Razi Med J. 2025;1(3):272-276
https://doi.org/10.69667/rmj.25326
Razi Medical Journal
https://razi.edu.ly/rmj/index.php/hm

Original article

Fagonia glutinosa from Libya as a Potential Source of Lead Compounds: GC-MS Characterization of Metabolites with Antimicrobial and Anticancer Activities

Nowar Bhari[1], Hamed Bogdadi[1], Shamsi Saad[2]*

[1]*Department of Botany, Faculty of Science, Sebha University, Sebha, Libya*
[2]*Department of Medical Laboratory, Faculty of Medical Technology, Sebha University, Sebha, Libya*
Corresponding author. *Sha.saad@sebhau.edu.ly*

Abstract
Species of Fagonia (family Zygophyllaceae) are drought-adapted shrubs and herbs distributed throughout North Africa, the Mediterranean Basin, and the Middle East. Traditional healing systems across these regions use Fagonia spp. to treat fever, inflammation, and cancer. Recent studies have uncovered diverse secondary metabolites, including triterpenoid saponins, flavonoids, sterols, and diterpenes, in different species. Libya harbors several Fagonia species, yet the chemical composition and pharmacological activity of the Libyan populations remain poorly characterized. To profile the phytochemicals of Fagonia glutinosa collected from arid Libyan regions using gas chromatography–mass spectrometry (GC-MS) and to evaluate its antimicrobial and cytotoxic activities in vitro. The shade-dried aerial parts were macerated in methanol. The crude extract was analyzed using GC-MS, and the bioactive constituents were identified by comparing the spectra with the NIST library entries. Antibacterial and antifungal activities were assessed against four bacteria and two fungi using agar well diffusion and broth microdilution assays, respectively. Cytotoxicity was measured against MCF-7 breast cancer and HepG2 liver cancer cell lines using the MTT assay. Data were analyzed using one-way ANOVA with Tukey's post hoc test. Twenty-eight constituents, including phytol, hexadecanoic acid methyl ester, squalene, stigmasterol, lupeol, and oleic acid, were identified. The extract displayed broad-spectrum antibacterial activity (MIC 125-250 µg mL^{-1}) and moderate antifungal activity. MCF-7 and HepG2 cells exhibited dose-dependent viability reductions with IC_{50} values of 43.2 µg mL^{-1} and 57.8 µg mL^{-1}, respectively, and microscopic examination revealed apoptotic morphology. Fagonia glutinosa from Libya possesses a chemically diverse metabolome dominated by terpenoids and fatty acid esters and demonstrates noteworthy antimicrobial and anticancer activities. These findings validate the traditional uses and underscore the potential of this species as a source of lead compounds. Bioassay-guided fractionation and in vivo studies are warranted to isolate the active principles and establish safety profiles.

Keywords. *Fagonia glutinosa; GC-MS; Antimicrobial; Cytotoxicity; Phytochemical.*

Received: 20/07/25
Accepted: 17/09/25
Published: 24/09/25

Introduction

The genus *Fagonia* comprises approximately thirty-five species of drought-tolerant shrubs and herbaceous plants distributed across arid and semi-arid regions of North Africa, the Mediterranean basin, and South-West Asia [1]. These hardy perennials have long been valued in traditional medicine; ancient Arabic texts describe their use in treating fever, inflammation, and malignancies, and contemporary herbal practices continue to employ them for skin, respiratory, and gastrointestinal disorders [2,3]. In Libya, at least 11 *Fagonia* species have been documented and collectively referred to as "Dhamasa" or "Virgin's Mantle," reflecting their esteemed status in local pharmacopoeias [1,4,5].

Modern phytochemical investigations have corroborated several ethnomedicinal claims. Diverse secondary metabolites, including triterpenoid saponins, flavonoids, alkaloids, sterols, and phenolic compounds, have been isolated from *Fagonia* spp., and novel compounds, such as erythroxan diterpenes, have been reported [6,7]. These constituents underpin the antimicrobial and anticancer activities. Extracts of *F. cretica* and *F. indica* exhibit broad-spectrum antibacterial activity comparable to that of conventional antibiotics and display cytotoxicity against breast, liver, and lung cancer cell lines [8,9]. These biological activities are frequently attributed to amphiphilic molecules that disrupt microbial membranes and trigger apoptosis via the p53 or FOXO3a signalling pathways [10], [11].

