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PHYTOCHEMICAL PROFILING, CYTOTOXIC POTENTIAL, AND NUTRITIONAL COMPOSITION OF *PROSOPIS JULIFLORA* AND *MORUS ALBA* EXTRACTS AND THEIR EFFECTS ON ANIMAL HEALTH

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Abstract

Bioactive substances of therapeutic and nutritional value can be found in medicinal plants. The phytochemical makeup, cytotoxic capability, and proximate nutritional profile of methanolic extracts from Prosopis juliflora and Morus alba were compared in this study. Standard analytical techniques were used to determine the approximate composition, and qualitative phytochemical screening for key secondary metabolites was carried out. A brine shrimp (Artemia salina) lethality assay was used to assess the cytotoxic activity at doses of 10, 100, and 1000 μg/mL⁻¹. GC–MS analysis was used to identify the chemical components. In comparison with M. alba (89.4%, 3.0%, 4.5%, and 3.0%, respectively), P. juliflora had greater nutritional value in terms of dry matter (95.5%), crude protein (9.1%), crude fibre (10.4%), and total ash (9.1%). Compared with P. juliflora (2.1%), M. alba had a higher crude fat content (4.0%). Both species contained alkaloids, flavonoids, phenols, coumarins, and saponins, according to the results of the phytochemical screening. In contrast to M. alba, which was characterized by acetone (49.54%), trichloromethane (21.30%), n-hexadecanoic acid (19.10%), and 9-octadecenoic acid methyl ester (8.00%), P. juliflora (81.96%) was characterized by GC–MS analysis, which revealed trichloromethane as the predominant constituent. P. juliflora caused 80% death, and M. alba caused 70% mortality at 1000 μg mL⁻¹, according to cytotoxicity tests that revealed a concentration-dependent increase in mortality. Significant effects of species (P = 0.0032) and concentration (P 0.001) on brine shrimp

Frontier in Medical & Health Research

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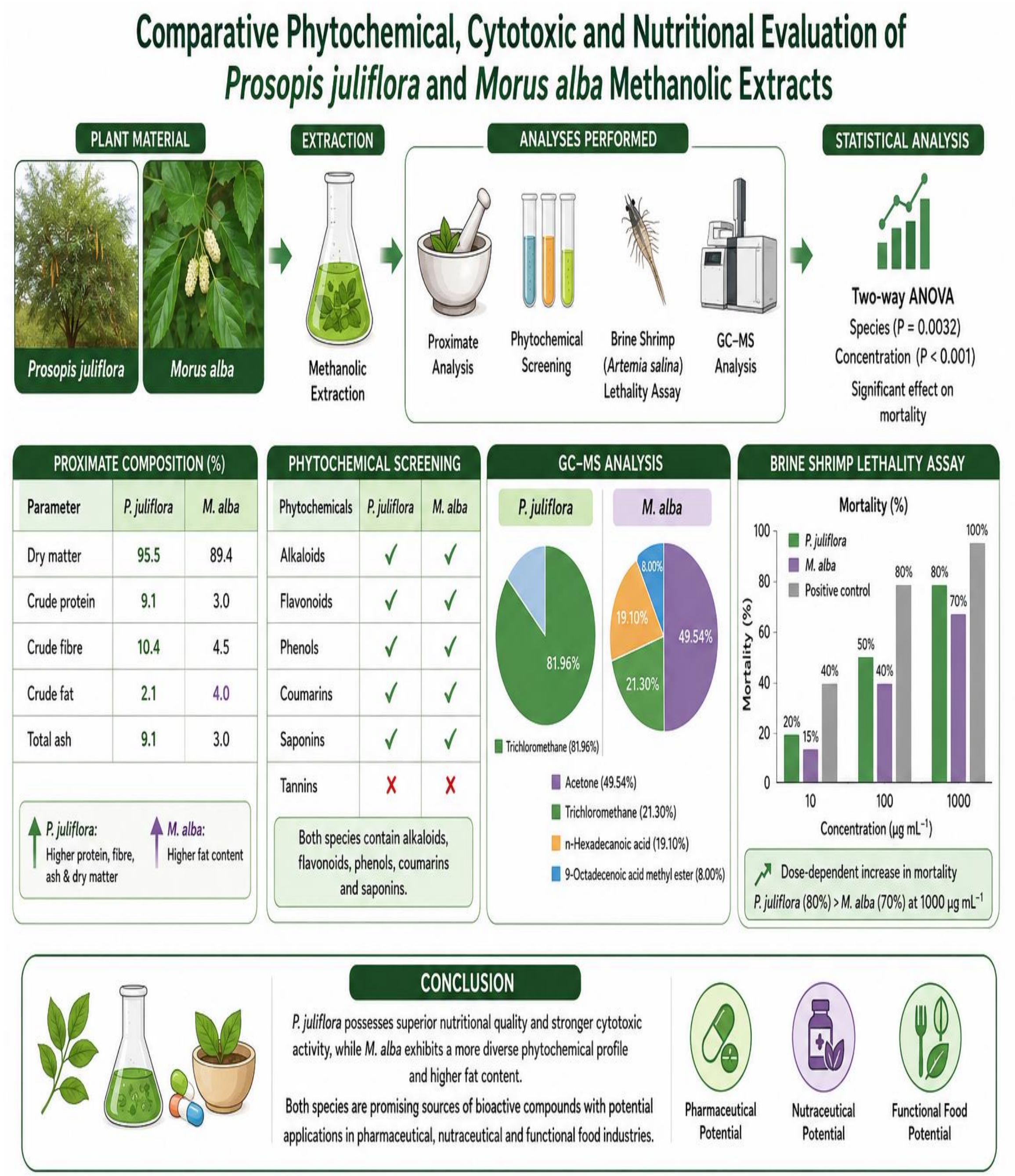
mortality were confirmed by two-way ANOVA. The results show that whereas M. alba has a more varied phytochemical profile and a higher fat content, P. juliflora has better nutritional quality and stronger cytotoxic activity. Both species are good sources of bioactive substances that can be used in the functional food, pharmaceutical, and nutraceutical industries.



