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Exploring the Therapeutic Potential of *Fragaria nubicola*: Antimicrobial, Phytotoxic, Cytotoxic, and Antioxidant Properties

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ABSTRACT

Background and Objective: *Fragaria nubicola* is a traditionally used medicinal plant, but its pharmacological properties remain underexplored. This study aimed to scientifically validate its antimicrobial, phytotoxic, cytotoxic, and antioxidant activities, focusing on the therapeutic potential of its root, fruit, and leaf extracts. **Materials and Methods:** Root, fruit, and leaf extracts of *F. nubicola* were prepared and evaluated using in vitro assays. Antimicrobial activity was tested against bacterial strains (*Klebsiella pneumoniae*, *Acinetobacter baumannii*) and fungal strains (*Aspergillus flavus*, *Aspergillus niger*) through zone of inhibition measurements. Phytotoxicity was assessed at concentrations of 10, 100, and 1000 µL, while cytotoxic potential was evaluated using brine shrimp lethality bioassays at 1000 ppm. For statistical analysis, ANOVA was used. Standard significance levels were set at $p<0.05$. **Results:** The root and leaf extracts showed maximum antifungal activity against *A. flavus*, while the fruit extract was most effective against *A. niger*. For antibacterial activity, the fruit and root extracts displayed significant zones of inhibition against *K. pneumoniae*, and the leaf extract was most effective against *A. baumannii*. Phytotoxic assays revealed maximum growth inhibition values of 67.7% (fruit extract at 10 µL), 81.3% (root extract at 100 µL), and 93.3% (leaf extract at 1000 µL). Cytotoxic tests indicated complete lethality of brine shrimp larvae at 1000 ppm for all extracts. **Conclusion:** The findings validate the traditional therapeutic uses of *F. nubicola* and demonstrate its significant antimicrobial, phytotoxic, and cytotoxic properties. These results support further exploration of its bioactive compounds for developing novel therapeutic agents.

KEYWORDS

Fragaria nubicola, antimicrobial properties, phytotoxicity, cytotoxicity, bioactive compounds, zone of inhibition, therapeutic potential, indigenous medicine

INTRODUCTION

Pharmacognosy is the branch of medical and pharmaceutical sciences that focuses on the study of crude drugs derived from natural sources. It encompasses authentication (macroscopic and microscopic), provenance (plant and animal origin), and quality control of raw medicinal substances. This field explores the botanical, organoleptic, physical, and pharmaceutical properties of crude drugs while aiming to identify, isolate, and screen active ingredients or bioactive molecules for various biological assays[1].

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Modern pharmacognosy has evolved through the integration of advanced scientific disciplines, including analytical chemistry, microbiology, plant tissue culture, biotechnology, and genetic engineering. These technologies have significantly expanded the scope of pharmacognostic research, enabling deeper exploration into the structure and function of bioactive compounds. Furthermore, allied fields such as botany, pharmacology, pharmaceutics, and phytochemistry contribute to the broader understanding and development of medicinal agents. Pharmacognosy also incorporates key interdisciplinary areas, such as ethnobotany, ethnomedicine, and ethnopharmacology, which investigate the traditional uses of medicinal plants in various cultures. Phytochemical analysis and molecular biology play critical roles in identifying novel compounds and understanding their mechanisms of action[2]. This holistic approach ensures the discovery, development, and standardization of natural products, reinforcing pharmacognosy as an indispensable pillar of drug discovery and development.

Pharmacognosy is the scientific discipline focused on the study of raw medicines obtained from four main natural sources: Plants, animals, minerals, and metals. Notably, plant-based resources contribute about 90% of crude medicines, while the remaining 10% come from the other three sources[3]. Pharmacognosy bridges traditional medicine and pharmaceutics, supporting pharmacists in the production of natural medicines. Advancements in extraction and purification techniques have enhanced pharmacognostic knowledge. Modern technologies, such as plant tissue culture, have been employed for the production of crude pharmaceuticals[4].

Medicinal plants remain the most accessible and prevalent source of active compounds for treating various diseases. Numerous biological assays have been developed to investigate the effects of plant extracts on living organisms[5]. Despite significant progress in plant-based research, there remains considerable potential for further development[6,7].

