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Phytochemical, Antioxidant and Antimicrobial Profiling of Saussurea Costus (Falc.) Lipsch., Root Extracts with GC-MS Profiling

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ABSTRACT: The plant Saussurea costus (Falc.) Lipsch. is recognised as an endangered plant species and is well recognized in the Indian system of traditional medicine. The goal of this study was to comparison phytochemical, antimicrobial, phenolic, flavonoid, and antioxidant analyses of root extracts of S. costus (SC) growing in the Kashmir region. Four different solvents based on polarity to extract and analyse the potential of these plant extracts. The results indicate higher entire phenolic content (TPC), flavonoid content (TFC), and stronger free radical scavenging action and potential DPPH and FRAP activity in methanolic extracts. The higher antimicrobial activity, with zones of inhibition, was observed in the order of Escherichia coli > Staphylococcus aureus > Candida albicans. All extracts showed moderate antioxidant and antimicrobial potential, but methanolic extracts of the roots have overall higher activity than ethyl acetate, followed by hexane and aqueous extracts in a concentration-dependent manner. The Gas Chromatography Mass Spectrometry (GCMS) profiling of root methanolic extracts helped determine different active secondary compounds that are accountable for the medicinal properties of S. costus root extracts at variable concentrations.

1. INTRODUCTION

The Himalayan areas of Kashmir are home to a variety of plants with medicinal properties that serve as both good nutritional and medicinal sources. Plants have long been used as effective and inexpensive remedies for various diseases, encouraging scientists to investigate their potential as modern drugs. The plant *Saussurea costus* L. is nonetheless an equally important therapeutic plant with a wide range of actions, used by native people as herbal remedies to treat various diseases. (Elnour and Abdurahman 2024; Koko et al. 2008; Cragg and Newman 2013; Abuzeid et al. 2014; Kumari et al. 2024; Eklund et al. 2010; Abdallah 2011; Zulkipli et al. 2015; Sukmawati et al. 2022). The Himalayan native *S. costus* is a perennial flowering herb locally called ‘kuth’ or ‘costa’ and belongs to the family Asteraceae, which has more than 400 reported species. (Rathore et al. 2021; Kumar and Pundir 2022; Shashank and Shreya 2023). The Traditional remedy of Chinese has rate for using this herb (*S. costus*) for various ailments, mostly as a pain reliever, and for skin diseases, antibacterial, digestive issues, hepatoprotective, and antifungal properties. Similar finding has been reported in Ayurveda and Unani medical system (Pandey et al. 2007; Mehta and Shah, 2021; Li et al. 2005) In this decade some secondary novel compounds have been extracted from *S. costus* & *S. lappa* with lots of medicinal uses, including compounds which acts as flavonoids, tannins, alkaloids, phenols, and terpenoids in different forms used to determine *in-vivo* and *in-vitro* anti-microbial, anti-inflammatory, anti-tumour, anti-oxidant, and ant-proliferative activities. (Deabes et al., 2021; Al-Saggaf et al. 2020; Kour et al. 2025; Vijayalakshmi et al. 2022). Previous research studies have separately proven that *S. costus* and *S. lappa* have numerous medicinal importances and pharmacologic abilities mostly for antimicrobial, anti-inflammatory, anticancer, antifungal and hepatoprotective properties (Chen et al. 1995; Venkataranganna et

al. 1998; Matsuda et al. 2000; Damre et al. 2003; Sharma 2019; Binobead et al. 2024; Ali et al. 2021; Elnour and Abdurahman, 2024). The best antibacterial action of *S. costa* against various microbial straining of *E. coli* & *S. aureus* has been reported in methanol extracts. The best fungal activity has been reported against *C. albicans* in the root ethanol extract (Al-Zayadi et al. 2023; Cragg and Newman, 2013). The root extract of *S. lappa sp.* against Numerous microbial strains of bacteria and fungi has proved significant antimicrobial potential against *S. epidermidis, S. mutans, S. aureus, S. typhi, S. typhimurium, P. aeruginosa, B. subtilis, E. coli, M. grisea, T. mentagrophytes,* and *T. rubrum* strains (Negi et al., 2013; Yu et al., 2007). In another study, the LCMS data obtained from ethanol and aqueous root extracts of *S. lappa* have proven effective in determining a variety of secondary metabolites and the biosynthesis of pharmaceutical byproducts and in studying phytochemistry and assessing its antimicrobial properties against Gram-positive and Gram-negative strains, mostly *S. aureus* & *salmonella* (Gwari et al., 2013; Deabes et al., 2021; Idriss et al., 2023). This study was conducted on root extracts of *S. costus* in order to investigate variations in phytochemical activity and antimicrobial screening in four different solvents, including methanol, hexane, ethyl acetate, and aqueous extracts, and the phytochemistry of root extracts using GCMS metabolite profiling, along with antioxidant and radical-scavenging potential.

2. MATERIALS AND METHODS

2.1. Plant materials

The Plant material of *S. costus* (SC) was gathered from the upper wild area of Pahalgam, district Anantnag, at an altitude of 3011.0±47.2 meters above mean sea level (AMSL), with a latitude of 34.028871 and a longitude of 75.369568. The Center of Biodiversity and Taxonomy, Department of Botany, University of Kashmir, was used to authenticate and document the plant *S. costus*. For authenticity, the herbarium was prepared and sent to the department with recipient specimen number KASH-9392.

2.2. Chemicals used

Methanol, ethyl acetate, hexane, water, nutritional agar, and Potato Dextrose Agar (PDA) were the solvents utilised in this investigation. Mueller-Hinton agar (MHA), and dimethyl sulfoxide (DMSO), were among the chemicals and reagents used in this experimental analysis and were bought from HI media (Mumbai, India). Additionally, ultrapure distilled water was used for all media preparations, and other microbiological reference chemicals used in this experiment were also acquired from HI media, with the highest-grade raw discs of ketoconazole and streptomycin, which are used as positive controls.

2.3. Preparation of solvent extractions

With the required adjustments, the solvent extraction process was modified from Dar et al. 2020. The *S. costus* material was shade-dried and later milled into fine powder. Root samples of *S. costus* species extracts were treated with several solvents, including hexane, ethyl acetate, methanol, and aqueous solvents, based on polarity. About 300 grams of dried plant material were imperilled to a Soxhlet extraction by 600–700 mL of each solvent, used in syphoning around a 24-72-hour period. A rotary evaporator was used to concentrate the resultant extracts. Whatman No. 1 filter paper (0.45 µm) was used to sieve them. 10 mg of yield from each extract was dissolved in 10% DMSO (dimethyl sulfoxide). Similar steps were repeated for other solvents. We created 20/40/80 mg/mL mother-stock solutions separately for each solvent to determine the antioxidant and antimicrobial potential. The stocks obtained stayed kept at 4°C for future lab usage in experiments.

