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Phytochemical and Bioactivity Profile of Selected Medicinal Plants from Chitwan, Nepal

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Abstract

Plants have been primary source of medicines from time immemorial. The herbal resources have inspired the scientific community to regularly develop new drugs to combat the issues of multidrug-resistant (MDR) pathogens and the negative side effects of the present therapeutics. The study aimed to estimate the qualitative and quantitative phytochemical composition, antioxidant potential, and antibacterial activity of Artemisia vulgaris, Boerhavia diffusa, Ocimum sanctum, and Tinospora cordifolia collected from Bharatpur. The plant materials were extracted with methanol and water by cold percolation. Qualitative phytochemical analyses were conducted using standard color differentiation reactions, while quantitative analyses involved absorbance-based measurements. Antioxidant potential was determined by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, while agar-well diffusion method was employed to estimate antibacterial activity against Staphylococcus aureus and Escherichia coli. Methanolic extracts exhibited higher phytochemical content and bioactivity. Among these, O. sanctum showed the highest total phenolic content (115.81 ± 3.58 mg GAE/g) and total flavonoid content (178.95 ± 21.95 mg QE/g), followed by B. diffusa, T. cordifolia, and A. vulgaris. O. sanctum also demonstrated the highest antioxidant potential with the lowest half-maximum inhibition concentration (IC_{50}) value (100.43 ± 0.38 µg/mL), showing a strong correlation of phenolic and flavonoid contents with DPPH radical scavenging ($p<0.05$). While methanolic O. sanctum extract exhibited the highest antioxidant potential, aqueous A. vulgaris demonstrated the most potent antibacterial property against both tested bacterial strains, suggesting different active compounds are responsible for these effects. The study provides valuable insights for developing efficient therapeutics from these plants through compound-level isolation, identification, and characterization.

Keywords Antibacterial activity, Antioxidant activity, Medicinal plants, Total flavonoid content, Total phenolic content.

1. Introduction

Conventional therapeutic systems have utilized plant-derived materials to treat various diseases for centuries. A huge proportion of the global population depends on

traditional medicine for primary healthcare (WHO, 2019). Reports have estimated that approximately 10% of the 7,000 flowering plant species of Nepal possess therapeutic value (Kalauni & Joshi, 2018). Despite being a great reserve of medicinal plants, only a limited number have been explored for their phytochemical and biological activity profiles (Sai et al., 2019). Moreover, the development of multidrug resistance among bacteria is becoming prevalent, rendering the existing drugs ineffective. The adverse side effects of these drugs are another matter of major concern (Ahmed et al., 2024). Nepalese medicinal plants may contain unique bioactive compounds having the potential to be developed into promising options in resolving these problems. This can be expected as they have been used effectively in the traditional systems of medicine for generations.

Artemisia vulgaris, *Boerhavia diffusa*, *Ocimum sanctum*, and *Tinospora cordifolia* represent some of the most commonly used plants in the conventional therapeutic practice of Nepal. *A. vulgaris*, called 'Titepati' in Nepal, is commonly known as 'common mugwort'. It belongs to the family of Asteraceae and possesses versatile therapeutic properties, exerting anti-inflammatory, antidiabetic, antiseptic, antimicrobial, and antidepressant effects. It, thus, has established itself as a valuable asset in traditional medicinal practices (Baruah et al., 2024; Sharma & Adhikari, 2023). It is used against various health problems such as psychoneuroses, insomnia, autonomic disorders, neurosis, restlessness, depression, and irritability in traditional medicinal practices. The phenols and flavonoids present as the major phytoconstituents in its methanolic extracts are responsible for antioxidant and antidiabetic properties (Sharma & Adhikari, 2023).

Boerhavia diffusa, commonly called 'Punarnava', is another medicinal plant employed in conventional practice, especially in 'Ayurveda, for treating jaundice, skin diseases, heart conditions, inflammation, constipation, and spleen enlargement (Sravani et al., 2024). The phytochemicals, including alkaloids, glycosides, terpenoids, flavonoids, saponins, tannins, and steroids in *B. diffusa*, have been reported in a study claiming the hepatoprotective property of its root is due to the presence of flavonoids (Sravani et al., 2024).

Ocimum sanctum, commonly called 'Holy basil', locally known as 'Tulsi', is reported to possess therapeutic potentials as an expectorant, analgesic, anticancer agent, antiasthmatic, antiemetic, diaphoretic, hepatoprotective, and hypotensive in traditional medicinal practices (Rahman et al., 2011). The key phytochemical constituents identified in *Ocimum sanctum* include eugenol, euginal (also known as eugenic acid), ursolic acid, carvacrol, linalool, limatrol, caryophyllene, methyl chavicol, sitosterol, and anthocyanins. These compounds are associated with bioactivity and various therapeutic potentials of the plant (Chetia et al., 2021; Rahman et al., 2011; Vijay et al., 2025).