Graphical abstract for phytochemical profiling, cytotoxic potential, and nutritional composition of *Prosopis juliflora* and *Morus alba* extracts and their effects on animal health

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INTRODUCTION

Medicinal plants have been utilized as significant sources of nutritional supplements and therapeutic substances. In recent years, growing concerns regarding the adverse effects of synthetic drugs have increased interest in plant-derived bioactive compounds. Alkaloids, flavonoids, tannins, saponins, phenolics, and glycosides are among the many secondary metabolites found in these natural products. These metabolites support a variety of biological activities, including antioxidant, antimicrobial, anti-inflammatory, and anticancer effects (Rashid et al., 2023; Lephatsi et al., 2023). To identify plants with medicinal and nutraceutical potential, phytochemical screening, cytotoxicity assessment, and nutritional evaluation have become crucial methods.

Mesquite, also called kikar or *Prosopis juliflora*, is a drought-tolerant leguminous tree that grows widely in tropical and subtropical climates. The plant has been widely used for fuelwood, fodder, soil stabilization, and traditional medicine because of its propensity to flourish in arid areas (Zhong et al., 2022; Afaq et al., 2024). Numerous pharmacological actions of P. juliflora have been attributed to the presence of flavonoids, alkaloids, phenolic compounds, tannins, and saponins in various portions of the plant (Pikhtirova et al., 2023; Bashir et al., 2022).

The flavonoid-rich composition of plants is primarily responsible for their antioxidant qualities, antifungal activity against several pathogenic fungi, and antibacterial activity against both gram-positive and gram-negative bacteria (Sharifi-Rad et al., 2023; Gupta, 2024). Moreover, alkaloid extracts of P. juliflora have demonstrated significant cytotoxic effects against leukemia cell lines, suggesting their possible role in anticancer drug development (Vogel et al., 2024).

White mulberry, or *Morus alba* L., is another important medicinal species that has been grown worldwide for many years. The plant, which is native to China, is prized for its biologically active chemicals and vital nutrients found in its leaves, fruits, bark, and roots (Asfaw et al., 2022; Xie et al., 2026). According to phytochemical research, M. *alba* contains flavonoids, phenolic acids, alkaloids, glycosides, derivatives of resveratrol, and a number of antioxidant compounds (Batiha et al., 2023; Fatima et al., 2024).

Important components with antidiabetic, hepatoprotective, neuroprotective, antibacterial, and anticancer properties include quercetin, rutin, chlorogenic acid, caffeic acid, morin, oxyresveratrol, and 1-deoxynojirimycin (Mehmood et al., 2025; Tricase et al., 2025). Mulberry leaves are beneficial for both human nutrition and animal feed applications since they are high in vitamins, minerals, proteins, and dietary fibre (Maqsood et al., 2022; Liu et al., 2025).

The growing incidence of cancer and the ongoing search for better treatment options have drawn significant attention to the study of the cytotoxic qualities of medicinal plants. An essential first step in finding physiologically active substances with possible anticancer properties is screening for cytotoxicity. Because of its ease of use, affordability, dependability, and good association with pharmacological activities such as anticancer, pesticidal, and antibiotic properties, the brine shrimp lethality assay is among the most popular bioassays (Zhivikj et al., 2022; Vogel et al., 2024). Plant extracts that are significantly deadly to the brine shrimp nauplii are frequently regarded as potential candidates for additional pharmacological research.

In addition to biological activity, nutritional content is an important consideration in determining the overall utility of medicinal plants. Proximate analysis provides information on moisture content, ash, crude protein, crude fibre, lipids, and carbs, all of which are important indicators of nutritional quality. Determining these factors helps to determine whether plant materials are acceptable for use in food, feed, and nutraceuticals. Prior study has emphasised the nutritional value of *P. juliflora* and M. *alba* due to their high protein, mineral, fibre, and bioactive phytochemical content (Ondiba et al., 2022; Saini et al., 2023).

The identification of phytochemicals found in medicinal plants has been further improved by the use of sophisticated analytical methods such

Frontier in Medical & Health Research
ISSN: (e) 3007-1607 (p) 3007-1593

as gas chromatography–mass spectrometry (GC–MS). The characterization of volatile and semivolatile substances is made possible by GC–MS analysis, which also offers important information about the chemical components responsible for the biological activities that have been observed (Murphy et al., 2024). When combined with cytotoxicity tests and qualitative phytochemical screening, GC–MS is an effective method for assessing the medicinal potential of plant extracts.

Prosopis juliflora and *Morus alba* have been studied separately for their nutritional and therapeutic qualities, but few comparison studies have investigated their phytochemical profiles, cytotoxic effects, and nutritional content. Thus, the goal of the current study was to evaluate the phytochemical components, cytotoxic potential, and approximate nutritional makeup of extracts from *M. alba* and *P. juliflora.* In this study, the biological and nutritional traits of the two species were also examined, and GC–MS analysis was used to determine the main bioactive chemicals. These results may aid in the creation of plant-based medicinal substances and offer proof of their application in the food, pharmaceutical, and nutraceutical sectors.

