Applied Chemical Engineering (2026) Volume 9 Issue 1 ISSN: 2578-2010 (O)
doi: 10.59429/ace.v9i1.5815

ORIGINAL RESEARCH ARTICLE

Methanolic extraction of three plants and their biological activity against different pathogenic bacteria

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ABSTRACT

The increased new and remerging infectious diseases, need to discovery and urgent of new microbial compounds having diverse chemical structures and novel mechanism have also been. The study focused on determining the antibacterial properties of pomegranate peels (Punica granatum), Cordia myxa fruits, and Citrullus colosynthis fruits, against pathogenic bacteria E.coli, Pseudomonas aeruginosa as Gram-negative and Staphylococcus aureus as Gram-negative. Also the antioxidant as free radical scavenge to the plants The plant parts were collected, shade dried and powdered and subjected to extraction by methanol maceration as solvents. The extracts were used to determine the antibacterial activity by agar well diffusion method and MIC assay. The results showed that the plant extracts inhibited the growth of both Gram-positive and negative bacteria, Gram-negative bacteria were more sensitive. The inhibition zone (in millimeters) was greater for Gram-negative bacteria (17 mm) than for Gram-positive bacteria (0 mm) with *Cordia myxa*. *Punica* peel and *Citrullus colocynthis* fruits extracts exhibited high biological activity against all types of bacteria at concentrations of 0.1, 0.5, and 1 mg/mL, respectively. *Cordia myxa* had the highest percentage of free radical scavenging, followed by *Punica* and then *Citrullus colocynthis.* On the basis of the Data obtained it is clear that plant extracts can be used as effective herbal cure against human pathogens.

Keywords: methanol extract; Punica; Citrullus colocynthis; Cordia myxa; biological activity; antioxidant

ARTICLE INFO

Received: 3 November 2025
Accepted: 15 December 2025
Available online: 22 January 2026

1. Introduction

A medicinal plant is any plant that contains substances in one or more of its organs that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs. This definition distinguishes between medicinal plants whose therapeutic properties and constituents have been scientifically proven and those regarded as medicinal but not yet thoroughly studied [1]. Many plants have been used in traditional medicine for years. Some appear to be effective, although there may not be sufficient scientific data, such as double-blind trials, to confirm their efficacy. These plants should qualify as medicinal plants. Pharmacists and pharmacologists use the term "crude drugs of natural or biological origin" to describe whole plants or parts of plants with medicinal properties [2].Also In recent years, there were an increasing in appearance of multiple resistances in human pathogenic microorganisms, this largely results from random use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases . This fact, forced scientists for searching for new antimicrobial substances [3].For the purpose of this

study a definition of medicinal plants should include the following: Plants used to extract pure substances for direct medicinal use or hemi-synthesis of medicinal compounds and antioxidant activity against free radicals (DPPH). The study deals with three plants were *Punica granatum* Peels, *Citrullus colocynthis* fruits *and Cordia myxa* Fruits.

Pomegranate Peel (Punica granatum) contains numerous phytochemicals, including, flavonoids, tannins, gallic acids and anthocyanins, have been associated with the medicinal potential of pomegranate peel. [4] According to various studies, the peels has higher concentration of biologically active components than the fruit , the punicalagin (α and β), ellagic acid,a notable increase in the levels of superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT), anticancer, antimicrobial, wound healing, and anti-inflammatory properties of pomegranate peel [5, 6].

The desert vine *Citrullus colocynthis* grows in sandy, arid soils. It resembles the watermelon, which belongs to the same genus. Native to the Mediterranean Basin and Asia, it is found along the western coast of North Africa, through the Sahara and Egypt, and as far east as India. It also grows along the northern coast of the Mediterranean Sea and the Caspian Sea. It also grows in southern European countries and on the islands of the Greek archipelago. On *Cyprus*, it has been cultivated on a small scale since the 14th century and is still exported today [7]. Previous studies have reported that aqueous and diluted acetone extracts from the roots, stems, and leaves of the *C. colocynthis* plant, as well as the fruit and seeds at three maturation stages, are active against Gram-positive and Gram-negative bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aureus* , and *Enterococcus faecalis*. *Staphylococcus aureus* is a dangerous gram positive bacterial pathogen which, not only evades the host's immune system but also can destroy the leucocytes especially neutrophils[8]. However, they have a more substantial effect on newer bacteria. The broth dilution method was used to measure the minimum inhibitory concentration (MIC), which prevents visible bacterial growth. The MIC was tested for concentrations ranging from 0.10 to 6.50 mg/mL. For aqueous extracts of immature fruits, the MIC was 0.20 mg/ml for *E. coli* and *P. aeruginosa*. Activity depends on strains, plant organs, stage of maturity, and extraction method [9].

The Assyrian plum, *Cordia myxa*, The tree, its leave and fruit have important applications in various societies, and the fruit is renowned for its medicinal properties. , Further research is necessary to improve our understanding of the fruit's postharvest quality attributes and characteristics, as well as to develop effective postharvest technologies that can maintain quality and extend shelf life [10]. A hydro-alcoholic extract of *C. myxa* fruit could serve as an alternative anti-inflammatory therapy for managing inflammatory conditions, or as a complementary therapy that allows patients to take smaller doses of conventional anti-inflammatory drugs, thereby minimizing these drugs' side effects [6]. The general steps of this study are methanol plant extractions. Study the evaluation of the extract's biological activity as an antibacterial and antioxidant.

