

Comparative phytochemical profiling and biological evaluation of hydroalcoholic extracts of medicinal plants and their polyherbal mixture.

Mohini Vishwas, Gunjan Jadon*, Awadh Kishor, Pratim Kumar Choudhury

Pacific College of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan-313001

Corresponding Author: Dr. Gunjan Jadon, Email: jadon_gunjan@yahoo.in

Abstract

Skin infections and inflammatory disorders are common health problems, and the search for safer plant-based therapies has increased in recent years. The present study aimed to evaluate the phytochemical composition, antioxidant, antimicrobial, and in vitro anti-inflammatory activities of hydroalcoholic extracts of *Cressa cretica*, *Fagonia indica*, *Plantago major*, and their polyherbal combination. The extracts were prepared by hydroalcoholic maceration and screened for phytochemical constituents. Total phenolic content (TPC), total flavonoid content (TFC), DPPH free radical scavenging activity, antimicrobial activity by agar well diffusion and minimum inhibitory concentration (MIC), and in vitro anti-inflammatory activity by the protein denaturation assay were evaluated using standard methods. The results showed that all extracts contained important phytochemicals such as phenolics, flavonoids, alkaloids, saponins, and terpenoids. Among the individual extracts, *Cressa cretica* showed comparatively better biological activity, while the polyherbal extract exhibited the highest TPC and TFC, strongest antioxidant activity with the lowest IC₅₀ value, larger zones of inhibition, lower MIC values, and better inhibition of protein denaturation. These findings suggest that the polyherbal extract possesses enhanced biological activity due to the combined action of bioactive phytochemicals. Therefore, it may serve as a promising natural candidate for the management of skin infections and inflammatory conditions

Keyword: Polyherbal extract; *Cressa cretica*; Antioxidant activity; Antimicrobial activity; Anti-inflammatory activity

How to cite this article: Mohini Vishwas Gunjan Jadon* Awadh Kishor Pratim Kumar Choudhury. Comparative phytochemical profiling and biological evaluation of hydroalcoholic extracts of medicinal plants and their polyherbal mixture.. Int J Drug Deliv Technol. 2026;16(85s):1764-1770. DOI: 10.25258/ijddt.16.85s.178.

Introduction

Skin infections and inflammatory disorders are among the most common health problems worldwide, affecting individuals of all age groups. These conditions include bacterial and fungal infections, wounds, dermatitis, eczema, acne, and other inflammatory skin diseases that impair skin integrity and reduce quality of life. Conventional treatment mainly relies on antibiotics, corticosteroids, and non-steroidal anti-inflammatory drugs (NSAIDs). Although these therapies are generally effective, their prolonged or indiscriminate use is often associated with adverse effects such as skin irritation, delayed wound healing, microbial resistance, and recurrence of infection.^{1,2} The increasing prevalence of antimicrobial resistance and the growing demand for safer and more affordable therapeutic options have renewed interest in medicinal plants as alternative or complementary treatments. Herbal medicines are rich sources of bioactive phytochemicals, including phenolic compounds, flavonoids, alkaloids, terpenoids, and tannins, which exhibit antioxidant, antimicrobial, and anti-inflammatory activities.³ These multiple pharmacological properties make medicinal plants promising candidates for the development of effective topical formulations for the management of skin infections and inflammatory disorders while potentially reducing the limitations associated with conventional synthetic drugs.^{3,4}

Cressa cretica L. (Rudanti) is a halophytic medicinal plant widely distributed in arid and saline regions of India. In traditional medicine, it has been used for the management of asthma, ulcers, diabetes, inflammation, and microbial infections.

Phytochemical investigations have revealed the presence of flavonoids, phenolic acids, coumarins, and triterpenoids that contribute to its antioxidant, antimicrobial, and anti-inflammatory properties.⁵ *Fagonia indica* (Dhamasa), an important medicinal herb of the family Zygophyllaceae, is extensively used in Ayurvedic and folk medicine for the treatment of wounds, skin disorders, fever, and inflammatory conditions. It contains various bioactive constituents, including flavonoids, saponins, alkaloids, and phenolic compounds, which have been reported to exhibit potent antioxidant, antimicrobial, and anti-inflammatory activities.⁶ *Plantago major* (Greater Plantain) is another well-known medicinal plant traditionally used for wound healing and the treatment of skin infections. The plant is rich in iridoid glycosides, phenolic acids, flavonoids, and polysaccharides, which are responsible for its broad spectrum of pharmacological activities.^{7,8} Since medicinal plants contain multiple bioactive constituents that act through different mechanisms, combining them in a polyherbal formulation may provide synergistic therapeutic effects, enhance biological efficacy, and simultaneously improve antioxidant, antimicrobial, and anti-inflammatory activities while minimizing the limitations associated with the use of a single plant extract.⁹

