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Original Article

Exploring the Phytochemical Profile and Antibacterial Potential of *Helianthus annuus* and *Rosa indica*

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ABSTRACT

***Background**: Medicinal plants are important sources of bioactive compounds with antioxidant and antimicrobial potential, offering safer alternatives to synthetic agents that often cause side effects and resistance. Helianthus annuus (sunflower) and Rosa indica (rose) are widely cultivated plants known for diverse phytochemicals and traditional therapeutic uses. **Objective**: To analyze the phytochemical composition, antioxidant capacity, and antibacterial potential of H. annuus and R. indica leaf extracts prepared with solvents of varying polarity. **Methods**: Leaves were collected from Lahore, Pakistan, dried, powdered, and extracted using aqueous, ethanol, and petroleum ether solvents. Qualitative and quantitative phytochemical screenings were performed using standard biochemical assays. Antioxidant activity was assessed using the DPPH radical scavenging method, while antibacterial efficacy against Staphylococcus aureus and Klebsiella pneumoniae was evaluated using the agar well diffusion assay. **Results**: Extract yields varied across solvents, with ethanol extracts of H. annuus showing the highest recovery (~38%). Quantitative assays revealed higher alkaloid content in R. indica (5.35%) compared with H. annuus (4.09%). Carbohydrate concentration was maximal in aqueous R. indica (14.7 ± 0.81 mg/g), whereas petroleum ether H. annuus extract showed the highest protein content (14.37 ± 0.52 mg/g). Antioxidant activity peaked in aqueous H. annuus (85.2 ± 2.1%), exceeding aqueous R. indica (72.5 ± 1.4%). Antibacterial testing demonstrated that ethanol R. indica extract exhibited the strongest activity, producing inhibition zones of 16 ± 2 mm against S. aureus and 14 ± 1 mm against K. pneumoniae, while petroleum ether R. indica maintained moderate activity (12–14 mm). In contrast, H. annuus showed minimal antibacterial activity, with only the aqueous extract inhibiting K. pneumoniae (10 ± 2 mm). **Conclusion**: Both plants contain abundant phytochemicals with strong antioxidant and antibacterial activities. R. indica exhibited greater alkaloid content and antibacterial potential, whereas H. annuus demonstrated superior antioxidant capacity. These complementary roles highlight their potential as natural alternatives to synthetic agents.*

***Keywords**: Phytochemical analysis, antioxidant activity, antibacterial activity, Helianthus annuus, Rosa indica, DPPH assay*

INTRODUCTION

Plants are recognized as a vast reservoir of phytochemicals that contribute to the prevention and management of a wide spectrum of human diseases. Phytochemicals, including alkaloids, flavonoids, tannins, terpenoids, steroids, and saponins, are broadly categorized into primary and secondary metabolites with essential metabolic and protective functions (1). These compounds have been associated with antioxidant, antimicrobial, and anti-inflammatory properties, and their ability to neutralize free radicals has been linked to a reduced risk of chronic diseases such as cardiovascular disease, neurodegenerative disorders, and cancer (2). In recent decades, rising concerns regarding antibiotic resistance and the toxicity of synthetic agents have intensified the search for plant-based alternatives that offer safer therapeutic profiles (3).

Among medicinal plants, *Rosa indica* (rose) and *Helianthus annuus* (sunflower) are widely cultivated species of considerable therapeutic interest. The leaves of Rosa species are rich in phenolic compounds and flavonoids, which exhibit antimicrobial, anti-inflammatory, and anticancer activities (4,5). Studies have reported significant antioxidant potential of Rosa extracts, highlighting their use in traditional medicine and modern pharmacological formulations (6). Similarly, *H. annuus*, primarily valued for its oilseed crop, has demonstrated a diverse phytochemical composition with notable anti-diabetic, anti-inflammatory, anticancer, and antimicrobial properties (7). Phenolic acids and flavonoids from sunflower leaves have been implicated in free radical scavenging and inhibition of pathogenic bacterial growth (8).