Microbial infections continue to cause high mortality rates, even with the annual development of new synthetic antifungal and antibacterial drugs. Pathogen resistance to these synthetic drugs has increased dramatically over the past two decades, compounded by the emergence of new diseases and undesirable side effects associated with certain antibiotics[8,9]. Less expensive and commonly used antimicrobial agents have become ineffective due to bacterial resistance[10]. In this context, herbal medications present a promising alternative, offering safety and efficacy compared to synthetic drugs[11]. Cancer chemotherapy destroys cancer cells with minimal harm to healthy tissues[12] selectively. Screening medicinal plants for cytotoxic activity against cancer involves bioassays, such as the brine shrimp lethality test. Initially proposed by Michael and refined by Vanhaecke *et al.*[13], this assay assesses toxicity by measuring the mortality of *Artemia nauplii* in the presence of plant extracts[14,15].

Antioxidants mitigate damage caused by reactive oxygen species (ROS) by scavenging free radicals. Plants are rich sources of antioxidant compounds, including flavonoids, phenolic acids, tannins, quinones, alkaloids, and terpenoids[16,17]. Among various antioxidant assays, the DPPH radical scavenging method is widely used for assessing anti-radical activity[18].

In Pakistan, abnormal weed growth poses a significant threat to cereal crops and other economically valuable plants, resulting in severe vegetation losses. Preventive strategies are essential to mitigate agricultural damage caused by weeds, which have a more significant impact than pests or insects. The *Lemna minor* bioassay is a widely recommended method for assessing the phytotoxic potential of plant extracts. *Lemna minor*, a simple aquatic angiosperm, is used as a test organism to evaluate vegetative growth inhibition and determine the toxic potential of plant extracts.

This study aims to investigate the antimicrobial, phytotoxic, cytotoxic, and antioxidant effects of root, fruit, and leaf extracts of medicinal plants, contributing to the development of natural therapeutics.

MATERIALS AND METHODS

Study area: *Fragaria nubicola* specimens were collected from the Thandiani Forest, District Abbottabad, Pakistan, in July, 2023. Thandiani Forest is classified as a moist temperate forest, recognized for its rich biodiversity of flora and fauna. It is located approximately 35 km northeast of Abbottabad, positioned between 34°17 to 34°10 N Latitude and 73°23 to 73°17 E Longitude, with an elevation range of 1,191-2,626 m above sea level. Muzaffarabad borders the forest to the east, Siren Forest to the West, the Kunhar River and Garhi Habibullah to the North, and the Abbottabad Sub-Forest Division to the South. The region experiences a moist temperate climate, characterized by an average annual precipitation of 1,424.1 mm.

Recent investigations in Thandiani have identified a high diversity of medicinal plants, highlighting the region's significant ethnobotanical value. Local communities continue to utilize these plant species for the treatment of various ailments, reflecting the deep-rooted traditional knowledge of herbal medicine in the area.

Extract preparation: The plant material was thoroughly washed with water, shade-dried, and ground into a fine powder using an electric grinder. For extraction, approximately 800 g of the dried powder were placed in flasks containing 2 L of methanol. The mixture was maintained at 24°C in the dark for 25 days, with shaking twice daily to ensure thorough extraction. After 25 days, the methanolic extract was filtered using Whatman filter paper No. 1. The plant residues were re-extracted by adding 500 mL of methanol, and the process was repeated three times to maximize yield. The combined filtrates were concentrated using a rotary evaporator at 45°C under vacuum pressure[19,20]. The crude methanolic extract was further diluted in Dimethyl Sulfoxide (DMSO) to prepare a stock solution at a concentration of 1 g of crude extract per 100 mL of DMSO.

Antibacterial activity: Several bacterial strains were selected for the study and maintained on nutrient broth to preserve their viability. The tested strains included one Gram-positive bacterium (*Staphylococcus aureus*) and three Gram-negative bacteria (*Acinetobacter baumannii, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*). Stock solutions and Luria Broth (LB) media were prepared following standard protocols. The bacterial strains were cultivated in a liquid broth medium. For this, 8 mg of bacterial inoculum was dissolved in 75 mL of sterile water, transferred to test tubes, and incubated for 24 hrs to allow adequate growth. The antibacterial activity of *F. nubicola* extracts (leaves, roots, and fruits) was assessed using the agar well diffusion method, as described by Liu Wells were created in agar plates inoculated with bacterial cultures, and the extracts were introduced into these wells. After incubation, clear inhibition zones around the wells indicated antibacterial activity. The diameter of the inhibition zones was measured in millimeters to quantify the effect of the extracts.

