *Silene indica* var. *indica* that places this species as a significant natural resource of scientific and cultural magnificence.

According to Khadim *et al.,* (2024), there is a lack of research on the ethnobotanical and medicinal potential of the plants of Asogaha Nallah and Maruk in the Haramosh Valley. The therapeutic uses, bioactive components, and conservation status of these local and indigenous plant species have not been thoroughly studied, despite the region's rich plant diversity. Therefore, the potential loss of the traditional knowledge about the medicinal plants in these locations highlights the necessity for scientific inquiry and documentation.A more comprehensive understanding of the ethnobotanical and medicinal significance of plants in Maruk and Asogaha Nallah can be gained by examining the *Sileneindica*. The particular species as well as the greater floristic community highlight how urgently traditional knowledge and scientific investigation must be combined in research to support the creation of natural medicines and conservation initiatives.

The study also aims to bridge this information gap by analyzing the medicinal plants of Asogaha Nallah and Maruk, identifying their bioactive components, and evaluating their pharmacological applications. Additionally, this study aims to support conservation and the sustainable use of these priceless plant resources by fusing ethnobotanical expertise with the most recent medicinal research.

Methodology:

Study area: The Haramosh valley is situated in the Karakoram Range and ranges in elevation from 2367 to 2637 meters. Its coordinates are 31°01'722" North latitude and 074°32'791" East longitude (Abbas *et al*., 2023). A map of the study area is shown in Fig 1. The valley, which lies 4 km from Gilgit city on the way to Skardu in the Baltistan region, is well-known for its glaciers, pure lakes, dense woods, high hills covered in valuable stones, and scenic meadows (Din *et al*., 2024). Abbas *et al*., (2023) demonstrated that there are vast arrays of medicinal herbs are available. The Himalayan, Karakoram, and Hindu Kush Mountain ranges only meet in Gilgit-Baltistan (Khan & Khatoon, 2008). These three enormous ranges come together at latitudes 35°–37° North and longitudes 72°–75° East at the meeting point of the Indus and Gilgit Rivers at Macpoon Das in the Haramosh Valley.

Taxonomy and diversity: The genus *Silene* L. (Caryophyllaceae) comprises approximately 700 species, predominantly distributed in temperate regions of the Northern Hemisphere, including parts of Africa, North America, and Eurasia. Among these, *Silene indica* var. *indica* is a prominent herbaceous species primarily found in the hilly regions of Northern Pakistan, India, Nepal, Bhutan, and the sub-Himalayan zone (Fassou *et al*., 2022; Mamadalieva *et al*., 2014). This species has distinct taxonomic traits that make it morphologically unique within the genus (Široký *et al*., 2001).

Growing to a height of 60 cm, *Silene indica* var. *indica* has upright or ascending stems that frequently branch basally. Its opposing, simple, narrowly lanceolate leaves are 3–6 cm long and can be either hairless or somewhat hairy (Ullah *et al*., 2018). Both glandular and eglandular pubescence are present on the stem and inflorescence. The 8–20 mm tubular-campanulate calyx has triangular apical teeth and 10–20 conspicuous nerves. The petals of the corolla are usually deeply split into narrow lobes, and they can be either pale pink or white. The plant has a superior ovary, a single style, a bifid stigma, and 6–15 stamens. The species can also be identified by its reniform, pisticulate seeds, which are about 1 to 1.5 mm in diameter (Jin *et al*., 2023; Zaman & Park, 2023).

Fruiting from May to August, *Silene indica* var. *indica* produces a capsule-shaped fruit that contains tiny brown seeds that are dispersed by wind or water. Fruiting occurs from August to September (Zaman & Park, 2023). Between 1,500 and 3,500 meters above sea level, this species can be found in rocky slopes, open woods, and alpine meadows (Naciri *et al*., 2017). Its known distribution has been extended to Afghanistan and Iran by recent studies (Gholipour, 2021).

