 https://doi.org/10.3311/PPch.38840

Periodica Polytechnica Chemical Engineering, 69(2), pp. 234–241, 2025

Phytochemical Profile of *Zygophyllum paulayanum* Extracts and their Promising Biological Activities

Mohan Lal[1*], Sachin Salunkhe[2], Amit Kumar[2], Dipankar Sutradhar[1]

[1] School of Advanced Sciences and Languages, VIT Bhopal University, Madhya Pradesh, 466114, India
[2] R&D, OmniActive Health Technologies, International Biotechnology Park, Hinjewadi, Phase-II, Pune, Maharashtra-411 057, India
[*] Corresponding author, e-mail: mohan.lal2019@vitbhopal.ac.in

Received: 20 November 2024, Accepted: 12 March 2025, Published online: 22 April 2025

Abstract

Zygophyllum paulayanum (syn. *Fagonia schweinfurthii*), a desert species known for its remarkable adaptation to harsh environmental conditions, has long been recognized for its medicinal properties, including antioxidant, wound healing, anti-inflammatory, antibacterial, anticancer, and antipyretic effects. This study aims to optimize the extraction process of its bioactive compounds using a range of solvents from non-polar to polar at different temperatures, followed by phytochemical screening. The hot ethanol extract was selected for further fractionation into non-polar to polar fractions, which were subsequently analyzed for phytochemical content and biological activities. The n-hexane fraction was found to be rich in fatty acids (24.71%), such as linoleic, palmitic, oleic, linolenic, stearic, lauric, and myristic acids, while the ethyl acetate fraction was abundant in polyphenols (22.14%). Notably, the ethyl acetate fraction exhibited the highest antibacterial activity, inhibiting strains of *Pseudomonas aeruginosa* (NCIM 5029, PAW1) and *Staphylococcus aureus* (NCIM 5021, S8). In addition to its antibacterial effects, both the n-hexane and ethyl acetate fractions showed positive responses in phytoestrogenic assays. Meanwhile, the ethanol fraction, rich in flavonoids (5.82%), tannins (4.95%), and alkaloids (18.50%), demonstrated the strongest antioxidant activity (92.08%) at concentration 100 µg/mL, water fraction rich in saponins (16.10%), exhibited the most potent anti-inflammatory effect (59.78%) at concentration 1000 µg/mL. The LC/QTOF-MS (liquid chromatography-quadrupole time-of-flight mass spectrometry) data of hot ethanol extract showed presence of phenol, flavonoids, saponins, alkaloids, and phytosterols cyclitol in plant. These findings highlight *Zygophyllum paulayanum* as a valuable source of bioactive compounds with promising therapeutic potential across various biological activities.

Keywords

Zygophyllum paulayanum, phytochemical profile, gas chromatography, HPLC-ESI-QTOF-MS, biological activities

1 Introduction

The species *Zygophyllum* and *Fagonia* have garnered global attention due to the presence of novel chemical constituents, including alkaloids, flavonoids, tannins, saponins, steroids, proteins, amino acids, carbohydrates, cardiac glycosides, starch, terpenoids, lignin, and phenols. *Zygophyllum paulayanum* (J. Wagner & Vierh.) Christenh. & Byng (Syn. *Fagonia schweinfurthii* (Hadidi) Hadidi ex Ghafoor) (Zygophyllaceae), a desert species, was taxonomically classified by Christenh. & Byng in 2018. It predominantly inhabits arid and semi-arid regions of Rajasthan, India, including the districts of Shri Ganganagar, Churu, Jhunjhunu, Sikar, Bikaner, Jodhpur, Barmer, and Jaisalmer. Commonly known as dhamasa or dhamasia [1], it is a small, spiny, erect undershrub with slender, terete, triate, glabrous branches. The leaves are opposite, 1-3 foliate, with petioles of variable length (3-30 mm), deeply striated, and slender. The stipules form two pairs of sharp, slender thorns, sometimes exceeding 12 mm, and the leaflets are linear, acute, and sessile or have very short petiolules [2].

This species has demonstrated numerous biological activities, including antioxidant, analgesic, astringent, anti-cancer, anti-asthmatic, and antimicrobial effects. It has traditionally been used for treating urinary discharges, toothaches, stomach troubles, kidney diseases, and as a febrifuge and prophylactic against smallpox. Other reported properties include wound healing, androgenic, anti-allergic, neuroprotective, endocrinological, antimicrobial, cytotoxic, and antitumor effects [3, 4], largely due to its rich phytochemical profile, which includes saponins, alkaloids, terpenoids, sterols, flavonoids, proteins,