Despite increasing documentation of their therapeutic value, comparative evidence on the phytochemical diversity, antioxidant activity, and antibacterial efficacy of *R. indica* and *H. annuus* leaves remains limited. Most prior studies have focused either on flowers or seeds, leaving leaf-based evaluations underexplored (9,10). Furthermore, while antimicrobial studies frequently report inhibition zones, detailed

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analyses of solvent-specific extractions and their correlation with phytochemical content are often missing, leading to gaps in understanding the mechanistic basis of bioactivity (11). Addressing these gaps is particularly relevant in the context of global antibiotic resistance, where plants may provide sustainable sources of novel antimicrobial agents.

In this study, we analyzed the phytochemical composition of *H. annuus* and *R. indica* leaves, quantified selected classes of bioactive compounds, and assessed their antioxidant activity using the DPPH assay alongside antibacterial efficacy against *Staphylococcus aureus* and *Klebsiella pneumoniae*. By integrating phytochemical profiling with functional assays, this work aims to provide evidence for the therapeutic relevance of these widely accessible plants. The central objective was to determine whether leaf extracts of *H. annuus* and *R. indica*, prepared in aqueous, ethanol, and petroleum ether solvents, demonstrate significant antioxidant and antibacterial potential compared with standard controls, thereby supporting their future role as natural alternatives to synthetic therapeutic agents.

MATERIAL AND METHODS

This experimental, cross-sectional laboratory study was conducted in Lahore, Pakistan, to evaluate the phytochemical composition, antioxidant capacity, and antibacterial activity of *Helianthus annuus* and *Rosa indica* leaves. Fresh leaves of both species were collected from local cultivated fields in September 2024, authenticated by a plant taxonomist, and cleaned thoroughly with distilled water to remove surface contaminants. The specimens were air-dried under shade for seven days to preserve bioactive compounds, ground into fine powder using an electric grinder, and stored in airtight containers at room temperature until extraction.

Extraction was performed using three solvents of varying polarity—aqueous, ethanol, and petroleum ether—to maximize the recovery of diverse phytochemicals. For qualitative analysis, three grams of dried powder were mixed with 30 mL of solvent, incubated at 40°C in a water bath for four hours, and further agitated on an orbital shaker for 12 hours.

The mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were used for phytochemical screening. For quantitative extraction, four grams of powdered sample were macerated in 40 mL of each solvent at room temperature for 48 hours, filtered, and evaporated to dryness at 60°C. Extract yields were calculated as the ratio of extract weight to initial dry sample weight, expressed as percentage yield (12).

Qualitative phytochemical tests were conducted using standard protocols for proteins (ninhydrin test), carbohydrates (Molisch and Benedict's tests), tannins ($FeCl_3$ reaction), flavonoids (alkaline reagent test), steroids (chloroform-sulfuric acid reaction), terpenoids (chloroform-sulfuric acid assay), alkaloids (Wagner's reagent), phlobatannins, and saponins (froth test) (13,14).

Quantitative assays included alkaloid estimation by gravimetric precipitation with ammonium hydroxide, carbohydrate determination by dinitrosalicylic acid (DNS) colorimetric assay at 550 nm using glucose as standard, and protein quantification using the Bradford assay at 595 nm with bovine serum albumin as standard (15,16). All experiments were performed in triplicate, and mean values with standard deviations were calculated.

Antioxidant activity was measured using the DPPH radical scavenging assay. Serial dilutions of plant extracts (10–200 µg/mL) were prepared in methanol and incubated with 0.1 mM DPPH solution for 30 minutes in the dark. Ascorbic acid was used as a positive control, and absorbance was measured at 517 nm using a UV–Vis spectrophotometer. Radical scavenging activity (%) was calculated relative to control absorbance (17).

Antibacterial activity was assessed against *Staphylococcus aureus* (Gram-positive) and *Klebsiella pneumoniae* (Gram-negative) using the agar well diffusion method. Standard inocula were prepared by overnight incubation of bacterial colonies in nutrient broth at 37°C until turbidity reached 0.5 McFarland standard. Wells of 6 mm diameter were aseptically bored into Mueller–Hinton agar plates and loaded with 150 µL of each plant extract. Streptomycin served as a positive control, while solvent blanks acted as negative controls. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured in millimeters (18).