Materials and Methods

Proximate analysis of *Prosopis juliflora* and *Morus alba*

To determine the compound constitution of *P. juliflora* and *M. alba.* The moisture substance was estimated by the Nancy-Trautmann strategy. Most celebrated Kjeldahl strategies are used to determine the unrefined protein percentage, and extraction of rough protein is completed with a Soxhlet device. Corrosive base treatments were used to estimate the percentage of unrefined fibres.

Moisture content

Nancy Trautmann and Tom Richard established a protocol for determining the moisture content of the two species. Right away, a little holder was weighed, and the compartment weighed 0.942 g; at this time, 1 g of moist *P. juliflora* and *M. alba* was collected. Powders were placed in that little compartment and dried in a burner at 105-110 °C for 24 hours; the sample was then weighed again, the weight of the holder was deducted, and the moisture content was calculated using the equation provided.

Mn=((Ww-Wd)/Ww x 100

Mn is the moisture content (%) of material n, Ww is the wet weight of the sample, and Wd is the weight of the sample after drying (Ashraf et al., 2013).

Dry matter %

Dry matter was determined according to the methods of Eric (2009) with the help of the following formula:

Dry matter % = 100- moisture content.

Protein %

The total nitrogen content of the samples was controlled by the miniaturized-scale Kjeldahl strategy. Finally, 1 g of ground material was placed in a processing carafe with 3 g of assimilation blend (mercury sulfate $HgSO_4$ and potassium sulfate K_2SO_4 at proportions of 1:9 and 20 mL of concentrated $H2SO_{4,\ \text{respectively})}$. the samples were bubbled in an absorption mechanical assembly for approximately 2 hours until the substance turned clear. The processed material was weakened to 250 mL. Ten ml was moved to the small-scale Kjeldahl refining device and refined to 50 mg of Zn residue and 10 mL of NaOH (40%). The distillate was gathered in a beneficiary containing 5 mL of 2% corrosive boric acid and methyl red as a pointer. The substance of the collector was titrated against the sulfuric acid corrosive to a light pink shading end-point. From the volume of the corrosive agent, the level of nitrogen was assessed, and protein was resolved with this equation.

Nitrogen %= volume of 0.1 N H2SO4 × 0.0014 × 250 × 100/weight of samples × 10

Protein %= Nx6.25

Fat

To estimate the lipid content, a dried sample (2 g) was separated with oil ether at 400 °C–600 °C in a Soxhlet device to evaluate the ether solvent segment. The extricated material was dried to a steady weight in a stove at 700 °C. The lipid

Frontier in Medical & Health Research
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substance content was determined utilizing the following formula:

Fat % = weight of ether extract × 100/weight of sample

Crude fibre

A known load of the test was bubbled through 1.25% NaOH, which was then trailed with 1.25% H2SO4 to break up the antacid and corrosive solvent parts. The buildup containing rough fibres was dried to a steady weight. The loss of weight on start in a mute heater at 500 degrees centigrade was utilized to compute the unrefined fibre as follows: Rough fibre% = misfortune in weight on ignitionx100/weight of the example.

Phytochemical analysis of plant extracts

Test for alkaloids

Mayer's reagent: In sixty ml of water, mercuric chloride (0.356 g) was broken up, and potassium iodide (5 g) was disintegrated in 20 mL of water. The two preparations were diversified, and the volume was made up to 1000 mL with refined H_2O. Dragendorff's reagent: Solution A: In 80 mL of refined water, basic tartaric corrosive (20 g) and bismuth nitrate (1.7 g) were broken up. Arrangement B: In 40 mL of refined water, potassium iodide (16 g) was disintegrated. Arrangements A and B were mixed at a 1:1 ratio. After 8 ml of 1% HCl was added, the sample was separated and warmed, and the plant was removed (0.5 g). The filtrate was treated independently with Dragendorff's reagent and Mayer's reagent. Precipitation and turbidity were observed to affect the proximity of the alkaloids.

Test for flavonoids

Separate plants (0.5 g) were mixed or shaken with petroleum-ether to remove the greasy materials. The remaining material was broken up in 20 mL of 80% ethanol and separated. Afterward, the filtrate was mixed with four ml of 1% KOH. Dim yellow shading was used to indicate the presence of flavonoids.

Test for coumarins

Separate plants (0.5 g) were placed in a small test tube and secured with filter paper moistened with 1 N NaOH. The test cylinder was put in bubbling H_2O for several minutes. The filter paper was detached and scanned for yellow fluorescence under UV light to demonstrate the nearness of the phytochemical coumarins.

Test for phenols

Concentrates of plants were treated with three to four drops of newly arranged $FeCl_3$. The appearance of light blue dusky shading reflected the proximity of the phenols.

Test for saponins

Plant removal (0.5 g) was broken down in a test tube and in bubbling water, which was then allowed to shake and cool together. Foam formation was observed to demonstrate the nearness of the saponins.

Test for tannins

In 20 mL of refined water, the plant removed (0.5 g) was bubbled in a test tube and sifted. To the filtrate, $FeCl_3$ (0.1%) was added. The presence of tanish green or bluish black indicated the presence of tannins. Test for glycosides. The concentrate (0.5 g) was shattered down in a rigid acidic (2.0 mL) corrosive solution comprising 1 drop of $FeCl_3$ (0.1%), after which it was rested with concentrated H_2SO_4 (1.0 ml). A darker circle at the boundary indicates the presence of glycosides.