Cite this article as: Lal, M., Salunkhe, S., Kumar, A., Sutradhar, D. "Phytochemical Profile of *Zygophyllum paulayanum* Extracts and their Promising Biological Activities", Periodica Polytechnica Chemical Engineering, 69(2), pp. 234–241, 2025. https://doi.org/10.3311/PPch.38840

amino acids, coumarin, and trace elements [5, 6]. There is also the possibility that essential oils from the species may show significant antimicrobial activity [7]. In our previous work on *Z. paulayanum*, we have reported the identification of kaempferol following hydrolysis of the ethanol extract and conducted biological studies both before and after hydrolysis, revealing key transformations in the phytochemical profile [8]. It has been observed that the hydrolysed extract of *Z. paulayanum* predominantly contains bioactive kaempferol glycosides while the unhydrolyzed extract primarily features kaempferol aglycone, known for its antioxidant, anti-inflammatory, antimicrobial, phytoestrogenic, and cytotoxic activities. The interesting outcome of active molecules in ethanol extract was exciting to explore this plant more to standardize the extraction, and screening of secondary metabolites with their potential biological activities. Building on this, our present work, we plan to optimize the extraction process by using different solvents and varying temperatures to maximize the yield of bioactive compounds. The best hot ethanol extract was fractionated from non-polar to polar fractions, which were then analyzed for their biological activities, including antioxidant, anti-inflammatory and antibacterial effects. This systematic approach allowed us to isolate and identify specific phytochemicals responsible for various therapeutic activities, highlighting the importance of optimized extraction in maximizing the efficacy of bioactive compounds. Our findings provide valuable insights into the phytochemical composition of *Z. paulayanum* and its potential medicinal applications.

2 Materials and methods

2.1 Chemical and reagent

Chemicals 2-diphenyl-2-picrylhydrazyl (DPPH) (TCI), phosphate-buffered saline, egg albumin and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) from Sigma Aldrich, solvents such as acetone (Qualigens), methanol, ethyl acetate, toluene, dimethyl sulfoxide (DMSO), water, acetic acid, formic acid, and trifluoroacetic acid (RANKEM), ethanol (Changshu) purity ≥99% were used in the analysis. Mixed tocopherol (Matrix) and diclofenac sodium (TCI) were used as controls. Standards like gallic acid 99.90% (Loba Chemie), tannic acid (Sigma Aldrich), rutin hydrate 95.40% (Merck), fatty acids: lauric acid 99%, myristic acid 99.5%, palmitic acid 99%, stearic acid 99.1%, oleic acid 98%, linoleic acid 98.8%, and linolenic acid 77.6% (TCI) were used for experiments and analysis purposes.

2.2 Instrumentations

The phytochemical composition, antioxidant and anti-inflammatory analysis of extracts were done using a UV-Vis spectrophotometer (Shimadzu 1800, Japan), fatty acids were tested with gas chromatography (PerkinElmer Clarus® 580, USA) against the standards and molecular mass was analyzed by HPLC/MS quadrupole time-of-flight instrument (Agilent Q-ToF G6540B, 1260 Infinity II HPLC, USA). The minimum inhibitory concentration (MIC) was tested using a microtiter plate reader (Spectra Max M2, Molecular Devices USA). Phytoestrogenic analysis was done using a microplate reader and 96 well plates.

2.2.1 HPLC-ESI-QTOF-MS condition

The mass spectrometric analysis was performed using an Agilent Q-ToF G6540B connected to an Agilent 1260 Infinity II HPLC. The mass spectrometer was operated in a positive electrospray ionization mode (Dual AJS ESI) and scanning was recorded from 50 to 1700 in MS mode [15]. Nitrogen was used for drying, the nebulizer gas temperature was 290 °C, the sheath gas temperature was 400°C and the pressure was 172369 Pa. The drying gas flow was 8.0 L/min and, sheath gas flow was 12 L/min. The voltage of the capillary was 4500 V, nozzle 1000 V, fragmentor 150 V and octapole RF peak 750 V. The mass data of molecule ions were processed through Mass Hunter Workstation (version B 09.00) software.

2.3 Plant material

The plant was collected on December 2021 from the desert region with a low rainfall record, village Meghana, Hanumangarh district, Tehsil-Nohar [29.18°N 74.77°E], Rajasthan, India (Fig. 1).

The species *Zygophyllum paulayanum* (J. Wagner & Vierh.) Christenh. & Byng (Syn. *Fagonia schweinfurthii* (Hadidi) Hadidi ex Ghafoor) (Zygophyllaceae) was authenticated by the Department of Botany, Agharkar Institute, Pune, Maharashtra, India, and the specimen (Voucher no: WP-294) was deposited in the institute for future reference.

2.4 Extract preparation and fractionation

Z. paulayanum plant was dried under the shade at room temperature and coarsely ground. 100 g of the powdered material was subjected to solvent extraction using n-hexane, ethyl acetate, ethanol, and water to evaluate the efficiency of different solvents in extracting bioactive compounds. The extractions were conducted at two temperature conditions ambient (25-30 °C) and

Fig. 1 Morphology of *Zygophyllum paulayanum*

elevated (60-65 °C) to assess the impact of temperature on extraction efficiency. Each extraction process consisted of three sequential washes using 500 mL of the respective solvent per wash, with each wash lasting 2 h, to maximize the yield of soluble components. The resulting extracts were dried under reduced pressure at 60±5 °C, and the extraction yields were measured to compare solvent efficiencies and temperature effect. The yield (Table S1 in Supplement) was calculated as per Eq. (1).