2.4. Instrument and Running conditions

The sample was put into a Shimadzu GC-MS-QP2010 machine. A GC SH I 5Sil MS Capillary through magnitudes of 30 m x 0.25 mm x 0.25 µm was utilised as the column. Spitless mode was used to inject the sample. The following operating criteria were established for GC MS: The oven temperature was maintained at 45°C for two minutes, then rose to 140°C at a rate of 5°C per minute, and finally touched 280°C, where it was maintained isothermally for ten minutes. Helium was utilised as the transporter gas at a movement degree of 1 mL, and the sample injection volume was 2 µL. The components of the extract were ionised at 70 eV. The GC ran for 9.10 to 52.0 minutes in total.s The structures of discovered chemicals were compared with those in the known NIST database using the NIST14.L library (2020). Retention durations and mass spectra matching entries in the NIST library at C:\Database\NIST20.L were used to identify compounds.

2.5. Antimicrobial screening

The strains were obtained from MTCC at the IMTECH Institute in Chandigarh, India. Pathogenic microbial strains were acquired. The bacterial and fungal test strains were cultivated on Mueller-Hinton Agar & Potato Dextrose Agar. They were stored at 4°C and sub-cultured every 15 days. 10% DMSO served as the negative regulator, while streptomycin and ketoconazole (20mg/mL discs) served as the positive control for bacteria and fungi. Three extract concentrations (20, 40, and 80mg/mL) were verified against *E. coli* (MTCC443), *S. aureus* (MTCC96), and *P. aeruginosa (MTCC1688)*, and *C. albicans* (ATCC24433), *C. glabrata* (ATCC2001), and *C. paropsilosis* (ATCC90018), which were cultivated in YEPD medium. After the plant treatments, the discs were incubated for 24 hours, and the readings were recorded.

2.6. Agar Well Diffusion Assay

The agar well diffusion technique was used to assess the antibacterial & antifungal qualities of root extracts of *S. costus spp.*, in a concentration-dependent pattern. The nutritional medium was Mueller-Hinton Agar for bacterial Potato Dextrose Agar for fungal growth. The root extracts of different strengths (20/40/80mg/mL concentration, with 40-50 µL volume used from each concentration per well) were applied to wells (5to7 mm diameter) that had been inoculated. With a sterile loop, positive controls streptomycin and ketoconazole) and DMSO as negative controls were used. Each plate was cultured for 16 to 48 hours at 35°C to 38°C in an incubation chamber. (**Fig. 1 & 2**). Additionally, the zone of inhibition was calculated in millimetres. Every test was performed uniformly in triplicate.

2.7. Evaluation of qualitative phytochemicals

Preliminary qualitative tests in different solvents for phytochemical screening of *Saussurea costus* samples were conducted using methanolic, hexane, ethyl acetate, and aqueous extracts to assess the current phytochemical profile. The qualitative standardized chemical tests were detected by Harborne (1998), with some modifications. Some primary and secondary metabolites were identified in root sample extracts of S. costus using various phytochemical analyses. (Mehta and Shah, 2021)

2.7.1. Terpenoid: - (Salkowski test), In a tube, about 5 mL of solvent extract was mixed with 2 mL of chloroform and then 3 mL of concentrated sulphuric acid, in a test tube. The reddish-brown color indicates the presence of terpenoids.

2.7.2. Saponins: - (Foam test), For this part, we use a test tube. First, add 2 mL of each extract to 2 mL of distilled water. Mix the mixture vigorously for 2 minutes. The formation of persistent foam indicated the occurrence of saponins.

2.7.3. Flavonoids: - (Lead acetate test), in a conical test tube, 2 mL of the extract was mixed with 2 to 3 drops of a strong lead acetate solution. The appearance of yellow colorization designates the occurrence of Flavonoids.

2.7.4. Steroids: - (Salkowski test used), a test in a long tube containing 2 mL of extract. Add 2 mL of chloroform and 2 mL conc. Sulphuric acid carefully. The appearance of a blue-to-green color in two liquid layers indicates the presence of steroids.

2.7.5. Tannins: - (Ferric chloride assay test), here 3 mL of sample extract was taken in a test tube; add 0.5mL of 5% ferric chloride. The presence of greenish precipitates indicated the presence of tannins.

2.7.6. Phenols: - (Ferric chloride assay test), In a test tube, 2 mL of sample extract was mixed with 2–3 drops of ferric chloride ($FeCl_3$). The green color indicated the presence of phenols.

2.7.7. Alkaloids: - (Dragendorff's assay test), in a test tube, 2-3 drops of Potassium bismuth iodide solution (reagent) were added to 2 mL of plant extract. A reddish-brown deposit specifies a positive outcome for alkaloids.

2.8. Calculating the total phenolic content (TPC)

The TPC was assessed by means of FC reagent (Folin-Ciocalteu mixture reagent) following the methodology of Ebrahimzaded et al. (2009, with minor modifications. Here, 100 µL of plant extract from each of the 20/40/80 mg/mL mother stocks in each solvent was added to 200 µL of 10% FC reagent; then, 100 µL of 20% sodium carbonate was added, and the mixture was diluted with double-distilled water to a final volume of 5 mL. All mixed thoroughly, then boiled for 2 minutes and left to cool. The absorption remained documented at 650 nm using a UV-Visible spectrophotometer. Here, gallic acid is used as a standard to determine the total phenolic acid content.

2.9. Calculating the total flavonoid content (TFC)

TFC is computed using an altered type of Hung and Morita's 2008 approach. Aluminum chloride (0.5 mL of 10% AlCl3), potassium acetate (2 mL of 1 M CH3COOK), and 0.4 mL of distilled water were mixed with 0.1 mL of plant extract from each solvent (SCM, SCE, SCH, and SCA) to create a total volume of 5 mL. After that, it was permitted to settle at room temperature for half an hour. For every extract, the same procedure was used. A UV spectrophotometer was used to measure the absorption at 415 nm. Rutin was utilized as a flavonoid to compute TFC. An adjustment arc was made by rutin as a reference.

2.10. 2,2-diphenyl-1-picrylhydrazyl, or DPPH

The DPPH assay was performed using Braca et al.'s (2018) methodology with minor modifications. Here, a 0.2 mM DPPH solution was prepared in methanol. Further, several concentrations of plant extracts (20 mg/ml, 40 mg/ml, and 80 mg/mL) in different solvents (SCM, SCE, SCH, SCA solvents) were added to the DPPH solution. The final solution was varied vigorously and kept in the dark for 15-20 minutes. The same method was followed for solvent extracts, and Vitamin C was used as a standard. A UV-visible spectrophotometer was subsequently used to measure the optical density at 517 nm. The IC50 values were determined for each solvent extract to see 50% inhibition of DPPH radicals against values with higher concentrations, and the regression equation $Y = mx + C$. IC_{50} values were analyzed from the linear regression equation using MS Excel 2010. The IC50 values are mentioned in Table 2.

$$\text{DPPH \% inhibition} = \frac{A1 - A2}{A1} \times 100$$

where A_1 is the absorbance value of the control (Vit-C) for the DPPH solution, and A_2 as absorbance value of the plant extracts for each solvent of *S. costus* species samples.