Antifungal activity: The antifungal activity of *Fragaria nubicola* extracts was evaluated against three fungal strains: *Candida albicans*, *Aspergillus niger,* and *Aspergillus flavus*. Sabouraud Dextrose Agar (SDA) medium was prepared following standard protocols[22] and dispensed into test tubes for subsequent antifungal activity testing. Terbinafine, a standard antifungal agent, was used as a positive control. The antifungal activity of the plant extracts was assessed using the agar well diffusion method, similar to the approach employed for antibacterial activity. Wells were created in SDA plates inoculated with fungal cultures, and the plant extracts were introduced into these wells. Zones of inhibition around the wells, indicating antifungal activity, were measured in millimeters.

Cytotoxic activity: The cytotoxic activity of the crude methanolic extracts of *Fragaria nubicola* was evaluated to assess their potential anticancer properties. The assay was conducted with minor adjustments to standard protocols. The extracts were subjected to cytotoxic activity testing to determine their effects on cell viability and potential anticancer efficacy.

Statistical analysis: For comparing the antibacterial or antifungal activity ANOVA was used. The ANOVA analyzes and compares the means of inhibition zones to determine statistically significant differences between treatments or conditions. For cytotoxic activity, nonlinear regression was used. Commonly used Standard significance levels in biological and pharmacological research were Set at $p<0.05$ (threshold indicates a 5% probability).

RESULTS

The antifungal activity of *Fragaria nubicola* extracts revealed that the root and leaf extracts exhibited the highest zone of inhibition against *Aspergillus flavus*, while the fruit extract demonstrated the most significant antifungal activity against *Aspergillus niger*. For antibacterial activity, both the fruit and root extracts showed strong inhibition against *Klebsiella pneumoniae*, whereas the leaf extract displayed the largest zone of inhibition against *Acinetobacter baumannii*.

The phytotoxic assay demonstrated that the fruit extract exhibited 67.7% growth inhibition at a concentration of 10 µL, while the root extract showed 81.3% growth inhibition at 100 µL. At the highest concentration of 1000 µL, the leaf extract achieved a maximum growth inhibition value of 93.3%.

Cytotoxic testing using brine shrimp lethality bioassays indicated that at a concentration of 1000 ppm, all brine shrimp larvae exposed to the *F. nubicola* extracts (leaves, roots, and fruits) were killed, confirming the extracts' cytotoxic potential.

Antifungal activity of *Fragaria nubicola:* The root extract of *Fragaria nubicola* demonstrated antifungal activity with a minimum zone of inhibition (R1) of 18.2 mm against *Candida albicans* and a maximum zone of inhibition (R1) of 26.4 mm against *Aspergillus flavus*. The antibiotic used as a control against *Candida albicans* exhibited a minimum zone of inhibition (R3) of 23 mm and a maximum zone of inhibition (R3) of 41 mm (Table 1).

The methanolic leaf extract exhibited the largest zone of inhibition (R2) of 26.1 mm against *Aspergillus flavus*, while the lowest growth inhibition (R3) was recorded at 13.7 mm against *Aspergillus niger*. The control antibiotic showed a minimum zone of inhibition (R3) of 23 mm against *Candida albicans*, and a maximum zone of inhibition (R3) of 41 mm against *Aspergillus flavus* (Table 2).

Table 1: Antifungal activity of *Fragaria nubicola* roots

	Root extract ZOI (mm)			Antibiotic ZOI (mm)				
Fungal strain	R1	R2	R3	R1	R2	R3	Means±SD (root)	Means±SD (antibiotic)
Aspergillus niger	24.5	24.2	25.2	32.3	30.1	29.7	24.6±0.77	30.7±0.75
Aspergillus flavus	26.4	23.7	22.7	37	38.2	41	23.6±0.895	38.73±1.06
Candida albicans	18.2	20	19.2	29	27	23	19.13±0.44	26.3±1.57