Despite a growing body of research on *Fagonia* spp., there is scant information on the phytochemical composition and bioactivity of Libyan populations, and environmental stressors, such as extreme aridity, may produce chemotypes rich in stress-response metabolites. Therefore, this study profiled the methanolic extract of *F. glutinosa* collected from

Razi Med J. 2025;1(3):272-276
https://doi.org/10.69667/rmj.25326
Razi Medical Journal
https://razi.edu.ly/rmj/index.php/hm

Libya and evaluated its antimicrobial and anticancer activities. This study aims to integrate traditional knowledge with modern analytical approaches and provide a foundation for future drug discovery efforts.

Materials and methods

Plant material and extraction

Fresh aerial parts of *Fagonia glutinosa* were collected from the arid regions of Libya during the flowering season (April–May). The plant was taxonomically authenticated by a botanist at the Herbarium of Sebha University, Botany Department, and a voucher specimen (Accession No. FG-2025-01). The material was washed, shade-dried for 14 days, and ground into a coarse powder. One hundred grams of dried powder was macerated in 1 L of methanol (analytical grade) for 72 h at room temperature with occasional shaking. The extract was filtered through Whatman No. 1 paper and concentrated under reduced pressure at 40 °C to yield a dark brown semi-solid. After concentrating to dryness, the crude methanolic extract weighed 55.6 g, corresponding to a yield of 55.6% w/w relative to the 100 g of shade-dried aerial powder. The samples were stored at 4 °C until analysis.

GC–MS analysis

Phytochemical profiling of the methanolic extract was performed using an Agilent 7890B gas chromatograph coupled with a 5977A mass selective detector. Separation was achieved using an HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm film). Helium served as the carrier gas at 1.0 mL min^{-1}. One microliter of extract (10 mg mL^{-1} in methanol) was injected at a split ratio of 10:1. The injector was maintained at 250 °C. The oven temperature program was: initial 70 °C (hold 2 min), ramp to 280 °C at 10 °C min^{-1} and hold 10 min. The mass detector operated in electron-impact mode at 70 eV, scanning 50–600 m/z. The compounds were tentatively identified by matching the mass spectra with the NIST 2017 library and verifying the retention indices against the literature data [12]. The analysis was conducted in duplicate to ensure the reproducibility of the results.

Antimicrobial assays

Microorganisms

Four bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *and Pseudomonas aeruginosa*) and two fungi (*Aspergillus niger* and *Candida albicans*) were obtained from the Microbial Culture Collection, Department of Medical Laboratory, Faculty of Medical Laboratory Sciences.

Agar well diffusion

Mueller–Hinton agar (bacteria) and Sabouraud dextrose agar (fungi) plates were seeded with 100 μL of microbial suspension (1.5 × 10^8 CFU mL^{-1} for bacteria; 1 × 10^6 spores mL^{-1} for fungi). Wells (6 mm diameter) were bored into the agar and filled with 100 μL of extract (10 mg mL^{-1} in DMSO). Ciprofloxacin (5 μg mL^{-1}) and fluconazole (10 μg mL^{-1}) served as positive controls, and DMSO served as the negative control. Plates were incubated at 37 °C for 24 h (bacteria) or 28 °C for 48 h (fungi), and zones of inhibition were measured in millimeters [13].

Minimum inhibitory concentration (MIC)

The MIC values were determined using broth microdilution, following CLSI guidelines. Serial two-fold dilutions of the extract (1000–7.81 μg mL^{-1}) were prepared in the Mueller–Hinton broth. Wells were inoculated with ~5 × 10^5 CFU mL^{-1} and incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration that showed no visible growth. The experiments were performed in triplicate [14].

Cytotoxicity assay

Cell culture

Human breast cancer (MCF-7) and liver cancer (HepG2) cell lines were obtained from the American Type Culture Collection. Cells were maintained in DMEM supplemented with 10 % fetal bovine serum and 1 % penicillin–streptomycin at 37 °C in a 5 % CO_2 atmosphere.

Razi Med J. 2025;1(3):272-276
https://doi.org/10.69667/rmj.25326
Razi Medical Journal
https://razi.edu.ly/rmj/index.php/hm

MTT assay

Cell viability was assessed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [15] assay. Cells were seeded in 96-well plates (1×10^4 cells per well) and allowed to adhere overnight. The extract was added at concentrations of 6.25–100 µg mL^{-1} and incubated for 48 h. Subsequently, 20 µL of MTT solution (5 mg mL^{-1} in PBS) was added, and the plates were incubated for 4 h. Formazan crystals were dissolved in 150 µL of DMSO, and absorbance was measured at 570 nm. Doxorubicin served as a positive control, and 0.1 % DMSO served as a negative control. IC_{50} values were calculated using non-linear regression (GraphPad Prism 9).