S. indica var. *indica* flourishes in rocky and alpine meadows, greatly enhancing the floristic richness of the area in Gilgit-Baltistan, because of its tolerance to a variety of environments and a moderate climate. Despite not being native, *S. indica* var. *indica* is important ethnobotanically because of its pale pink flowers and tubular-campanulate calyx (EL-Banhawy *et al*., 2020). *Silene indica* var. *indica* must be accurately identified or to be distinguished from related species like *Silene vulgaris*. The calyx lengths (14–18 mm) and floral colors of both species are similar, but *S. vulgaris* (Moench) Garckeis completely glabrous, whereas *S. indica* var. *indica* has glandular and eglandular pubescence, calyx deeply green nerves, corolla lobes fimbriate. They also have different seeds; *Sileneindica* var. *indica* has pusticulate seeds, whereas *S. vulgaris* with whitish green nerves, corolla bifid, grooved seeds (Sandoval-Ortega *et al*., 2019). These distinguishing characteristics highlight how crucial accurate taxonomy is within the *Silene* genus (Ali-Shtayeh *et al*., 2022; Jafari *et al*., 2020) Fig. 2 (a-e).

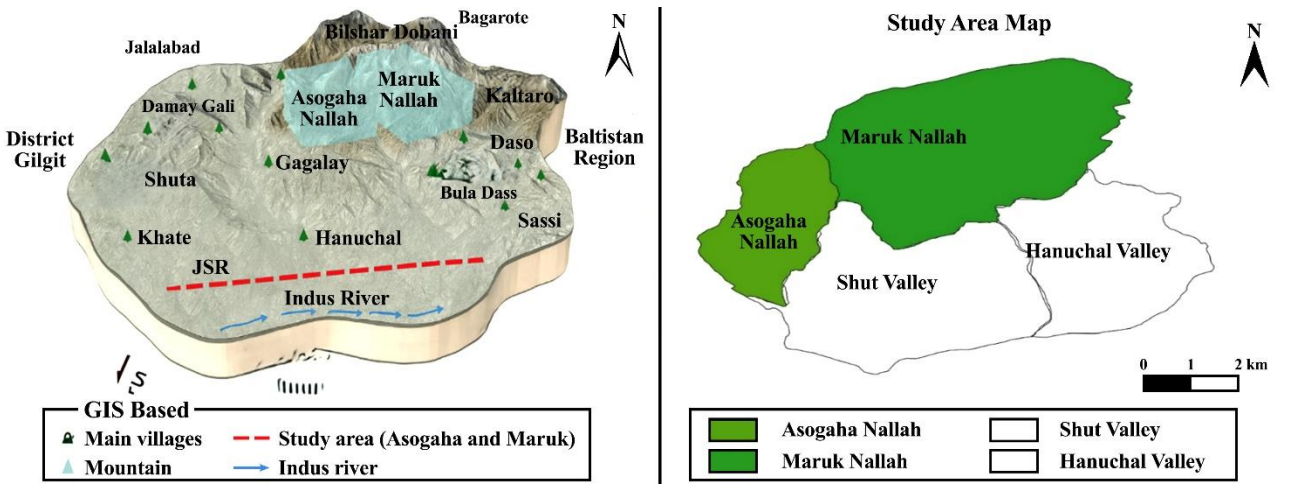

Fig. 1. Study Area Map of Maruk and Asogaha Nallah, Haramosh, District Gilgit.

Fig. 2. a & b, *Silene indica* var. *indica* (photograph by Hasnain Abbas); c, d & e, The image shows the characteristic slender stem, glandular and eglandular plant parts, developing buds, and the distinctive white, deeply divided petals typical of the species (a, b). A *Silene vulgaris* image shows the glabrous stem, calyx inflated in fruit bladdery, bifid corolla lobes (c, d, e).

Key to species

+ Calyx 12–15 mm long with light nerves, broadly triangular; stamens 10–14; corolla whitish, lobes bifid; seeds grooved, plant parts glabrous ……. *Silene vulgaris*
- Calyx 14–18 mm long prominent green nerves, ovate; stamens 6–15; corolla lobs fimbriate seeds pusticulate,plant parts glandular-eglandular ……..……... *Silene indica* var. *indica*