Tinospora cordifolia, commonly called 'Gurjo', 'Giloe' or 'Guduchi' is another plant of great medicinal importance in traditional medicine (Garg & Garg, 2018; Modi et al., 2021). It is useful in relieving fatigue, dyspepsia, fever, diarrhea, constipation, urinary

diseases, burning pain, and blood accumulation. It is also believed to be beneficial in jaundice, cancer, snake bite, and scorpion sting, and also possesses immunomodulatory, analgesic, and antidiabetic properties (Chaudhary et al., 2024; Singh et al., 2021). The plant is reported to be rich in a variety of phytochemicals, particularly phenolics and flavonoids, including 1-deoxy-inositol and brucine, which are effective against various diseases due to their antioxidant, antidiabetic, anticancer, antidepressant, analgesic, anti-inflammatory, and antitumor properties (Garg & Garg, 2018; A. Mishra et al., 2013; P. K. Mishra et al., 2017).

So, all of these plants are traditionally employed for treating various health ailments. They are also used similarly by the local community in the Bharatpur area of Chitwan district of Nepal. However, scientific validation of the therapeutic potential of these locally grown plants is limited and needs to be established through systematic phytochemical analysis and bioactivity assessment.

The curative properties of plants are primarily believed to be due to their secondary metabolites, particularly phenolic compounds and flavonoids. These bioactive compounds exhibit various therapeutic properties, including antioxidant, antimicrobial, anti-inflammatory, and anticancer behaviors (Kumar & Pandey, 2013). Total phenolic content (TPC) and total flavonoid content (TFC) are, therefore, important indicators of the therapeutic potential of plant extracts. The phytochemicals and hence bioactivity of the plant species depend on the geographical location, the extraction scheme used, and the climatic conditions of harvesting, including the micronutrient profile of the soil (Khalil et al., 2020; Pandey et al., 2017). Therefore, this study aims to scientifically evaluate the phytochemical contents and bioactivities of the selected medicinal plants from the Bharatpur area of Chitwan district of Nepal, to provide scientific justification for their traditional uses. The findings may lay the foundation for future studies involving the separation, isolation, and characterization of the novel bioactive compounds that can be developed as future drug candidates with high efficacy and minimal or zero adverse side effects.

2. Materials and Methods

2.1 Plant Materials Collection and Identification

Plant materials of *Artemisia vulgaris*, *Boerhavia diffusa*, *Ocimum sanctum*, and *Tinospora cordifolia* were collected in March 2021 from Birendra Multiple Campus and surrounding areas in Bharatpur, Chitwan district, Nepal. The collected plant materials were authenticated in the laboratory of Botany, Birendra Multiple Campus, by Botanical expert. Fresh plant materials thereafter were cleaned, washed, and shade-dried at room temperature. Fine powder of the dried plant materials was obtained by grinding in an electric grinder. The powdered materials were stored in air-tight containers for further analysis.

2.2 Preparation of the Extracts

The plant extracts were prepared using the cold percolation method (Khelurkar et al., 2017). 40 g each of the powdered plant materials was mixed with 120 mL of methanol and distilled water in separate conical flasks, continuously shaken for 72 hours at room temperature using a mechanical shaker, followed by filtration through Whatman filter paper No. 1. The filtrates were finally concentrated by evaporation at 45°C using a rotary evaporator and stored at 4°C until further analysis.

2.3 Preliminary Phytochemical Screening

Qualitative phytochemical screening of the extracts was performed using standard color differentiation methods based on Harborne's protocols (Harborne, 1980). The presence of various secondary metabolites, including alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, and phenolic compounds, was revealed through specific color reactions.

2.3 Determination of Total Phenolic Content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent method with gallic acid as the standard (Singleton & Rossi, 1965). The method adopted is a slightly modified form as adopted by Alam & Sharma (Alam & Sharma, 2020). A calibration curve (Figure 1) was obtained as an absorbance versus concentration plot, measuring the absorbance of 12.5, 25, 50, and 100 µg/mL gallic acid solutions. Solutions of 125, 250, 500, and 1000 µg/mL plant extracts were prepared, and their absorbance was measured at 760 nm using a UV-Visible spectrophotometer. For the absorbance measurement, 1 mL of each solution of GA or sample was added to 5 mL of 10% FCR and 4 mL Na_2CO_3 solution, and the resultant blue colored mixture solution was homogenized and warmed at 40 ^{0}C in a water bath for 30 minutes. Then, the absorbance was measured in triplicate at 760 nm against a blank for each solution (Ismail et al., 2020; Pathak & Niraula, 2019). TPC was calculated using the linear regression equation: y = mx + C from the calibration curve using the following formula and expressed as gallic acid-equivalent milligram per gram of dry extract (mg GAE/g).

$$C = \frac{C_1 . 1000}{C_2}$$

Where, C = Total phenolic content in the extract, equal to mg GAE/g

C_1 = Concentration of GA estimated from the calibration curve in mg/mL

C_2 = Concentration of the plant extract in mg/mL

2.5 Determination of Total Flavonoid Content (TFC)

TFC was estimated by the aluminum chloride colorimetric method with quercetin as the standard (Zhishen et al., 1999). A calibration curve (Figure 4) was obtained measuring the absorbance of quercetin (QE) solutions of concentrations: 12.5, 25, 50, and

100 µg/mL at 510 nm and TFC was calculated from the plot using the linear regression (y = mx + C) measuring the absorbance of the plant extract solutions of concentrations: 125, 250, 500, and 1000 µg/mL at 510 nm. For the absorbance measurement, 1 mL of each solution of QE or sample was added to 4 mL of distilled water, into which 0.3 mL 5% $NaNO_2$ was added, then, after 1 minute, 0.3 mL 10% $AlCl_3$, and in 6 minutes, 2mL 1 M NaOH, followed by immediate addition of 2.4 mL of distilled water. TFC was calculated using the following equation and expressed as quercetin-equivalent milligram per gram of dry extract (mg QE/g).

$$C = \frac{C_1 . 1000}{C_2}$$

Where, C = Total flavonoid content (TFC) in the extract, equal to mg QE/g

C_1 = Concentration of QE estimated from the calibration curve in mg/mL

C_2 = Concentration of the plant extract in mg/mL

All measurements were carried out thrice.

