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Amrit Research Journal December 2025, Vol. 6, Issue 1, 15-25
ISSN: 2738-9553
RESEARCH ARTICLE

Unveiling the Bioactive Potential: Phytochemical Profiling and Biological Activities of *Syzygium nervosum* Bark

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(Received: September 21, 2025, Received in revised form: November 27, 2025, Accepted: December 8, 2025, Available online: December 19, 2025)

DOI: https://doi.org/10.3126/arj.v6i1.87503

Highlights

- Dichloromethane extract had the highest phenolic content
- Hexane extract showed the most flavonoids
- Ethyl acetate extract was found to exhibit the highest antioxidant and antibacterial activity
- *Syzygium nervosum* bark shows strong therapeutic potential

Abstract

Since ancient times, plant-based remedies have played a vital role in managing health complications. This study investigates the phytochemical composition, antioxidant, and antibacterial potential of diverse solvent extracts of *Syzygium nervosum* collected from the Kaski district of Nepal. Among the tested fractions, the dichloromethane extract showed the highest phenolics (81.15 ± 0.02 mg GAE/g), followed by hexane (78.79 ± 0.10 mg GAE/g), ethyl acetate (72.00 ± 0.08 mg GAE/g), and aqueous fractions (19.25 ± 0.02 mg GAE/g). In contrast, the hexane extract showed the highest flavonoid content (233.54 ± 1.00 mg QE/g), followed by dichloromethane (219.54 ± 0.60 mg QE/g), ethyl acetate (156.94 ± 0.53 mg QE/g), and methanol (141.94 ± 0.92 mg QE/g). Antibacterial screening by agar well diffusion revealed that the ethyl acetate fraction showed moderate activity against *Escherichia coli* (14.0 mm), *Staphylococcus aureus* (13.0 mm), and *Pseudomonas aeruginosa* (9.0 mm). Antioxidant activity, determined by DPPH assay, was highest for ethyl acetate (IC_{50} = 27.92 µg/mL), followed by dichloromethane, hexane, methanol, and aqueous fractions. The aqueous extract exhibited the weakest activity. Overall, *Syzygium nervosum* demonstrates promising bioactive potential, supporting its future application in developing therapeutic agents against oxidative stress and microbial infections.

Keywords: *Syzygium nervosum, total phenolic content, DPPH, agar well diffusion, total flavonoid content*

Introduction

A large number of pharmaceuticals used for the management of several health complications originate from plants, and nearly 3/4th of the global population relies on treatments derived from plant extracts (Tayab et al., 2021). Many individuals in developing countries still depend on traditional medicine for primary healthcare due to their low cost, easy access, and cultural compatibility (Maroyi, 2013). Different parts of medicinal herbs, such as seeds, roots, leaves, fruits, bark, flowers, or even

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the entire plant, contain active secondary metabolites. This potent class of secondary metabolites,such as alkaloids, tannins, polyphenols, steroids, flavonoids, saponins, etc.,possesses unique therapeutic assets and is incorporated in the design of natural drugs (Jamshidi-kia et al., 2018; Joshi & Prabhakar, 2020). The widespread commercial availability of herbal health products in drug stores, supermarkets, food stocks, and health workshops reflects the upsurge of consumer choice of natural products over Western drugs (Wyk & Prinsloo, 2020).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are sets of highly sensitive chemicals formed by the incomplete reduction of oxygen and nitrogen during cellular metabolism. They act as a two-edged sword in different cellular processes of the living organisms (Liu et al., 2021). Redox homeostasis normally balances the concentration of free radicals, which is essential for normal cellular functions, including transcriptional activation and signal transduction pathways. On the other hand, excess accumulation causes oxidative stress, which damages cell functions and contributes to diseases like cancer, diabetes, and neurodegenerative disorders (Dwivedi et al., 2022). Both enzymatic antioxidants (catalase, superoxide dismutase, and peroxidase) and non-enzymatic compounds (ascorbic acid, tocopherol, glutathion, and biothiols) function as ROS scavengers and prevent the reactions of free radicals with cell membrane, proteins, carbohydrates, nucleic acids, and lipids (Jin & Kang, 2024; Sies & Cadenas, 1985). The upsurge of multidrug-resistant (MDR) bacteria poses a severe challenge to worldwide community health by reducing the efficacy of antibiotics, increasing disease severity, and impacting the economy. If antimicrobial resistance (AMR) continues to escalate at its current rate, it could lead to the deaths of about 10 million people each year by 2050. Therefore, it is essential for contemporary scholars and researchers to urgently explore potent antibacterial agents to combat infections (Shresta et al., 2021).