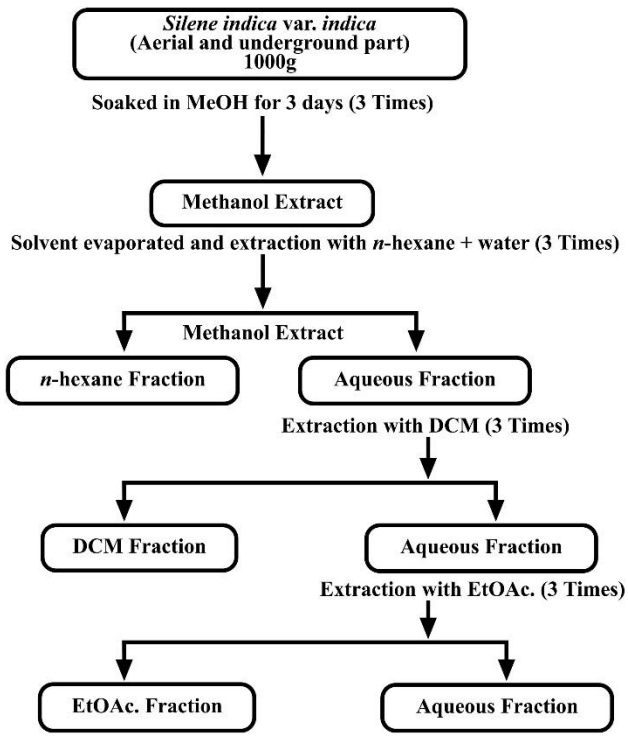

Fig. 3. Scheme for extraction and preparation of different fractions of *Silene indica.*

Preparation of plant material: *Silene indica* var. *indica* plants were cleaned and washed with tap water to get rid of any surface scum. Once the moisture had completely evaporated, the plants were set to air dry in a shaded area to get rid of any last bits of moisture and prevent them from rotting. The plant material was processed into a uniformly fine powder using an electric grinder by avoiding any clumps (Bitwell *et al*., 2023).

Extraction of plant material: Hundred g of dried powdered *Silene indica* var. *indica* material was placed in a conical flask with 100–150 ml of methanol. The flask was shaken occasionally to mix the ingredients, and it was allowed to macerate for 72 hours at room temperature (Engels *et al*., 2019). The soluble phytoconstituents were released during the maceration procedure, which also broke down and softened the plant cell wall. After that, the methanolic extract was sieved three times using Whatman No. 1 filter paper to get rid of any remaining plant material. To obtain crude extract, the extract was reduced at 40°C using a rotary vacuum evaporator (BuchiR-200), and it was then stored at -20°C for fractionation (Pandey *et al*., 2015).

Fractionation of methanolic extract: In fractionation process, several solvents including methanol (MeOH), n-hexane, dichloromethane (DCM), and ethyl acetate (EtOAc), and water were used for extracting bioactive compounds of plant material.

The crude methanolic extract of *Silene indica* var. *indica* was used for fractionation as described by Suresh *et al.,* (2020) and Tariq *et al.,* (2015). The crude extract was passed through n-hexane and water twice, the n-hexane soluble fraction and the aqueous fraction (n-hexane insoluble) were separated. Dichloromethane (DCM) was added to the n-hexane insoluble fraction twice, yielding both soluble and insoluble DCM fractions. Finally, as illustrated in Figure 3, the DCM insoluble fraction was run through ethyl acetate to separate the soluble and insoluble fractions. After the solvents were removed using a rotary evaporator, all the fractions were condensed and stored in glass vials pending additional research.

Preliminary phytochemical screening

1. Carbohydrate analysis

(i) Molish's test (total carbohydrate): For the Molisch's test, take 2 mL of the extract in a test tube and add2 drops of a freshly prepared alcoholic α-naphthol (20%) solution. Subsequently, 2 mL of H_2SO_4 (sulfuric acid) concentrated were carefully mixed to the mixture to create a layer underneath it. At the junction of the two layers, a reddish-violet ring was appeared, signifying the presence of carbohydrates. Nevertheless, adding too much alkali caused this ring to disappear (Das *et al*., 2014).

(ii) Benedict's test (reducing sugar): Procedure of (Hernández-López *et al*., 2020) was applied to detect the presence of carbohydrate content. 5 mL of Benedict's solution and 0.5 mL of extract were combined in a test tube to create a reaction mixture. After that, the mixture was placed in a water bath and boiled for five minutes. The appearance of a brick-red precipitate indicated the presence of carbohydrates in the extract.