2.11. FRAP, or ferric reducing antioxidant power

With a few minor modifications, the FRAP test was conducted using the Banzia et al. (2018) technique. The FRAP assay uses 30 mL of FRAP solution, which contains TPTZ (tripyridyl-s-triazine), an acetate buffer, and ferric chloride. The ferric ions (Fe3+) are reduced to ions (Fe2+) by the TPTZ solution. When the FRAP reaction occurs, a blue color appears, indicating that the reaction is happening.

2.12. Antimicrobial Profile Analysis

The antimicrobial profiling of *S. costus* root extracts obtained with different solvents (SCM, SCH, SCE, SCA) was evaluated against three bacterial and fungal strains each. These include fungal strains such as *Candida albicans* (CA), *Candida glabrata* (CG), and *Candida paropsilosis* (CP), as well as bacterial strains such as *S. aureus* (SA), *E. coli* (EC), and *Pseudomonas aeruginosa* (PA). With the support of Agar well diffusion method, antibacterial and antifungal activities were plotted against observed zone of inhibition (ZOI) which were calculated in mL, against 20/40/80 mg/mL, concentrations gradient mannerly, with the highest efficacy at 80 mg/mL for most extracts among four solvents in order of SCM>SCH>SCE>SCA, with streptomycin as positive control (Control 1=C1), against bacterial strains and ketoconazole (Control 3 = C4), against fungal strains. At concentrations of 20 mg/mL each, with DMSO as a negative control (Control 2 = 0), against all microbial strains. All data were generated from three replicates for each experiment and each strain.

2.13. Statistical examination

The mean and standard deviation (mean ± SD) were used to examine data collected in triplicate (n=3). Here, the two-way ANOVA (Tukey's test) was used to assess differences in solvent extracts among samples. Results were further analyzed using GraphPad Prism (8.9) software to simplify presentation and identify statistically significant differences ($p \leq 0.05$). The IC_{50} values were calculated using a regression equation against control samples.

3. RESULTS

3.1. Qualitative Phytochemical Analysis Determination

The phytochemical analysis of *S. costus* root samples was conducted using approved analytical techniques in four solvents. The analysis unveiled the occurrence of terpenoids, steroids, flavonoids, saponins, tannins, phenols, and alkaloids. However, root

samples extracted with methanol (M) were more effective than those extracted with hexane (H), ethyl acetate (E), or aqueous (A).

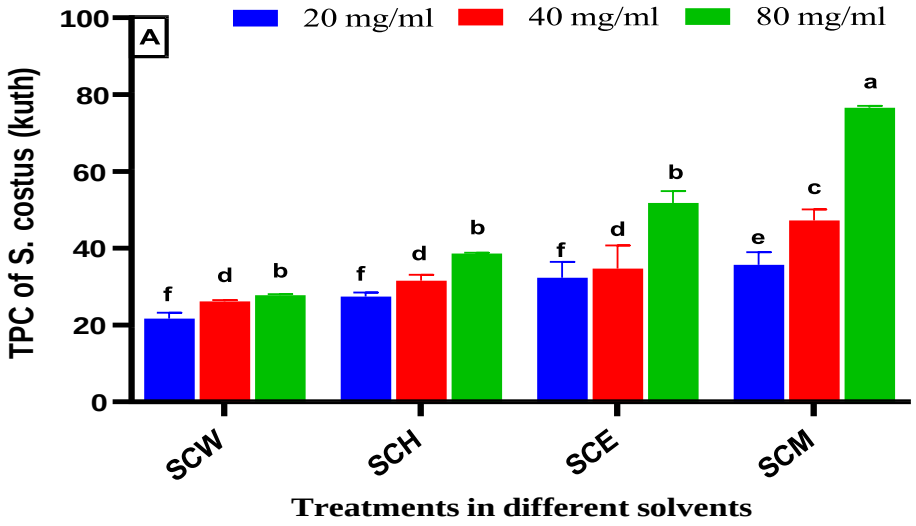
Table. 1. Qualitative phytochemical screening of *S. costus* root extracts in four solvents.

Saussurea costus					
S. No.	**Tests**	**Roots samples**			
	Solvents	**M**	**E**	**H**	**A**
1.	**Terpenoids**	+++	++	++	+
2.	**Saponins**	++	+	+	-
3.	**Flavonoids**	+++	++	++	++
4.	**Steroids**	+++	+	+	-
5.	**Tannins**	++	+	-	+
6.	**Phenols**	+++	++	++	++
7.	**Alkaloids**	+++	++	+	-

3.2. Determination of Quantitative Phyto-chemical Analysis

3.2.1. Calculating the flavonoid content (TFC = mg RE/g DW) and total phenolic content (TPC = mg GAE/g DW).

The entire phenolic and flavonoid quantity of the *S. costus* root sample extracts is depicted in **Fig. 1. (A, B).** The outcomes clearly showed that the highest TPC was seen in root samples of methanolic solvent (76.57 ± 0.4 mg GAE/g DW), followed by ethyl acetate (51.81 ± 3.1 mg GAE/g DW), then hexane (31.64 ± 0.3 mg GAE/g DW), and aqueous (27.76 ± 0.3 mg GAE/g DW) at 80 mg/mL concentration. A similar pattern was observed, with the highest TFC in methanolic solvent (92.58 ± 1.1 mg RE/g DW), hexane (53.9 ± 0.96 mg RE/g DW) and aqueous (43.82 ± 2.1 mg RE/g DW). With the change in concentration and solvents against *S. costus* extracts, there was a change in phenolic and flavonoid content. At higher concentrations, high levels of phenolic and flavonoid content are recorded. Here, the TPC & TFC show a positive correlation (Gallic acid (GAE), and Rutin (RE) as standard). **(Fig. 1. A. B).**



Natural Resources for Human Health
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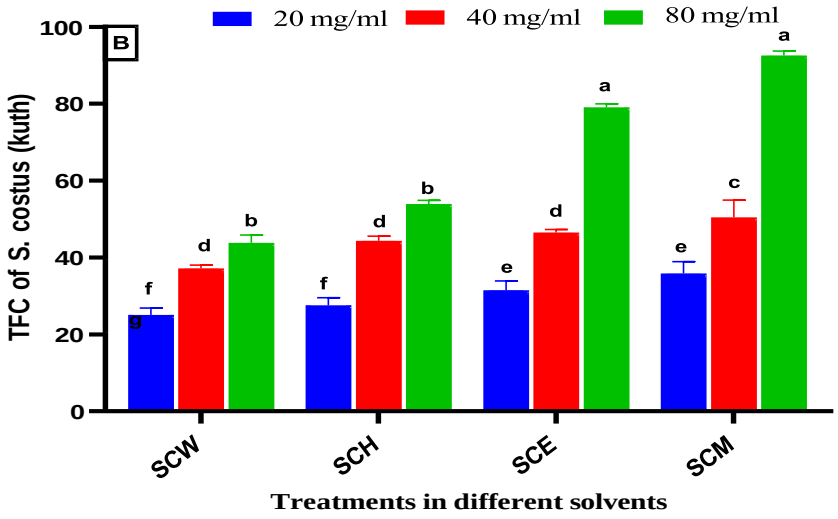


Fig. 1. (A. B) depicts total phenol and total flavonoid (TPC & TFC) content in different solvents (Methanol > Ethyl acetate > Hexane > Aqueous) of *S. costus* in different concentrations.