Biosynthesis of secondary metabolites in plants is induced by various biotic and abiotic stresses, including environmental situations, edaphic features, seasonal variations, and pathogen/herbivore invasions (Sampaio et al., 2016). The chemical assembly and pharmacological actions of secondary metabolites show significant specificity, being greatly influenced by multiple biotic and abiotic issues, including the plant organ, harvesting season, state of maturity, and soil micronutrient profiles (Muscolo et al., 2024).Nepal is a geographically diverse Himalayan country with various climatic regions that hosts over 5,500 flowering plant species. It has ample sociocultural diversity that has fostered widespread traditional use of different flora for medicinal purposes (Shrestha et al., 2022). Contemporary ethnobotanical research in Nepal has extended beyond the documentation to underscore conservation strategies, sustainable management practices, and analysis of cultural heritage ethics (Khakurel et al., 2022).

Syzygium nervosum (syn. Cleistocalyx operculatus) belongs to the Myrtaceae family that contains nearly 1,200-1,800 species all over the world. It is indigenous to tropical and subtropical regions extending across China, India, Nepal, and other Southeast Asian countries, Australia, Africa, Europe, and Latin America (Araújo et al., 2024; Nigam et al., 2012). Thisis a moderately sized deciduous tree growing up to the heights of 15 meters, distinguished by its brownish-black bark. The plant bears elliptical leaves measuring 5-10 cm in width and 8-20 cm in length, featuring visible secondary venation. Its inflorescences bear pale green flowers that develop from small oval buds into bell-shaped blossoms, each comprising a grey calyx and violet corolla. These aromatic flowers subsequently produce spherical black fruits. In Nepal, it is known as Kyamuna and recognized as Jambolan, black olive, and Malay apple in other regions (Uddin et al., 2022). In Chinese traditional medicine, leaves and barks have been used for skin ulcers and scabies. The leaves and root extractions are used in the treatment of pimples, inflammation of the breast, influenza, dysentery, and constipation (Wang et al., 2016). In the Vietnamese traditional medicine, the leaves and floral buds have been traditionally prepared as a medicinal infusion for the treatment of multiple gastrointestinal and respiratory complaints, including gastritis, febrile illnesses, jaundice, dysentery, and diarrhoea. Many studieshave reportedthat the plant exhibits significant antioxidant, antibacterial, anti-inflammatory, and antiosteoclastogenic effects (Thanh et al., 2024). Malla et al. (2014) documented the traditional use of bark extract from this species for treating muscular swelling in cattle. The study reported ethnomedicinal applications of leaf extract for pharyngeal disorders. Ripe fruits are eaten by the local people of the Parbat district of Nepal.

Despite its rich ethnomedicinal history, the bioactive potential of *Syzygium nervosum* bark remains underexplored in Nepal. This study, therefore, seeks to unlock its therapeutic promise by investigating the phytochemical composition and evaluating the antioxidant and antibacterial activities of the methanol extract and its fractions collected from Kaski, Nepal.

Materials and Methods

Chemicals and microbial strains

Usual solvents like dichloromethane, n-hexane, methanol, ethyl acetate, and FCR were procured from Thermo Fisher Scientific India Pvt. Ltd., gallic acid from Central Drug House, Pvt. Ltd., India, Dimethyl Sulphoxide (DMSO) from Merck, and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), Mueller-Hinton Agar (MHA), Mueller-Hinton Broth (MHB), and Quercetin from HiMedia were purchased. Bacteria Strains used in this study: *Staphylococcus aureus* (ATCC 25235)*, Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 25922). All samples were obtained from the Department of Microbiology, Prithvi Narayan Campus, Pokhara.