2.6 DPPH Radical Scavenging Assay

Antioxidant potential was estimated as the activity to scavenge 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical (Brand-Williams et al., 1995). Varying concentrations (20, 40, 60, 80, and 100 µg/mL) of plant extracts, as well as ascorbic acid (taken as standard antioxidant) were prepared, and 2 mL of each was added to 2 mL 0.2 mM DPPH solution, incubated in the dark for 30 minutes at room temperature followed by the absorbance measurement at 517 nm in a UV-Visible spectrophotometer. DPPH radical scavenging activity was computed as follows:

$$\% \text{ Radical Scavenging Activity} = [(A_0 - A_s)/A_0] \times 100$$

Where A_0 and A_s are the absorbance of DPPH with control (solvent) and with the test sample, respectively.

IC_{50} (concentration to scavenge 50% of DPPH radicals) values were determined from the dose-response curves of % radical scavenging effect vs concentration.

2.7 Antibacterial Activity Assay

Antibacterial activity was assessed using the agar-well disc diffusion method (Murray et al., 2007). *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) were the bacterial strains used in the study. Bacterial cultures were grown on nutrient agar plates, and wells were made using a sterile cork-borer. 50 µL and 100 µL of solution of plant extracts (100mg/mL) in dimethyl sulfoxide (DMSO) were added to the wells. The plates were incubated at 37°C for 24 hours. Antibacterial activity was assessed by measuring the zone of inhibition (ZOI); 30 µg pellet of chloramphenicol being used as positive control while DMSO as negative control.

2.8 Statistical Analysis

All measurements were carried out thrice, and data were recorded as mean ± standard deviation (SD) values. Software such as Microsoft Excel and OriginPro was used in the collection, analysis, and graphical representation of data observed for TPC, TFC, and antioxidant activity. Linear regression analysis was employed to establish calibration curves for TPC and TFC determinations. Pearson's correlation coefficients (R^2) were calculated to assess the linearity of the calibration curves. Statistical significance was considered at $p < 0.05$ and determined using OriginPro.

3 Results and Discussions

The present study revealed the presence of various phytochemicals and bioactivities of the medicinal plants under investigation, and also showed significant variations in the phytochemical content and bioactivities.

3.1 Phytochemical Screening

Qualitative phytochemical screening revealed the presence of various bioactive constituents, including alkaloids, flavonoids, terpenoids, tannins, saponins, glycosides, and phenolic compounds in different plant extracts (Table 1), showing richer secondary metabolites in methanolic extracts compared to aqueous extracts. The results are consistent with the previous studies reporting the higher quantities of the phytochemicals in methanol sufficient enough to produce the detectable color change during the screening tests (Pandey et al., 2017; Sharma & Adhikari, 2023). Methanol extracts secondary metabolites to a greater extent due to its intermediate solubility, facilitating the dissolution of a wider range of secondary metabolites compared to water. The presence of diverse secondary metabolites in the preliminary phytochemical screening supports the traditional uses of these plants and their potential therapeutic applications (Altemimi et al., 2017).

Table 1. Phytochemical screening of methanol and aqueous extract of selected plant materials

S. N.	Phytochemicals	Plant Extracts						
		Methanol Extract				Aqueous Extract		
		AV	BD	OS	TC	AV	BD	TC
1	Alkaloids	+	+	-	-	-	-	-
2	Coumarins	-	+	-	+	-	-	-
3	Flavonoids	+	+	+	+	-	-	-
4	Glycosides	+	+	+	+	+	+	+
5	Polyphenols	+	+	+	-	-	-	-
6	Quinones	-	-	+	-	-	-	-
7	Reducing sugars	+	+	+	+	+	+	+

8	Saponins	-	+	+	-	-	-	-
9	Terpenoids	+	+	+	+	+	+	+
10	Steroids	+	+	+	+	+	+	+
11	Tannins	+	+	+	-	-	-	-
12	Anth. Glycosides	-	-	+	-	+	+	-

AV = *A. vulgaris*, BD = *B. diffusa*, OS = *O. sanctum*, TC = *T. cordifolia*,

3.2 Total Phenolic Content (TPC)

The TPC analysis revealed significant variations among the tested plant species and extraction solvents (Table 2). Methanolic extracts consistently showed higher TPC values compared to their aqueous counterparts. Among the methanolic extracts, *O. sanctum* exhibited the highest TPC (115.81 ± 3.58 mg GAE/g), followed by *B. diffusa* (90.347 ± 8.26 mg GAE/g), *T. cordifolia* (74.95 ± 8.12 mg GAE/g), and *A. vulgaris* (54.14 ± 6.93 mg GAE/g). Figure 2 and Figure 3 show comparative bar diagrams of TPC of methanolic and aqueous extracts of the plant materials, respectively.