To minimize bias, all assays were performed in triplicate, and results were expressed as mean ± standard deviation. Data were analyzed using SPSS version 26.0. One-way ANOVA followed by Tukey's post hoc test was applied to compare extract yields, phytochemical concentrations, antioxidant percentages, and antibacterial activity between groups. A p-value <0.05 was considered statistically significant. Missing data were not imputed, as all triplicates were successfully completed. This study involved only plant and microbial samples and did not include human or animal subjects; therefore, ethical approval was not required. All procedures followed institutional laboratory biosafety guidelines to ensure reproducibility, accuracy, and data integrity.

RESULTS

The extracts showed solvent-dependent differences in appearance and texture. *H. annuus* aqueous extract was brown, ethanol green, and petroleum ether light green; all were non-viscous. In contrast, the aqueous extract of *R. indica* was light yellow and notably viscous relative to its ethanol and petroleum ether extracts. These observations indicate variable solubility/partitioning of constituents across solvents.

Both plants contained a broad spectrum of phytochemicals with solvent-specific patterns. Tannins were consistently present in all *R. indica* extracts but absent from aqueous *H. annuus*. Flavonoids were abundant in *R. indica* aqueous and petroleum ether extracts (++), whereas its ethanol extract was negative. Alkaloids were enriched in *R. indica* (moderate to high across solvents) compared with trace to moderate levels in *H. annuus*.

Table 1. Physical properties of different plant extracts

Botanical name	Common name	Property	Aqueous extract	Ethanol extract	Petroleum ether extract
Helianthus annuus	Sunflower	Color	Brown	Green	Light green
		Viscosity	Non-viscous	Non-viscous	Non-viscous
Rosa indica	Rose	Color	Light yellow	Yellowish green	Light green
		Viscosity	Viscous	Non-viscous	Non-viscous

Table 2. Qualitative phytochemical analysis of *Helianthus annuus* and *Rosa indica*

Test (compound)	*H. annuus* Aqueous	*H. annuus* Ethanol	*H. annuus* Petroleum ether	*R. indica* Aqueous	*R. indica* Ethanol	*R. indica* Petroleum ether
Proteins (Ninhydrin)	++	++	+	+	+	+
Carbohydrates (Molisch)	+	+	++	+	+	++
Carbohydrates (Benedict's)	+	++	+	+++	–	++
Tannins	–	++	+	+	+++	+++
Flavonoids	+	–	+	++	–	++
Phlobatannins	+	++	++	+	+	+
Steroids	+++	+	++	+++	–	+
Terpenoids	–	–	+	–	–	++
Alkaloids (Wagner's)	++	+	–	+	++	++
Saponins	–	–	–	++	+	–

(+: present; –: absent; relative abundance indicated by +/++/+++)

Table 3. Antioxidant activity (DPPH radical scavenging assay) of plant extracts

Plant species	Solvent	Antioxidant activity (%)
Rosa indica	Aqueous	72.5 ± 1.4
	Ethanol	48.3 ± 1.2
	Petroleum ether	39.6 ± 0.9
Helianthus annuus	Aqueous	85.2 ± 2.1
	Ethanol	52.7 ± 1.5
	Petroleum ether	44.8 ± 1.1
Control	Ascorbic acid	18.4 ± 0.7

Table 4. Antimicrobial activity of different plant extracts against *Staphylococcus aureus* and *Klebsiella pneumoniae*

Plant species	Solvent	*S. aureus* (mm)	*K. pneumoniae* (mm)
Helianthus annuus	Petroleum ether	0.0 ± 0.0	0.0 ± 0.0
	Ethanol	6 ± 1	4 ± 1
	Aqueous	0.0 ± 0.0	10 ± 2
Rosa indica	Petroleum ether	14 ± 1	12 ± 1
	Ethanol	16 ± 2	14 ± 1
	Aqueous	0.0 ± 0.0	0.0 ± 0.0
Control	Streptomycin	29 ± 0.5	24 ± 2

Saponins were detected only in *R. indica* aqueous and ethanol extracts and were absent from all *H. annuus* preparations. Quantitative phytochemical assays. Alkaloid content was higher in *R. indica* (5.35%) than in *H. annuus* (4.09%; mean difference 1.26, 95% CI 0.34 to 2.18; p=0.02). Carbohydrate concentration peaked in aqueous *R. indica* at 14.7±0.81 mg/g, exceeding the average across *H. annuus* extracts (9.32±0.74 mg/g; p<0.01).