Although *Cressa cretica*, *Fagonia indica*, and *Plantago major* have been individually investigated for their phytochemical composition and various pharmacological activities, comparative studies evaluating their hydroalcoholic extracts under identical experimental conditions are limited. Moreover, there is a lack of scientific evidence

regarding the biological potential of their polyherbal combination, particularly in relation to antioxidant, antimicrobial, and anti-inflammatory activities. Most published studies have focused on a single plant or a specific pharmacological property, while comprehensive comparative evaluation of these medicinal plants and their combined extract remains insufficient¹⁰⁻¹¹. Therefore, further investigation is required to establish whether a polyherbal approach offers any additional therapeutic advantage over the individual extracts.

Based on this background, the present study was designed to prepare hydroalcoholic extracts of *Cressa cretica*, *Fagonia indica*, and *Plantago major*, individually and as a polyherbal combination, and to comparatively evaluate their phytochemical profile, total phenolic and flavonoid contents, antioxidant activity, antimicrobial potential, and in vitro anti-inflammatory activity. The findings of this study are expected to provide scientific evidence supporting the therapeutic potential of the selected medicinal plants and their polyherbal combination for the management of skin infections and inflammatory disorders.

Materials and Methods

2.1 Plant Materials

Fresh whole plants of *Cressa cretica* L., *Fagonia indica* Burm.f., and *Plantago major* L. were collected from different regions of Rajasthan, India. The collected plant materials were authenticated by a qualified taxonomist. The plant materials were washed thoroughly with distilled water to remove dust and other impurities, shade-dried at room temperature for about 15 days, and then coarsely powdered using a mechanical grinder. The powdered samples were stored in airtight containers at room temperature until further use.

2.2 Preparation of Hydroalcoholic Extracts

The shade-dried plant materials of *Cressa cretica*, *Fagonia indica*, and *Plantago major* were separately powdered using a mechanical grinder. Each powdered sample was extracted by the cold maceration method using a hydroalcoholic solvent (ethanol:water, 80:20 v/v). The plant powder was soaked in the solvent for 15 days at room temperature with occasional shaking to ensure proper extraction of phytoconstituents. After maceration, the extracts were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary vacuum evaporator at a temperature below 45°C. The concentrated extracts were further dried to obtain semisolid masses and stored in airtight containers at 4°C until further analysis. A polyherbal extract was prepared by mixing equal quantities of the dried hydroalcoholic extracts of the three plants. The percentage yield of each extract was calculated using the weight of the dried extract with respect to the initial weight of the powdered plant material and expressed as percentage (% w/w).^{12,13}

2.3 Preliminary Phytochemical Screening

The hydroalcoholic extracts of *Cressa cretica*, *Fagonia indica*, *Plantago major*, and the polyherbal extract were subjected to preliminary phytochemical

screening using standard qualitative methods. The extracts were screened for the presence of major secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, terpenoids, steroids, proteins, carbohydrates, and fixed oils. This preliminary analysis was carried out to identify the major classes of bioactive compounds that could be responsible for the observed antioxidant, antimicrobial, and anti-inflammatory activities of the extracts.¹⁴

2.4 Determination of Total Phenolic Content

The total phenolic content (TPC) of the individual plant extracts and the polyherbal extract was determined using the Folin-Ciocalteu colorimetric method described by Singleton and Rossi with slight modifications. Gallic acid was used as the reference standard for preparing the calibration curve. The calibration curve was constructed using gallic acid solutions in the concentration range of 25–125 µg/mL. The absorbance of the reaction mixture was measured at 765 nm using a UV-Visible spectrophotometer, and the results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg gallic acid/g). All measurements were carried out in triplicate.¹⁵