Experimental

Sample collection

Mature barks of *S. nervosum* were taken out from the community forest zone of Pokhara Metropolitan-32, Kaski, Sundaridanda, Nepal in December 2024. The plant sample was collected without hindering the natural ecosystem and biodiversity. For Botanical authentication, we submitted a herbarium specimen to Dr. Hom Nath Pathak, Assistant Professor of Botany at Prithvi Narayan Campus, Pokhara.

Extraction and fractionation

The plant bark was carefully washed with sterile distilled water. The collected plant material was then sliced into minor pieces and shade-dried for four weeks. The dried bark was pulverized in a mechanical grinder into fine powder and stored in a plastic zip-lock bag. The extract was set using 80% methanol in a Soxhlet apparatus. Specifically, 150 g of powdered material was mixed with 500 mL of solvent and extracted for several cycles at 45 °C until a clear solution circulated in the apparatus (Khade et al., 2023). The volume of the extract was reduced by evaporating in a water bath at 45 ºC and stored in a refrigerator at 4ºC until use (Ahmed et al., 2014). The extraction yield was calculated by using the formula:

$$\text{Yield (\%)} = \frac{\text{Weight of extract (g)}}{\text{Weight of plant powder (g)}} \text{ X } 100$$

Phytochemical screening

The methanolic extract and all solvent fractions of *Syzygium nervosum* bark were subjected to preliminary phytochemical screening to identify various chemical groups using standard testing methods (Aiyegoro & Okoh, 2010; Kaur et al., 2016). Examinations were performed for alkaloids, carbohydrates, glycosides, polyphenols, flavonoids, tannins, reducing sugars, phytosterols, carboxylic acid coumarins, saponins, quinine, and terpenoids.

Estimation of total phenolic content and total flavonoid content

The Folin-Ciocalteu method was used to determine the total phenolic content (TPC) of the plant extract and its various fractions (Karki et al., 2024). A gallic acid (GA) standard curve was plotted, and the TPC was quantified as mg GAE/gm by regression analysis. Briefly, 1 mL of the GA solutions of different concentrations and the test samples (5 mg/mL) were separately mixed with 5 mL of FCR and 4 mL of 7% Na_2CO_3. The mixture was shaken well and placed in the dark for 30 minutes for incubation. The absorbance was noted at 765 nm using a UV-Vis spectrophotometer against a blank. Similarly, total flavonoid content (TFC) was assessed by the $AlCl_3$ colorimetric method (Kalauni et al., 2023; Pawar & Dasgupta, 2018). A quercetin standard curve was constructed, TFC was assessed by the regression analysis, and articulated in mg QE/g. A stock solution of quercetin was set in DW (1mg/mL) and diluted to various concentrations. All of the solutions (1 mL) were mixed with 0.3 mL of 10% $AlCl_3$, 0.3 mL of 5% $NaNO_2$, and 2 mL of 5% NaOH was added, and after 6 minutes total volume was made 10 mL with DW. The same composition was set for each of the fractions and the extract. The final solutions were placed in the dark for 30 minutes, and the absorbance was recorded against a blank at 415 nm.

In-vitro antioxidant activity

Antioxidant capacity of ME and its fractions was evaluated using DPPH method with ascorbic acid as a standard (Ainsworth & Gillespie, 2007; Huyut et al., 2017). It involved the following procedure. Each of the test samples/ascorbic acid were dissolved

in DMSO to prepare solutions of different concentrations (500, 200, 100, 50, 25, and 12.5 µg/mL) in separate test tubes and labelled properly. For each sample, 2 mL of the solution was mixed with 2 mL of 1 mM DPPH and placed in the dark for 30 minutes. Subsequently, the absorbance was measured at 517 nm using a spectrophotometer. The DPPH radical scavenging activity (%) at varying concentrations was determined by means of the following formula:

$$\text{Radical Scavenging Capacity (\%)} = \left[\frac{(\text{Ab}-\text{As})}{\text{Ab}}\right] \times 100$$

Where,

A_b = Absorbance of control, A_s = Absorbance of sample

Finally, the concentration stopping 50% of the solution (IC_{50}) was calculated by the linear regression analysis.