Table 2. Total Phenolic Content (TPC) of methanol and aqueous extract of different plant species

S. N.	Plant Species	TPC (mg GAE/g) ± SD	
		Methanol Extract	Aqueous Extract
1	*A. vulgaris*	54.14 ± 6.93	20.046 ± 5.16
2	*B. diffusa*	90.38 ± 8.26	14.72 ± 1.87
3	*O. sanctum*	115.81 ± 3.58	N/A
4	*T. cordifolia*	74.95 ± 8.12	24.61 ± 1.60

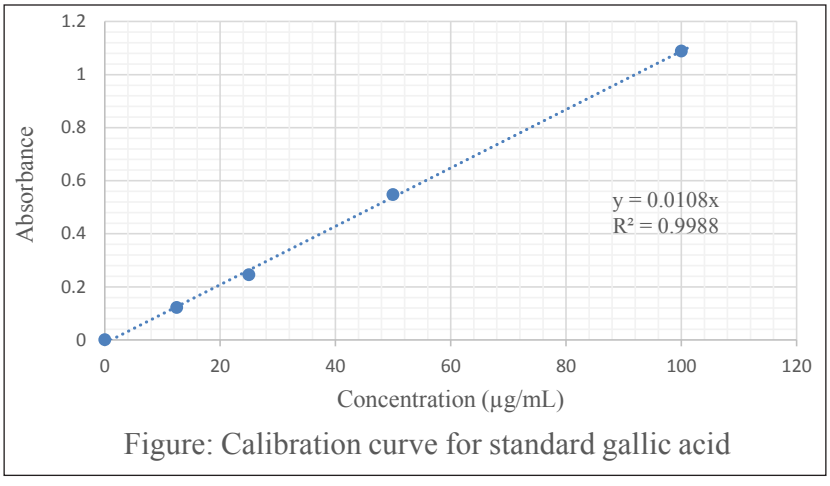

Figure 1. Gallic Acid Calibration curve

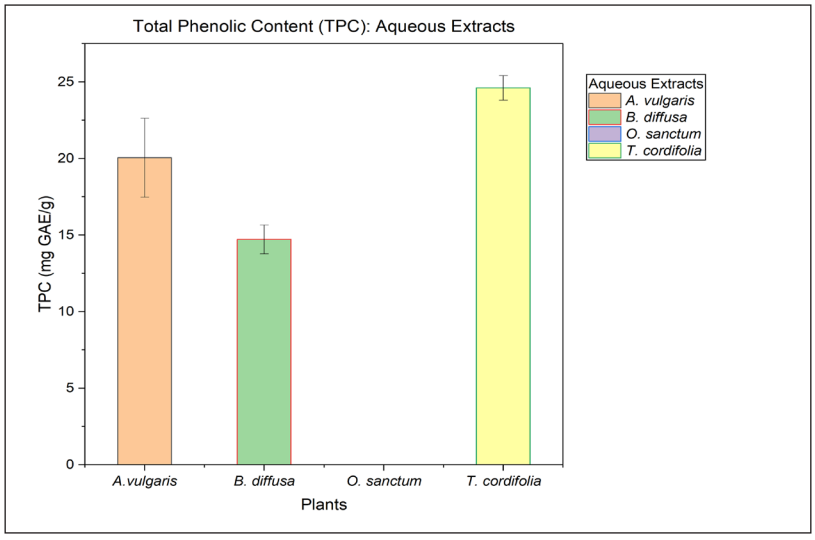

Figure 2. Total Phenolic Content (TPC) of methanolic extracts of different plant species

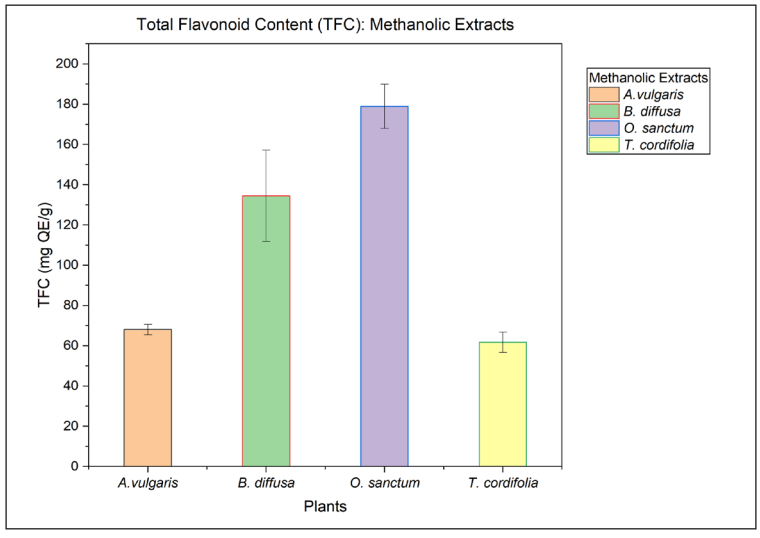

Figure 3. Total Phenolic Content (TPC) of aqueous extracts of different plant species

3.3 Total Flavonoid Content (TFC)

Similar to TPC, methanolic extracts demonstrated higher TFC values than aqueous extracts (Table 3). *O. sanctum* methanolic extract showed the highest TFC (178.947 ± 21.95 mg QE/g), followed by *B. diffusa* (134.474 ± 45.45 mg QE/g), *A. vulgaris* (68.026 ± 5.15 mg QE/g), and *T. cordifolia* (61.711 ± 10.214 mg QE/g). Among aqueous extracts, *B. diffusa* (18.026 mg QE/g) and *T. cordifolia* (17.895 mg QE/g) showed higher values than *A. vulgaris* (8.158 mg QE/g). Figure 5 and Figure 6 show the comparative bar diagrams of TFC of methanolic and aqueous extracts of the plant materials, respectively.