3.2.2. Determination of Ferrous reducing antioxidant power activity

The FRAP is an easy, quick antioxidant assay used with a variety of plant samples, including *S. costus* root extracts. It is a reduction assay in which the solution changes from yellow to light green, indicating that the samples contain antioxidant compounds that reduce ferric (Fe^{3+}) to Ferrous (Fe^{2+}) ions. The capacity of reducing ferric was found to grow with increases concentration of *S. costus* extracts and highest reduction power is observed in methanolic solvent extracts (83.05 ± 0.5 µmol Fe (II)/g DW), after which ethyl acetate (61.09 ± 0.8 µmol Fe (II)/g DW), than hexane (42.43 ± 0.8 µmol Fe (II)/g DW), and aqueous (35.02 ± 0.7 µmol Fe (II)/g DW), calculated against a controlled sample (Vitamin-C) (87.17 ± 1.03 µmol Fe (II)/g DW) at 80 µL concentration. At the higher concentration, there is significant FRAP activity, and it is positively correlated with TPC and TFC. **(Fig. 2. (C)**.

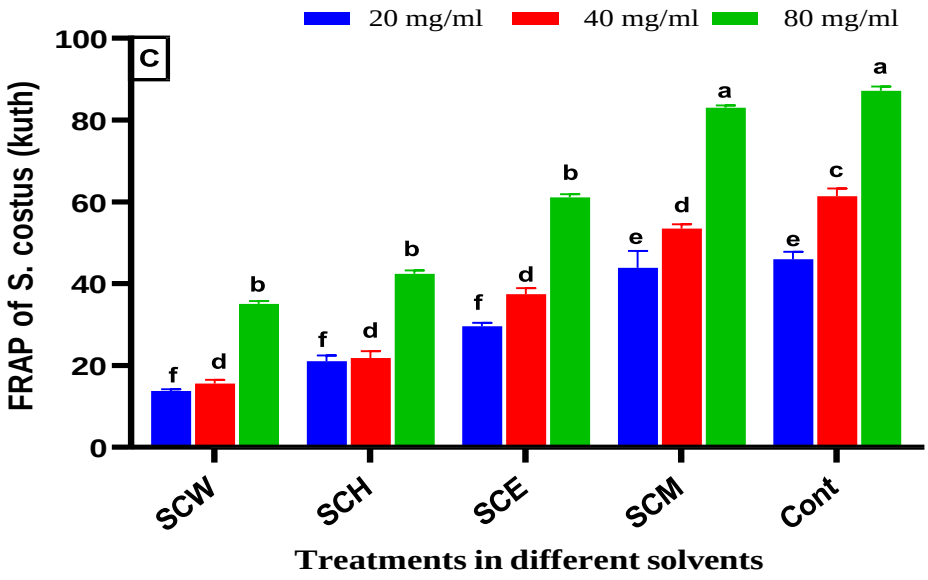


Fig. 2. (C). depicts ferrous reducing antioxidant power (FRAP) of *S. costus* in different solvents (Methanol > Ethyl acetate > Hexane > Aqueous) in different concentrations.

3.2.3. DPPH analysis (2,2-diphenyl-1-picrylhydrazyl) scavenging activity measurement

The free DPPH radical scavenging potential indicates that significant amounts of S. costus extract can donate atoms (hydrogen) to neutralize the stable DPPH radical. The assay shows that free radical scavenging activity is dose- and solvent-dependent. The methanol solvent showed the highest scavenging potential, with the highest percentage inhibition of 69.19 ± 2.5 %, followed by ethyl acetate at 59.46 ± 2.2 %, then hexane at 38.90 ± 1.5 %, and aqueous extracts at 25.51 ± 1.07 %, at an 80 mg/ml concentration each. This study indicates that among the four solvents (M, H, E, A), the strongest free radical scavenging activity was recorded in methanol extracts at higher concentration. The DPPH results show a strong correlation with FRAP results. The IC_{50} values also indicated that the methanol solvent is a better performer than ethyl acetate, hexane, and aqueous extracts. **(Fig 3. (D). Table 2).**

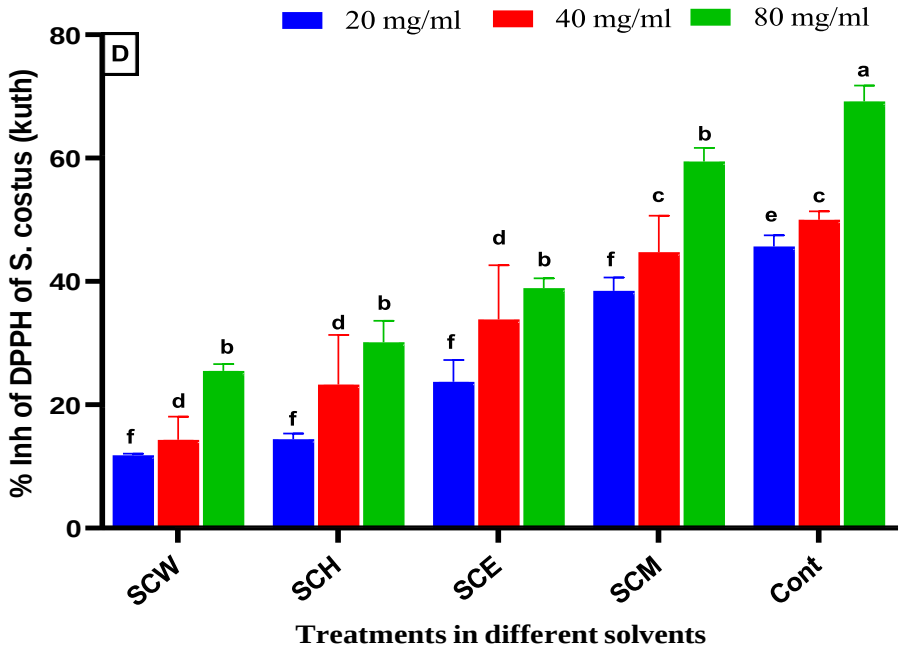


Fig. 3. (D) depicts DPPH activity (2,2-diphenyl-1-picrylhydrazyl) of *S. costus* in different solvents (Methanol > Ethyl acetate > Hexane > Aqueous) at different concentrations.

Table 2. Fifty per cent inhibitory (IC50) of different *S. costus* root extracts (mg/mL) in different solvents.