Antibacterial activity

The agar-well diffusion method was used for testing antibiotic susceptibility by following the guidelines of National Committee for Clinical Laboratory Standards (NCCLS) (Atef et al., 2019; Murray et al., 2007). Mueller Hinton Agar plates were used to analyse antibacterial activity. Pure colonies of each bacterium were grown in sterile saline until a turbidity adjusted to McFarland's standard 0.5 (1.5 x 10^8 CFU/mL). Then the inoculum was spread uniformly to each plate using a sterilised cotton swab. Wells of 6 mm in diameter were created in the agar medium with a disinfected borer for the loading of test samples. Test samples of 5 mg/mL aliquots were loaded to individually well. There was a control sample for every bacterial strain in which the extract was replaced with pure solvents. The plates were labelled, parafilm sealed, and put in an incubator at 37°C for 24 hours. On the next day, each plate was taken out and the Zone of Inhibition (ZOI) were measured by using a ruler.

Results and Discussion

Extraction yield

The actual extraction of bioactive compounds is affected by the affinity of the solvent to the phytochemicals. Water, ethanol, and methanol are better solvents for extracting polar compounds like phenolics and flavonoids (Olabanji et al., 2023). Because of the chemical variety of the phytochemicals in the plants, solvents like acetone, methanol, hydromethanol, hydroethanol, chloroform, hexane, etc., are also used by scholars. In this study, we used 80% methanol and extracted using a Soxhlet apparatus, and the extraction yields are shown in **Table 1**. Methanol gave an extraction yield of 11.56% and the extract was fractionated. Most of the polar compounds, like sugars, amino acids, and some phenols, are extracted with water or ethanol, ethyl acetate and semi-polar compounds, like lipids and terpenes, are extracted with hexane, DCM. Hexane and DCM fractions are found in a little bit higher proportions than EF indicates, with the higher proportions of semi-polar substances in the extract than highly polar secondary metabolites. It might be attributed to the efficiency of separation as well (Abubakar & Haque, 2020)bioactive fractions, or compounds obtained from medicinal plants are used for different purposes, the techniques involved in producing them are generally the same irrespective of the intended biological testing. The major stages included in acquiring quality bioactive molecule are the selection of an appropriate solvent, extraction methods, phytochemical screening procedures, fractionation methods, and identification techniques. The nitty-gritty of these methods and the exact road map followed solely depends on the research design. Solvents commonly used in extraction of medicinal plants are polar solvent (e.g., water, alcohols).

Table 1. Extraction yield of methanol and its fraction *of S. nervosum*.

Solvent extract/fractions	Yield (%)
Methanol (ME)	11.56
Hexane fraction (HF)	8.08
DCM fraction (DF)	8.23
Ethyl acetate fraction (EF)	3.62
Residue (RF)	11.06

Phytochemical screening *of S. nervosum*

Phytochemical screening of the crude extract and its different solvent fractions displayed the occurrence of numerous bioactive composites, which are revealed in **Table 2**. The results showed the presence of phenolics, Carbohydrates, quinones, and tannins in all of the samples. Alkaloids are found in the ME only. These compounds exhibit significant therapeutic activities like anti-diabetic, antimicrobial, anti-cancer, antidepressant, etc. (Khade et al., 2023).

Table 2. Outcomes of phytochemical screening of *S. nervosum* extracts.