Collection and extraction of plant material

The plant material was taken from the Sargodha district in Pakistan. The solid and fresh portions of the species were also collected and cleaned or washed with water. The samples collected at the research facility were dried at room temperature. The dry samples were minced to a high grade, and chloroform and methanol were absorbed exclusively for 7 days in round bottom flagons using filtration. The concentrations of the two species were collected at 40 °C using a vacuum rotary evaporator at the research facility. The concentrations were assessed, named, and stored at 4 °C for subsequent testing.

ISSN: (e) 3007-1607 (p) 3007-1593

Cytotoxic potential of the two invasive species

Brine shrimp lethality assay

The potential for cytotoxicity was explored by measuring the lethality of saline water-treated shrimp as per the convention of Rehman et al. (2005). Saline solution-dried shrimp eggs were taken into a slightly parceled container containing counterfeit ocean H_2O (38 g/L, pH=8.5). Brackish water nauplii shrimp with a 2^{nd} step were utilized to accomplish the test. In this testing, each and every concentrate in 3 fixations (1000, 100 and 10 ppm) was placed into minor sterile vessels in triplicate (9 vials/remove). At room temperature, approximately 10 shrimps were added to every vessel using Pasteur's pipette. After twenty-four hours, the vessels were maintained under light, and the stayers were tallied. The subsequent information was surveyed by utilizing a formula; all the data were assessed by an investigation (LdP Line Software) to regulate the "Lethality Dose 50" (LC50) at ninety-five certainty intervals.

GC–MS Analysis

The chemical constituents of the methanolic extracts of *Prosopis juliflora* and *Morus alba* were analysed using gas chromatography–mass spectrometry (GC–MS). The analysis was performed on a GC–MS system equipped with a capillary column (30 m × 0.25 mm × 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. The injector temperature was maintained at 250 °C, and 1 µL of sample was injected in split mode. The oven temperature was initially set at 60 °C for 2 min, increased at a rate of 10 °C min^{-1} to 280 °C, and held at that temperature for 10 min. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV with a scan range of m/z 40–600. The ion source and transfer line temperatures were maintained at 230 °C and 280 °C, respectively.

The identification of phytochemical constituents was accomplished by comparing the obtained mass spectra with those available in the National Institute of Standards and Technology (NIST) mass spectral library. The relative percentage composition of each compound was calculated from the peak area of the chromatogram. The identified compounds were subsequently evaluated for their possible biological and pharmacological significance on the basis of published literature.

Results

Analysis of the proximate composition of *Prosopis juliflora*

Prosopis juliflora has a relatively low moisture content (8.3%) and a high dry matter content (95.51%). 9.1% crude protein, 2.1% crude fat, 10.4% crude fibre, and 9.1% total ash were found in the plant. This species' high protein, fibre, and ash content highlights its nutritional significance and raises the possibility of using it as a useful source of minerals and nutrients (Table 1, Figure 1a).

Table 1: Proximate composition analysis of *Prosopis juliflora*

Nature of sample	*Prosopis juliflora*
Dry matter%	95.51%
Moisture%	8.3%
Crude protein%	9.1%
Crude fat %	2.1%
Crude fibre%	10.4%
Total Ash %	9.1%

***Prosopis juliflora* GC–MS Analysis Peak Report**

The GC–MS analysis of *Prosopis juliflora* revealed a number of chemical components. With 81. the overall peak area, trichloromethane was the most common chemical. Smaller amounts of other chemicals were found. The prevalence of trichloromethane suggests that it plays a

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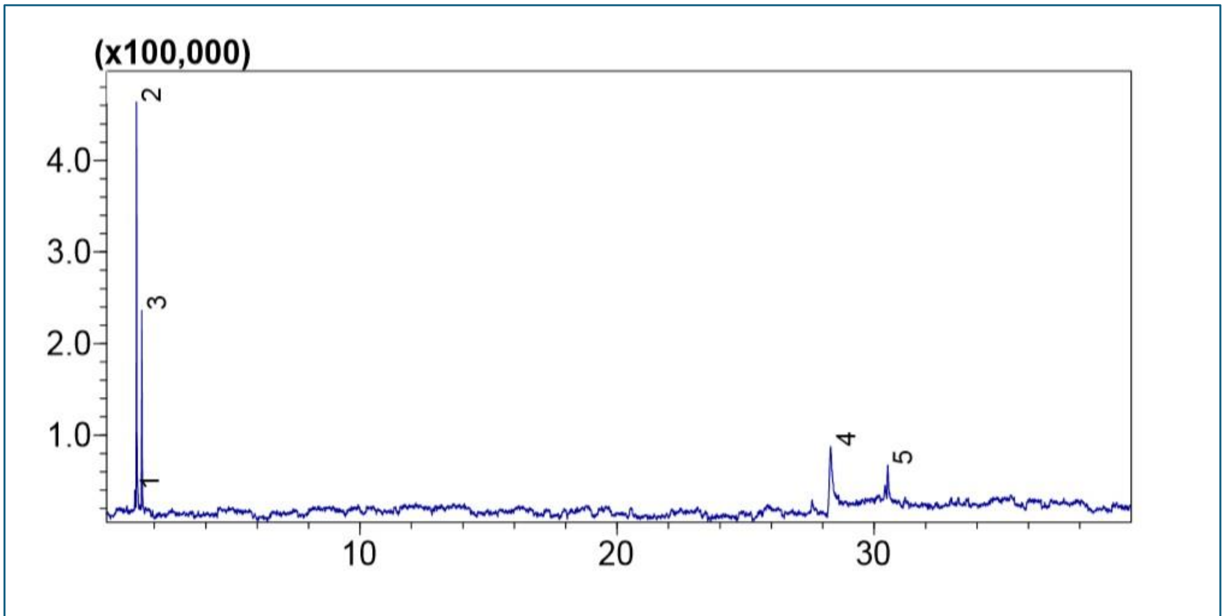
significant role in the extract's phytochemical composition (Figure 1b).