(iii) Fehling's test (reducing sugar): This approach involved combining 2 mL of the extract with 1 mL of Fehling's solutions A and B in a test tube. The mixture was then heated for five minutes on a water bath. Carbohydrates were present in the extract because a brick-red precipitate formed (Hernández-López *et al*., 2020; Das *et al*., 2014).

2. Glycosides

Mineral acids (diluted H_2SO_4 or HCl) were used to hydrolyze the extract in order to determine its free sugar concentration. The extract's entire sugar content was ascertained by hydrolyzing it. An increase in the extract's sugar content indicated the presence of glycosides (Johannessen *et al*., 2005).

3. Proteins

(i) Biuret's test: Kancherla *et al.,* (2019) employed the method to check for the presence of proteins by means of adding 5-8 drops of NaOH (sodium hydroxide 10%w/v) solution and 1-2 drops of $CuSO_4$ (copper sulphate 3%w/v) solution in 1 mL pre-warmed pant extract. The extract developed a violet-red color, indicating the presence of proteins.

(ii) Millon's test: One milliliter of the extract was dissolved in one milliliter of distilled water as part of the procedure (Abbas *et al*., 2023) used to ascertain whether proteins were present. Later on, five or six drops of Millon's reagent were added. Proteins were identified in the extract by the development of a white precipitate that turns red when heated.

4. Oil test: A small amount of the extracts was hard-pressed between two filter paper sheets. The presence or absence of oil and lipids was found with petroleum ether, benzene, and methanolic extracts as oil stains on paper (Abbas *et al*., 2023).

5. Flavonoids estimation

Lead acetate test: Few drops of lead acetate (10%) solution were added in 2 mL of the plant extract to carry on the lead acetate test. The extract contained flavonoids, as shown by the presence of a yellow precipitate as expressed (Godlewska *et al.,* 2023).

6. Steroids and triterpenoids analysis

Salkowski test: Triterpenoids and Steroids presence were estimated according to the procedure of Jacob *et al.,* (2021). The plant extract is mixed with 2 mL of chloroform, and then filtered. Shake the filtrate after adding a few drops of strong sulfuric acid. A while is given for the mixture to stand. If the lower layer becomes red in color, it means that steroids are present, and if it becomes yellow in color, it means that triterpenoids are either present or absent.

7. Test for saponins

Frothing test: To ascertain whether saponins were present, two milliliter of the extract was dissolved in ten milliliter of distilled water vigorously shaken for five minutes. The long-lasting existence of stable foam is an indication that saponins are present (Thangjam *et al.,* 2020).

8. Alkaloids determination

(i) Dragendroff 's test: Dragendroff's assay was employed illustrated by Abbas *et al.,* (2023) to find out the presence of alkaloids in methanolic extract of plant. For this purpose2 mg of the extract was combined with 5mL of distilled waterthen after adding three drops of 2 M HClacidic reaction was started. Dragendroff's reagent (1mL) was added to this reaction mixture.The mixture contained alkaloids as evidenced by the formation of an orange or orange-red precipitate.

(ii) Mayer's test: Mayer's reagent was used to confirm the existence of alkaloids in the plant extract via the protocol explained by Sabdoningrum *et al.,* (2021). Mayer's reagent was applied in little drops to 2 mg of the methanolic extract. In the investigation a white or pale yellow precipitate was developed in the test tube which indicated the alkaloids presence.

(iii) Wagner's test: Wagner's reagent test was carried out by means of adding 1mL of HCL (hydrochloric acid) with a few drops of Wagner's reagent in 2 mg of the methanolic extract. Appearance of yellow or brown precipitation showed alkaloids presence in the extract (Abbas *et al*., 2023).

(iv) Hager's test: Saturated picric acid solution is used to prepare Hager's reagent. For this analysis, 2 mg of methanolic extract was taken in test tube and put little drops of Hager's reagent as the methodology given by Suganandam *et al.,* (2022). If a yellow precipitate appeared, the extract was known to contain alkaloids.