Phytochemical/test method	ME	HF	DF	EF	RF
Alkaloids (Hager's test, Wagner's test, Meyer's test)	+				
Carbohydrates (Molisch's test)	+	+	+	+	+
Reducing sugars (Fehling's test)	+	+	+	+	+
Flavonoids (Ferric chloride test, lead acetate test)	+	-	+	+	+
Phenolic compounds (Iodine test, ferric chloride test)	+	+	+	+	+
Tannins (Braymer's test, NaOH test)	+	+	+	+	+
Saponins (Froth test)	+	+	+	+	-
Phytosterols (Salkowski's test:)	-	-	-	-	-
Triterpenoids (Salkowski's test:)	-	-	-	-	-
Quinones (Alc. KOH test)	+	+	+	+	+
Terpenoids ($CHCl_3$ test)	-	+	+	+	-
Coumarins (NaOH test)	-	+	-	-	+

Note: (+) indicates presence and (-) indicates absence.

Total phenolic and total flavonoid content

Phenolics, a significant class of secondary metabolites, serve as antioxidants by counteracting free radicals. These phenolic compounds are categorised by redox properties, permitting them to serve as potential antioxidants. Therefore, it is vital to quantify the phenolic content in plant extracts that display substantial biological properties. The TPC of methanol extract and its fractions were intended by plotting a calibration curve of absorbance versus concentration. In this experiment, we determined TPC by using the calibration curve ($y = 0.0073x + 0.0392$) as shown in **Figure 1a**.

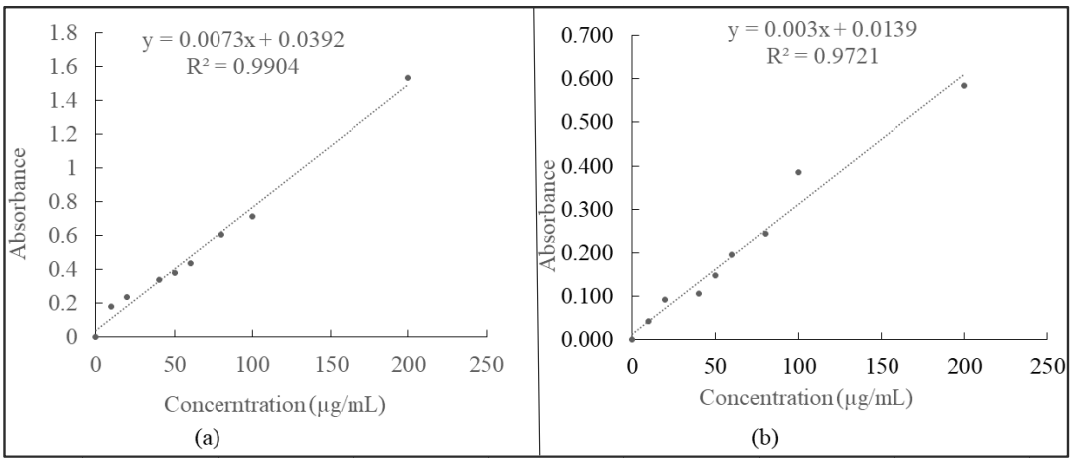

Fig. 1. (a) Gallic acid calibration curve (b) Quercetin calibration curve.

The experiment presented the uppermost TPC of 81.15 ± 0.02 mg GAE/g in the DCM fraction,next HF 78.79 ± 0.00,EF 72.00 ± 0.08, RF 19.25 ± 0.02 mg GAE/g. The results indicate that most of the phenolics are extracted with DCM and ethyl acetate. The Soxhlet method with DCM as the solvent is more effective for extracting lipid-class compounds as compared to other solvents (Teixeira et al., 2022).The total flavonoid contents (TFC) in various solvent extracts of S. nervosum bark were quantified as quercetin equivalents (mg QE/g) using the standard curve (y=0.003x+0.0139) as shown in Figure 1b. The maximum flavonoid content was detected in the hexane extract (233.54 ± 1.00 mg QE/g), afterward the DCM extract (219.54 ± 0.60 mg QE/g), ethyl acetate extract (156.94 ± 0.53 mg QE/g), and methanol extract (141.94 ± 0.92 mg QE/g). The residue was found to have the lowest flavonoid content of 60.54 ± 0.40 mg QE/g. The ethanol leaf extract of the plant of Vietnamese origin was reported to have a total phenol and flavonoid content of67.55 ± 2.28 mg/g and 93.78 ± 6.04 mg/g, respectively (Lan & Tran, 2024).