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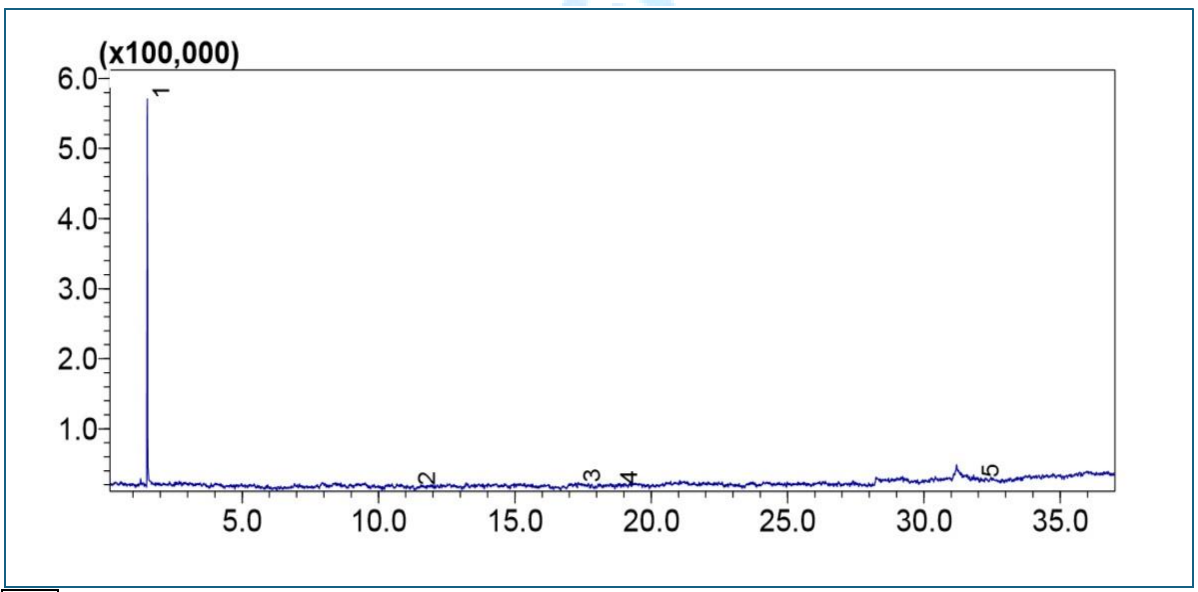

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Figure 1 (1a–1b): GC–MS analysis of (1a) *Prosopis juliflora* and (1b) *Morus alba*

Analysis of *Morus alba's* Proximate Composition

The proximate composition of *Morus alba* revealed a moisture content of 2.6% and a dry matter content of 89.4%. 3.0% crude protein, 4.0% crude fat, 4.5% crude fibre, and 3.0% total ash were found in the species. Compared with *Prosopis juliflora*, *Morus alba* had a higher crude fat content but lower protein, fibre, and ash concentrations (Table 2).

Table 2: Proximate composition analysis of Morus alba

Nature of sample	*Morus alba*
Dry matter%	89.4%
Moisture%	2.6%
Crude protein%	3%
Crude fat %	4%
Crude fibre%	4.5%
Total Ash %	3%

Comparative Proximate Composition of *Morus alba* and *Prosopis juliflora*

Clear distinctions between the two species were shown by a comparative investigation. *Morus alba* had a higher crude fat content, but *Prosopis juliflora* had higher dry matter, crude protein, crude fibre, and total ash contents. These results suggest that the nutritional profile of *Prosopis juliflora* is relatively richer (Table 3).

Table 3: Comparative proximate composition of Prosopis juliflora and Morus alba. The values are expressed as the mean ± standard error (SE) of three replicates.

Parameter	*Morus alba* (Mean ± SE)	*Prosopis juliflora* (Mean ± SE)
Dry Matter (%)	89.40 ± 1.96	95.50 ± 2.02
Moisture (%)	2.60 ± 0.92	8.30 ± 1.33
Crude Protein (%)	3.00 ± 0.87	9.10 ± 1.21
Crude Fat (%)	4.00 ± 0.87	2.10 ± 0.29
Crude Fibre (%)	4.50 ± 1.15	10.40 ± 1.39
Total Ash (%)	3.00 ± 0.87	9.10 ± 2.08

***Morus alba* GC–MS Analysis Peak Report**

Five main components were found in *Morus alba* by GC–MS analysis. The most prevalent component was acetone (49.54%), followed by trichloromethane (21.30%), n-hexadecanoic acid (19.10%), and 9-octadecenoic acid methyl ester (8.00%). The variety of substances found indicates that *Morus alba* has a number of bioactive components that can support its biological activity (Figure 2a).

The line graph shows the relative proximal makeup of *P. juliflora* and M. alba. Prosopis juliflora (95.51%) and *M. alba* (89.40%) both have high dry matter contents. Both species had relatively low moisture contents; however, compared with *M. alba* (2.60%), Prosopis juliflora was present in greater proportions (8.30%).