9. Test for tanins

Gelatin test: A solution of 1% w/v gelatin in water with 10% sodium chloride is made. To the test residue, a small amount of this solution was applied. A white precipitate will form that indicated the presence of tannins (Jacob *et al.,* 2021).

10. Test for phenols:

Ferric chloride test: The test residue is heated in water, filtered, and then two milliliters of ferric chloride solution are added to the filtrate, which is then monitored. The development of blue and green hues suggests the existence of phenolic compounds (Jacob *et al.,* 2021).

Antioxidant bioassay

DPPH free radical scavenging activity: *Silene indica* var. *indica* antioxidant bioassay was evaluated using DPPH Free Radical Scavenging Activity by using the method described by Rahman *et al*., (2016). To make the fractions for each plant, first was mixed 1 mL of DMSO with 12 mg of each fraction (Ethyl acetate (EtOAc), Methanol (MeOH), Dichloromethane (DCM), and n-Hexane) in an Eppendorf tube (Jamil *et al*., 2012).DPPH (30 µM) working reagent was freshly prepared in DMSO and kept in dark bottle to avoid photo-oxidation. In a reaction test tube, 250 µL of each aliquot of distinct fraction from Eppendorftube was mixed with 800µL100mMTris-HCl buffer (pH 7.4) and add 1 mL of working DPPH then vortex 2-5 sec. Note the absorbance at 517 nm and test was carried out in triplicate. Before obtaining the absorbance, the spectrophotometer was adjusted as zero through blank test tube containing 1mL of extraction solvent (DMSO). Control absorbance was recorded by 1 mL DPPH solution combined with 1 mL DMSO. The standard antioxidant compound (Ascorbic acid) solution was used with equivalent concentration as aliquot to parallel the antioxidant inhibition activity.

$$\text{DPPH inhibition (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \text{ x } 100$$

Antibacterial activity

Preparation of plant extract concentrations: Antimicrobial activity of different partitioned crude extract (n-hexane, dichloromethane (DCM), ethyl acetate (EtoAc), methanol) (Fig. 3) of *Silene indica* var. *indica* was calculated. To do this, a 500 mg/ml stock solution was created of each fraction as a standard concentration. From the stock solution, separate concentrations, such as 100, 200, 300, 400 mg/ml were prepared.

Culture of pathogenic bacterial strains: Two common pathogenic bacteria, *Escherichia coli* (E. coli) gram-negative and *Enterococcus* (gram +ve), were selected as the target pathogens for the bioassay. Slanetz and Bartley agar media was selected for *Enterococcus* specie while CHRO Magar (Dungan & Bjorneberg, 2021) was chosen for *Escherichia coli* (*E. coli*). The plates were kept at 37°C for 1-2 days. Furthermore, these pathogenic strains were developed on Müller-Hinton agar plates from the original culture.

Agar well diffusion technique: The minimum inhibitory concentration (MIC) of the partitioned fractions (MeOH, EtOAc, *n*-hex and DCM) of *S. indica* var. *indica* extracts was determined by measuring the zone of inhibition using the agar well diffusion technique (Erhonyota *et al.,* 2023). Sterile Muller-Hinton agar was poured in petri-plates and kept at 4 °C for 15 minutes to solidify the agar. Following solidification, a sterile cork borer was used to aseptically perforate a well of approximately 5 mm in diameter on each agar plate, creating seven wells. The test organisms (*E. coli* and *Enterococci*) were carefully streaked by swab of bacterial suspension. To allow the inoculums to diffuse into the agar, the plates were placed at room temperature for an hour. Following this, 100µL volumes of extracts at varying concentrations (100, 200, 300, 400, and 500µg/mL) were put into each well that was properly labeled (Youmbi *et al.,* 2020; Lacmata *et al.,* 2012).

After the extracts were incubated for 48 hours at 37°C, the plates were carefully examined for any zones of inhibition surrounding the extract wells. Using a millimetre scale, the clearance zone of inhibition surrounding each well was assessed. The antibacterial properties of different extract concentrations were expressed by the diameter (mm) of the zone of inhibition (Demgne *et al*., 2022; Omosa *et al*., 2022).