Table 3. Results of antioxidant capacity, total flavonoid content, and Total phenolic content of *C. nervosum.*

Test samples	TPC (mg GAE/g)	TFC (mg QE/g)	IC_{50} (Antioxidant)
ME	46.83 ± 0.06	141.94 ± 0.92	37.91 ± 0.40
HF	78.79 ± 0.00	233.54 ± 1.00	33.11 ± 0.32
DF	81.15 ± 0.02	219.54 ± 0.60	30.02 ± 0.00
EF	72.00 ± 0.08	156.94 ± 0.53	27.92 ± 0.00
RF	19.25 ± 0.02	60.54 ± 0.400	38.24 ± 0.11
Ascorbic acid	-	-	23.73 ± 0.05

Note: Values are the Mean ± SD, n = 3.

In-vitro antioxidant activity

The antioxidant capacity was assessed by assessing the DPPH free radical scavenging capacity of the extract, taking ascorbic acid as a control. DPPH is dark coloured crystalline compound, which is a chemically steady free radical is extensively used to assess the capability of the samples to function as free-radical scavengers and hydrogen donors. This method is a fast, straightforward, and cost-effective approach for evaluating antioxidant activity (Baliyan et al., 2022). The sample concentration shows a linear relationship with decolourization intensity, which is quantified based on the electron-scavenging ability of the test samples. The dose-dependentdisparity of (%) scavenging ability of DPPH free radical with concentration (µg/mL) of all the tested samples is shown in Figure 2. The sigmoid-shaped curves indicate the highest effect of ascorbic acid and comparable effects of other samples. Among the tested samples, EF showed the maximum antioxidant capacity with an IC_{50} value of 27.92 ± 0.00 µg/mL, followed by DF, HF, ME, and RF with the IC_{50} values of 30.02 ± 0.00, 33.11 ± 0.32, 37.91 ± 0.40 µg/mL, respectively. The RF was found to exhibit the lowest antioxidant capacity with the highest IC_{50} value of 38.24 ± 0.11µg/mL **(Table 3)**. Evaluation of antioxidant capacity of methanol leaf extract of S. nervosum by DPPH method exhibited the strongest activity with an IC50 =11.76 µg/mL, followed by ethyl alcohol extract (IC_{50} =16.95 µg/mL), and the lowestcapacity was shown by the ethyl acetate extract, which is comparable to our study (T. H. D. Nguyen, 2023). Similarly, the free radical scavenging capacity of the ethanol leaf extract of the plant exhibited noteworthy antioxidant activity with an IC_{50} = 14.50 ± 0.71 µg/mL (Lan & Tran, 2024).The

stronger biological activity observed in the more polar fractions suggests that polar solvents successfully extract the active polar compounds. Specifically, the ethyl acetate and dichloromethane fractions, which were richer in phenolics and flavonoids, demonstrated the greatest antioxidant and antibacterial effects. Similar results were reported by Tourabi et al. (2023), where the hydroethanolic extract exhibited the highest biological activity because of the excess concentration of active compounds.