Table 2: Antifungal activity of leaf of *Fragaria nubicola*

	Leaves ZOI (mm)			Antibiotic ZOI (mm)				
Fungal strain	R1	R2	R3	R1	R2	R3	Means±SD (root)	Means±SD (antibiotic)
Aspergillus niger	18.6	14.2	13.7	32.3	30.1	29.7	15.5±1.46	30.7±0.75
Aspergillus flavus	24.3	26.1	21.4	30	38.2	41	23.9±1.199	36.4±3.01
Candida albicans	18.7	23.1	19.2	30	32	23	20.3±1.29	28.8±2.52

Table 3: Antifungal activity of fruit of *Fragaria nubicola*

	Fruit ZOI (mm)			Antibiotic ZOI (mm)				
Fungal strain	R1	R2	R3	R1	R2	R3	Means±SD (root)	Means±SD (antibiotic)
Aspergillus niger	20.6	21.5	24.2	32.6	30.1	31.3	22.1±0.98	31.33±0.88
Aspergillus flavus	18.3	19.5	22.6	27.4	38.2	41	20.13±1.15	35.53±3.84
Candida albicans	13.3	14.6	11.2	23	30	32	13.03±0.86	28.33±2.52

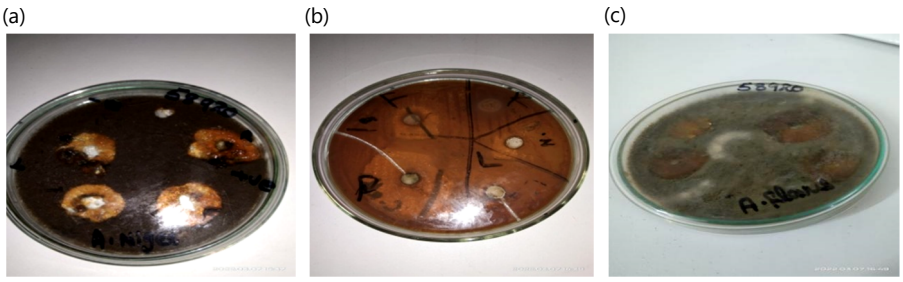

Fig. 1(a-c): Antifungal activity of *Fragaria nubicola*, (a) *A. niger*, (b) *C. albicans* and (c) *A. flavus*

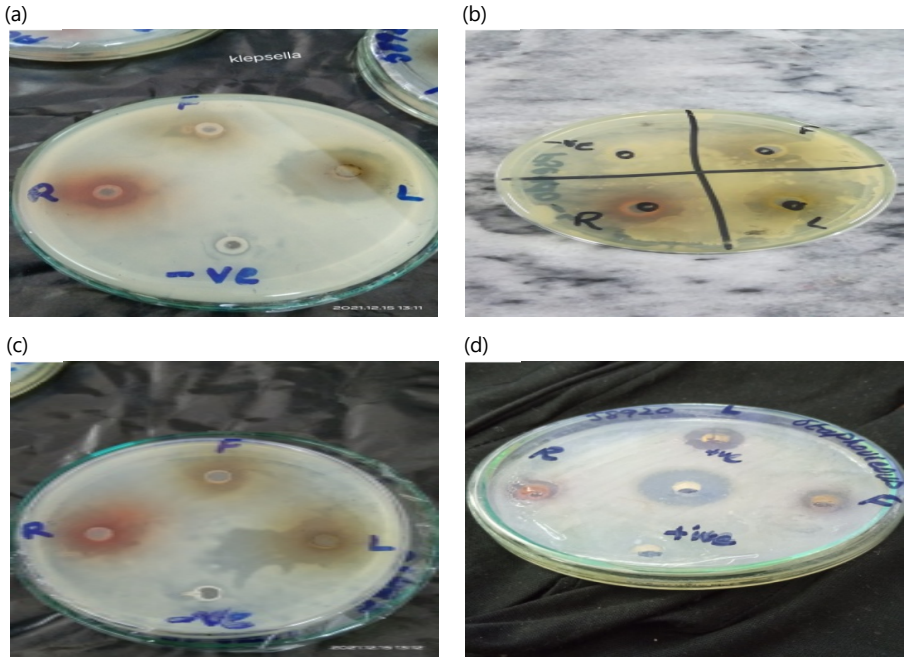

Fig. 2(a-d): Antibacterial activity of *Fragaria nubicola* (Lindl.ex Hook.f.) Lacaita, (a) *Klebsielly pneumonia*, (b) *Pseudomonas aeruginosa*, (c) *Acinetobacter baumannii* and (d) *Staphylococcus aureus*