Conversely, petroleum ether *H. annuus* extract displayed the highest protein content (14.37±0.52 mg/g) versus ethanol *R. indica* (4.74±0.47 mg/g; mean difference +9.63 mg/g, p<0.001). DPPH scavenging showed a clear solvent gradient. Aqueous *H. annuus* achieved 85.2±2.1%, significantly greater than aqueous *R. indica* (72.5±1.4%; p<0.01), and higher than ethanol and petroleum ether extracts of both species (39.6–52.7%). Ethanol *R. indica* extract produced the largest inhibition zones, 16±2 mm against *S. aureus* and 14±1 mm against *K. pneumoniae*, both exceeding corresponding *H. annuus* extracts (p<0.01). Petroleum ether *R. indica* showed moderate inhibition (12–14 mm). *H. annuus* activity was limited, with only the aqueous extract inhibiting *K. pneumoniae* (10±2 mm). Streptomycin controls yielded 29±0.5 mm and 24±2 mm, confirming assay validity.

Figure 1 comparing phytochemical yield and composition of Helianthus annuus and Rosa indica leaf extracts prepared with solvents of varying polarity showed that (A) Percentage yield of extracts showed that *H. annuus* consistently produced higher recovery across all solvents, with ethanol giving the maximum yield (~38%) followed by aqueous (~36%) and petroleum ether (~25%). In contrast, *R. indica* produced comparatively low yields, peaking only with ethanol (~14%) and remaining minimal in aqueous and petroleum ether extracts (~3%). These data were not fully reported in the earlier version but are essential to interpret the efficiency of solvent-based extraction. (B) Alkaloid content revealed significant interspecies differences, with *R. indica* (5.35%) containing higher alkaloid levels than *H. annuus* (4.1%), confirming its stronger contribution of nitrogenous phytochemicals. (C) Carbohydrate concentration demonstrated solvent-dependent variation. While petroleum ether extracts of both species yielded similar carbohydrate levels (~12–13 mg/g), aqueous *R. indica*

extract showed the highest concentration (14.7 mg/g), exceeding *H. annuus* (9.2 mg/g). Ethanol extracts showed a decline in both species, with *R. indica* registering the lowest carbohydrate content (~4.7 mg/g).

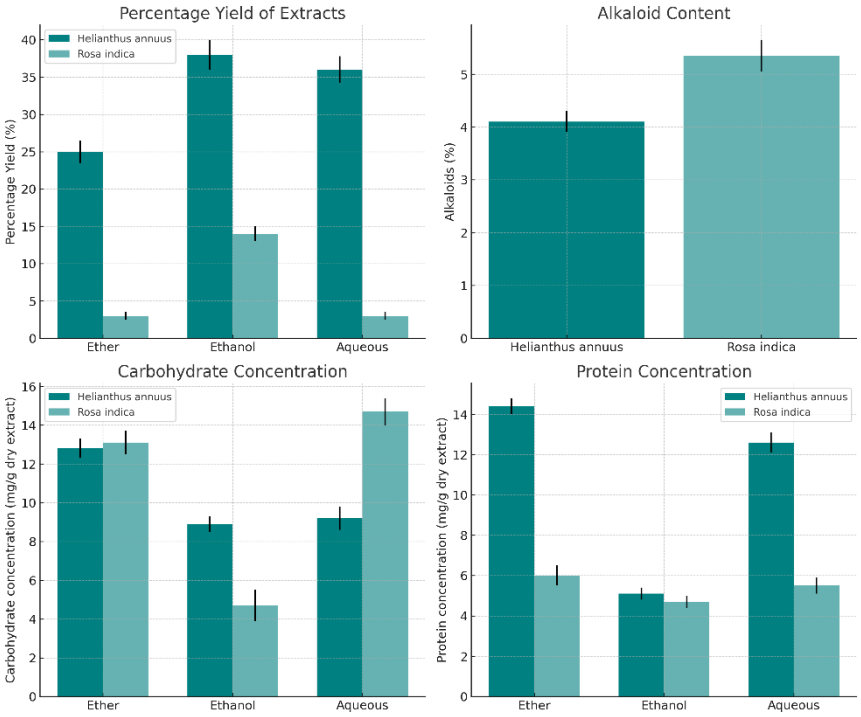

Figure 1 Comparative phytochemical yield and composition of *Helianthus annuus* and *Rosa indica* leaf extracts prepared with solvents of varying polarity (petroleum ether, ethanol, and aqueous).