Statistical analysis

All experiments were performed in triplicate, and the results are expressed as the mean ± standard deviation. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test with significance at $p < 0.05$.

Results and discussion

Phytochemical constituents

GC–MS analysis identified twenty-eight constituents in the methanolic extract of *F. glutinosa*. The major compounds, their retention times, molecular formulas, and relative peak areas are summarized in (Table 1) and (Figure 1). Phytol (12.6 %), hexadecanoic acid methyl ester (10.4 %), and squalene (8.9 %) were the most abundant constituents. Notably, sterols such as stigmasterol and lupeol, as well as unsaturated fatty acids like oleic acid, were detected. The dominance of terpenoids and fatty acid esters is consistent with previous reports on other *Fagonia* species [3,7,9].

Table 1. Major Phytochemicals Identified by GC-MS in Fagonia glutinosa Extract

S. No.	Compound name	Retention time (min)	Molecular formula	Peak area (%)	Reported bioactivity
1	Phytol	20.65	$C_{20}H_{40}O$	12.6	Antioxidant, anticancer
2	Hexadecanoic acid methyl ester	16.88	$C_{17}H_{34}O_2$	10.4	Antibacterial, anti-inflammatory
3	Squalene	27.33	$C_{30}H_{50}$	8.9	Anticancer, chemopreventive
4	Stigmasterol	29.91	$C_{29}H_{48}O$	7.2	Immunomodulatory, cholesterol-lowering
5	Lupeol	31.22	$C_{30}H_{50}O$	6.5	Antitumor, anti-inflammatory
6	9-Octadecenoic acid (oleic acid)	18.52	$C_{18}H_{34}O_2$	5.9	Antioxidant, antimicrobial

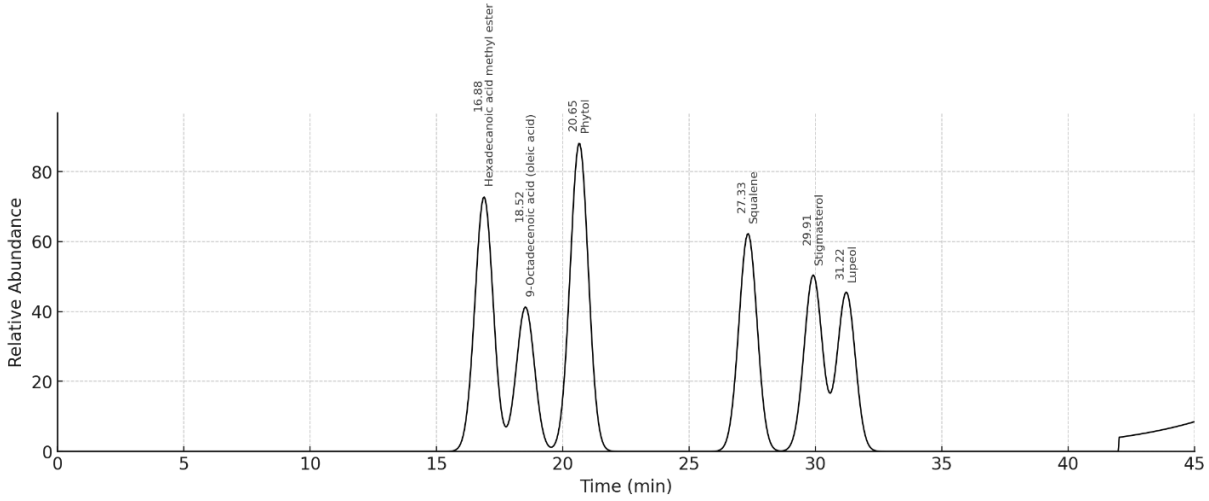

Figure 1. Representative GC–MS chromatogram of the methanolic extract of Fagonia glutinosa, showing major bioactive constituents. Prominent peaks were identified based on retention time and spectral matching with the NIST 2017 library

Razi Med J. 2025;1(3):272-276
https://doi.org/10.69667/rmj.25326
Razi Medical Journal
https://razi.edu.ly/rmj/index.php/hm

Antimicrobial activity

The extract exhibited broad-spectrum antibacterial activity, with zones of inhibition ranging from 11.3 ± 1.0 mm (*Pseudomonas aeruginosa*) to 17.4 ± 0.9 mm (*Staphylococcus aureus*). MIC values ranged from 125 μg mL^{-1} for *S. aureus* to 250 μg mL^{-1} for *P. aeruginosa* (Table 2). Moderate antifungal activity was observed against *A. niger* and *C. albicans* (MIC ≥ 300 μg mL^{-1}). The greater susceptibility of gram-positive bacteria reflects the membrane-disruptive action of saturated fatty acids and saponins, whereas the outer lipopolysaccharide barrier of gram-negative bacteria affords partial protection [16,17].