2.5 Determination of Total Flavonoid Content

The total flavonoid content (TFC) was determined by the aluminium chloride colorimetric method with slight modifications of the reported procedure. Quercetin (QE) was used as the reference standard for preparing the calibration curve. The calibration curve was constructed using quercetin solutions in the concentration range of 25–125 µg/mL. After completion of the reaction, the absorbance was measured at 510 nm using a UV-Visible spectrophotometer, and the flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g). All analyses were performed in triplicate.¹⁶

2.6 Antioxidant activity by DPPH Radical Scavenging method

The antioxidant activity of the hydroalcoholic extracts and the polyherbal extract was evaluated by the DPPH free radical scavenging assay with slight modifications of the reported method. Stock solutions of the extracts (1000 µg/mL) were prepared in methanol and diluted to obtain concentrations of 25, 50, 75, and 100 µg/mL. Each test solution was mixed with freshly prepared 0.5 ml DPPH solution and incubated in the dark for 30 min. The absorbance was measured at 517 nm using a UV-Visible spectrophotometer, and L-ascorbic acid was used as the reference standard. The percentage radical scavenging activity and IC₅₀ values were calculated.¹⁷

2.7 Antimicrobial Activity

2.7.1 Agar Well Diffusion Method

The antimicrobial activity of the hydroalcoholic extracts of *Cressa cretica*, *Fagonia indica*, *Plantago major*, and the polyherbal extract was evaluated by the agar well diffusion method. Mueller-Hinton agar plates were inoculated with standardized cultures of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. Wells of 6 mm diameter were

prepared aseptically, and each well was filled with the extract solution (100 mg/mL). Ciprofloxacin and fluconazole were used as the standard antibacterial and antifungal drugs, respectively. The plates were incubated at 37°C for 18–24 h, and the zone of inhibition was measured in millimetres. All experiments were performed in triplicate.^{18,19}

2.7.2 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the extracts was determined by the broth serial dilution method. Two-fold serial dilutions of each extract were prepared in Mueller–Hinton broth, followed by inoculation with standardized microbial suspension (0.5 McFarland standard). The tubes were incubated at 37°C for 18–24 h, and the lowest concentration showing no visible microbial growth was recorded as the MIC. All experiments were carried out in triplicate.^{18,20}

2.8 In Vitro Anti-inflammatory Activity by Protein Denaturation Assay

The in vitro anti-inflammatory activity of the hydroalcoholic extracts and the polyherbal extract was evaluated by the inhibition of heat-induced bovine serum albumin (BSA) denaturation method with slight modifications of the reported procedure. Different concentrations of the extracts (25, 50, 75, and 100 µg/mL) were mixed with 5% aqueous BSA solution, and the pH was adjusted to 6.3. The reaction mixture was incubated at 37°C for 20 min, followed by heating at 57°C for 20 min. After cooling, phosphate buffer (pH 6.3) was added and the absorbance was measured at 660 nm using a UV–Visible spectrophotometer. Diclofenac sodium was used as the reference standard. The percentage inhibition of protein denaturation and IC₅₀ values were calculated. All experiments were performed in triplicate.^{21,22}

Result and Discussion

3.1 Percentage Yield of Hydroalcoholic Extracts

The percentage yield of the hydroalcoholic extracts obtained from *Cressa cretica*, *Fagonia indica*, *Plantago major*, and the polyherbal mixture is presented in **Figure 1**. Among the individual plants, *Cressa cretica* showed the highest extraction yield (10.2% w/w), followed by *Plantago major* (6.4% w/w) and *Fagonia indica* (4.1% w/w). The higher extractive yield of *Cressa cretica* indicates the presence of a comparatively greater amount of hydroalcohol-soluble phytoconstituents.

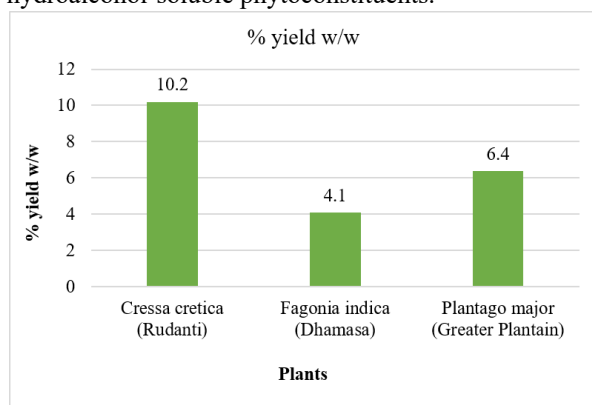


Figure1: Percentage yield of hydroalcoholic extracts

3.2 Preliminary Phytochemical Screening

The results of the preliminary phytochemical screening are presented in **Table 1**. All three hydroalcoholic extracts and the polyherbal extract showed the presence of important secondary metabolites, including alkaloids, flavonoids, phenolic compounds, saponins, and terpenoids. Tannins were detected in *Cressa cretica* and *Fagonia indica* but were absent in *Plantago major*. Glycosides and proteins were not detected in any of the extracts.