In a similar vein, compared with *M. alba* (3.00%), *P. juliflora* has a significantly greater crude protein content (9.10%), demonstrating its superior protein nutritional value. *P. juliflora* recorded a crude fibre percentage of 10.40%, whereas *M. alba* recorded a crude fibre content of 4.50%. On the other hand, compared with *P. juliflora* (2.10%), *M. alba* had a greater crude fat content (4.00%). *P. juliflora* had a higher total ash content (9.10%) than *M. alba* did (3.00%), which is a measure of mineral composition. With the exception of crude fat, *P. juliflora* generally presented higher values for the majority of proximate metrics, indicating its relatively richer nutritional composition (Figures 2a 7 2b).

Cytotoxic Effects of Plant Extracts on Baby Brine Shrimps

The graph shows the cytotoxic effects of extracts from *Prosopis juliflora* and *Morus alba* against juvenile brine shrimp (*Artemia salina*) at various

Frontier in Medical & Health Research
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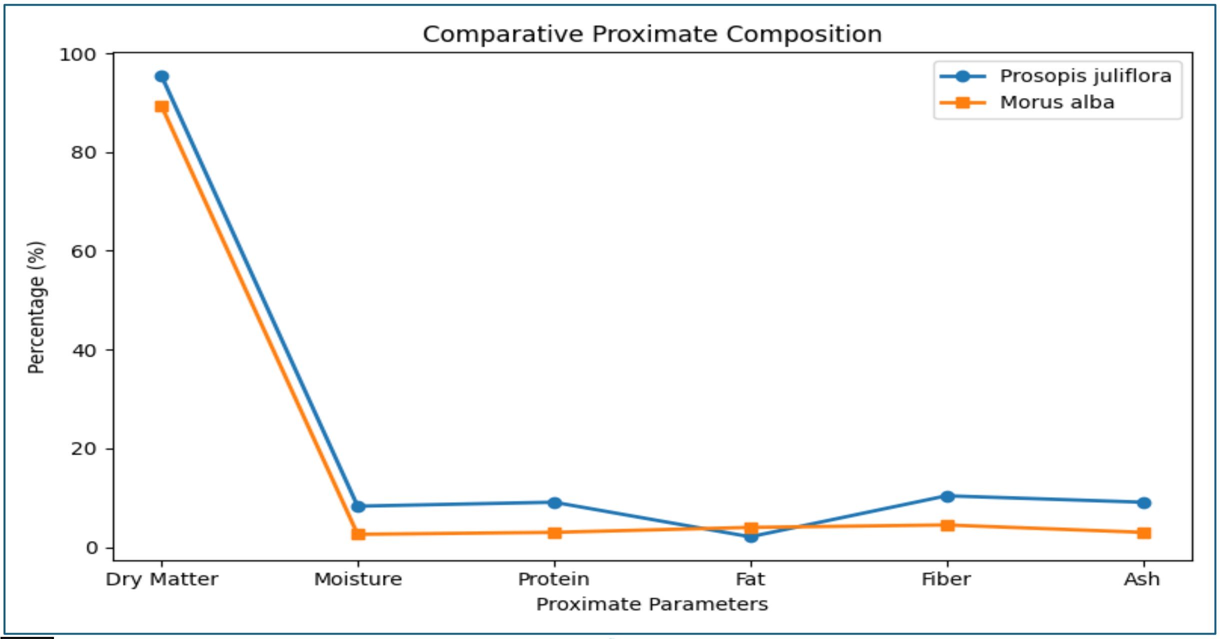
concentrations (10, 100, and 1000 µg/mL). A dose-dependent cytotoxic effect was indicated by the mortality percentage gradually increasing with increasing extract concentration.

P. juliflora resulted in 10% mortality at 10 µg/mL, but *M. alba* resulted in no mortality. For *P. juliflora* and *M. alba,* mortality increased to 40% and 30%, respectively, at 100 µg/mL. For *P. juliflora* and *M. alba,* the greatest concentration (1000 µg/mL) caused 80% and 70% mortality, respectively. At 10, 100, and 1000 µg/mL, the positive control demonstrated the greatest cytotoxic effect, resulting in 40%, 80%, and 100% death, respectively. These results show that both plant extracts have the potential to be cytotoxic, with *P. juliflora* showing somewhat higher toxicity than *M. alba* at all tested dosages. The presence of bioactive substances that can have harmful effects on brine shrimp nauplii is suggested by the increase in mortality with increasing concentration (Figure 2b).