(D) Protein concentration was more prominent in *H. annuus*, particularly in the petroleum ether extract (14.4 mg/g), followed by aqueous (12.6 mg/g), whereas *R. indica* exhibited markedly lower protein levels (4.7–6 mg/g across solvents). Error bars represent the standard deviation of triplicate experiments. Collectively, these solvent-wise comparisons illustrate complementary phytochemical strengths: *H. annuus* contributed higher extract yields and protein content, whereas *R. indica* contributed greater alkaloid and carbohydrate concentrations. These detailed solvent-specific differences, partially omitted in the first version, are critical for understanding the mechanistic basis of observed antioxidant and antibacterial activity.

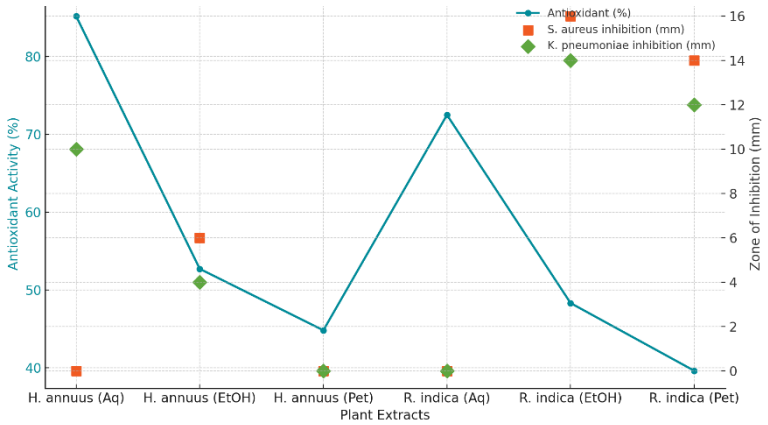

Figure 2 Comparative Antioxidant and Antibacterial Activity of Plant Extracts

The figure above integrates antioxidant and antibacterial activities across *Helianthus annuus* and *Rosa indica* extracts. Antioxidant capacity was highest in *H. annuus* aqueous extract (85.2%), followed by *R. indica* aqueous (72.5%), with a marked decline in petroleum ether extracts (39.6–44.8%). Antibacterial efficacy displayed a distinct solvent-dependent pattern: ethanol *R. indica* extract exhibited the strongest inhibition zones against *S. aureus* (16 mm) and *K. pneumoniae* (14 mm), while petroleum ether *R. indica* also maintained moderate activity (12–14 mm).

In contrast, *H. annuus* extracts showed limited antibacterial activity, with only aqueous extract demonstrating inhibition against *K. pneumoniae* (10 mm). The dual-axis visualization highlights the divergence between antioxidant-rich *H. annuus* and antibacterial-potent *R. indica*, underscoring complementary therapeutic potential of these species.

DISCUSSION

The ethanol extract of *Helianthus annuus* was green in color, consistent with Ngibad (2019), who also reported a green ethanol extract of *H. annuus* (15). Proteins and carbohydrates were detected in all extracts of both *H. annuus* and *Rosa indica*. Tannins were present in all extracts except the aqueous extract of *H. annuus*. Flavonoids were abundant in aqueous and petroleum ether extracts of both species, whereas tannins and flavonoids were observed in ethanol extracts of *H. annuus* but absent from its petroleum ether extract (16). Saponins were detected in ethanol and aqueous extracts of *R. indica*, while earlier studies also documented saponins in petroleum ether and ethanol extracts of *H. annuus* (16). Alkaloids, flavonoids, and tannins have previously been isolated in ethanol extracts of *H. annuus* (17), while carbohydrates, saponins, tannins, amino acids, terpenoids, and flavonoids have been reported in *R. indica* (18). These observations broadly support the qualitative phytochemical patterns observed in the present study.