Based on maximum phytochemical composition, the hot ethanol extract was fractionated from non-polar to polar fractions using solvents like n-hexane, ethyl acetate, ethanol and water. Each fractions were dried under reduced pressure at 60±5 °C and the yield (Table S2 in Supplement) was calculated as per Eq. (1):

$$\%\text{Yield} = \frac{x(\text{g})}{y(\text{g})} \times 100\,, \tag{1}$$

where x(g) is the extract mass in g, and y(g) is the dried sample mass in g.

2.5 Phytochemicals determination

2.5.1 Total phenolic content

The total polyphenol content (TPC) was determined using a method described by ISO [9] using gallic acid as standard. Extract samples were prepared in methanol at a concentration of 0.20 mg/mL to 1 mL of the sample solution. 5.0 mL of 10% Folin Ciocalteu's phenol reagent and 4 mL of 7.5% Na_2CO_3 were added, and the mixture was allowed to react for 30 min. The absorbance of the solution was measured at 765 nm and the total polyphenol content TPC (%w/w) was calculated according to Eq. (2):

$$\%\text{TPC} = \frac{\text{Abs}(x) \times \text{dil}(\text{std}) \times \text{pur}(\text{std})}{\text{Abs}(\text{std}) \times \text{dil}(x)}\,, \tag{2}$$

where Abs(x) is the absorbance of the sample at 765 nm, dil(std) is the dilution of standard, pur(std) is the percentage purity of standard, Abs(std) is the absorbance of standard and dil(x) is the dilution of sample.

2.5.2 Total flavonoid content

The total flavonoid content (TFC) was determined using the $AlCl_3$ method [10]. The samples were prepared in 90% methanol with 2.0 mg/mL concentration. 1 mL sample solution was added in a 10 mL volumetric flask, followed by 3 mL 5% $NaNO_2$ solution, 0.3 mL 10% $AlCl_3$ solution, and 2 mL 1M NaOH solution. Then the flask was filled up with water, shaken vigorously and incubated for 30 min. The absorbance was recorded at 415 nm. Rutin hydrate was used as standard. The TFC content (%w/w) was calculated as per Eq. (3):

$$\%\text{TFC} = \frac{\left(\frac{\text{Abs}(x) - c}{m(\text{std})}\right) \times 100 \times \left(\frac{50}{25}\right) \times 100}{x(\text{g}) \times 10^6}\,, \tag{3}$$

where Abs(x) is the absorbance of the sample solution, c is the intercept, m(std) is the slope of the standard curve and x(g) is the mass of the sample.

2.5.3 Total tannin content

The total tannin content (TTC) was determined using the method described in [11] and tannic acid was used as standard. Samples were prepared in a concentration of 1 mg/mL in water. 1.25 mL Folin-Denis reagent and 2.5 mL Na_2CO_3 solution were added. The solution was mixed well and incubated for 30 min. The absorbance was measured at 760 nm. The TTC was calculated as per Eq. (4). TTC was expressed as milligrams of tannic acid equivalents per gram of dried sample. The results were determined by a correlation coefficient and linear regression.

$$\%\text{TTC} = \frac{\left(\frac{\text{Abs}(x) - c}{m(\text{std})}\right) \times 100 \times \left(\frac{50}{25}\right) \times 100}{x(\text{g}) \times 1000000}\,, \tag{4}$$

where Abs(x) is the absorbance of the sample solution, c is the intercept, m(std) is the slope of the standard curve and x(g) is the mass of the sample.

2.5.4 Total alkaloid content

Total alkaloid content (TAC) was determined using the gravimetric method [12]. Samples were prepared in a concentration of 2.5 mg/mL using 10% acetic acid in ethanol and stored for 4 h. The filtrate was kept in a water bath to concentrate it to 1/4 of the initial volume, and concentrated ammonium hydroxide was added drop by drop to the solution until precipitation. The liquid was filtered and the residue was washed with diluted NH_4OH and dried. The TAC content (%w/w) was calculated as per Eq. (5):

$$\%\text{TAC} = \frac{dr(\text{g})}{x(\text{g})} \times 100\,, \tag{5}$$

where dr(g) is the dried residue in g and x(g) the extract mass in g.

2.5.5 Total saponin content

The total saponin content (TSC) was determined using the AOAC (1990) method [13]. 5.0 g of sample was extracted using 100 mL methanol at 100 °C. Total three washes were performed for 15-20 min each and the extract was concentrated to 20 mL. The solution was allowed to cool, 150 mL acetone was added and then left for 30 min. The solution was filtered and dried at 105 °C for 3 h. The TSC content (%w/w) was calculated as per Eq. (6).

$$\%\text{TSC} = \frac{dr(\text{g})}{x(g)} \times 100\,, \tag{6}$$

where dr(g) is the mass of the dried residue in g and x(g) is the extract mass in g.