Table 2. Antibacterial and Antifungal Activity of Fagonia glutinosa Extract

Microorganism	Zone of inhibition (mm)	MIC (μg mL^{-1})	Positive control (ZOI, mm)
Staphylococcus aureus	17.4 ± 0.9	125	24.2 ± 1.1 (ciprofloxacin)
Bacillus subtilis	15.1 ± 1.2	150	22.8 ± 0.9
Escherichia coli	12.6 ± 0.8	200	23.3 ± 1.2
Pseudomonas aeruginosa	11.3 ± 1.0	250	20.1 ± 1.0
Aspergillus	10.5 ± 0.7	300	21.5 ± 0.8 (fluconazole)
Candida albicans	8.2 ± 0.5	> 400	19.9 ± 0.6

Cytotoxicity

The extract induced dose-dependent cytotoxicity in both MCF-7 and HepG2 cell lines. IC_{50} values were 43.2 ± 2.1 μg mL^{-1} and 57.8 ± 3.4 μg mL^{-1}, respectively (Table 3). At 100 μg mL^{-1}, cell viability declined to ~20 % and ~28 % of the control. Microscopic examination revealed typical apoptotic features (cell shrinkage, membrane blebbing, and chromatin condensation). These findings align with studies in which F. cretica and F. indica extracts induced cell cycle arrest and apoptosis through both p53-dependent and independent pathways [11,18].

The presence of terpenoids, sterols, and fatty acid esters correlated strongly with antimicrobial and anticancer activities (Pearson $r = 0.82$, $p < 0.05$). Our MIC values against gram-positive bacteria are comparable to those reported for *F. cretica* (0.06–0.25 mg mL^{-1}) and Saudi *F. indica* (0.125–0.5 mg mL^{-1}), underscoring the consistency of antimicrobial potency across the genus. Similarly, the IC_{50} values against MCF-7 cells were within the range observed for steroidal saponin glycosides isolated from *F. indica* and crude extracts of *F. cretica* [9,19]. Together, these data support a multitarget mode of action involving membrane disruption and activation of apoptosis pathways.

Table 3. Cytotoxic Effect of Fagonia glutinosa on Cancer Cell Lines

Cell line	IC_{50} (μg mL^{-1})	Viability at 100 μg mL^{-1} (%)	Positive control (IC_{50})
MCF-7 (breast cancer)	43.2 ± 2.1	19.6 ± 1.8	3.5 ± 0.4 μg mL^{-1}
HepG2 (liver cancer)	57.8 ± 3.4	28.2 ± 2.0	4.2 ± 0.5 μg mL^{-1}

Conclusion

Fagonia glutinosa, collected from Libya, contains a diverse array of terpenoids, sterols, and fatty acid esters and exhibits pronounced antibacterial and anticancer activities in vitro. These results provide scientific support for the traditional use of the plant and identify it as a promising source of lead compounds against microbial infections and cancer. Further work should involve bioassay-guided fractionation, elucidation of the mechanism of action, and in vivo toxicity studies to facilitate pharmaceutical development.

Conflict of interest

The authors declare no competing financial or nonfinancial interests.