Table 4: Antibacterial activity of *Fragaria nubicola* fruit

	Fruit ZOI (mm)			Antibiotic ZOI (mm)				
Bacterial strain	R1	R2	R3	R1	R2	R3	Means±SD (root)	Means±SD (antibiontic)
Pseudomonas aeruginosa	13	17.2	20.5	27	25	29	16.9±1.83	27±1.41
Staphylococcus aureus	14	16.7	21	30	29	31	17.2±1.76	30±0.70
Klebsiella pneumonia	15	20	22	21	23	29	19±1.88	24.33±2.16
Acinetobacter baumannii	12	16	18	27	26	29	15.33±1.57	27.33±2.33

The fruit extract dissolved in methanol exhibited antifungal activity with the most significant effect against *Aspergillus niger* (24.2 mm in R3) and the least effect against *Candida albicans* (11.2 mm in R3). The largest zone of inhibition in R3 was 41 mm against *Aspergillus flavus*, while the lowest zone of inhibition against *Candida albicans* upon antibiotic exposure was 23 mm (Table 3, Fig. 1a-c).

Antibacterial activity of *Fragaria nubicola:* The methanol-soluble fruit extract of *Fragaria nubicola* exhibited antibacterial activity against *Klebsiella pneumoniae,* with the highest zone of inhibition (R1) of 12 mm. In comparison, antibiotic treatment against *Staphylococcus aureus* resulted in a maximum zone of inhibition of 31 mm (R3) and a minimum zone of inhibition of 21 mm (R1) for *Klebsiella pneumoniae*. DMSO used as a negative control, showed no significant effect on bacterial growth (Table 4).

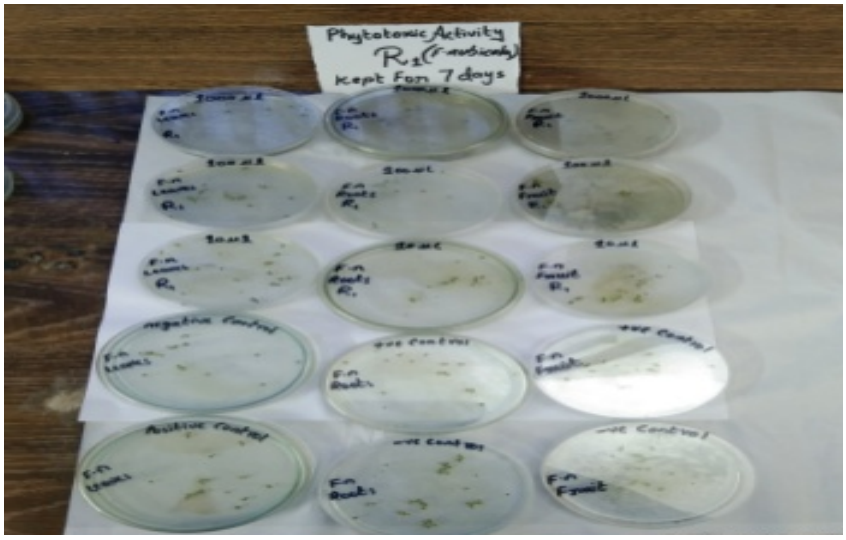

Fig. 3: Phytotoxic activity of *Fragaria nubicola* experimental results

Table 5: Antibacterial activity of *Fragaria nubicola* root

	Root ZOI (mm)			Antibiotic ZOI (mm)				
Bacterial strain	R1	R2	R3	R1	R2	R3	Means±SD (root)	Means±SD (antibiotic)
Pseudomonas aeruginosa	16	20.5	22.5	27	25	29	19.66±1.74	27±1.414
Staphylococcus aureus	15.6	20.7	21	32	27	31	19.1±1.64	26.66±2.68
Klebsiella pneumonia	18.7	22	30	37	30	39	23.56±3.01	35.33±2.52
Acinetobacter baumannii	19	28	22	27	33	29	23±2.35	29.66±1.55

The root methanolic extract demonstrated an overall maximum zone of inhibition in R3 (30 mm) against *Klebsiella pneumoniae*, with a minimum zone of inhibition in R1 (15.6 mm) against *Staphylococcus aureus*. For the antibiotic control, the minimum zone of inhibition (R1) was 27 mm against Pseudomonas aeruginosa and *Acinetobacter baumannii*, while the maximum inhibition zone was 37 mm (R1) against *Klebsiella pneumoniae*. The DMSO, the negative control, did not inhibit bacterial growth (Table 5).