The presence of phenolics and flavonoids suggests that these extracts possess good antioxidant potential, while alkaloids, saponins, and terpenoids are known to contribute to antimicrobial and anti-inflammatory activities. The combination of these phytochemicals in the polyherbal extract may produce a broader therapeutic effect through synergistic interactions among the different bioactive compounds.^{5,7,23}

Table 1: phytochemical screening test of of hydroalcoholic Extracts

S.N.	Constituents	<i>C. cretica</i>	<i>F.indica</i>	<i>P. major</i>
1	Alkaloids	+	+	+
2	Flavonoids	+	+	+
3	Phenols	+	+	+
4	Tannins	+	+	-
5	Saponins	+	+	+
6	Steroids & terpenoids	+	+	+
7	Glycosides	-	-	-
8	Carbohydrates	+	+	+
9	Antraquinones	-	+	-

(+) indicates presence of phytochemicals; (-) indicates absence of phytochemicals

3.3 Total Phenolic Content (TPC)

The total phenolic content of the hydroalcoholic extracts and the polyherbal extract was determined by the Folin–Ciocalteu method, and the results are presented in **Table 2**. Among the individual extracts, *Cressa cretica* showed the highest total phenolic content, followed by *Fagonia indica* and *Plantago major*. The polyherbal extract exhibited the highest phenolic content among all the samples, indicating that combining the three plant extracts increased the overall concentration of phenolic compounds.

Phenolic compounds are well known for their antioxidant properties because they can donate hydrogen atoms or electrons to neutralize free radicals. Therefore, the higher phenolic content observed in the polyherbal extract may contribute to its better antioxidant and biological activities.²⁴

Table 2: Total phenolic content of plant extracts and polyherbal mixture

S. No.	Sample	Total Phenolic Content (mg Gallic acid/g extract)
1	Cressa cretica (C)	56.84 ± 1.36
2	Fagonia indica (F)	45.52 ± 1.12
3	Plantago major (P)	52.73 ± 1.28
4	Polyherbal mixture (M)	59.67 ± 1.42

3.4 Total Flavonoid Content (TFC)

The total flavonoid content of the hydroalcoholic extracts and the polyherbal extract was estimated by the aluminium chloride colorimetric method, and the results are presented in **Table 3**. Among the individual plant extracts, *Cressa cretica* showed the highest flavonoid content, followed by *Fagonia indica* and *Plantago major*. The polyherbal extract exhibited the highest total flavonoid content among all the samples, indicating that the combination of the three medicinal plants resulted in an increased level of flavonoids.

Flavonoids are one of the major groups of plant secondary metabolites and are well known for their antioxidant, antimicrobial, and anti-inflammatory properties. Therefore, the higher flavonoid content observed in the polyherbal extract support the concept that combining medicinal plants can provide a broader range of phytoconstituents and may produce a synergistic therapeutic effect.

Table 3: Total flavonoid content of plant extracts and polyherbal mixture

S. No.	Sample	Total Flavonoid Content (mg Quercetin/g extract)
1	Cressa cretica (C)	31.42 ± 0.94
2	Fagonia indica (F)	24.56 ± 0.81
3	Plantago major (P)	28.73 ± 0.88
4	Polyherbal mixture (M)	34.81 ± 1.03

3.5 DPPH Radical Scavenging Activity

The antioxidant activity of the hydroalcoholic extracts and the polyherbal extract was evaluated by the DPPH free radical scavenging assay. The percentage inhibition at different concentrations and the IC₅₀ values are presented in **Table 4** and **Figure 2**. All the extracts showed concentration-dependent free radical scavenging activity. Among the individual extracts, *Cressa cretica* exhibited the highest antioxidant activity, followed by *Fagonia indica* and *Plantago major*. The polyherbal extract showed the highest

percentage inhibition at all tested concentrations and produced the lowest IC₅₀ value, indicating the strongest antioxidant potential. L-ascorbic acid showed the maximum scavenging activity and served as the reference standard.