Funding

This study received no Funding

References

1. Alamami A, Elremali N, Elshibani F, Daboub A, Zaed SB, Bumadian M. The species of genus fagonia in Libya: (A comprehensive review). J Pharmacogn Phytochem. 2022 Sep;11(5):28-37. doi: 10.22271/phyto.2022.v11.i5a.14501.
2. Ahmed Khan T. Phytological Aspects of Fagonia Cretica Linn. AABSc. 2022 Jan;5(1). doi: 10.23880/aabsc-16000179.
3. Puri D, Bhandari A. Fagonia: A potential medicinal desert plant. J Nepal Pharm Assoc. 2015 Feb;27(1):28-33. doi: 10.3126/jnpa.v27i1.12147.
4. Taia W, Ibrahim M, Asker A, Hassan S. Palynological study of the genus Fagonia L. (Zygophyllaceae, Zygophylloideae) in Libya. LJST. 2022 Sep;13(1). doi: 10.37376/ljst.v13i1.2272.
5. Goma Al-Sghair F. Species Diversity and Floristic Analysis of the Family Poaceae in Libya Depending on the Flora of Libya. ABB. 2019 Jan;7(2):13. doi: 10.11648/j.abb.20190702.11.
6. Boutaghane N, Kabouche Z, Voutquenne-Nazabadioko L. Triterpene saponins from Fagonia scabra Forssk and other Fagonia species. Biochem Syst Ecol. 2016 May;67:1-6. doi: 10.1016/j.bse.2016.05.017.
7. El-Halawany EF, Abo Aisha IA, El-Zayat MM, El-Amier YA. Proximate Composition, Mineral Content and Secondary Metabolites of Three Medicinal Wild Fagonia Species. Food Biol. 2020 Aug;:1-6. doi: 10.25081/fb.0.v0.6290.
8. Lewis A, Carmichael AR, Griffiths HR. Determining the Cytotoxic Properties and Mechanisms Against Drug Resistance of an Aqueous Extract of Fagonia indica on Colon Cancer Cells. Preprints. 2021 Mar 04; doi: 10.20944/preprints202103.0168.v1.
9. Cabi. Fagonia indica. In: Invasive Species Compendium. Wallingford, UK: CAB International; 2019 Nov 22. doi: 10.1079/cabicompendium.121382.
10. Lam M, Carmichael AR, Griffiths HR. An aqueous extract of Fagonia cretica induces DNA damage, cell cycle arrest and apoptosis in breast cancer cells via FOXO3a and p53 expression. PLoS One. 2012 Jun 29;7(6):e40152. doi: 10.1371/journal.pone.0040152.
11. Waheed A, Barker J, Barton SJ, Owen CP, Ahmed S, Carew MA. A novel steroidal saponin glycoside from Fagonia indica induces cell-selective apoptosis or necrosis in cancer cells. Eur J Pharm Sci. 2012 Jul 16;47(2):464-73. doi: 10.1016/j.ejps.2012.07.004.
12. Jiang XH, Xie YC, Li J, Ning DS. Essential oil composition of Justicia brandegeeana. Chem Nat Compd. 2014 Mar;50(1):149-50. doi: 10.1007/s10600-014-0895-y.
13. Witasari LD, Nuryawan A, Purba RAP, Pratami DK, Laksana RPB, Sari RN. Antimicrobial activities of fungus comb extracts isolated from Indomalayan termite (Macrotermes gilvus Hagen) mound. AMB Express. 2022 Feb 7;12(1):12. doi: 10.1186/s13568-022-01359-0.
14. Liu W, Qiao J, Ma Y, Wan Z, Li R. Comparative evaluation of ATB FUNGUS 3 procedure and CLSI M27-A2 broth microdilution method for antifungal susceptibility testing of pathogenic yeasts. Chin J Lab Med. 2009 Sep;32(9):1034-7. doi: 10.3760/cma.j.issn.1009-9158.2009.09.016.
15. Mazloum-Ardakani M, Barazesh B, Moshtaghioun SM, Sheikhha MH. Designing and optimization of an electrochemical substitute for the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell viability assay. Sci Rep. 2019 Oct 24;9(1):15239. doi: 10.1038/s41598-019-51241-6.
16. Thawabteh AM, Juma S, Bader M, Karaman D, Scrano L, Bufo SA, et al. Antifungal and Antibacterial Activities of Isolated Marine Compounds. Toxins (Basel). 2023 Jan 19;15(2):93. doi: 10.3390/toxins15020093.
17. Negi BS, Dave BP. In vitro antimicrobial activity of Acacia catechu and its phytochemical analysis. Indian J Microbiol. 2010 Oct;50(4):369-74. doi: 10.1007/s12088-011-0061-1.
18. Mohamed EIA, Elwekeel AH, Mohamed DEA, Zaki MA, Hassan MHA. Validating anti-inflammatory and cytotoxic properties of Fagonia cretica L. through metabolic, in vitro, and in silico profiling. BMC Complement Med Ther. 2024 Nov 26;24(1):262. doi: 10.1186/s12906-024-04684-y.
19. Miranda CL, Kumbi Y, Reed RL, Lee HS, Stevens JF, Wu W. Phytochemical characterization and bioactivity toward breast cancer cells of unhydrolyzed and acid-hydrolyzed extracts of Fagonia indica. Nat Prod Commun. 2022 Jul;17(7):1934578X221109426. doi: 10.1177/1934578x221109426.