Results

Phytochemical analysis: The following Table 1.described the results of the phytochemical screening of the crude methanolic extract MeOH of *Silene indica*. It shows the absence of glycosides, tannins, tri-terpenoids, reducing sugar, and the presence of many secondary metabolites that were identified for their pharmacological characteristics, such as saponins, alkaloids, proteins, amino acids, oils, steroids, carbohydrates, phenols, and flavonoids.

The result of bioassay screening is shown in the Table 1 which described different types of compounds that were present in the sample. A particular class of chemicals is represented by each row, which includes information on the reagents added, the expected reactions, and the observed outcomes. Essential oils, alkaloids, flavonoids, proteins, amino acids, terpenoids, saponins, steroids, and phenols all showed positive results (+), indicating their existence based on expected reactions such as the production of oil spots, colour changes, or the emergence of precipitates or bubbles. On the other hand, negative results (-) were noted for reducing sugars, glycosides, tri-terpenoids, tannins, and carbohydrates, suggesting that these substances were absent because there were no expected responses. These results facilitate the sample's characterization and provide pertinent information for prospective uses in a variety of sectors by offering insightful information about the sample's chemical makeup.

The Table 2 and Figure 4 displayed the results of the antioxidant bioassay conducted on several *S. indica* var. *indica* fractions. The mean values of the three trials with standard deviations (SD) are provided for each fraction. These statistics display the antioxidant activity as a percentage suppression of oxidative processes, in relation to a standard antioxidant. The n-Hexane fraction exhibited the highest inhibition at 71.24%, followed by the aqueous fraction at 60.7%. The DCM, ethyl acetate, and methanol fractions showed lower inhibition rates of 32.43%, 19.49%, and 23.61%, respectively. These results indicated varying antioxidant capacities among the fractions, which could influence their potential uses in different areas like functional foods or traditional medicine.

Table 1. Qualitative analysis of phytochemical in plant extract of *Silene indica.*

S. No.	Class of compounds	Added reagents	Expected results	Results
1.	Essential oils	Filter paper + extract	Oil spot formed	+
2.	Alkaloids	Dragendroff test	Orange-red precipitate	+
3.	Flavonoids	Lead acetate test	Yellow color formed	+
4.	Proteins	Million's reagent	Red color formed	+
5.	Glycosides	Ammonia + Chloroform	Pink color	-
6.	Amino acids	Ninhydrin test	Deep blue or pink	+
7.	Terpenoids	Chloroform+sulphuric acid	Reddish-brown	+
8.	Tri-terpenoids	Chloroform sulphuric acid + Acetic anhydride	reddish violet colour formed	-
9.	Tannis	Chloroform Acetic anhydride + Sulphuric acid	Green colour formed	-
10.	Saponins	Distilled water + extract	Soapy bubbles	+
11.	Steroids	Sulphuric acid +chloroform	Brown ring	+
12.	Carbohydrates	Extract + iodine sol	Blue-violet color	+
13.	Reducing sugars	Fehling sol A & B	Red precipitate	-
14.	Phenols	Ferric chloride test	Blue green	+

A (+) sign indicates the appearance of primary metabolites while a (-) sign indicates the non-appearance of secondary metabolites