The improved antioxidant activity of the polyherbal extract may be attributed to the combined presence of phenolic compounds and flavonoids from all three medicinal plants. These phytoconstituents can donate hydrogen atoms or electrons to neutralize DPPH free radicals, thereby reducing oxidative stress²⁵. The higher total phenolic and flavonoid contents observed in the polyherbal extract were also consistent with its lower IC₅₀ value, suggesting a positive relationship between phytochemical content and antioxidant activity.

Table 4: Percentage DPPH Radical Scavenging Activity of Plant Extracts and Polyherbal Mixture

S. No.	Sample	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL	IC50 (µg/mL)
1	Cressa cretica (C)	35.35	53.61	68.54	77.38	45.08
2	Fagonia indica (F)	15.57	25.78	48.95	67	76.45
3	Plantago major (P)	31.28	42.97	63.3	73.61	58.65
4	Polyherbal mixture (M)	40.04	59.68	73.38	84.48	37.67

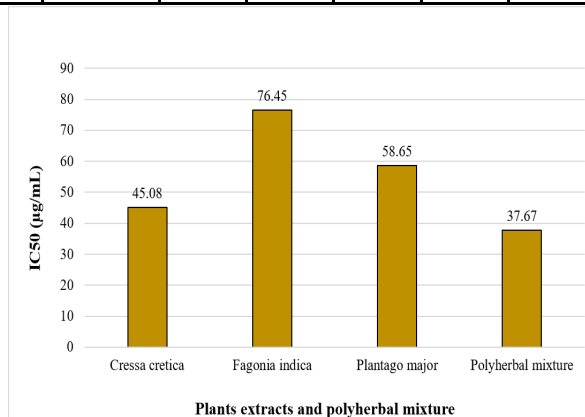


Figure 2: Comparative IC₅₀ (µg/mL) value of individual Plants extracts and the polyherbal mixture.

3.6 Antimicrobial Activity (Zone of Inhibition)

The antimicrobial activity of the hydroalcoholic extracts and the polyherbal extract was evaluated against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* by the agar well diffusion method. The results are presented in **Table 5** and **figure 3**. All the extracts showed antimicrobial activity against the tested microorganisms, although the degree of inhibition varied among the extracts. The polyherbal extract produced the largest zones of inhibition against all the tested organisms, followed by *Cressa cretica*, *Fagonia indica*, and *Plantago major*. Ciprofloxacin and fluconazole showed the highest inhibition zones and were used as the reference standards.

Among the tested microorganisms, Gram-positive bacteria were found to be more susceptible than Gram-negative bacteria. The comparatively lower activity against *E. coli* and *P. aeruginosa* may be due to the presence of an outer membrane, which limits the penetration of many phytochemicals. The enhanced antimicrobial activity of the polyherbal extract may be attributed to the combined action of phenolics, flavonoids, alkaloids, saponins, and terpenoids present in the three plant extracts. The results indicate that the polyherbal combination possesses broader antimicrobial activity than the individual extracts, suggesting a possible synergistic effect among the bioactive constituents.²⁶⁻²⁷

Table 5: Zone of inhibition (mm) of plant extracts and polyherbal mixture at 100 mg/mL

Microorganism	<i>Cressa cretica</i>	<i>Fagonia indica</i>	<i>Plantago major</i>	polyherbal mixture	Standard
<i>Staphylococcus aureus</i>	16.2 ± 0.4	12.8 ± 0.5	14.9 ± 0.4	18.7 ± 0.5	29.1 ± 0.4
<i>Staphylococcus epidermidis</i>	15.4 ± 0.5	11.9 ± 0.4	14.2 ± 0.5	17.8 ± 0.4	28.3 ± 0.5
<i>Escherichia coli</i>	13.8 ± 0.4	10.6 ± 0.3	12.9 ± 0.4	16.1 ± 0.5	27.4 ± 0.4
<i>Pseudomonas aeruginosa</i>	11.9 ± 0.5	9.5 ± 0.4	11.2 ± 0.4	13.9 ± 0.4	25.8 ± 0.5
<i>Candida albicans</i>	14.2 ± 0.4	10.9 ± 0.4	13.1 ± 0.5	16.5 ± 0.4	25.2 ± 0.4

