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Phytochemical Profiling and Bioactivity Assessment of *Colebrookea oppositifolia* Sm. from the High Altitudes of Nepal's Far Western Region

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Abstract

Traditional methods have made considerable use of herbal therapy to treat a variety of illnesses. This study aimed to quantify the levels of phenolic compounds and flavonoids in extracts made with two distinct solvents and evaluate their antibacterial qualities. Methanolic extract exhibited the highest concentration of bioactive compounds, with a total phenolic content (TPC) of 125.68 ± 2.67 mg gallic acid equivalents (GAE) per gram and a total amount of flavonoids (TFC) of 35.28 ± 3.22 mg quercetin equivalents (QE) per gram. Antibacterial assays revealed that both methanolic and extracts of hexane extracts efficiently inhibited *Shigella sonnei*, producing a zone of inhibition measuring 12 mm. These findings underscore the significant bioactive potential of the plant extracts under investigation, particularly those obtained using methanol and hexane solvents. Such properties render them promising candidates for the development of novel therapeutic agents aimed at treating infectious diseases.

Keywords : *Colebrookea oppositifolia* Sm., TPC, TFC, antibacterial

Introduction

Medicinal plants have been used to treat and manage ailments since ancient times because they contain therapeutic elements that have long been essential to human health. These plants contain bioactive chemicals that are dispersed across several plant organs, which either make them direct therapeutic agents or provide a basis for drug development (Sofowora, 1996). The necessity for more research into these natural resources is highlighted by the broad classification of medicinal plants into two groups: those with scientifically proven therapeutic properties and those believed to be therapeutic based on conventional wisdom but lacking in-depth scientific research (Ojha et al., 2025).

Natural remedies made from microorganisms, plants, and animals have long been utilized in traditional medicine. People have been using plants as medicine for at least 60,000 years, based on fossil evidence. The evolutionary development of chemical diversity in these natural chemicals has developed a wide range of physiologically active molecules with promising therapeutic potential over millions of years (Moura & Albuquerque, 2012). Phytochemicals are bioactive substances that plants manufacture that play a vital role in plant physiology, including growth, pollination, and pathogen defense (Molyneux et al., 2007). Additionally, they aid in shielding plants from external stresses, including UV radiation and infections (King & Young, 1999).

Phytochemicals can be classified into two groups: primary metabolites and secondary metabolites. Proteins, carbohydrates, and nucleic acids are examples of primary metabolites that are essential to plants' essential metabolic functions (Leitzmann, 2016). Secondary metabolites, on the other hand, like terpenoids, flavonoids, and phenolics, are produced via specific pathways and are frequently associated with antiviral, antifungal, and antibacterial properties (Bourgaud et al., 2001). The potential of these secondary chemicals to counteract oxidative stress, a factor linked to the development of degenerative disorders such including stomach ulcers, atherosclerosis, and cancer, makes them highly interesting for pharmaceutical investigation (Mwamatope et al., 2020).

Despite its widespread use, relatively little research has been done on the phytochemical constitution and biological features of *Colebrookea oppositifolia* Sm's root section. This study will evaluate the biological activities of the root section and investigate the phytochemical content to fill this study gap and broaden our understanding of the possible medical benefits of *Colebrookea oppositifolia* Sm.

Description of *Colebrookea oppositifolia* Sm.

Common names for *C. oppositifolia* Sm. include Bhaman, Dhursul, Binmeuli, and Indian squirrel tail. It is primarily found in India, Nepal, and Bhutan. As members of the Lamiaceae family, the fragrant plants are used as a traditional medicine. It has a white

tomentose stem with several branches and elliptic-lanceolate leaves that are ternately whorled. The plant has four stamens, a small corolla tube, a hairy calyx, and white panicled spikes that resemble inflorescences. Typically, the plant can be found up to 1500 meters above sea level, and the ovary is bilocular (having two locules). It is renowned for having no smell and a bitter taste (Ishtiaq et al., 2020). The current study was primarily concerned with the collection, identification, and biological and phytochemical activities of the plant extracts.

Materials and Methods

Chemicals

For this study, Merck and Fischer Scientific supplied two high-purity, analytical-grade solvents, methanol and hexane, to guarantee dependable and efficient extraction. HiMedia and LOBA CHEMI Pvt. Ltd. provided the necessary reagents, such as Folin-Ciocalteu (FC) reagent, as well as microbiological culture media, such as Nutrient Agar, Mueller-Hinton Agar, and Mueller-Hinton Broth. Accuracy and reliability in the microbiological assays and phytochemical analyses were ensured by the use of quality solvents, reagents, and culture media.

Plant Collection and Identification

From the Far Western region of Nepal, the root section of *C. oppositifolia* Sm. was gathered (Sayal Gaupalika-02, Doti). The specimen was authenticated by the Central Department of Botany, Tribhuvan University. For aesthetic purposes, the plant is shown in Figure 1.

Figure 1

Colebrookea oppositifolia Sm.