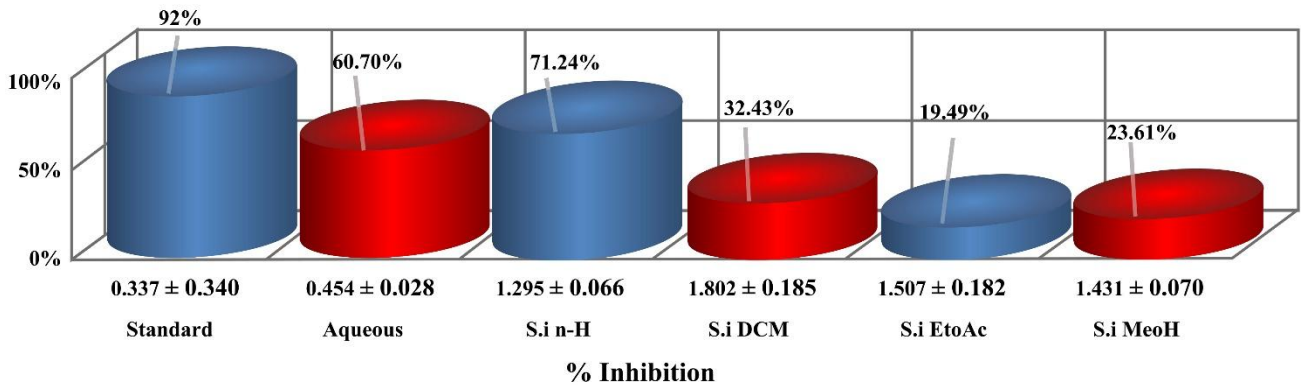

Fig. 4. DPPH free radical scavenging activity of four extracts mean of the three trials.

Table 2. DPPH free radical scavenging activity of different fractionates of *S. indica.*

S. No.	Fractions	Mean	% Inhibition
1.	Standard	0.337 ±0.034	92%
2.	Aqueous	0.454 ± 0.028	60.7%
3.	n-Hexane	1.295 ± 0.066	71.24%
4.	DCM	1.802 ± 0.185	32.43%
5.	EtoAc	1.507 ± 0.182	19.49%
6.	MeoH	1.431 ± 0.070	23.61%

Means values expressed in column with ± SD of three replicates at ($p<0.05$)

Table 3. Zone of inhibition of *E. coli* using various concentrations of *Silene indica's* fractionates.

Plant	Zone of % inhibition of *E. coli*				
	Concentration	MeOH	EtOAc	*n*-hex	DCM
S. indica	100	0	94.73	78.94	84.21
	200	0	0	N/A	N/A
	300	52.63	0	N/A	N/A
	400	63.15	N/A	N/A	N/A
	500	89.47	N/A	N/A	N/A

Table 4. Zone of inhibition of *Enterococci* using various concentrations of *Silene indica's* fractionates.

Plant	Zone of % inhibition of *Enterococci*				
	Concentration	MeOH	EtOAc	*n*-hex	DCM
S. indica	100	0	0	70.8	80.1
	200	0	0	N/A	N/A
	300	57.14	61.9	N/A	N/A
	400	70.1	N/A	N/A	N/A
	500	80.1	N/A	N/A	N/A

An overview of the antibacterial activity of *S. indica* var. *indica* extract against *E. coli* and *Enterococci* is given. The results demonstrate that growth patterns differ based on the concentrations of the extract and solvent. Specifically, at 100µg concentration, the MeOH fraction exhibited 0% inhibition against *E. coli*, whereas EtOAc, n-hex, and DCM demonstrated 90.7%, 78.94%, and 84.21% inhibition, respectively. For *Enterococci*, MeOH demonstrated 0% inhibition, EtOAc demonstrated 0%, n-hexane demonstrated 70.8% inhibition, and DCM demonstrated 80.1% inhibition. At 200µg and 300µg concentration, neither pathogen exhibited any inhibition against MeOH, EtOAc, n-hex, or DCM but MeOH fraction of 300µg concentration showed 52.63% inhibition against *E. coli*. Similarly,MeOH showed 57.14% inhibition and EtOAc 61.9% inhibition against *Enterococci*. There was no DCM or n-hex available. At 400µg of concentration, MeOH demonstrated 63.15% inhibition against *E. coli*; however, EtOAc, n-hex, and DCM were not available. When it came to *Enterococci*, MeOH exhibited 70.1% inhibition, while DCM, n-hex, and EtOAc were unavailable. The highest inhibition was noted at 500µg concentration, MeOH showed 80.1% and 89.47% inhibition respectively as shown in Tables 3 and 4 against *E. coli* and *Enterococci*. EtOAc, n-hex, and DCM showed no activity against either pathogen.