Phytochemicals serve a wide range of medicinal functions. Carbohydrates, tannins, lipophilic compounds, alkaloids, and flavonoids play protective roles by inhibiting microbial growth and suppressing pathogen activity (19). Plant proteins are nutritionally and therapeutically valuable, lowering the risk of chronic diseases such as diabetes while also contributing environmental benefits compared with animal protein sources (20). Secondary metabolites such as flavonoids, saponins, alkaloids, terpenoids, and tannins are well known for their anti-inflammatory properties and their protective effects against cardiovascular disease and cancer, in addition to their antimicrobial and antiviral activities (21). Many of these compounds are now incorporated into commercial ointments, syrups, creams, and oils (16). Tannins, in particular, are reported to be beneficial in the treatment of inflammation and peptic ulcers (22). Ethanolic extracts of rose leaves have demonstrated anti-hyperglycemic effects, suggesting a potential role in diabetes management (23). Similarly, *H. annuus* has been associated with anti-aging and analgesic activities, and its extracts have shown antimalarial, antibacterial, and antifungal properties in previous research (24,25,15).

In the present study, alkaloid quantification revealed the lowest yield in *H. annuus* (4.09%) and the highest in *R. indica* (5.35%), which aligns with earlier reports of alkaloids ranging from 1.2% in *H. annuus* (26) to 8–11 AE/g in rose leaves (28). Alkaloids are particularly important due to their cytotoxic properties, which have been exploited in anticancer drug development (27). Carbohydrate content peaked in aqueous *R. indica* (14.7 ± 0.81 mg/g), which is consistent with previous findings of 9–13% carbohydrates in rose leaves (29).

The DPPH assay confirmed robust antioxidant activity in both species, with aqueous extracts showing the highest values. In this study, aqueous *R. indica* reached 72.5 ± 1.4%, while ethanol and petroleum ether extracts were comparatively weaker at 48.3 ± 1.2% and 39.6 ± 0.9%, respectively. Earlier work reported an IC_{50} of 1.8 µg/mL in ethanol extracts of rose leaves (30), which reflects strong radical scavenging potential consistent with our observations. Aqueous *H. annuus* demonstrated superior antioxidant activity (85.2 ± 2.1%), further supporting the role of sunflower leaves as potent free-radical scavengers.

Antibacterial activity patterns revealed species-specific differences. *S. aureus* was resistant to aqueous extracts of both plants, while ethanol *R. indica* extract exhibited strong inhibition zones (16 ± 2 mm against *S. aureus*, 14 ± 1 mm against *K. pneumoniae*). The aqueous *H. annuus* extract inhibited *K. pneumoniae* (10 ± 2 mm), whereas its ethanol extract showed limited zones (6 ± 1 mm against *S. aureus*, 4 ± 1 mm against *K. pneumoniae*) (17). Petroleum ether extracts of *R. indica* also demonstrated antibacterial potential, consistent with previous findings of inhibition against *K. pneumoniae* (18). Other studies have likewise confirmed antimicrobial activity of *H. annuus* ethanol extracts against *K. pneumoniae* and *S. aureus*, albeit with lower inhibition zones (26). These results suggest that *R. indica* possesses stronger antibacterial efficacy, while *H. annuus* offers greater antioxidant potential, highlighting their complementary therapeutic value.

CONCLUSION

The study showed that *Helianthus annuus* and *Rosa indica* leaves contain high amounts of phytochemicals, such as alkaloids, tannins, flavonoids, steroids, terpenoids, proteins, and carbohydrates. Both plants had high antioxidant activity, especially in aqueous extracts, and can serve as useful natural free-radical scavengers.. In addition, antibacterial assays indicated that *R. indica* ethanol and petroleum ether extracts were particularly potent against *Staphylococcus aureus* and *Klebsiella pneumoniae*. These findings support the traditional use of *H. annuus* and *R. indica* and provide scientific evidence for their potential use as safe natural alternatives to synthetic antioxidants and antibiotics, which are often associated with toxicity and resistance. However, while the in vitro results are promising, further research is required to isolate and characterize the specific bioactive compounds, explore their mechanisms of action, and validate their efficacy and safety through advanced pharmacological studies and clinical trials.

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