Extract Preparation

After being properly cleaned and shade-dried to preserve its phytoconstituents, the plant material was ground into a fine powder in a grinding mill in order to ensure

a consistent consistency for use later. 200 mL of methanol and hexane were used to dissolve around 10 grams of *C. oppositifolia* Sm. powder. The mixes underwent three days of maceration, which involved constant shaking every 24 hours. The extracts were filtered following maceration, and the filtrates were then concentrated at a controlled temperature of 40–45 °C using a rotary evaporator (Ellwood et al., 2014).

Qualitative Phytochemical Analysis

Standard procedures were used for qualitative phytochemical screening to determine the different metabolites found in the root of *C. oppositifolia Sm* (Bhardwaj et al., 2024). Phytochemicals such as terpenoids, phenolic compounds, alkaloids, lipids, glycosides, flavonoids, carbohydrates, and saponins were the main focus of the screening. An overview of the bioactive components in the plant extract is obtained by this analysis.

Estimation of Total Phenolic Content (TPC)

According to Lu et al., the Citocalteu-Folin colorimetric technique was used to ascertain the plant extracts' total phenolic content (TPC) (Lu et al., 2011). In duplicate, 80 µL of 1M Na_2CO_3, 100 µL of 10% Folin-Ciocalteu reagent (diluted 1:10), and 20 µL of plant extract were added to 96-well plates. The reaction mixture was incubated for 30 minutes at room temperature until it became blue. The absorbance at 765 nm was then measured using a spectrophotometer. Using a gallic acid standard curve that ranged from 7.5 to 100 µg/mL, TPC was measured in milligrams of gallic acid equivalent per gram of extract dry weight (mg GAE/g).

Estimation of Total Flavonoid Content (TFC)

The total flavonoid content of the plant extracts was ascertained using the aluminum chloride method, as described by Ahmed et al. (Kocak et al., 2017). 96-well plates were filled with 20 µL of plant extract, 100 µL of distilled water, and 60 µL of ethanol in triplicate. Next, 10 µL of a 1M potassium acetate (CHCOOK) solution and 10 µL of a 10% aluminum chloride (AlCl3) solution were added. The reaction mixture was incubated at room temperature for 30 minutes. The absorbance at 415 nm was then measured using a spectrophotometer. The total flavonoid content was measured using a standard calibration curve for standard quercetin, which spans from 7.5 to 100 µg/mL. The results were reported as milligrams of quercetin equivalent per gram of extract dry weight (mg QE/g).

2Evaluation of Antimicrobial Activity

Using the agar well diffusion method, antibacterial activity was assessed on Mueller-Hinton Agar (MHA) plates (Jaishi et al., 2024). *Escherichia coli* (ATCC 25312), *Staphylococcus aureus* (ATCC 43300), and *Shigella sonnei* (ATCC 25931) were among the test microorganisms that were cultivated in Mueller-Hinton Broth (MHB) and

incubated for 24 hours at 37 °C. The broth was turbidized to a 0.5 McFarland standard to maintain a constant bacterial density. The plant extract (50 µL) was applied to the agar plates after wells were made in them using a cork borer. 50% DMSO was added to the negative control wells, while 50% neomycin was added to the positive control wells. For 18 to 24 hours, the Petri dishes were incubated at 37 °C following 15 minutes of diffusion. The antibacterial effectiveness of the plant extract was assessed by measuring and observing the zones of clearance following incubation.

Statistical Analysis

Microsoft Excel was used to collect and analyze data from the Gen5 Mi-Microplate Reader. Total flavonoid content (TFC) and total phenolic content (TPC) results were displayed as mean ± standard deviation. The data gathered from the studies were thoroughly evaluated thanks to this analytical method.

Results

Qualitative Phytochemical Analysis

Table 1 shows the findings of a qualitative phytochemical screening of the *C. oppositifolia* Sm. extract in two different solvents.

Table 1

Plant extract screening for qualitative phytochemicals

Phytochemicals	Test types	Results
Alkaloids	Dragendorff's test	+
Carbohydrates	Molisch's test	+
Reducing sugars	Fehling test	+
Glycosides	Borntrager's test	+
Amino acids	Xanthoproteic test	+
Flavonoids	Alkaline reagent test	+
Phenols	$FeCl_3$ test	+
Tannins	Braymer's test	+
Terpenoids	Salkowski's test	+

Note. (+) = present, (-) = absent

Total Phenolic Content (TPC)

Table 2 displays the two solvent extracts' phenolic content. of *C. oppositifolia* Sm. The table below shows that the total phenolic content (TPC) value of the methanolic extract was greater at 125.68 ± 2.67 mg GAE/g, whereas the TPC value of the hexane extract was lower at 46.28 ± 3.14 mg GAE/g (Ojha & Sharma, 2025). These findings demonstrate how the amounts of phenolic compounds varied somewhat between the two solvent extracts.

Table 2

TPC of plant extract in methanol and hexane

Plant Extracts	TPC (mg GAE/g)
Methanolic	125.68 ± 2.67
Hexane	46.28 ± 3.14

Total Flavonoid content (TFC)

Table 3 below shows the flavonoid content from root extracts of *C. oppositifolia* Sm. The methanolic extract demonstrated a slightly higher total flavonoid content (TFC) value of

35.28 ± 3.22 mg QE/g, whereas the hexane extract showed a decreased TFC value of 16.26 ± 4.35 mg QE/g. These results show that there is no longer any difference in the concentrations of flavonoids between the two extracts of solvents.