2.5.6 Fatty acid analysis

Fatty acids were identified using gas chromatography with flame ionization detection (GC-FID) [14]. 100-200 mg samples were prepared with 4 mL 0.5 M methanolic NaOH solution and refluxed at 70 °C for 20 min until fat globules disappeared. BF_3-methanol complex (5 mL) was added and refluxed again for 10-15 min, and 4 mL of n-heptane was added through the condenser and refluxed continued for the next 1 min. Saturated NaCl (15 mL) was added to allow layer separation. The upper layer was passed through anhydrous Na_2SO_4 and 1 mL solution was made up to 25 mL with n-heptane. Samples were injected into gas chromatography. The column used DB-FFAP (30 m × 0.25 mm, 0.25 µm) with a flow of 2.4 mL/min. The system suitability of fatty acid analysis was not more than 5.0% (% RSD-relative standard deviation). Fatty acid content (%w/w) was calculated as per Eq. (7):

$$\%\text{Fatty acids} = \frac{\text{Ar}(x)}{\text{Ar}(\text{std})} \times \frac{\text{Std}(\text{mg})}{\text{std}(\text{ml})} \times \frac{x(\text{ml})}{x(\text{mg})} \times cf\,, \tag{7}$$

where Ar(x) is the area of the sample, Ar(std) is an area of standard, std(mg) is mass of standard in mg, std(ml) is dilution of standard in ml, x(ml) dilution of sample, x(mg) mass of sample and cf is conversion factor.

2.6 Evaluation of biological activities

2.6.1 Antioxidant activity

The *in vitro* antioxidant activity was determined using DPPH and the spectrophotometric method [16]. The extract samples were prepared in concentrations of 5.0, 15.0, 25.0, 50.0 and 100.0 µg/mL using methanol. The 0.1 mM DPPH solution was prepared in methanol and added 0.5 mL of this DPPH solution into each sample. The samples were mixed thoroughly and incubated in the dark for 30 min. The absorbance of the solution was measured at 517 nm by using UV spectroscopy. The remaining DPPH was calculated as per Eq. (8). The mixed tocopherol was used as a positive control.

$$\text{Remaining DPPH}(\%) = \frac{(\text{Abs}_0 - \text{Abs}_t)}{\text{Abs}_0} \times 100\,, \tag{8}$$

where Abs_0 is the absorbance of the control and Abs_t is the absorbance of the test compounds.

2.6.2 Anti-inflammatory activity

The *in vitro* anti-inflammatory activity was assessed as the inhibition of the albumin denaturation method [17]. The extract samples were prepared in concentrations of 250.0, 500.0 and 1000.0 µg/mL using methanol, 1% egg albumin, 2.8 mL of phosphate-buffered saline (PBS, pH 6.8), 2 mL of the standard drug (diclofenac sodium). The solutions were mixed thoroughly and heated at 70 °C for 5 min. All samples were cooled and the absorbance was measured at 660 nm. The inhibition was calculated as per Eq. (9).

$$\%\text{Inhibition} = \frac{\text{Abs}(c) - \text{Abs}(x)}{\text{Abs}(c)}\,, \tag{9}$$

where Abs(c) is the absorbance of the control and Abs(x) is the absorbance of the sample solution.

2.6.3 Phytoestrogenic effect of the extracts on the breast cancer cell line

The number of viable MCF-7 cells were determined according to the method described in Refs. [18, 19]. A total of 2000 cells per well were plated in a 96-well plate and incubated in a 5% CO_2 incubator set at 37 °C. The cells were treated with the test products diluted in DMSO after reaching 40-50% confluence. An appropriate positive control (*β*-estradiol) was included, and DMSO was used as a vehicle (negative control). The plates were incubated in a 5% CO_2 incubator at 37 °C for 72 h. Then, 10 µL of MTT (5 mg/mL in PBS) was added to each well and incubated for an additional 4 h. After removing the MTT, 100 µL of DMSO was added to dissolve the formazan crystals, and absorbance was measured at 570 nm using a microplate reader. The %Cytotoxicity is expressed as per Eq. (10) and similarly, %Cell viability was expressed as per Eq. (11):

$$\%\text{Inhibition} = \frac{\text{Abs}(c) - \text{Abs}(x)}{\text{Abs}(c)}, \tag{10}$$

where Abs(*c*) is the absorbance of the negative control and Abs(*x*) is the absorbance of the sample solution.

$$\%\text{Cell viability} = 100 - \%\text{Cytotoxicity} \tag{11}$$

2.6.4 Determination of minimum inhibitory concentrations (MICs) of the compounds against multidrug-resistant (MDR) pathogens

A stock solution of all the samples was prepared at a concentration of 100 mg/mL in DMSO and MIC's of all the compounds were determined using the broth microdilution method [20] against *P. aeruginosa* NCIM 5029, *P. aeruginosa* PAW1, *S. aureus* NCIM 5021, and *S. aureus* S8 as per the CLSI guidelines (CLSI, USA, 2018). Briefly, overnight grown culture was taken and optical density (OD) was adjusted to 10^5 CFU/mL in Luria Bertani (LB) broth and added to the microtiter plate. The samples were added to the microtiter plate in concentrations ranging from 0.039-20 mg/mL by serial dilution. The wells containing only medium and inoculated medium were considered as negative and positive controls, respectively. DMSO was also used as a control. The microtiter plates were incubated at 37 °C and after 24 h of incubation absorbance was read at 540 nm using a microtiter plate reader (Spectra Max M2, Molecular Devices, USA).