IC50 values						
DPPH	**I**	**II**	**III**	**Equation**	**R value**	**IC50**
SCW	11.84	14.32	25.52	y = 0.1883x + 6.24	R^2 = 0.9738	232.39
SCH	14.42	23.28	30.13	y = 0.1989x + 11.005	R^2 = 0.9314	196.05
SCE	23.73	33.85	38.91	y = 0.1878x + 21.205	R^2 = 0.8619	153.33
SCM	38.48	44.76	59.46	y = 0.2818x + 31.13	R^2 = 0.9986	66.96
Control	45.66	50	69.19	y = 0.3237x + 36.065	R^2 = 0.9748	43.05

3.3. Determination of Antifungal and Antibacterial potential on different strains

The antibacterial and antifungal activities of *S. costus* were determined in root extracts using four different solvents, with three strains per activity, and an antibiotic serving as a positive control. **(Fig. 4, 5).** These activities were evaluated on the Zone of Inhibition (ZOI) obtained by the strains EC, SA, PA, CA, CG, and CP against positive controls such as streptomycin and ketoconazole, with DMSO as a negative control, and measured in millimeters (mm). **(Fig. 4, 5).** In this study, among four

solvents, methanol has the highest potency, followed by hexane, ethyl acetate, and aqueous. The maximum potency was recorded at 80 mg/mL, followed by 40 mg/mL and 20 mg/mL among the six microbial strains tested. Here, the potential bacterial strains with the highest zone of inhibition were seen in EC = 27.33±1.1mm; SA = 26.67 ± 0.5mm; PA = 25.67 ± 0.5mm, and fungal strains as CA = 22.66 ± 1mm; CG = 22 ± 1mm; & CP = 22.66±1mm, at 80 mg/mL concentration. Similarly, the effective microbial strains were observed at a higher level of significance in *E. coli*, followed by *P. aeruginosa* and *S. aureus*, and, among the fungal strains, high sensitivity was observed in *C. albicans*, followed by *C. glabrata* and *C. parapsilosis*, against the extracts. These activities were observed to be concentration-dependent; the higher the root extract concentration, the stronger the antimicrobial activity. Additionally, every test was conducted out in three times, and all data sets remained prepared appropriately.

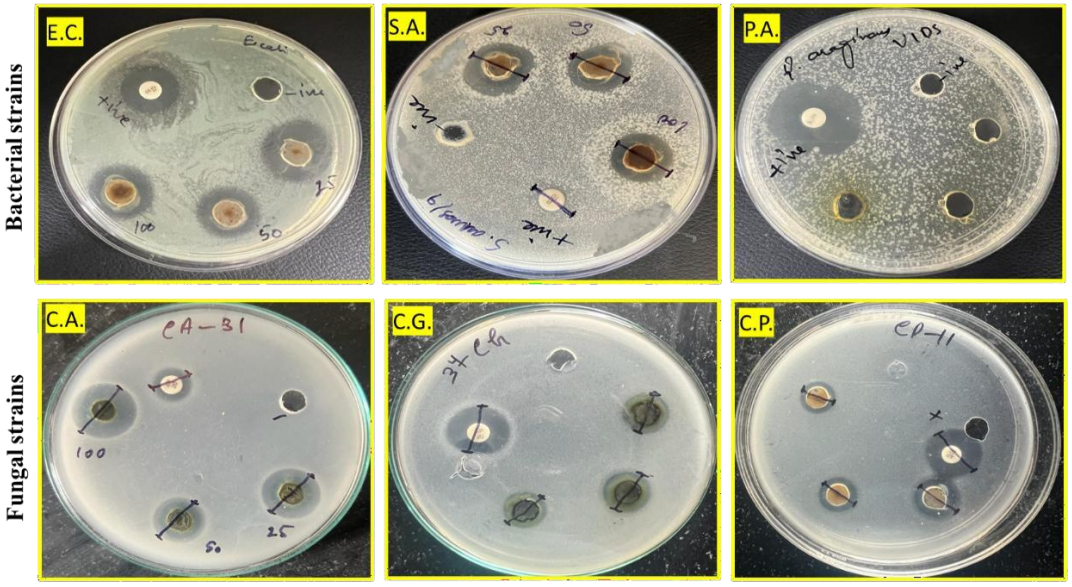
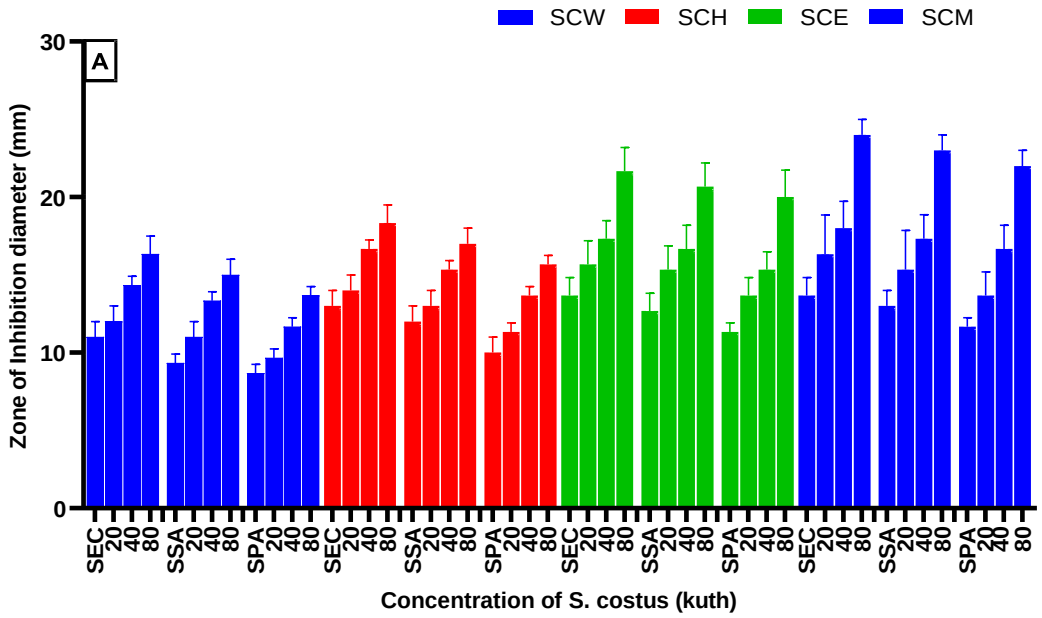


Fig. 4. (A. B). (A) The antibacterial and (B) antifungal activity of the root methanolic sample extract of *Saussurea costus* (SCM) on various strains, EC, SA, PA, CA, CG, and PA.