Table 3. Total Flavonoid Content (TFC) of Methanol and Aqueous Extract of Different Plant Species

S. N.	Plant Species	TFC (mg QE/g) ± SD	
		Methanol Extract	Aqueous Extract
1	*A. vulgaris*	68.026 ± 5.15	8.158 ± 0.53
2	*B. diffusa*	134.474 ± 45.45	18.026 ± 1.38
3	*O. sanctum*	178.947 ± 21.95	N/A
4	*T. cordifolia*	61.711 ± 10.214	17.895 ± 2.72

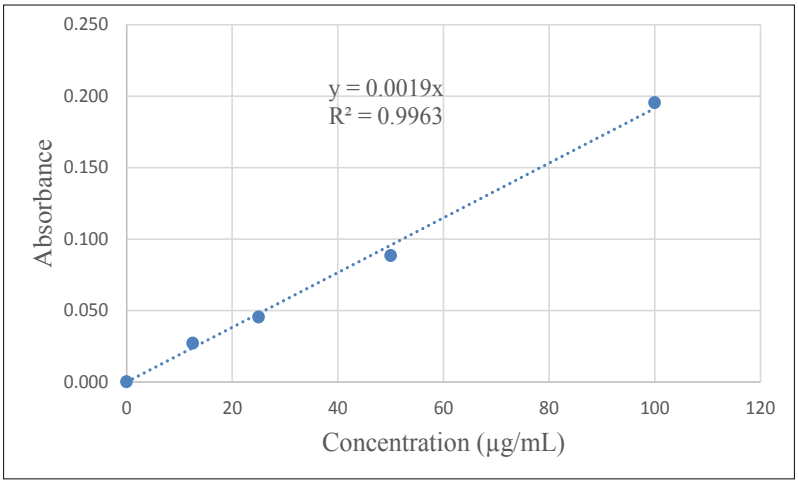

Figure 4. Quercetin calibration curve

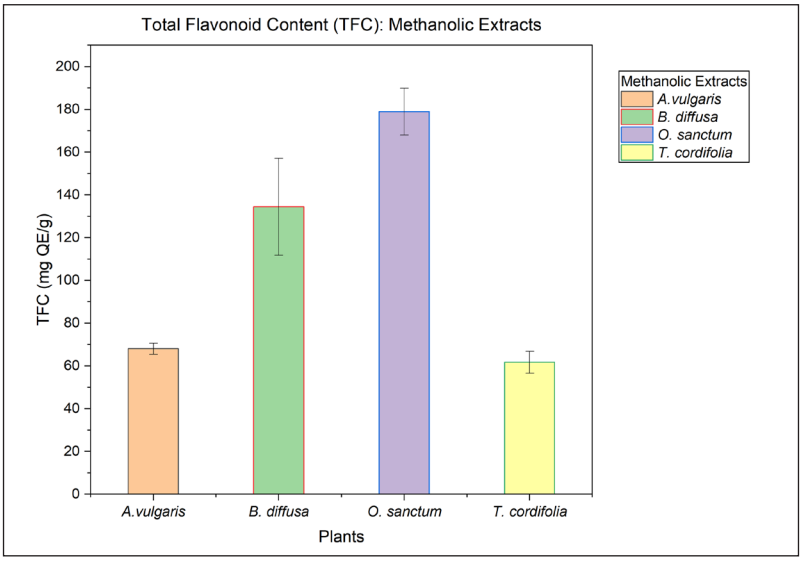

Figure 5. Total Flavonoid Content (TFC) of methanolic extracts of different plant species

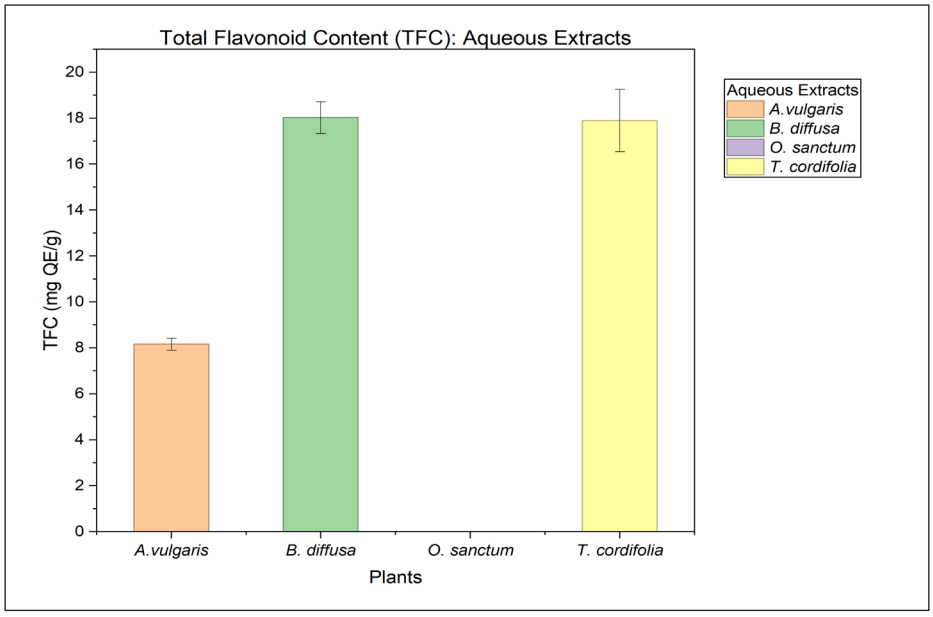

Figure 6: Total Flavonoid Content (TFC) of aqueous extracts of different plant species