Discussion

Silene spp., are particularly famous for its contributions to pharmacology and ethnobotany in many parts of the world (Ullah *et al*., 2019). In this research result of phytochemical screening of *Silene indica* var. *indica* showed that all essential primary metabolites i.e. carbohydrates, protein/amino acids and essential oil were positively present in *Silene indica*. Traditionally, *Silene indica* var. *indica* has been used to treat a wide range of diseases as digestive difficulties, lung infections, and inflammation (Abbas *et al*., 2024; Din *et al*., 2024). Yahaya *et al*., (2020) demonstrated that secondary metabolites act as defensive force against various stresses and medical disorders in living organism. This study examined the presence of many potent secondary metabolites that served as defensive compounds such as alkaloids showed as orange-red precipitate by Dragendroff test and confirmed by Mayer's, Wagner's and Hager' tests. Heinrich *et al*., (2021) described that alkaloids are vital for plants and human beings serving as powerful defensive compounds against pathogens and imperative components in drug discovery. Flavonoids presences were visible as yellow colour formation with lead acetate, phenolic compounds were verified by ferric chloride test and saponins appeared as soapy bubbles. Plants containing the high content of flavonoids could be valuable as antibacterial activity (Sharma *et al*., 2020). A result of tannins test was negative. These secondary metabolites may be responsible for the pharmacological characteristics (i.e.antibacterial, anti-inflammatory, and antioxidant activities) of the species. A comprehensive phytochemical screening showed that the *Silene indica* var. *indica* contain bioactive compounds with a range of pharmacological characteristics.

Plant extracts have been tested for their potential to scavenge free radicals or donate hydrogen to assess the anti-oxidative activity using the DPPH radical assay (Baliyan *et al.,* 2022). The antioxidant bioassay revealed significant activity in each of the *Silene indica* var. *indica*

extract's components. Result given in Table 3 displayed the higher percentage of inhibition (60.7%) in aqueous fraction as showing potent antioxidant properties. The n-Hexane fraction (71.24%) also showed significant antioxidant activity, suggesting the presence of bioactive compounds that can scavenge free radicals and reduce oxidative stress-related damage.

Phenolic compounds are distinguished phytochemicals which exhibited significant health benefits including anticancer, antibacterial, and anti-inflammatory properties and ubiquitous in several medicinal plants (Rahman *et al.,* 2020). These are key molecules of defense system that have characteristic antioxidant properties which combat harmful free radicals in the body (Rahman *et al.,* 2017). Plants have developed these chemicals to combat oxidative stress, and humans can also benefit from them when they consume it (Rauf *et al.,* 2024; Rahman *et al*., 2021). Similar finding was observed by Smahane *et al*., (2015) who study bioactive compounds of *S. vulgaris* extract which exhibited notable antioxidant properties that measured by ABTS, DPPH, FRAP, HRSA assays.

Silene indica var. *indica* extract showed promising antibacterial action against *E. coli* and *Enterococci* in the antibacterial bioassay, as evidenced by significant inhibition zones. The ethyl acetate (EtOAc) fraction had the highest percentage of inhibition against *E. coli* (90.7%) at a concentration of 100µg, suggesting that it has the potential to be an effective antibacterial agent. Similarly, at 500µg, the methanol (MeOH) fraction demonstrated up to 80.1% inhibition against *Enterococci*. These results validate the traditional use of *Silene indica* var. *indica* extract in medicine and show that it could treat bacterial infections. Roy *et al*., (2022) documented that flavonoid presences exhibited antimicrobial, antiviral, antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic qualities of plant extract. Numerous pharmaceutical companies have used these plants to produce flavonoids because of their numerous therapeutic uses. Alike Ullah *et al*., (2019) concluded that *Silene conoidea* L., serve as a medicinal plant in several ethnobotanical applications because of its numerous phytochemicals, including flavones, glycosides, conoidene which are responsible for therapeutic nature.

Thus results of phytochemical analysis, antioxidant activity, and antibacterial efficacy of *Silene indica* var. *indica* increase its significance for therapeutic application in the medical and healthcare sectors. To pinpoint the precise bioactive substances causing these pharmacological effects and investigate their mechanisms of action, more investigation is needed. Moreover, additional clinical research is required to confirm *Silene indica's* potential as a medication for a range of conditions.

Conclusion

The comprehensive analysis of *S. indica* var. *indica* reveals its rich phytochemical profile, significant antioxidant activity, and promising antibacterial properties that have never been reported previously. Present research findings suggest that *S. indica* var. *indica* could be a valuable source of primary and secondary natural bioactive compounds for therapeutic applications. Future research should focus on isolating specific bioactive compounds and understanding their mechanisms of action.

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