The leaf extract of *F. nubicola*, dissolved in methanol, exhibited antibacterial activity against *Klebsiella pneumoniae, Acinetobacter baumannii, Staphylococcus aureus,* and *Pseudomonas aeruginosa*. The largest zone of inhibition was observed in R3 for *A. baumannii* (24 mm), and the smallest zone of inhibition was observed for *P. aeruginosa* in R1 (15.5 mm). Antibiotic treatment showed a maximum zone of inhibition of 39 mm (R3) against *Klebsiella pneumoniae*. As expected, DMSO did not inhibit bacterial growth (Table 6, Fig. 2a-d).

Phytotoxic assay of *Fragaria nubicola:* The methanolic extract of *Fragaria nubicola* demonstrated significant growth inhibition in *Lemna minor* at all tested concentrations. At 10 µL, the fruit extract exhibited 62% growth inhibition, while at 100 µL, the inhibition increased to 78.1%, and at 1000 µL, it reached 85.5%. The leaf extract showed a growth inhibition of 60.7% at 10 µL, 64.6% at 100 µL, and 93.3% at 1000 µL. The root extract displayed a growth inhibition of 67.7% at 10 µL, 81.3% at 100 µL, and 91.3% at 1000 µL. These results confirm the phytotoxic potential of *F. nubicola* extracts and their capacity to adversely affect the growth of *Lemna minor* (Table 7, Fig. 3).

Cytotoxic activity of *Fragaria nubicola:* The cytotoxic potential of the methanolic extract of *F. nubicola* leaves was investigated using brine shrimp larvae (Table 8). At a concentration of 1000 ppm, all the shrimp larvae were dead. At 100 ppm, nine shrimp larvae were dead and one remained alive, while at 10 ppm, three shrimp larvae survived, and seven were dead. The highest mortality rate (100%) was recorded at 1000 ppm, indicating that the leaf extract contains active cytotoxic components.

Table 6: Antibacterial activity of *Fragaria nubicola* leaf

	Leaf ZOI (mm)			Antibiotic ZOI (mm)				
Bacterial strain	R1	R2	R3	R1	R2	R3	Means±SD (root)	Means±SD (antibiotic)
Pseudomonas aeruginosa	15.5	20.5	22	22	29	27	19.33±1.81	26±1.88
Staphylococcus aureus	16	19	20	32	30	31	18.33±1.10	31±0.70
Klebsiella pneumonia	20	18.7	22	37	28	39	20.23±0.82	34.66±3.15
Acinetobacter baumannii	18	22	24	27	30	32	21.33±1.57	29.66±1.27

Table 7: Phytotoxic activity of *Fragaria nubicola*

		No. of fronds (before)			No. of fronds (after)			Negative control				
Plant parts	Concentrations	R1	R2	R3	R1	R2	R3	R1	R2	R3	Mean value	GI (%)
Leaves	10 µL	28	28	27	7	10	16	30	30	30	11	60.7
	100 µL	30	28	20	12	9	11	30	30	30	10.6	64.6
	1000 µL	30	38	38	2	3	1	30	30	30	2	93.3
Roots	10 µL	28	28	28	11	8	10	30	30	30	9.66	67.7
	100 µL	28	28	28	4	6	7	30	30	30	5.66	81.3
	1000 µL	26	33	30	4	3	1	30	30	30	2.6	91.3
Fruit	10 µL	26	23	28	12	11	10	29	29	29	11	62
	100 µL	28	29	28	7	6	6	29	29	29	6.3	78.1
	1000 µL	26	28	24	4	2	4	29	29	29	3.33	88.5