The higher values of TPC and TFC observed in methanolic extracts compared to aqueous extracts can be attributed to the superior dissolving efficiency of methanol for phenolic compounds and flavonoids. Methanol's intermediate polarity makes it an effective solvent for extracting a wide range of bioactive compounds (Altemimi et al., 2017). The rankings of TPC and TFC of the tested plant species are consistent with the recent regional studies reporting high values for *O. sanctum* and species variability due to location and extraction method (Baruah et al., 2024; Chaudhary et al., 2024; Pathak & Niraula, 2019; Sharma & Adhikari, 2023; Sravani et al., 2024). The findings also support the effective use of the plants against various diseases, especially those related to oxidative stress and inflammation; however, further confirmation is required through clinical investigations.

3.4 Antioxidant Activity

The aqueous extracts failed to change the purple color of DPPH, showing their inactivity as antioxidants. In contrast, methanolic extracts demonstrated significant antioxidant activity with IC_{50} values ranging from 100.43 ± 0.38 to 229.58 ± 1.50 µg/mL (Table 4). However, when compared with ascorbic acid (IC_{50} = 12.23 ± 0.19), the antioxidant potency of the plant extracts is considerably lower. This is expected, as the plant extracts constitute a complex mixture of numerous phytochemicals rather than a single purified antioxidant compound, resulting in higher IC_{50} values (Rice-Evans et al., 1996).

Table 4: Comparison of IC_{50} values of methanolic extracts of different plants with standard ascorbic acid

Sample	IC_{50} (µg/mL) ± SD
Ascorbic acid	12.23 ± 0.19
Artemisia vulgaris	229.58 ± 1.50
Boerhavia diffusa	192.62 ± 2.98
Ocimum sanctum	100.43 ± 0.38
Tinospora cordifolia	180.07 ± 2.25

The decreasing order of antioxidant potential among the series: *O. sanctum* > *T. cordifolia* > *B. diffusa* >*A. vulgaris*, paralleled TPC and TFC in the methanolic extracts. Similar correlations between TPC or TFC and DPPH radical scavenging activity in the recent studies of the selected plants, also support the findings in the present study (Baruah et al., 2024; Chaudhary et al., 2024; Pathak & Niraula, 2019; Sharma & Adhikari, 2023; Sravani et al., 2024). The species-specific variability of the values found, on comparison with the previous studies, might be due to the difference in the location and extraction method (Sharma & Adhikari, 2023).

Figure 7 shows comparative bar diagram of the IC_{50} values of ascorbic acid and the methanolic plant extracts. Figure 9 shows the concentration-response curves (used to calculate the IC_{50} values) created from the related concentration-dependent response for percentage DPPH radical scavenging observed, as shown in Table 5 (graphically represented as bar diagram in Figure 8).

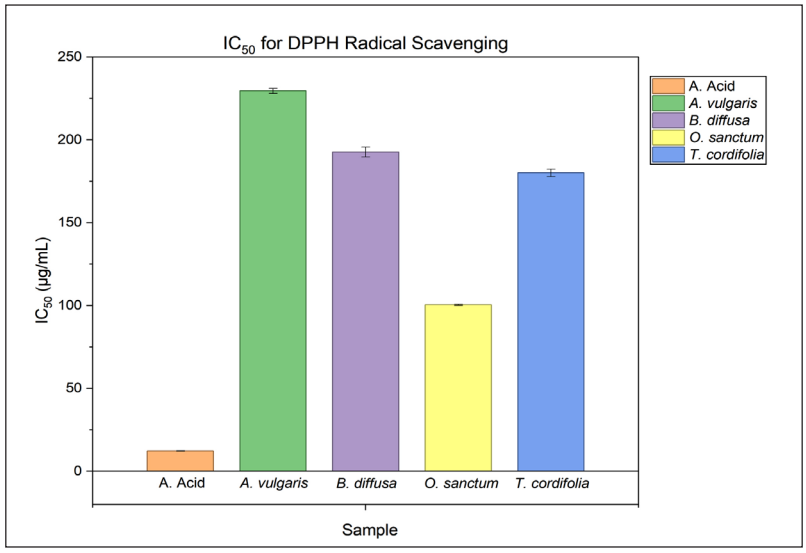

Figure 7: Half-maximal inhibitory concentration (IC_{50}) of Ascorbic acid and methanolic extracts of the plant materials

Table 5. Percentage DPPH radical scavenging of the samples at different concentration