2.7 Statistical analysis

The yield, phytochemical content, and biological activities data were performed in triplicate. The results are presented as mean ± standard deviation (SD).

3 Results and discussion

The species *Z. paulayanum* was collected from desert area of Rajasthan, India. The whole plant (as shown in Fig. 1) was extracted with solvents of different polarity such as n-hexane, ethyl acetate, ethanol and water. The extraction was carried out under ambient (25-30 °C) and hot (60-65 °C) conditions to maximize the yield of phytochemicals. Optimization tools are crucial for determining optimal process conditions, with hot extraction generally yielding higher amounts of phytochemicals [21]. The highest yield was obtained from the hot water extract (16.80% ± 0.56), followed by ethanol (13.28% ± 0.12), ethyl acetate (3.00% ± 0.17), and n-hexane (2.34% ± 0.05), compared to extractions at ambient temperature. Following extraction optimization, all extracts were analyzed for their phytochemical composition, including polyphenols, flavonoids, tannins, saponins, and alkaloids. The ethyl acetate extract showed the highest polyphenol content (22.15% ± 1.85), while the ethanol extract contained the most flavonoids (4.02% ± 0.05), tannins (2.18% ± 0.22), and alkaloids (12.45% ± 0.50). The highest saponin content (9.62% ± 0.15) was found in the water extract (as shown in Table S1 in Supplement).

Among the extracts, ethanol provided the highest overall phytochemical composition, except for saponins. The presence of bioactive compounds in various *Fagonia* species has been reported [22, 23], but quantifying these bioactive constituents is essential. Modern research emphasizes the importance of active constituent quantification to standardize herbal formulations [24].

Since the ethanol extract had the highest phytochemical content, it was further fractionated from non-polar to polar fractions using n-hexane, ethyl acetate, ethanol, and water fractions subsequently. All fractions were evaluated for the phytochemical composition (Table S2 in Supplement). In the n-hexane fraction, oily bioactives were extracted in which a total of seven fatty acids were identified by gas chromatography. The total fatty acids content was 24.71%, in which the maximum was linoleic acid 10.44% followed by palmitic acid 6.73%, oleic acid 3.11%, linolenic acid 2.27%, stearic acid 1.10%, lauric acid 0.62% and myristic acid 0.44% (Table S3 in Supplement). Maximum

polyphenols (22.15% ± 0.18) were found in ethyl acetate fraction. The n-hexane and ethyl acetate fractions exhibited positive phytoestrogenic activities in the biological assay (Table S4 in Supplement). Some studies suggest that fatty acids can alter the inflexibility of tumour cell membranes which causes cell death [25]. As shown in Table 1, the ethyl acetate showed highest MICs at 5, 5, 1.25 and 2.5 mg/mL against *P. aeruginosa* NCIM 5029, *P. aeruginosa* PAW1, *S. aureus* NCIM 5021, and *S. aureus* S8.

There is the possibility that the presence of polyphenols [26] or secondary metabolites can defend against bacteria, fungi and viruses [27, 28]. Similarly, the different fractions of *Fagonia olivieri* showed activity against *Staphylococcus aureus* in the range of 3 to 10 mg/mL, and *Pseudomonas aeruginosa* in the range of 3 to 15 mg/mL [29]. Ethanol fraction, rich in flavonoids 5.82% ± 0.04, tannins 4.95% ± 0.28, and alkaloids 18.50% ± 3.67 gave the highest *in vitro* antioxidant activity of 92.08 ± 0.99 at a concentration 100 µg/mL (Table S5 in Supplement). The ethanol fraction gave effective antioxidant activities, which might be due to the presence of high content of flavonoids, tannins and alkaloids which are well-known antioxidant agents [30]. The water fraction was rich in saponins (16.10% ± 0.14), and showed maximum anti-inflammatory activity of 59.78 ± 1.01 at a concentration of 1000 µg/mL (Table S6 in Supplement). It was reported, that the presence of kaempferol and saponins or their glycosides could be responsible for such activities [31, 32].

Based on the above activities the hot ethanol extract was analysed for molecular mass distribution to check the presence of possible phytochemicals. The mass spectrum analysed in positive mode. In total eighteen bioactive compounds were identified in aglycon and glycone form. Phytochemicals, particularly flavonoids, are primarily found in their glycoside forms in nature, although they have been identified in both glycosidic and aglycone forms [33]. In phenols the carvacrol $(M+Na)^+$ and in flavonoids, in aglycone form were identified quercetin, kaempferol $(M+H)^+$, myricetin, apigenin, $(M+H_2O)^+$, taxifolin $(M+Na)^+$ and in glycoside form epigallocatechin gallate, apigenin 7-O-glucoside $(M+H_2O)^+$, catechin 7-glucoside, kaempferol 7-O-glucoside, apigenin-6,8-C-glucoside $(M+H)^+$. As reported in Table 2, in saponins pinatol $(M+Na)^+$, ursolic acid $(M+H)^+$, hederagenin 28-O-beta-D-glucopyranosyl ester, and diosgenin $(M+H_2O)^+$ were identified. The harmine $(M+H_2O)^+$ was identified as alkaloid, beta-sitosterol $(M+H_2O)^+$ as phytosterol and cyclitol (cyclic polyol) the pinitol $(M+Na)^+$. The molecules identified by LC/QTOF-MS in ethanol extract are also known for biological activities such as antioxidant, anti-inflammatory, anti-microbial and phytoestrogenic.