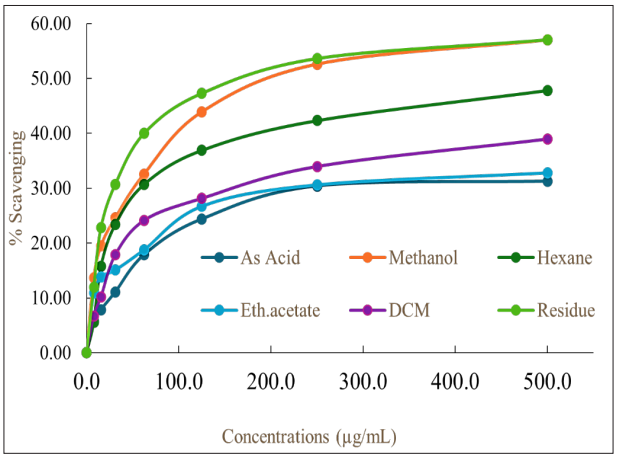

Fig. 2. Variation of concentration with % scavenging capacity of *S. nervosum.*

Antibacterial activity

The antibacterial capacity was assessed by the agar well diffusion technique. The antibacterial properties of various solvent extracts of *S. nervosum* were tested against three bacterial strains: *Pseudomonas aeruginosa, Escherichia coli*, *and Staphylococcus aureus,* and the results are shown in **Figure 3**. The extracts confirmed prominent antibacterial action, and the zones of inhibition (ZOI) for each bacterium were measured to assess the effectiveness of the extracts. The EF showed higher antibacterial activity against *E. coli, P. aeruginosa*, and *S. aureus* with zone of inhibition 14.0 mm, 9.0 mm, and 13.0 mm, respectively. The ME was neutral to against *S. aureus*. The ME and DF showed weak activity against *E. coli* and *P. aeruginosa* with a zone of inhibition (7.0 mm). The antibacterial activity of DF against *E.coli* was lower with zone of inhibition (7.0 mm). The residue does not have antibacterial activity against three bacterial strains.

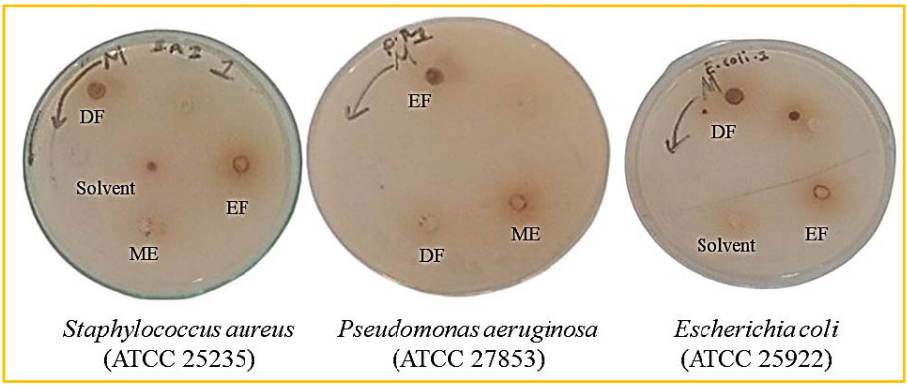

Fig. 3. Antibacterial test slides of different fractions of *S. nervosum.*

In a study carried out by P. T. M. Nguyen et al. (2017) on *C. operculatus* extract revealed significant antibacterial action was revealed against Gram-positive *S. aureus, Bacillus subtilis*, and *Streptococcus mutans* GS-5 and three multi-resistant bacteria, *Staphylococcus*, *Staphylococcus epidermis* 847, and *S. haemolyticus* 535, a North German epidermis strain..

Table 4. List of similar studies and findings to the current research.

Author / Year	Plant Part Studied	Extraction / Methods Used	Key Phytochemicals Reported	Reported Biological Activities	Remarks / Gaps Identified
Pham et al., 2020	Leaves, seeds, bark (review)	Review of ethnomedicinal and phytochemical studies	Phenolics, flavonoids, terpenoids, sterols	Antioxidant, antimicrobial, anti-inflammatory, cytotoxic	Comprehensive review; minimal bark-specific profiling available
(Uddin et al., 2022)	Syzygium spp. (includes *S. nervosum*)	Systematic review	Flavonoids, terpenoids, phenolic acids	Antidiabetic, antimicrobial, anticancer (across species)	Highlights the need for isolating active compounds from bark
(Dung et al., 2008)	Flower buds (the most studied organ)	GC-MS, ethanol extraction	Essential oils (eugenol derivatives)	Antioxidant, anti-inflammatory	Not directly bark-related; useful for understanding species chemistry
(Lan & Tran, 2024)	Leaves	Methanol/ethyl acetate fractionation,	Flavonoids phenolics	Antidiabetic, anti-inflammatory, ant anti-cholesterol	No bark analysis; shows strong flavonoid presence in the species
(Narkprasom et al., 2015)	Seeds	Microwave-assisted Solvent extraction	Phenolic acids, flavanones	Estimation of phenols and antioxidant activity	Extraction method improves the Total Phenolic compounds from Making Seeds
(Yahaya et al., 2025)	*S. samarangense*	Root bark extract	flavonoids, terpenoids, phenolics	Antibacterial, Spectroscopic characterization	Study includes multiple Syzygium species; limited *S. nervosum* focus
Current study	Stem bark	Methanol extract and fractions	Phenolics, flavonoids, alkaloids	antioxidant, antibacterial	Study suggests the potential for bark