Sample	% DPPH radical scavenging activity Concentration (µg/mL) ± SD				
	20	**40**	**60**	**80**	**100**
Ascorbic acid	82.90 ± 0.50	96.91 ± 0.24	97.08 ± 0.14	97.10 ± 0.06	97.18 ± 0.18
A. vulgaris	5.51 ± 0.81	9.33 ± 0.25	11.18 ± 0.42	16.54 ± 0.50	23.37 ± 0.29
B. diffusa	2.51 ± 0.25	5.08 ± 0.07	14.01 ± 0.31	20.18 ± 0.40	25.78 ± 0.11
O. sanctum	23.49 ± 0.09	27.29 ± 0.31	33.85 ± 0.34	41.30 ± 0.09	46.87 ± 0.10
T. cordifolia	17.45 ± 0.49	17.70 ± 0.42	22.10 ± 0.29	24.81 ± 0.36	28.66 ± 0.27

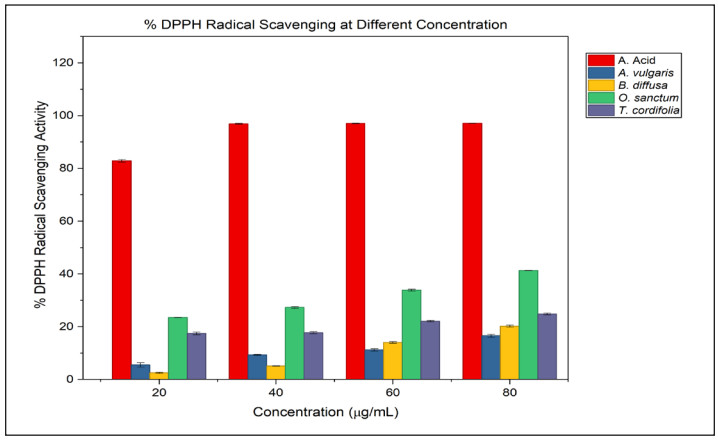

Figure 8. % DPPH radical scavenging of the samples at different concentration

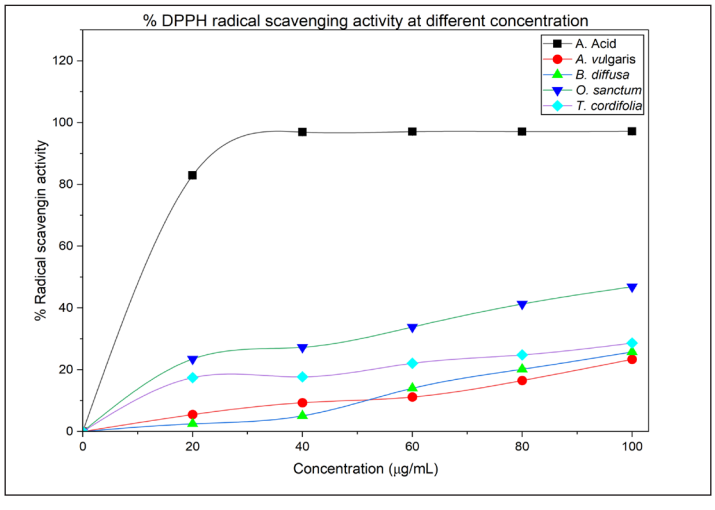

Figure 9. Variation of percentage DPPH radical scavenging activity with the concentration of Ascorbic acid and methanolic extracts of the plant materials

The strong correlation ($p < 0.05$) between TPC or TFC and antioxidant activity (IC_{50} values) indicates that the compounds responsible for exerting the antioxidant properties

are primarily phenolic compounds. This relationship is well-established in the literature as phenolic compounds possess multiple hydroxyl groups that can donate hydrogen atoms to neutralize free radicals (Rice-Evans et al., 1996).

The IC_{50} values obtained for the methanolic extracts (100.43 ± 0.38 to 229.58 ± 1.50 µg/mL) indicate moderate antioxidant activity compared to the standard ascorbic acid (12.23 ± 0.19 µg/mL). The comparatively weaker antioxidant activity of the plant extracts was observed relative to the standard ascorbic acid since the extracts represent complex mixtures of bioactive compounds rather than a solo antioxidant compound of high concentration. The mixture, instead, may provide synergistic effects leading to additional health benefits beyond antioxidant activity. Although the plant extracts showed moderate DPPH radical scavenging activity compared to ascorbic acid, they have demonstrated promising antioxidant potential against various diseases related to oxidative stress, with *O. sanctum* having the highest potency.

3.5 Antibacterial Activity

Antibacterial activity assessment revealed variable responses against the tested bacterial strains (Table 6). With the volume of 50 µL, limited antibacterial activity was observed. *A. vulgaris* aqueous extract showed growth inhibition against *E. coli* (ZOI: 9 mm), while *A. vulgaris* methanolic extract (ZOI: 18 mm) and *T. cordifolia* aqueous extract (ZOI: 13 mm) showed activity against *S. aureus*. With the extract volume of 100 µL, more pronounced antibacterial effects were observed.

A. vulgaris aqueous extract exhibited the strongest activity against both bacterial strains: *E. coli* (ZOI: 15 mm) and *S. aureus* (ZOI: 24 mm). *B. diffusa* methanolic extract showed weak activity against *E. coli* (ZOI: 8 mm). Antibacterial activity of some extent was observed with all of the methanolic extracts tested against *S. aureus*, with *A. vulgaris* showing the highest activity (ZOI: 24 mm). Some images of antibacterial activity tests showing zones of inhibition are given below in Figure 10.