Table 8: Cytotoxic activity of *Fragaria nubicola*

		No. of shrimps			Shrimps alive			Shrimps dead			Dead (%)		
Plant parts	Concentrations	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
Leaves	10 ppm	10	10	10	1	3	1	9	7	9	90	70	90
	100 ppm	10	10	10	1	1	0	9	9	10	90	90	100
	1000 ppm	10	10	10	0	0	0	10	10	10	100	100	100
Fruit	10 ppm	10	10	10	2	1	1	8	9	9	80	90	90
	100 ppm	10	10	10	1	1	0	9	9	10	90	90	100
	1000 ppm	10	10	10	0	0	0	10	10	10	100	100	100
Root	10 ppm	10	10	10	1	2	2	9	8	8	90	80	80
	100 ppm	10	10	10	0	1	0	10	9	10	100	90	100
	1000 ppm	10	10	10	0	0	0	10	10	10	100	100	100

Table 9: Antioxidant activity of *fragaria nubicola* leaves, fruits and roots

Plant part used	Concentrations (mg/mL)	DPPH (%) Inhibition (µg/mL) Mean±S.E.M	IC_{50}
Fruit	1000	70.22±0.08	175
	500	58.36±0.30	
	250	52.15±0.20	
	125	48.28±0.40	
Root	1000	73.66±0.02	200
	500	60.10±0.10	
	250	55.12±0.16	
	125	49.50±0.11	
Leaf	1000	74.50±0.40	205
	500	60.82±0.16	
	250	48.60±0.26	
	125	49.93± 0.51	

Similarly, the cytotoxic effect of the methanolic extract of *F. nubicola* fruit was tested on brine shrimp larvae. At 10 ppm, one shrimp was alive and nine were dead; at 100 ppm, two shrimp were alive and eight were dead. All shrimp were dead at 1000 ppm, with the maximum mortality rate (100%) observed at this concentration. These results suggest that the fruit extract possesses cytotoxic components, further supporting its cytotoxic potential.

The methanolic extract of *F. nubicola* root was also tested for cytotoxicity on brine shrimp larvae. At 10 ppm, only one shrimp was alive, with the remaining nine dead, and all shrimp were dead at a concentration of 1000 ppm. The root extract demonstrated strong cytotoxicity, suggesting the presence of potent cytotoxic compounds.

Antioxidant activity of root, fruit, and leaf of *F. nubicola:* The antioxidant potential of the medicinal plant *F. nubicola* was evaluated in its different parts using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay. The results indicated that the plant extract exhibited significant antioxidant activity, as evidenced by the color change of DPPH from purple to yellow. This change signifies the plant's ability to scavenge free radicals, further supporting its antioxidant potential (Table 9). The DPPH% inhibition increased with concentration for all plant parts, with the highest inhibition observed at 1000 mg/mL. The fruit exhibited the lowest IC_{50} value (175 µg/mL), indicating the strongest antioxidant activity, followed by the root (200 µg/mL) and leaf (205 µg/mL).

DISCUSSION

Fragaria nubicola, a member of the Rosaceae family, is traditionally used in indigenous medicine for treating a wide range of ailments such as skin conditions, cardiovascular diseases, wounds, and gastrointestinal disorders. This study assesses the pharmacognostic properties and explores the biological activities of the fruits, roots, and leaves of *F. nubicola*. The research focuses on examining the phytochemical, elemental, and powder drug characteristics, along with evaluating the plant's phytotoxic, cytotoxic, antibacterial, and antioxidant activities[3,20,21].

The methanolic extracts of *F. nubicola* leaves, roots, and fruits demonstrated significant biological activities against a variety of pathogenic microorganisms. Specifically, the extracts exhibited potent antibacterial effects against both gram-negative (*Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae*) and gram-positive (*Staphylococcus aureus*) bacterial strains, in addition to displaying antifungal activity against *Aspergillus niger, Aspergillus flavus*, and *Candida albicans*. These findings align with previous research, including that of Pangeni *et al.*[23], who utilized the disc diffusion method and observed similar antibacterial properties in *F. nubicola* leaves.

Specifically, the methanolic fruit extract exhibited a zone of inhibition of 22 mm against *Klebsiella pneumoniae* and 12 mm against Acinetobacter baumannii. The root extract demonstrated effective inhibition with zones of 28 mm against *Acinetobacter baumannii* and 12 mm against *Staphylococcus aureus*. The leaf extract showed zones of 24 mm for *Acinetobacter baumannii* and 15.5 mm for Pseudomonas aeruginosa. These findings highlight *F. nubicola* as a promising source for the treatment of a wide variety of bacterial infections[24].