Table 1 MICs of Ethanol Ext and fractions

Sample name	MICs (mg/mL)			
	P. aeruginosa NCIM 5029	*P. aeruginosa* PAW1	*S. aureus* NCIM 5021	*S. aureus* S8
n-Hexane fraction	10	10	2.5	2.5
Ethyl acetate fraction	5	5	1.25	2.5
Ethanol fraction	10	20	20	10
Water fraction	>20	20	20	>20
DMSO	10	20	>20	>20

4 Conclusion

In conclusion, the medicinal plant identified under the recent nomenclature *Z. paulayanum*, collected from the desert area of Rajasthan, India has been studied for its phytochemical and biological screening. The analysis indicates the presence of bioactive compounds, including fatty acids, phenol, flavonoids, tannins, saponins, alkaloids and cyclitol (cyclic polyol), which demonstrate its potential antioxidant, anti-inflammatory, antibacterial, anticancer, and phytoestrogenic activities. This study highlights the significance of selective solvent use for isolating specific bioactive compounds and correlating them with their biological effects. Thus, *Z. paulayanum* emerges as a valuable source of phytochemicals with promising therapeutic potential for healthcare applications. This study also suggests that this species has a good source of bioactives that could be commercialized for different applications. In future studies, this extract could be explored for more bioactive identification and their role in biological systems.

Acknowledgements

Authors thank VIT Bhopal University (Madhya Pradesh) and OmniActives Health Technologies (R&D), Pune (Maharashtra) for support during this study.

Conflict of interest

The authors declare that there is no conflict of interest.

Table 2 Identification of possible phytochemicals in *Z. paulayanum* ethanol extracts fractions by HPLC-ESI-QTOF-MS method using positive ionization mode

Bioactive name	RT (min)	m/z (adduct mass)	Adduct	Formula	Ref.
		Phenol			
Carvacrol	3.83-3.95	173.22	$(M+Na)^+$	$C_{10}H_{14}O$	[6]
		Flavonoids			
Kaempferol	25.58-25.88	287.27	$(M+H)^+$	$C_{15}H_{10}O_6$	[5, 7, 8]
Kaempferol 7-O-glucoside	19.72-19.95	449.11	$[M+H]^+$	$C_{21}H_{20}O_{11}$	[5, 7, 8]
Apigenin	19.72-19.95	288.23	$(M+H_2O)^+$	$C_{15}H_{10}O_5$	[5, 7]
Apigenin 7-O-glucoside	33.52-34.02	450.41	$(M+H_2O)^+$	$C_{21}H_{20}O_{10}$	[7]
Apigenin-6,8-C-glucoside	18.95-19.28	595.16	$[M+H]^+$	$C_{27}H_{30}O_{15}$	[5, 7]
Quercetin	25.58-25.88	303.23	$(M+H)^+$	$C_{15}H_{10}O_7$	[5, 7]
Taxifolin	14.87-14.94	327.20	$[M+Na]^+$	$C_{15}H_{12}O_7$	[5, 7]
Myricetin	18.59-18.83	336.12	$[M+ H_2O]^+$	$C_{15}H_{10}O_8$	[32]
Catechin 7-glucoside	25.58-25.88	453.33	$[M+H]^+$	$C_{21}H_{24}O_{11}$	[5, 7]
Epigallocatechin gallate	16.44-16.87	476.30	$(M+H_2O)^+$	$C_{22}H_{18}O_{11}$	[31]
		Saponins			
Ursolic Acid	23.67-24.00	457.36	$(M+H)^+$	$C_{30}H_{48}O_3$	[7]
Hederagenin 28-O-beta-D-glucopyranosyl ester	17.82-18.07	652.41	$(M+H_2O)^+$	$C_{36}H_{58}O_9$	[5, 7]
Diosgenin	15.99-16.00	432.28	$(M+H_2O)^+$	$C_{27}H_{42}O_3$	[7]
		Alkaloids			
Harmine	22.85-23.15	212.25	$(M+H_2O)^+$	$C_{13}H_{12}N_2O$	[7]
		Phytosterols			
beta-Sitosterol	25.58-25.88	414.70	$(M+H_2O)^+$	$C_{29}H_{50}O$	[5, 7]
		Cyclitol (cyclic polyol)			
(Pinatol) Pinitol	37.30-37.83	217.15	$(M+Na)^+$	$C_7H_{14}O_6$	[7]

References

[1] Rathore, M. K., Sharma, M. C., Goyal, M., Singh, G. K., Nagori, B. P. "Pharmacognostical studies on root of Fagonia schweinfurthii Hadidi", International Journal of Pharmaceutical & Biological Archives, 2(5), pp. 1514–1517, 2011.