Natural Resources for Human Health
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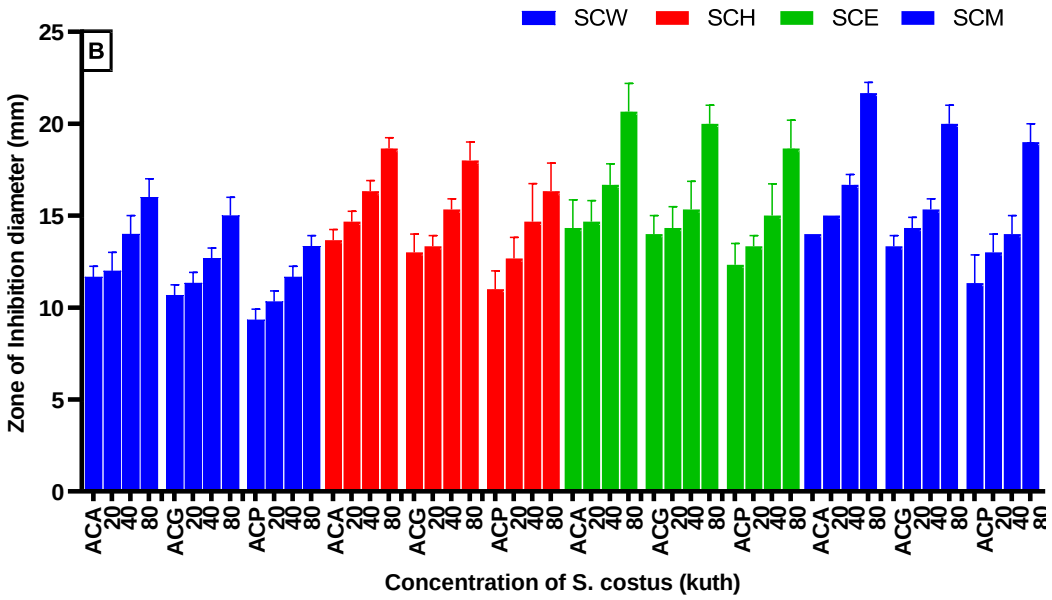

Fig. 5. (A. B). Depicts the Anti-bacterial and antifungal properties of root extracts of S. *costus* examined in different solvents (Methanol > Ethyl acetate > Hexane > Aqueous) against various bacterial strains, as EC, SA, and PA, *and* against fungal strains, CA, CG, and CP. At concentrations of 20, 40, and 80 mg/mL, with positive controls as streptomycin (Control 1=C1), ketoconazole (Control 2 = C2), and DMSO as a negative control =0.

3.4. Gas Chromatography Mass Spectrometry Analyses (GCMS)

In this study, the methanolic sample extract exhibited the highest activity among the three solvents, suggesting this may be due to the significant presence of secondary metabolites, as shown in Table 3. Here, the majority of secondary compounds extracted from the root sample of *S. costus* sp. by GC-MS analysis exhibit effective antioxidant, phytochemical, and antimicrobial activities. Thus, suggested to be a powerful medicinal plant with praiseworthy potential. The chromatogram for this study is shown in Fig. 7, and the list of compounds is provided in **Table 3** and **Fig. 6.**

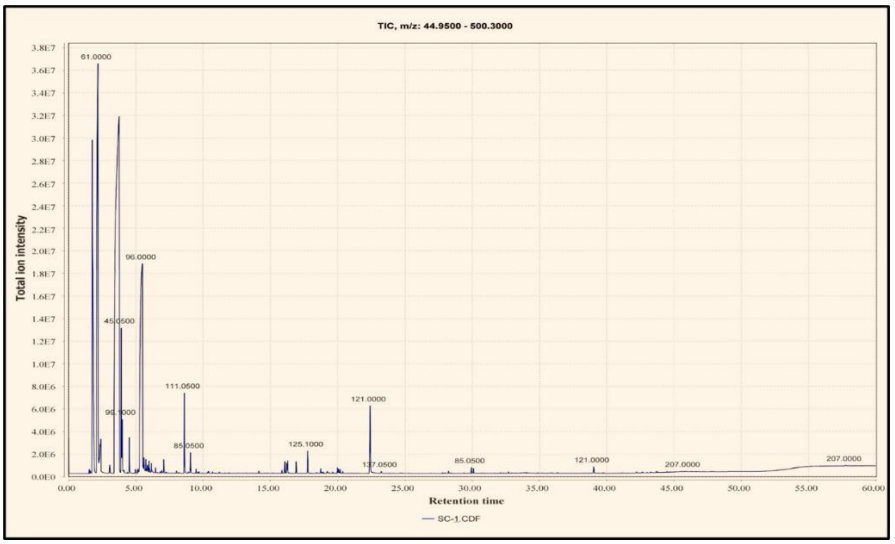

Fig. 6. Chromatogram of Gas chromatography mass spectrometry analyses (GCMS) of methanolic root extract of *S. costus spp.*

Table 3. Gas chromatography mass spectrometry analyses (GCMS) of methanolic root extract of *S. costus spp.*

S. No.	Secondary metabolites of S. costus (Kuth)	Retention Time	Peak Area (%)
1)	Methylene chloride	1.7	9.58
2)	Pentanoic acid, 3-methyl-4-oxo-	2.1	15.08
3)	Silane, dimethoxydimethyl-	2.3	0.82
4)	Acetic acid	2.3	0.72
5)	Acetic acid, [(phenylmethoxy)imino]-, trimeth	3.0	0.15
6)	Methyl Isobutyl Ketone	3.7	46.14
7)	2-Pentanol, 4-methyl-	3.9	1.81
8)	Acetamide, N-[(dimethylamino)methylidene]-	3.9	1.16
9)	Propanoic acid, 2-methyl-	4.1	0.04
10)	Trimethylsilyl ethaneperoxoate	4.3	0.02
11)	3-Penten-2-one, 4-methyl-	4.5	0.47
12)	Furfural	5.4	16.22
13)	2-Propanone, (1-methylethyl) hydrazone	5.5	0.19
14)	Vinyl butyrate	5.7	0.16
15)	Cyclohexane, 1,3,5-trimethyl-	5.8	0.15
16)	3-Hexanol, 2-methyl-	5.9	0.18
17)	Butanal	6.1	0.17
18)	Methanol, TBDMS derivative	7.0	0.21
19)	2-Pentene, 1-ethoxy-4-methyl-, (Z)-	7.2	0.03
20)	3-Aminopyrazine 1-oxide	8.5	1.33
21)	2-Furancarboxaldehyde, 5-methyl-	8.8	0.03
22)	4-Heptanone, 2,6-dimethyl-	9.0	0.34
23)	Glutaric acid, 2,4-dimethylpent-3-yl propyl est	16.0	0.2
24)	Prop-2-yn-1-yl 2-methylbutanoate	16.2	0.17
25)	1-Methyl-1-(2-butoxy)-1-silacyclohexane	16.2	0.21
26)	2,3,4,5-Tetra-methylcyclopent-2-en-1-ol	16.9	0.19
27)	2,3,4,5-Tetra-methylcyclopent-2-en-1-ol	17.7	0.38
28)	4'-Hydroxy-2,2-dimethylpropiophenone	22.4	1.62
29)	alpha-Isobutylionone	39.0	0.16