The essential oil isolated by hydrodistillation of the flower buds of the plant was found to comprise a total of 55 compounds on GC-MS analysis. The oil showed significant antibacterial susceptibility against different bacterial strains, including multiantibiotic-resistant bacteria (MARB), methicillin-resistant *S. aureus* (MERSA), and vancomycin-resistant *Enterococci* (VRE), with their minimum inhibitory concentration (MIC) values extending from 1-20 µL/mL. Similarly, the ethanol extract also showed significant MIC and minimum bactericidal activity (MBC) values reaching from 0.25 -32 milligrams/mL against different Gram-positive and Gram-negative bacteria (Dung et al., 2008). The dried flower bud of the plant was used to investigate the antibacterial activity against different bacteria. The hexane extract showed the strongest growth-inhibitory effect against *Helicobacter pylori* strains ATCC 51932 and ATCC 43504, as well as *Salmonella enterica* serovar *Typhimurium* ATCC 13311, with MIC values of 0.06, 0.13, and 0.4 mg/mL, respectively (Thanh et al., 2024). These results indicate that the plant is a good source of important bioactive secondary metabolites that could be used as a prospective source of natural medicines. The present investigation into the plant's stem bark is consistent with several related studies, some of the relevant of which are summarized in Table 4. Investigating phytochemicals provides an eco-friendly and sustainable route for drug discovery, as plant-derived metabolites are naturally occurring, biodegradable, and require fewer hazardous chemicals during extraction and screening (Newman & Cragg, 2020). Many modern drugs originate from plant secondary metabolites, demonstrating the value of phytochemistry in identifying bioactive, biocompatible lead compounds. This green approach aligns with global efforts to reduce environmental impact while accelerating the development of safer therapeutics (Chen et al., 2016).

Conclusions

The present study highlights the rich phytochemical profile of *S. nervosum* bark, revealing the occurrence of diverse bioactive compounds, including alkaloids, flavonoids, polyphenols, tannins, saponins, and terpenoids. Quantification of total phenolic and flavonoid contents confirmed significant levels of antioxidant constituents, while antibacterial assays demonstrated promising activity, supporting its traditional medicinal use. Importantly, this study confirms that the polarity of the solvent considerably influences the composition and concentration of secondary metabolites, making solvent choice a critical factor for effective extraction. The findings not only establish a robust scientific basis for the therapeutic potential of *S. nervosum* bark but also provide valuable insight for designing greener approaches to isolate, characterize, and evaluate bioactive compounds of herbal origin. Further pharmacological and toxicological studies are necessary to fully elucidate its mechanisms, bioavailability, and clinical relevance, paving the way for natural

Acknowledgements

The authors extend their gratitude to Prithvi Narayan Campus, Tribhuvan University, Pokhara, Nepal, for providing laboratory facilities.

Author Contributions

Lekha Nath Khanal: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing-original draft preparation. **Sujan Neupane:** Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing-original draft preparation. **Purna Prasad Dhakal:** Conceptualization, Data curation, Writing-review and Editing, Resources. **Mani Ram Kandel:** Conceptualization, Data curation, Writing-review and Editing, Resources, Supervision, Project handling

Conflicts of Interest

The authors declare no conflict of interest.

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