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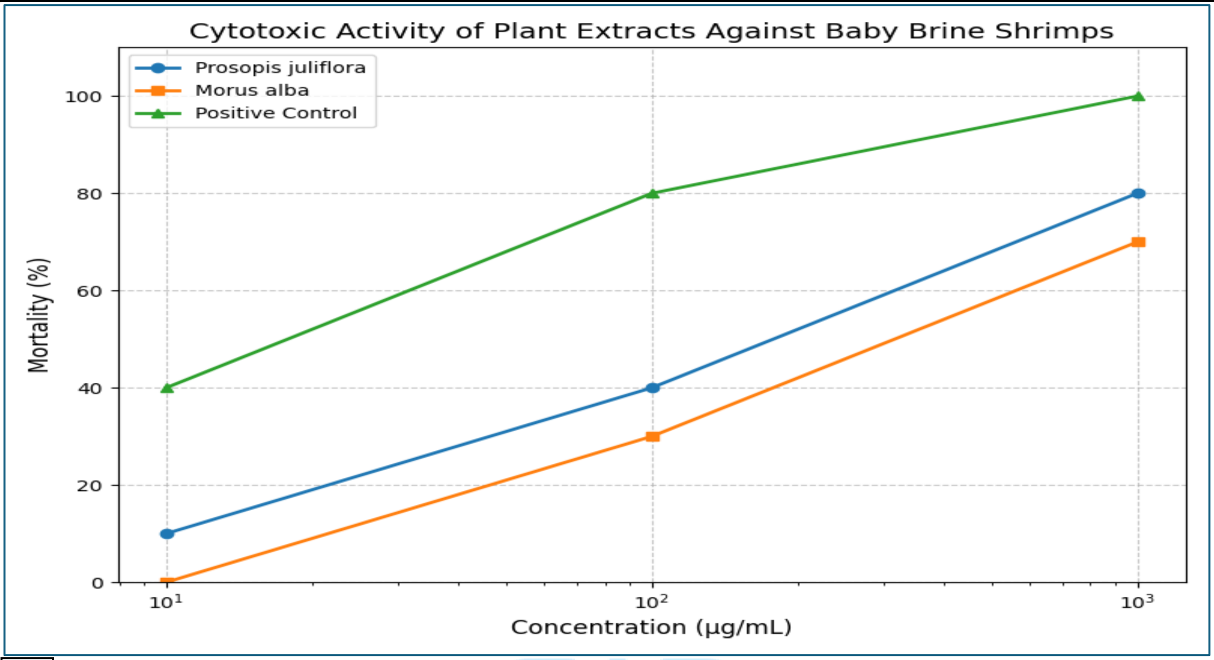

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Figure 2 (2a–2b): Comparative analysis of the proximate compositions of *Prosopis juliflora* and *Morus alba* (2a). Cytotoxic activity of plant extracts against baby brine shrimp (*Artemia salina*) at different concentrations (2b).

Effects of Plant Species and Extract Concentrations on Brine Shrimp Mortality: Two-Way Analysis of Variance (ANOVA)

Both plant species and extract concentration significantly affected brine shrimp mortality according to two-way ANOVA. While species also had a substantial effect on mortality ($P = 0.0032$), concentration had a highly significant effect ($P < 0.001$). Nevertheless, the species–concentration interaction was not significant ($P = 1.0000$), suggesting that both species responded similarly to all the concentrations tested (Table 4).

Table 4: Two-way analysis of variance (ANOVA) showing the effects of plant species and extract concentration on the mortality (%) of baby brine shrimps (Artemia salina).

Source of Variation	DF	SS	MS	F value	P value
Species	1	450	450	13.5	0.0032
Concentration	2	14800	7400	222	0
Species × Concentration	2	0	0	0	1
Error	12	400	33.33	–	–
Total	17	15650	–	–	–

Qualitative Phytochemical Evaluation of *Morus alba* and *Prosopis juliflora*

Both plant species contained alkaloids, flavonoids, saponins, coumarins, and phenols, as determined by qualitative phytochemical screening. Both extracts lacked tannins. The presence of these phytochemicals suggests that *Prosopis juliflora* and *Morus alba* have therapeutic potential and may account for their biological and cytotoxic effects (Table 5).

Frontier in Medical & Health Research
ISSN: (e) 3007-1607 (p) 3007-1593

Table 5: Qualitative phytochemical analysis of Prosopis juliflora and *Morus alba*

Phytochemical	*Prosopis juliflora* extract	*Morus alba* extract
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	-	-
Coumarins	+	+
Phenols	+	+

Discussion

Nutritional Significance and Proximate Composition

Prosopis juliflora had more dry matter (95.50%) than *Morus alba* did (89.40%), according to the proximate analysis, suggesting less moisture retention and improved stability for processing and storage. Similar results have been reported for drought-resistant legumes (Wanzala & Minyoso, 2025), whose dry matter generally increases because of the lower water content in arid settings. Improved feed value and industrial application are frequently linked to high dry matter content (Melesse et al., 2023; Ma & Hu, 2024).

P. juliflora exhibited a significantly higher crude protein content (9.10%) than *M. alba* (3.00%), indicating that it could be a better plant-based protein source. Previous research has shown that Prosopis species can fix nitrogen, which may have contributed to their enhanced protein buildup. According to previous study, Prosopis species can fix nitrogen, which explains why they store more protein. M. alba, on the other hand, is valued for its beneficial secondary metabolites rather than protein content, despite reports of moderate protein levels (Bhatla & Lal, 2023; Kumar et al., 2022).

Additionally, compared with *M. alba*, *P. juliflora* has more crude fibre (10.40%) (4.50%), which is consistent with previous research indicating that woody legumes have a greater lignocellulosic content (Gayathri & Uppuluri, 2022). Dietary fibre is associated with a lower incidence of metabolic diseases and is essential for digestive health (Bulsiewicz, 2023; Deehan et al., 2024).

M. alba, on the other hand, had a greater crude fat content (4.00%) than P. juliflora did (2.10%), suggesting a relatively richer lipid profile. This could be explained by the presence of fatty acid derivatives found in the GC–MS analysis, such as 9-octadecenoic acid methyl ester and n-hexadecanoic acid, which are recognized for their energy-rich and bioactive characteristics (Khalid et al., 2024).

Additionally, *P. juliflora* had a higher total ash content (9.10%), indicating greater mineral accumulation. High ash content is generally linked to increased availability of micronutrients, especially potassium, calcium, and magnesium (Mohanty et al., 2025).