Table 6. Antibacterial activity screening of different plant extracts

Plant species	Extract from	Bacteria	Zone of Inhibition (ZOI) mm		
			Extract volume		**Chloramphenicol (positive control)**
			100µL	**50µL**	
A. vulgaris	methanol	*E. coli*	10	-	26
		S. aureus	24	18	44
	aqueous	*E. coli*	15	9	25
		S. aureus	-	-	33
B. diffusa	methanol	*E. coli*	8	-	26
		S. aureus	8	-	41
	aqueous	*E. coli*	-	-	25
		S. aureus	-	-	38

T. cordifolia	methanol	*E. coli*	-	-	28
		S. aureus	7	-	25
	aqueous	*E. coli*	-	-	24
		S. aureus	15	13	40
O. sanctum	methanol	*E. coli*	-	-	26
		S. aureus	11	-	40

Bioactive compounds exert their antibacterial effects primarily by disrupting the essential bacterial cellular processes–such as compromising membrane integrity, inhibiting nucleic acid or protein synthesis, and blocking metabolic pathways–ultimately leading to cell death (Pandey et al., 2017). The antibacterial activity results reveal distinct patterns in the antimicrobial potential of the tested plants. Whilst there is some extent of antibiotic activity shown by each of the methanolic extracts against *S. aureus*, their activities against *E. coli* were either weaker or absent. This is expected, as gram-negative bacteria possess an additional outer layer rich in lipopolysaccharide that restricts penetration by many phytochemicals (Kwansa-Bentum et al., 2023). Interestingly, the aqueous *A. vulgaris* extract demonstrated the highest activity against *E. coli*, suggesting the presence of water-soluble antibacterial agents in this species. This warrants further clarification through targeted fractionation.

The observed differential antibacterial patterns across the plant species indicate the variability in their bioactive constituents and their specificity towards particular microorganisms, which merits further investigation. Variation in susceptibility between *S. aureus* and *E. coli* to the same extract may be attributed to structural differences in the bacterial cell envelope and permeability (Pandey et al., 2017). Overall, these findings support the traditional medicinal use of the plants in treating infectious diseases, as also reported in recent studies, and underscore the need for additional evaluations using broad bacterial panels to guide the development of advanced antibacterial agents with minimal adverse side effects.

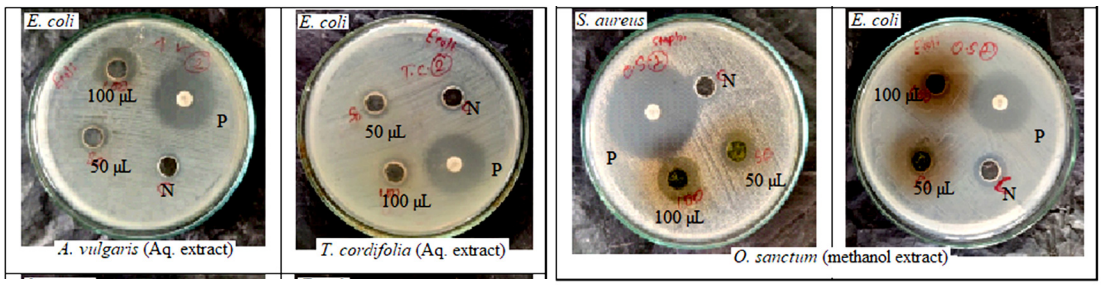

Figure 10: Some images of antibacterial activity tests (P = Positive control, N = Negative control)

4. Conclusions

The study began with the collection of selected plants from the Bharatpur area of Chitwan District, Nepal, to evaluate their qualitative and quantitative phytochemical contents, antioxidant, and antibacterial activities. The presence of various phytochemicals with reported pharmacological properties been revealed. Methanolic extract of *O. sanctum* demonstrated the highest values of TPC, TFC, and IC_{50}, underscoring its rich source of antioxidant phenolic compounds responsible for its important therapeutic potential for maintaining immunity and fighting against diseases related to oxidative stress. Antibacterial response varied with the solvent used and the species of tested microorganisms. While some extent of bioactivity was exerted by each of the methanolic extracts against *S. aureus*, the aqueous extract of *A. vulgaris* showed prominent antibacterial effects against both *E. coli* and *S. aureus*. The difference in the specific bioactive compounds and the permeability of the bacterial cell membrane might be the prominent cause of the observed differences. Antioxidant potential of the plant extracts was significantly correlated ($p<0.05$) with phenolic content. Higher phenolic content along with the higher antioxidant activity, though, highlights the higher therapeutic potential; it does not always mean to have corresponding antibacterial potency and thus emphasizes the need for compound-level investigation, which is beyond the scope of the present study due to limited time, funds, and resources.

Overall, the results validate effective medicinal uses of these plants in traditional practice and conclude *O. sanctum* as a particularly rich source of phenolic compounds and *A. vulgaris* as a promising source of antibacterial compounds. Future work should focus on fractionation of the plant extracts to isolate and identify the bioactive compound, along with the in-vivo bioactivity analysis to develop the advanced therapeutics with minimum adverse side-effects.

Declaration of Competing Interests: It is declared that there are no competing interests with anybody regarding the research work.

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