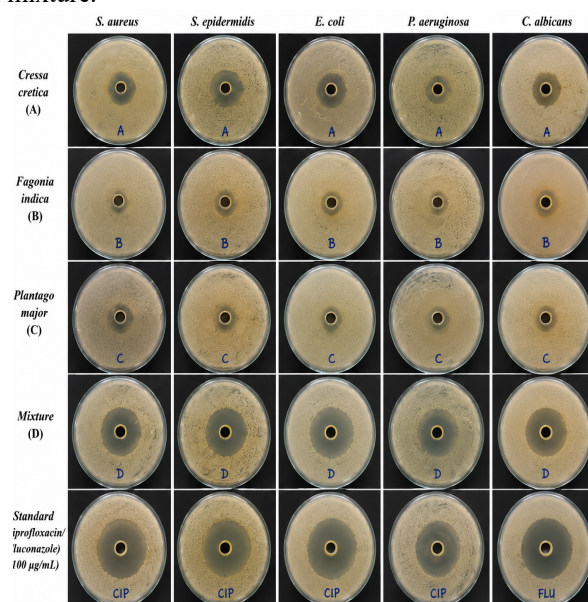


Figure 3: Zone of inhibition exhibited by extracts, polyherbal mixture and standards

3.7 Minimum Inhibitory Concentration (MIC) of Antimicrobial Activity

The minimum inhibitory concentration (MIC) of the hydroalcoholic extracts and the polyherbal extract against the selected microbial strains is presented in **Table 6** and **figure 4**. The MIC values varied among the individual extracts depending on the test organism. Among the individual plants, *Cressa cretica* showed lower MIC values than *Fagonia indica* and *Plantago major*, indicating better antimicrobial activity. The polyherbal extract exhibited the lowest MIC values against all the tested microorganisms, showing that it was more effective than the individual extracts in inhibiting microbial growth.

The lower MIC values of the polyherbal extract suggest that combining the three medicinal plants enhanced their antimicrobial efficacy. This improved activity may be due to the synergistic action of different phytochemicals, such as phenolics, flavonoids, alkaloids, saponins, and terpenoids, which act through multiple mechanisms to inhibit microbial growth. Similar studies on polyherbal formulations have also reported that combinations of medicinal plant extracts produce lower MIC values than individual extracts, indicating enhanced antimicrobial potential.²⁸

Table 6: Minimum inhibitory concentration (MIC) of extracts (mg/mL)

Microorganism	<i>Cressa cretica</i>	<i>Fagonia indica</i>	<i>Plantago major</i>	polyherbal mixture
<i>Staphylococcus aureus</i>	1.56	3.12	1.56	0.78
<i>Staphylococcus</i>	1.56	3.12	1.56	0.78

<i>epidermidis</i>				
<i>Escherichia coli</i>	3.12	6.25	3.12	1.56
<i>Pseudomonas aeruginosa</i>	6.25	12.50	6.25	3.12
<i>Candida albicans</i>	3.12	6.25	3.12	1.56