In addition to its antibacterial properties, the methanolic extracts also exhibited strong antifungal activity. The root, fruit, and leaf extracts showed notable inhibition against *Candida albicans* (26.4 mm), *Aspergillus flavus* (26.1 mm), and *Aspergillus niger* (24.2 mm), indicating the potential of *F. nubicola* as an antifungal agent[4].

Phytotoxicity testing revealed that *F. nubicola* extracts have the potential to inhibit plant growth, particularly in *Lemna minor*. This finding suggests that the plant could be explored for its use as a natural herbicide or as a control agent for invasive plant species. The phytotoxic effects were concentration-dependent, reinforcing the plant's potential in agricultural applications[25].

Cytotoxicity assays conducted on brine shrimp larvae demonstrated the plant's potential anticancer properties. The leaf extract exhibited a 100% mortality rate at a concentration of 1000 ppm, providing further evidence of its cytotoxic potential. This result supports the hypothesis that *F. nubicola* contains bioactive compounds with anticancer activity[26].

Furthermore, the antioxidant activity of the methanolic extracts was evaluated using the DPPH radical scavenging assay[27]. The leaf extract showed the highest antioxidant potential, with significant free radical scavenging activity at a concentration of 500 ppm. Both the root and fruit extracts also demonstrated noteworthy antioxidant effects, particularly the fruit extract at 500 ppm. These findings are consistent with the work of Bahukhandi *et al.*[4], who reported strong antioxidant activity in *F. nubicola* from India.

Fragaria nubicola demonstrates considerable pharmacological potential, with notable antimicrobial, cytotoxic, phytotoxic, and antioxidant properties. These findings validate its traditional uses and suggest that *F. nubicola* could serve as a valuable natural resource for the development of therapeutic agents. Further studies and clinical evaluations are necessary to fully understand the therapeutic potential of this plant[25].

Based on the findings of this study, future research should explore the phytotoxic properties of *F. nubicola*, which shows the potential for developing natural herbicides to sustainably manage weeds and reduce crop yield losses in agriculture. Its cytotoxic and antimicrobial properties highlight its promise for developing novel therapeutic agents, including anticancer drugs and antibiotics, warranting further studies to isolate and characterize the active compounds and investigate their mechanisms of action[4]. To fully understand its therapeutic potential, *in-vivo* and *in-vitro* studies are needed to assess the bioavailability, toxicity, and efficacy of its bioactive compounds in living systems. Comprehensive research is also necessary to identify and quantify its bioactive compounds, facilitating the development of standardized extracts for pharmaceutical applications[26]. Additionally, raising awareness about the sustainable use and conservation of *F. nubicola* is crucial, with efforts to educate local communities on its benefits and proper methods for harvesting and preserving the plant.

CONCLUSION

The methanolic extract of *F. nubicola* exhibits significant antibacterial and antifungal activities, positioning it as a valuable source for the development of novel antimicrobial agents capable of combating a wide array of pathogenic microorganisms. The findings from this study highlight the plant's potent phytotoxic effects, particularly in its impact on *Lemna minor*, suggesting that *F. nubicola* could be used as a natural herbicide to control weeds and reduce crop losses. Furthermore, the plant contains active cytotoxic components, as evidenced by the cytotoxicity assays, indicating its potential in cancer treatment. Additionally, the plant's strong antioxidant capacity further supports its potential for promoting health and preventing oxidative stress-related diseases. Overall, *F. nubicola* offers promising therapeutic properties that warrant further investigation for its application in pharmaceutical and agricultural industries.

SIGNIFICANCE STATEMENT

Fragaria nubicola exhibits strong antimicrobial, phytotoxic, cytotoxic, and antioxidant properties. Its extracts effectively inhibit fungal (*Aspergillus spp*.) and bacterial (*Klebsiella pneumoniae*, *Acinetobacter baumannii*) growth. The leaf extract shows high phytotoxicity (93.3% inhibition of *Lemna minor*), while all extracts display cytotoxicity against brine shrimp larvae. Its potent antioxidant activity highlights its potential for pharmaceutical and agricultural applications.

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