[2] Rathore, A. S., Lohar, V., Kumar, R., Choudhary, V., Bhandari, A. "Chemical composition and anti-inflammatory activity of various extracts of Fagonia schweinfurthii Hadidi", Medical Chemistry & Drug Discovery, 3(1), pp. 30–36, 2012.

[3] Puri, D., Bhandari, A. "Fagonia: a potential medicinal desert plant", Journal of NPA, 27(1), pp. 28–33, 2014. https://doi.org/10.3126/jnpa.v27i1.12147

[4] Sharma, A. "Primary Metabolite Profiling and Potential Antioxidants Activity from Fagonia cretica (Stem and Roots)", Journal of Drug Delivery and Therapeutics, 9(4-A), pp. 376–380, 2019. https://doi.org/10.22270/jddt.v9i4-A.3498

[5] Alamami, A., Elshibani, F., Elremali, N., Daboub, A., Zaed, S. B., Bumadian, M. "The species of genus *fagonia* in Libya: (A comprehensive review)", Journal of Pharmacognosy and Phytochemistry, 11(5), pp. 28–37, 2022. https://doi.org/10.22271/phyto.2022.v11.i5a.14501

[6] Djellouli, M., Berghioua, A. "Chemical composition, antioxidant and antimicrobial activity of *Fagonia longispina* (Zygophyllaceae) of Algerian", Biodiversitas Journal of Biological Diversity, 22(6), pp. 3448–3453, 2021. https://doi.org/10.13057/biodiv/d220653

[7] Malavika, P. S., Singh, V., Kumar, Y. "The chemistry and pharmacology of *Fagonia* genus: a review", International Journal of Scientific Development and Research, 6(7), pp. 332–343, 2021.

[8] Lal, M., Sutradhar, D. "Extraction of kaempferol derivatives from *Zygophyllum paulayanum* and its diverse biological activities", Natural Product Research, pp. 1–5, 2024. https://doi.org/10.1080/14786419.2024.2375310

[9] ISO "ISO 14502-1:2005, Determination of substances characteristic of green and black tea. Part 1: Content of total polyphenols in tea – Colorimetric method using Folin-Ciocalteu reagent", International Organization for Standardization, Geneva, Switzerland, 2005.

[10] Patil, N. B., Adsul, V. B., Khatiwora, E., Kale, A. A., Tambe, S. P., Deshpande, N. R. "Spectroscopic Determination of Total Phenolic and Flavonoid Contents of Tribulus Terrestris Fruits", International Journal of ChemTech Research, 4(3), pp. 899–902, 2012.

[11] Kavitha, D., Padmini, R., Chandravadivelu, G., Magharla, D. D. "Phytoconstituents Screening and Antioxidant Activity of *Syringodium isoetifolium* Leaf Extracts", Indian Journal of Pharmaceutical Sciences, 84(5), pp. 1309–1322, 2022. https://doi.org/10.36468/pharmaceutical-sciences.1028

[12] Jain, P., Sharma, H. P., Basri, F., Priya, K., Singh, P. "Phytochemical analysis of Bacopa monnieri (L.) Wettst. and their anti-fungal activities", Indian Journal of Traditional Knowledge, 16(2), pp. 310–318, 2017. https://doi.org/10.13140/RG.2.2.17307.46882

[13] Eleazu, C. O., Eleazu, K. C., Awa, E., Chukwuma, S. C. "Comparative Study of the Phytochemical Composition of the Leaves of Five Nigerian Medicinal Plants", Journal of Biotechnology and Pharmaceutical Research, 3(2), pp. 42–46, 2012. https://doi.org/10.9734/JAMPS/2016/21816

[14] Zhang, H., Wang, Z., Liu, O. "Development and validation of a GC–FID method for quantitative analysis of oleic acid and related fatty acids", Journal of Pharmaceutical Analysis, 5(4), pp. 223–230, 2015. https://doi.org/10.1016/j.jpha.2015.01.005

[15] Kumar, S., Singh, A., Kumar, B. "Identification and characterization of phenolics and terpenoids from ethanolic extracts of *Phyllanthus* species by HPLC-ESI-QTOF-MS/MS", Journal of Pharmaceutical Analysis, 7(4), pp. 214–222, 2017. https://doi.org/10.1016/j.jpha.2017.01.005

[16] Abbas, M. W., Hussain, M., Akhtar, S., Ismail, T., Qamar, M., Shafiq, Z., Esatbeyoglu, T. "Bioactive Compounds, Antioxidant, Anti-Inflammatory, Anti-Cancer, and Toxicity Assessment of *Tribulus terrestris*—In Vitro and In Vivo studies", Antioxidants, 11(6), 1160, 2022. https://doi.org/10.3390/antiox11061160

[17] Nagavekar, N., Singhal, R. S. "Enhanced extraction of oleoresin from *Piper nigrum* by supercritical carbon dioxide using ethanol as a co-solvent and its bioactivity profile", Journal of Food Process Engineering, 41(1), e12670, 2018. https://doi.org/10.1111/jfpe.12670

[18] Rasmussen, T. H., Nielsen, J. B. "Critical parameters in the MCF-7 cell proliferation bioassay (E-Screen)", Biomarkers, 7(4), pp. 322–336, 2002. https://doi.org/10.1080/13547500210132907