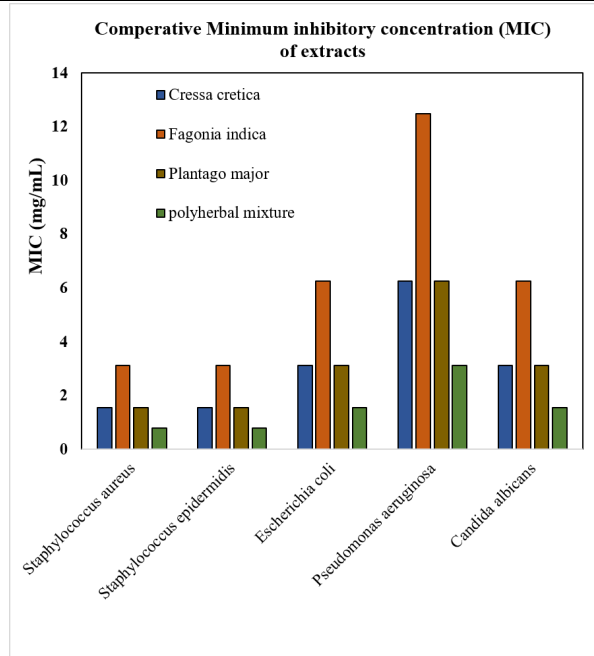


Figure 4: Comparative MIC values of extracts and polyherbal mixture

3.8 In Vitro Anti-inflammatory Activity

The in vitro anti-inflammatory activity of the hydroalcoholic extracts and the polyherbal extract was evaluated by the protein denaturation assay. The percentage inhibition at different concentrations and the IC₅₀ values are presented in **Table 7** and **Figure 5**. All the extracts showed concentration-dependent inhibition of protein denaturation. Among the individual extracts, *Cressa cretica* exhibited the highest anti-inflammatory activity, followed by *Fagonia indica* and *Plantago major*. The polyherbal extract showed the highest percentage inhibition at all tested concentrations and the lowest IC₅₀ value, indicating better anti-inflammatory activity. Diclofenac sodium, used as the standard drug, showed the highest inhibition of protein denaturation.

The better anti-inflammatory activity of the polyherbal extract may be due to the combined action of phenolic compounds, flavonoids, and other bioactive phytochemicals present in the three medicinal plants. These compounds help stabilize proteins against heat-induced denaturation, which is considered an important mechanism in reducing inflammation. The results also agreed with the antioxidant study, where the polyherbal extract showed higher phenolic and flavonoid contents and stronger free radical

scavenging activity. This suggests that the antioxidant and anti-inflammatory activities of the polyherbal extract are closely related.^{21-22,29}

Table 7: Percentage inhibition of protein denaturation

Sample	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL
Cressa cretica	34.90 ± 0.84	52.79 ± 0.92	68.04 ± 1.08	78.01 ± 1.14
Fagonia indica	20.97 ± 0.62	31.52 ± 0.78	47.95 ± 0.96	60.70 ± 1.08
Plantago major	29.77 ± 0.73	43.84 ± 0.84	61.73 ± 1.04	72.29 ± 1.10
Polyherbal mixture	39.74 ± 0.92	58.80 ± 1.06	74.93 ± 1.18	85.34 ± 1.22
Diclofenac sodium	48.68 ± 0.96	68.62 ± 1.12	85.04 ± 1.24	94.72 ± 1.30

Values are expressed as Mean ± SD (n = 3).

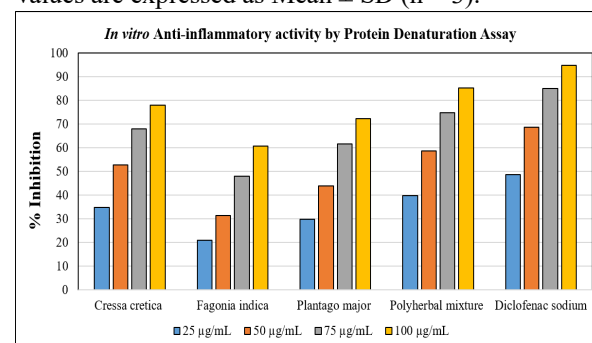


Figure 5: Concentration-dependent inhibition of protein denaturation by hydroalcoholic extracts, polyherbal mixture and diclofenac sodium

Conclusion

The present study demonstrated that the hydroalcoholic extracts of *Cressa cretica*, *Fagonia indica*, and *Plantago major* possess important phytochemicals with promising biological activities. Among the individual extracts, *Cressa cretica* showed comparatively better activity, while the polyherbal extract exhibited the highest phenolic and flavonoid contents, strongest antioxidant activity, better antimicrobial effect, lower MIC values, and maximum inhibition of protein denaturation. These findings suggest that combining the three medicinal plants enhanced their biological activity through a synergistic effect. Therefore, the developed polyherbal extract may be considered a promising natural candidate for the management of skin infections and inflammation.