Table 3

TFC of hexane and methanolic extract

Plant Extracts	TFC (mg QE/g)
Methanolic	35.28 ± 3.22
Hexane	16.26 ± 4.35

Antimicrobial Properties

The antibacterial activity of the plant extracts against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Shigella sonnei* and *Escherichia coli*) bacteria was evaluated using the agar well diffusion method. Table 4 presents the antimicrobial test results, and Figure 2 shows the results. Both the methanolic and hexane extracts demonstrated a good 12 mm zone of inhibition against *Shigella sonnei*. These results imply that different extracts have differing degrees of antibacterial activity against the studied microorganisms.

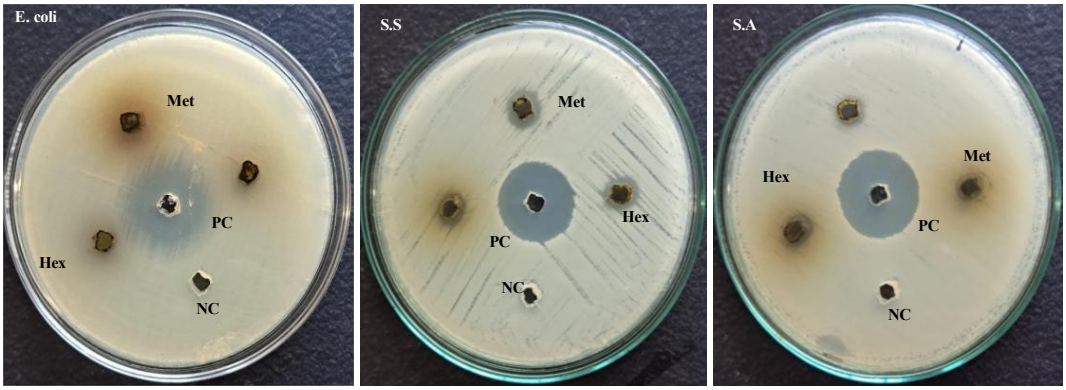
Table 4

Zone of Inhibition (ZOI) of plant extracts against bacteria

Plant Extracts	Bacteria	ZOI of Sample (mm)
Methanolic	*Staphylococcus aureus*	11
	Escherichia coli	7
	Shigella sonnei	12
Hexane	*Staphylococcus aureus*	11
	Escherichia coli	6
	Shigella sonnei	12

Figure 2

Antibacterial test slides against several bacterial strains, with SA standing for Staphylococcus aureus, E. coli for Escherichia coli, and SS for Shigella sonnei.



Discussion

The investigation found that the methanolic extract of *C. oppositifolia* Sm. contains a significantly higher amount of phenolic 125.68 ± 2.67 mg GAE/g and flavonoid 35.28 ± 3.22 mg QE/g content than the hexane extract. The methanolic extract's superior capacity to extract high-molecular-weight phenolic chemicals over hexane may be the cause of its higher phenolic content (Brglez Mojzer et al., 2016). Likewise, as flavonoids are a subgroup of phenolics, the increased amount of flavonoids seen in the methanolic extract might be related to the total quantity of phenolic chemicals (Andersen & Markham, 2005). The collection site is cold, has rich soil, receives a moderate water supply, and gets little sunshine. Numerous studies have shown that these factors affect the biosynthesis of secondary metabolites. Environmental changes can impact the production of some medicinal plant species because ecological conditions and climate have an impact on the concentration of secondary metabolites (SMs) in these plants (Pant et al., 2021). The crude methanolic and hexane extracts also showed substantial antibacterial activity, as indicated by significant zones of inhibition, especially against *Shigella sonnei* and *Staphylococcus aureus*. These outcomes are in line with earlier research on *C. oppositifolia* Sm. methanolic extract, which showed similar antibacterial activity against the same bacterial strains (Ahmed et al., 2009). The presence of secondary metabolites like rutin and flavonols is probably what causes the antibacterial action (Sharma et al., 2021). Furthermore, substances including vitamins, minerals, carotenoids, saponins, and enzymes might possibly be involved in the antibacterial actions that have been noted (Nascimento et al., 2000).

Conclusion

To sum up, *Colebrookea oppositifolia* Sm. exhibits significant therapeutic value

because of its diverse phytochemical makeup, especially its high concentration of flavonoids, phenolics, and other bioactive substances. Total flavonoid content (TFC) and total phenolic content (TPC) were higher in the methanolic extract than in the hexane extract. Effective antibacterial activity against *Shigella sonnei* was demonstrated by both extracts. These results support the potential of *C. oppositifolia* Sm. roots as a drug development candidate by indicating that they have a favorable phytochemical and biological profile.

It is positioned as a promising source for the creation of new therapeutic medicines due to its demonstrated antibacterial capabilities. To clarify the pharmacological mechanisms and increase the plant's therapeutic significance in contemporary medicine, more thorough research is necessary.

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