[19] Odum, J., Tittensor, S., Ashby, J. "Limitations of the MCF-7 Cell Proliferation Assay for Detecting Xenobiotic Oestrogens", Toxicology in Vitro, 12(3), pp. 273–278, 1998. https://doi.org/10.1016/s0887-2333(97)00115-x

[20] Mulani, M. S., Kamble, E. E., Kumkar, S. N., Tawre, M. S., Pardesi, K. R. "Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review", Frontiers in Microbiology, 10, 539, 2019. https://doi.org/10.3389/fmicb.2019.00539

[21] Yim, H. S., Chye, F. Y., Rao, V., Low, J. Y., Matanjun, P., How, S. E., Ho, C. W. "Optimization of extraction time and temperature on antioxidant activity of *Schizophyllum commune* aqueous extract using response surface methodology", Journal of Food Science and Technology, 50(2), pp. 275–283, 2013. https://doi.org/10.1007/s13197-011-0349-5

[22] Prajapati, R., Davra, K., Kalariya, M., Sailor, G., Jain, V. "Pharmacognostic and phytochemical evaluation of the *Fagonia arabica* stem-a potent Indian medicinal plant", International Journal of Pharmacognosy, 7(7), pp. 193–197, 2020.

[23] Sharma, S., Bhandari, A., Puri, D., Sharma, R., Verma, R. Kumar, A. "Pharmacognostical and phytochemical evaluation of *Fagonia schweinfurthii Hadidi*", World Journal of Pharmaceutical Research, 3(1), pp. 619–628, 2013.

[24] Jain, B. K., Joshi, Y. "Phytochemical analysis of *Fagonia schweinfurthii* Hadidi", Journal of Pharmaceutical Research International, 33(59A), pp. 803–811, 2021. https://doi.org/10.9734/JPRI/2021/v33i59A34332

[25] Arrieche, D., Olea, A. F., Jara-Gutiérrez, C., Villena, J., Pardo-Baeza, J., García-Davis, S., Viteri, R., Taborga, L., Carrasco, H. "Ethanolic Extract from Fruits of *Pintoa chilensis*, a Chilean Extremophile Plant. Assessment of Antioxidant Activity and In Vitro Cytotoxicity", Plants, 13(10), 1409, 2024. https://doi.org/10.3390/plants13101409

[26] Sulieman, A. M. E., Alanaizy, E., Alanaizy, N. A., Abdallah, E. M., Idriss, H., Salih, Z. A., Ibrahim, N. A., Ali, N. A., Ibrahim, S. E., Abd El Hakeem, B. S. "Unveiling Chemical, Antioxidant and Antibacterial Properties of *Fagonia indica* Grown in the Hail Mountains, Saudi Arabia", Plants, 12(6), 1354, 2023. https://doi.org/10.3390/plants12061354

[27] Archana, A. N., Sabale, S. G., Kale, B. S. "*In vitro* antimicrobial activity of *Fagonia schweinfurthii* Hadidi from Northern Western Ghats, India", Journal of Pharmacognosy and Phytochemistry, 11(3), pp. 283–292, 2022.

[28] Dinesh, P., Deepak, C., Anil, B., Praveen, G. K., Mohd, Y. "Formulation and its In-vitro Anti-fungal Study of *Fagonia schweinfurthii* Hadidi Extract Cream", Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences, 4(5), 386, 2018. https://doi.org/10.26479/2018.0405.30

[29] Rashid, U., Khan, M. R., Jan, S., Bokhari, J., Shah, N. A. "Assessment of phytochemicals, antimicrobial and cytotoxic activities of extract and fractions from *Fagonia olivieri* (Zygophyllaceae)", BMC Complementary and Alternative Medicine, 13(1), 167, 2013. https://doi.org/10.1186/1472-6882-13-167

[30] Ganpat, S. S., Shantilal, K. B. "Antioxidant Potential of *Fagonia schweinfurthii* Hadidi from the Northern Western Ghats India", International Journal of Pharmaceutical Sciences Review and Research, 76(1), pp. 28–32, 2022. https://doi.org/10.47583/ijpsrr.2022.v76i01.006

[31] Kanwal, N., Siddiqui, A. J., Haq, F. U., El-Seedi, H. R., Musharraf, S. G. "Two-stage mass spectrometry approach for the analysis of triterpenoid glycosides in *Fagonia indica*", RSC Advances, 8(71), pp. 41023–41031, 2018. https://doi.org/10.1039/c8ra08350a

[32] Younas, A., Hussain, L., Shabbir, A., Asif, M., Hussain, M., Manzoor, F. "Effects of *Fagonia indica* on Letrozole-Induced Polycystic Ovarian Syndrome (PCOS) in Young Adult Female Rats", Evidence-Based Complementary and Alternative Medicine, 2022(1), 1397060, 2022. https://doi.org/10.1155/2022/1397060

[33] Tuszyńska, M. "Validation of the Analytical Method for the Determination of Flavonoids in Broccoli", Journal of Horticultural Research, 22(1), pp. 131–140, 2014. https://doi.org/10.2478/johr-2014-0016