References

1. Ki V, Rotstein C. Bacterial skin and soft tissue infections in adults: A review of their epidemiology, pathogenesis, diagnosis, treatment and site of care. Can J Infect Dis Med Microbiol. 2008;19(2):173–84.
2. Wang YF, Que HF, Wang YJ, Cui XJ. Chinese herbal medicines for treating skin

- and soft-tissue infections. *Cochrane Database Syst Rev.* 2014;(7):CD010619.
3. Kędzia B, Granica S, et al. Medicinal plants for skin disorders: Phytochemistry and pharmacological insights. *Molecules.* 2025;30(15):3281.
4. Yeşilada E, et al. Medicinal plants used traditionally for skin-related problems in the South Balkan and East Mediterranean region—A review. *Front Pharmacol.* 2022;13:866047.
5. Mimani T, Ray A, Patel V, et al. A review on *Cressa cretica* Linn.: A halophytic plant. *Pharmacogn Rev.* 2011;5(10):221–26.
6. Qureshi H, Asif S, Ahmed H, Al-Kahtani HA, Hayat K. Chemical composition and medicinal significance of *Fagonia cretica*: A review. *Nat Prod Res.* 2016;30(6):625–39.
7. Adom MB, Taher M, Mutalabisin MF, et al. Chemical constituents and medical benefits of *Plantago major*. *Biomed Pharmacother.* 2017;96:348–60.
8. Samuelsen AB. The traditional uses, chemical constituents and biological activities of *Plantago major* L. A review. *J Ethnopharmacol.* 2000;71(1–2):1–21.
9. Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: Concept of Ayurveda. *Pharmacogn Rev.* 2014;8(16):73–80.
10. Aladejana EB. Biological Properties of Polyherbal Formulations: A Review of their Antimicrobial, Anti-Inflammatory, Antioxidant, and Toxicological Activities. *Pharmacogn J.* 2023;15(5):933–963.
11. Abrar A, Zafar A, Fatima M, et al. Mechanistic insight into the synergistic antimicrobial potential of *Fagonia indica* Burm.f. extracts with cefixime. *Saudi Pharm J.* 2024;32:101893.
12. Chanda S, Kaneria M. Assessment of effect of hydroalcoholic and decoction methods on extraction of antioxidants from selected Indian medicinal plants. *Asian Pac J Trop Biomed.* 2012;2(3):195–202.
13. Al-Rashidi RA, Nasrallah H, Bouazzi A, Zaïri A. Preparation of plant extract through hydroalcoholic maceration. *Bio-protocol.* 2025. Available from: [Bio-protocol](#)
14. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis.* 3rd ed. London: Chapman & Hall; 1998.
15. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic.* 1965;16(3):144–158.
16. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal.* 2002;10(3):178–182.
17. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci Technol.* 1995;28(1):25–30.
18. Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically (CLSI M07).* 12th ed. Wayne, PA: CLSI; 2024.
19. Perez C, Pauli M, Bazerque P. An antibiotic assay by the agar well diffusion method. *Acta Biol Med Exp.* 1990;15:113–115.
20. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing.* Wayne, PA: CLSI; 2024.
21. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *J Pharm Pharmacol.* 1968;20(3):169–173.
22. Sakat S, Juvekar AR, Gambhire MN. In vitro antioxidant and anti-inflammatory activity of *Methanol extract of Oxalis corniculata* Linn. *Int J Pharm Pharm Sci.* 2010;2(1):146–155.
23. Sulieman AME, Alanaizy E, Alanaizy NA, Abdallah EM, Idriss H, Salih ZA, et al. Unveiling chemical, antioxidant and antibacterial properties of *Fagonia indica* grown in the Hail Mountains, Saudi Arabia. *Plants (Basel).* 2023;12(6):1354.
24. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods Enzymol.* 1999;299:152–178.
25. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. *J Food Sci Technol.* 2011;48(4):412–422.
26. Chali DA, Feyisa SG, Kenea MA, et al. Phytochemical screening and evaluating in vitro antibacterial and antifungal activity of 80% methanolic *Impatiens rothii* root extract. *BMC Complement Med Ther.* 2024.
27. Joshi B, Sah GP, Basnet BB, et al. In vitro antimicrobial activity of *Acacia catechu* and its phytochemical analysis. *Indian J Microbiol.* 2011;51(3):369–374.
28. Chotchuan P, Phumisantiphong U, et al. In vitro synergistic inhibitory effects of plant extract combinations on bacterial growth of methicillin-resistant *Staphylococcus aureus*. *Antibiotics (Basel).* 2023;12:1605.
29. Ramesh M, et al. In vitro anti-inflammatory activity of *Ficus racemosa* bark using albumin denaturation method. *Anc Sci Life.* 2019;38(4):219–223.