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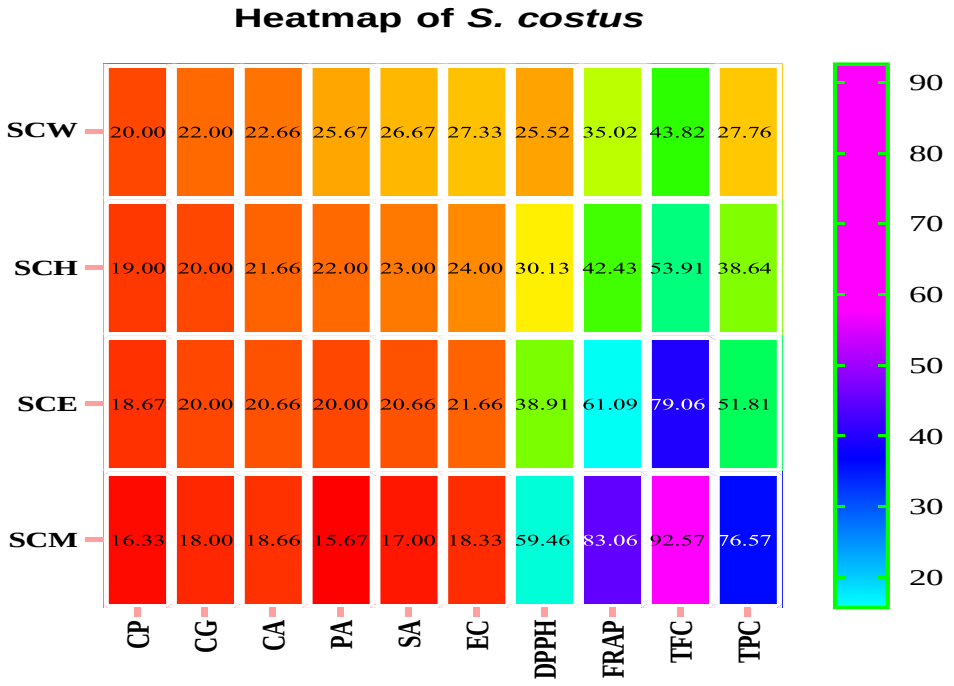

Fig. 7. Correlation Heatmap analysis between antioxidants and antimicrobial activities of root extracts of *S. costus* samples

Table 4: Correlation matrix of root samples of *S. costus* extracts between antioxidant and antimicrobial activities.

Variables	TPC	TFC	FRAP	DPPH	EC	SA	PA	CA	CG	CP
TPC	1									
TFC	0.96	1								
FRAP	0.99	0.98	1							
DPPH	0.99	0.94	0.98	1						
EC	-0.98	-0.97	-0.97	-0.95	1					
SA	-0.98	-0.97	-0.97	-0.95	0.99	1				
PA	-0.98	-0.96	-0.97	-0.96	0.99	0.99	1			
CA	-0.99	-0.96	-0.99	-0.99	0.98	0.98	0.99	1		
CG	-0.94	-0.88	-0.91	-0.92	0.96	0.97	0.97	0.95	1	
CP	-0.98	-0.90	-0.96	-0.98	0.95	0.95	0.97	0.98	0.96	1

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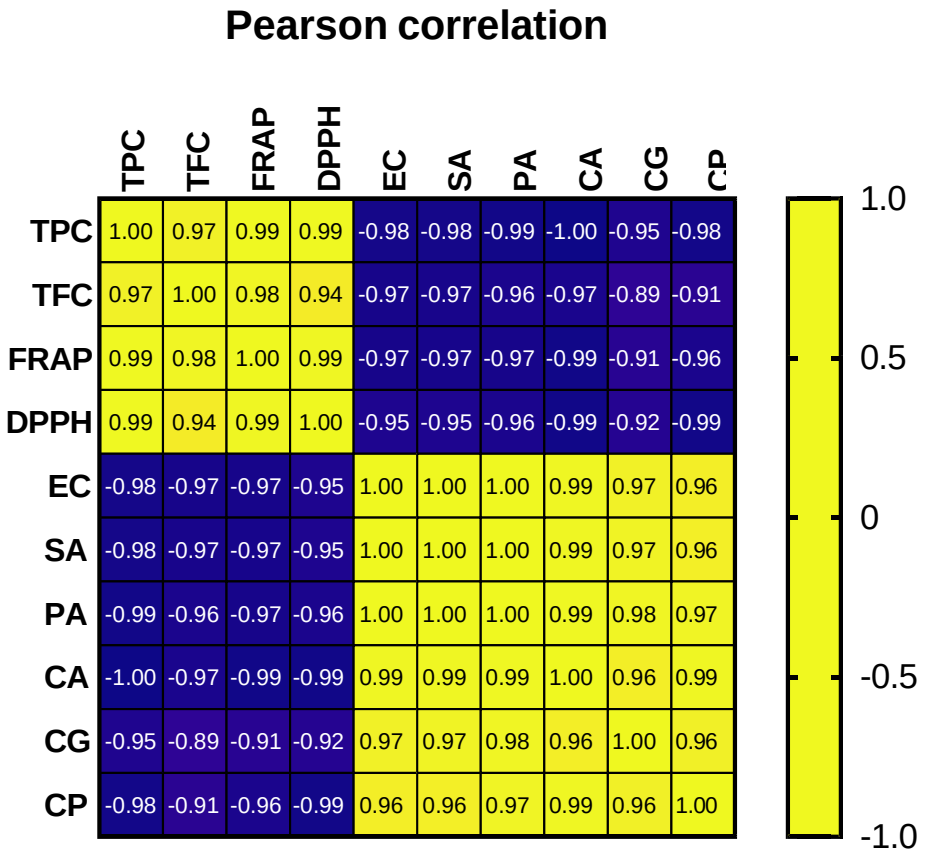

Fig. 8. Pearson correlation between antioxidants and antimicrobial activities of root extracts of *S. costus* samples.

4. DISCUSSION

In this study, *S. costus* root samples underwent quantitative and qualitative phytochemical screening, and antibacterial and antifungal profiling were performed using four solvents of increasing polarity: methanol, hexane, ethyl acetate, and aqueous extracts. According to this study, the phytochemicals present in good quantities include flavonoids, phenols, alkaloids, and terpenoids, mostly in root methanolic extracts, followed by ethyl acetate extracts, and least in hexane and aqueous extracts. The highest was 80 mg/mL, followed by 40 mg/mL and 20 mg/mL. In another study, a similar pattern was recorded in another species of *S. lappa sp.*, in ethanolic extract, which reduces the adverse effects of glucocorticoids by acting as a natural antioxidant and anti-inflammatory agent (Al-Saggaf et al., 2020; Abd El-Rahman et al., 2020).