Flavonoids and phenolic substances are well known to scavenge free radicals and lessen oxidative stress (Akbari et al., 2022; Rudrapal et al., 2022). The potential use of alkaloids in cancer treatments is supported by their cytotoxic and antitumour properties (Olofinsan et al., 2023). Additional antibacterial and anti-inflammatory properties are provided by saponins and coumarins (Wijesekara et al., 2024; Kathar Basha & Dhamodaran, 2026).

Chemical Composition and GC–MS Analysis

The chemical profiles of both plant extracts were thoroughly characterized by GC–MS analysis. In addition to minor components, trichloromethane was found to be the predominant chemical (81.96%) in P. juliflora. Similar phytochemical studies have indicated that the predominance of volatile halogenated chemicals may be attributed to extraction-related artifacts or solvent-associated residues (Odero et al., 2022; Khalid et al., 2024).

Acetone (49.54%), trichloromethane (21.30%), n-hexadecanoic acid (19.10%), and 9-octadecenoic acid methyl ester (8.00%) were among the most varied chemical profiles

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displayed by M. *alba*. The antibacterial, anti-inflammatory, and anticancer effects of fatty acid derivatives such as palmitic acid and oleic acid esters are well known (Alqurashi et al., 2022)

Cytotoxic Activity (Assay for Brine Shrimp)

Both plant extracts showed dose-dependent cytotoxic effects in the brine shrimp lethality assay. Stronger bioactivity was indicated by the greater toxicity of P. juliflora (80% death at 1000 μg/mL) than that of M. *alba* (70% mortality) (Saidu, 2023). These findings corroborate previous research showing that alkaloids and phenolic compounds in Prosopis species have strong cytotoxic and anticancer potential (Murugesh et al., 2026; Choudhari et al., 2025).

Reports of the bioactive substances of M. *alba*, such as oxyresveratrol and DNJ (1-deoxynojirimycin), which have anticancer and antioxidant qualities, are in line with the reported cytotoxicity (Tricase et al., 2025). The presence of dose-dependent bioactive phytochemicals in both species is confirmed by increasing mortality with increasing concentrations (Sethi et al., 2025).

Comparative Assessment

Overall, *Morus alba* displayed richer lipids and more varied phytochemical profiles, but *Prosopis juliflora* showed superior nutritional qualities (protein, fibre, and ash). Nonetheless, both species showed strong cytotoxic activity, suggesting possible therapeutic value. The consistency of the bioactivity patterns is further supported by the absence of an interaction effect in the ANOVA results, which indicates that both species react to concentration variations in a similar way. These results are consistent with earlier research showing that rather than primary metabolites alone, plant secondary metabolites play major roles in cytotoxic and therapeutic activities (Chinou, 2008).

Taken together, the present findings demonstrate that, owing to their rich phytochemical composition and significant cytotoxic activity, *Prosopis juliflora* and *Morus alba* possess considerable nutritional and pharmacological potential. The superior protein, fibre, and mineral contents of *P. juliflora* suggest its suitability as a valuable nutritional and feed resource, while the diverse chemical constituents identified in M. *alba* indicate a broader spectrum of bioactive compounds with possible therapeutic applications.

The presence of alkaloids, flavonoids, phenols, coumarins, and saponins in both species further supports their biological importance, as these metabolites are widely recognized for their antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (Akbari et al., 2022; Rudrapal et al., 2022; Olofinsan et al., 2023; Wijesekara et al., 2024). Moreover, the concentration-dependent cytotoxic responses observed in the brine shrimp assay reinforce the potential of these plants as sources of pharmacologically active molecules, which is consistent with previous reports on the therapeutic significance of secondary metabolites in medicinal plants (Choudhari et al., 2025; Sethi et al., 2025; Chinou, 2008). Therefore, the isolation, characterization, and validation of the active constituents of both species warrant further investigation, which may contribute to the development of novel nutraceutical and pharmaceutical products.

Conclusion

This study demonstrated that both *Prosopis juliflora* and *Morus alba* are valuable medicinal plants that possess important nutritional and bioactive properties. *P. juliflora* had a superior nutritional composition, with relatively high levels of protein (9.1%), fibre (10.4%), and ash (9.1%), indicating its potential as a nutrient-rich plant resource. M. *alba,* on the other hand, had a higher crude fat content (4.0%) and a wider range of phytochemically active chemicals according to the results of the GC–MS analysis. Qualitative screening supported the therapeutic significance of alkaloids, flavonoids, phenols, coumarins, and saponins by confirming their presence in both species. Significant dose-dependent cytotoxic effects were detected in the brine shrimp lethality assay, with *P. juliflora* showing greater activity (80% death at 1000 μg mL^{-1}) than M. *alba* did (70% mortality). Taken together, these findings indicate that both species have great potential for use as natural sources of bioactive chemicals in pharmaceutical, nutraceutical, and functional food applications. To confirm their therapeutic potential, more research involving the isolation of active ingredients and in vivo biological assessment is advised.

Declarations

Ethics approval

All the protocols used in this study were approved by the Institutional Ethics and Guideline Committee of the University of Lahore, Lahore, Pakistan. All the experimental methods of this study followed all the appropriate guidelines and regulations.

Consent for publication

All the subjects gave their “informed consent” for the publication of details within the text (“informed consent”) to be published in the above Journal and Article. Written “informed consent” was obtained from all the authors for the publication of this manuscript.

Availability of data and materials

The data generated are provided within the manuscript and are available from the author upon reasonable request.

Competing Interests

All the authors declare that there are no competing interests.

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Authors' contributions

Jahanzib Rasheed wrote the review and analysed the work; Aroosh Shabbir supervised the study; and all the authors contributed to the manuscript.

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