The present investigation supported *S. costus* as a potent antioxidant with high total phenol and flavonoid content. A comparative analysis revealed that methanol root extracts had the highest TPC and TFC, followed by ethyl acetate extracts, while hexane and aqueous extracts had the lowest values. The lowest actions were observed at 40 mg/mL and 20 mg/mL, whereas the highest TPC and TFC were observed at 80 mg/mL. **(Fig. 1. (A. B))** Many studies support the idea that rich flavonoid and phenolic compounds are the best antioxidant sources and free radical scavengers. Earlier studies have also shown that *S. costus* is a strong antioxidant and a free radical scavenger (Hassan & Masoodi, 2020). In another study, higher flavonoid and phenolic content have been described in *S. costus* and *S. lappa* extracts in different solvents (Akl & Younos, 2024; Binobead et al., 2024). In this study, the plant *S. costus* root extracts exhibit strong free radical scavenging activity, as demonstrated by FRAP and DPPH assays, supporting their high antioxidant activity and enhanced free radical quenching. Methanol and ethyl acetate extracts show high FRAP and DPPH activity compared to hexane and aqueous extracts. The strongest DPPH and FRAP activities were concentration-dependent, peaking at approximately 80 mg/mL and exceeding those at 40 mg/mL and 20 mg/mL. **(Fig. 2C)** The high levels of secondary metabolites are responsible for strong DPPH and FRAP activities. Similar

findings have been reported in *in-vitro* studies with *Saussurea costus* using gold nanoparticles as an antioxidant agent in various assays, demonstrating strong DPPH and FRAP activities in root solvent extracts via green nanoparticle synthesis (Binobead et al., 2024; Almayouf et al., 2024; Adel et al., 2025). **(Fig. 3D)** In another *in-vivo* study on animal models using an ethanol solvent, strong antioxidant activity in *S. lappa* plant root extracts has been supported (El Sheikh et al., 2025). Furthermore, another study has shown that root samples of *S. costus* are rich in phytochemicals and exhibit strong antioxidant and radical-scavenging activities. (Hussien et al., 2024; Putry et al., 2024).

It was observed that root extracts exhibited greater antibacterial activity than antifungal activity, as evidenced by the order of solvents (Methanol > Ethyl acetate > Hexane > Aqueous). Here, the methanolic root extract exhibited improved antimicrobial action compared to ethyl acetate, hexane, and aqueous solvent root extracts, with the highest action recorded against bacterial strains, such as *E. coli*>*S. aureus* >*P. aeruginosa*, and fungal strains, including *C. albicans*>*C. paropsilosis*>*C. glabrata*, **(Fig. 4, 5**). In previous studies, a similar result was observed in *Saussurea costus*, which showed potent antimicrobial activity against various *Escherichia* spp., *Salmonella* spp., *Candida spp., Pseudomonas spp., Bacillus spp.,* and *Staphylococcus spp.* (Idriss et al., 2023; Ya & Jian, 2013; Elnour and Abdurahman, 2024; Ahmed & Coskun, 2023). Here, the root extracts of *S. costus* have shown equally effective results in Gram-positive and Gram-negative species. A similar type of antibacterial results were seen in plant species like *S. costus* in separate studies, against both Gram-positive and Gram-negative species across various bacterial strains (Al-Zayadi et al., 2023; Zaky et al., 2022; Alnahdi et al., 2017; Shaikh et al., 2022; Reda et al., 2024; Akani et al., 2020). Similar kinds of results were observed in other plant species with noteworthy antibacterial activity against *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, and *E. faecium*, with the highest interactions with 55 sample combinations of oil extracts in *Viola odorata* species (Orchard et al., 2023; Zeng et al., 2025; Ahmed and Coskun, 2023). Likewise, results are also recorded in *Saussurea lappa sp.*, which has tremendous antimicrobial, antifungal, and antioxidant activity, with numerous pharmacological activities (Elnour & Abdurahman, 2024). Additionally, another study matches our study on *Saussurea lappa sp.*, with the best antibacterial potential in root ethanol extracts compared with aqueous extracts, with zero effect on *Salmonella spp.* strains at varying (100 µg/mL) concentrations (Omer et al 2019; Soliman et al. 2022; Airharam et al 2012; Idriss et al. 2023).

In this study, GC-MS data summarising *Saussurea costus* root methanol extracts identified a diversity of compounds with antioxidant, antimicrobial, and anticancer properties, as demonstrated using *in-vitro*, *in-silico*, and *in-vivo* types of assays **(Table 3, & Fig. 6.).** A similar set of Phytoconstituents for ethanol and aqueous extracts has been reported, comprising 37 compounds identified by GC-MS analysis. In another study of *S. lappa* and *S. costus*, the researchers identified 40 compounds in each ethanol extract, with terpenes as the major class. (Maurer & Grieder, 1997; Gwari et al., 2013; Negi et al., 2013). Here, data from this study on root samples of *S. costus* show a strong correlation in antioxidant activities across assays and a positive correlation between antifungal and antibacterial activities. **(Table 4, Fig. 6, 7.).** In a different research, some compounds with significantly detected in the ethanol and aqueous extract 9,12,15-octadecatrienoic acid, cyclodecacyclotetradecene, 6-ethyenlhexahydro-6-methylene-7, isosteviol methyl ester, 14,15-didehydro, cyclodecacyclo- tetradecene,14,15-didehydroxy, propanediol, hydroxymethyl-2-nitro, 2(3H)-benzofuranone, 6-ethenythexhy- droxy, bicycle-decane, 2-methylene-5-1-methylvinyl-8, cyclohexane,1,2-diethenyl-4-(1-methyle.2(3H)-benzofuranone, docosahexaenoic acid, androstan-17-one, cholest-7-en-3-ol,4-meth-yl-, 3-ethyl-3-hydroxy, and 3-oxatricyclo-triaconta-1) were identified with significant activities (Shoji et al., 1986; Govindan & Bhattacharaya, 1977; Maurer & Grieder, 1997; Banerjee et al., 2017; Omer et al., 2019). Among the solvents used here, the methanolic extract showed the highest levels of significant phytochemicals, antioxidants, antifungal, and antibacterial potential, which may be due to unique secondary metabolites identified in GC-MS analysis **(Table 3)** and their absence in other solvents such as hexane > ethyl acetate > aqueous extracts. The metabolite list generated from GC-MS analysis of the root sample of S. costus is rich in flavonoids, phenols, and terpenoids, thereby aligning with our quantitative photochemical study. The presence of rich metabolites, especially terpenes, phenols, and flavonoids, has a synergistic effect on plant activity (Mehta & Shah, 2021; Gwari et al., 2013; Idriss et al., 2023; Omer et al., 2019). This study should be further validated using animal models and in silico analyses to substantiate *S. costus* as a useful medicinal herb and its utility in the modern cosmetic & pharmaceutical industries.

5. CONCLUSION

This study demonstrated that *S. costus* root extracts exhibit notable phytochemical and antimicrobial activities, with varying results across four solvents. The *S. costus* species methanol extracts from root samples have shown potent activity against various microbial strains, with rich phenolic and flavonoid content and free radical scavenging activity, which are attributed to the presence of potent secondary compounds identified by GC-MS analysis. Strong antibacterial activity against *S. aureus* and *E. coli*

strains was observed, as was antifungal activity against C. albicans across different doses. Nonetheless, we found that *S. costus* extracts worked well against both Gram-positive and Gram-negative bacterial strains. supporting their classification as the best bactericidal and fungicide agent, thus proving them to be the best antimicrobial agents. This study can be a base for future pharmaceutical and biotechnological studies to validate its true herbal medicinal nature by isolating active compounds and *in-vivo* study validation using animal models.

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Authors' contributions: SAS: completed all of the experiments and document writing; **FA**: analyzed data and drafted some parts of the manuscript; **AG**: finalized, conceptualized, and administered the overall work; **CM**: supervised, formatted, and finalized the manuscript through contributions from further authors. I would like to thank **Mr. Fayeem Aadil** for his selfless help with interpreting my data and drafting parts of the manuscript.

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