1.1. Antioxidant activity

Free radicals are naturally produced in the human body as a result of normal metabolism and different endogenous processes. Due to their high reactivity as oxidants and inhibitors of enzymes, free radicals lead to the oxidation of biomolecules, such as proteins, lipids, DNA, and amino acids, which ultimately results in cell damage and consequently cell death. Thus, for proper physiological function in the body, free radicals and antioxidants must be balanced[11]. Antioxidant phytochemicals exist widely in fruits, vegetables, cereal grains, edible macrofungi, microalgae, and medicinal plants, such as Polyphenols , carotenoids, phenolic and flavonoids compounds which help to enhance its antioxidants capacity, Crude extracts of plants by emersion extraction method with solvents such as methanol [12].

2. Methodology

2.1. Collect and prepare plant extracts

The fruits and peels of the selected plants were collected from the field, cleaned, washed with distilled water, and air-dried in the laboratory. The fruits of Cordia myxa (20 g), C. colosynthis (20 g) and the peels of P. granatum (20 g), then were ground into a fine powder using an electric grinder after drying them at room temperature. Then, each powder was extracted using 200 mL of methanol and left for 24 hours. The mixture was filtered through Whatman filter paper No. 5, and the flow-through was dried in an oven at 40 °C for two to three days. The substances were extracted and weighed. Then, 1 g of each extract was dissolved separately in 100 mL of methanol to obtain a 1% stock solution [13, 14].

2.1.1. Chemical detection of the active components in plant extracts[15]

1. Phenols

a.Ferric Chloride reagents : The extracts were treated with three to four drops of ferric chloride solution. The formation of a bluish-black color indicates the presence of phenols.

b. Lead acetate: The 50 mg extract was dissolved in 5 ml of distilled water. Then, 3 ml of 10% lead acetate solution was added. The formation of a bulky white precipitate indicates the presence of phenol compounds.

2. Alkaloids:

a.Dragendroff Reagent: About 1 ml of each extract stock was treated with a few drops of Dragendroff's reagent. The formation of an orange-colored precipitate indicates the presence of alkaloids.

b. Mayer's reagent:

Five milliliters of each plant extract were treated with one milliliter of Mayer's reagent. The development of turbidity and white sediment indicates the presence of alkaloids.

3. Glycoside: One milliliter of the extract from each plant part was mixed with five milliliters of Benedict's reagent. The appearance of a red sediment indicates the presence of reducing sugars.

4.Flavonoids: Five milliliters of each extract were treated with one milliliter of potassium hydroxide alcohol. The development of a yellow sediment indicates the presence of flavonides.

Saponins: The presence of saponins is indicated by the appearance of foam for a long time as a result of stirring the aqueous plant solution in a test tube.

5. Tannins: Add five milliliters of distilled water to five milliliters of extract. The mixture was heated at 80-100°C for 10 minutes in a water bath. After filtering, 5-6 drops of 1% ferric chloride were added. A dark green color indicates the presence of tannins.

6. Cumarins: Place 5 ml of plant extract in a test tube. Cover the tube with filter paper wetted with 1 M sodium hydroxide solution. Put the tube in a boiling water bath for 5 minutes.

2.2. Collecting of pathogenic bacteria

Clinical bacteria obtained from swabs of burned patients were selected as indicator isolates for the antimicrobial activity of the methanol extract: *E.coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The isolates were identified using a range of biochemical and morphological techniques [16] and were finally confirmed using the automated bacterial identification instrument Vitek-2 Compact System GP and GN card. The stored isolates were in brain heart infusion broth with 20% glycerol at −20 °C. The isolates were activated in culture at BHIA and incubated at 37 °C for 24 hours before use.

2.2.1. Antibacterial activity of methanol plant extract

The three different plants was evaluated against two types of pathogenic bacteria, both Gram-positive and Gram-negative (**Table 1**), using the agar well diffusion method [2]. A standardised suspension of each tested bacterium (1.5 x 10^8 CFU/ml) was prepared using the McFarland standard (0.5N), and then swabbed separately onto sterile Muller-Hinton agar (MHA) plates using sterile cotton swabs. This approach was repeated for each tested bacterium. Using a sterile cork borer, four holes were punched in each culture plate. One of the holes contained 100 µl of methanol as a positive control, and 100 µl of methanol extract at concentrations of 0.1, 0.5 and 1gm/ml were added to the remaining three holes. The same method was used for the methanol extract of *Cordiamyxa* at the same concentrations. The same method was used for the *Citrullus colosynthis* methanol extract at the above concentrations. The culture plates were then incubated at 37 °C for 24 hours, after which the clear zone of inhibition around each hole was measured [5].

Antioxidant activities: Various concentrations of extracts (100, 75, 50 and 25 mg/ml) were mixed with 5.0 ml of methanolic solution containing DPPH radicals (0.004% w/v) to determine the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity. The mixture was shaken vigorously and left to stand in a microtiter plate for 30 minutes in the dark until stable absorbance values were obtained. The reduction of the DPPH radical was determined by reading the absorbance at 517 nm. Radical-scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the following equation: % RSA=(AOPPH - As)/AOPPH x 100.

where As is the absorbance of the solution when the sample extract is added at a particular level, and AOPPH is the absorbance of the DPPH solution [17,18].

Statistical analysis: The experiments were conducted and analysed as factorial experiments with three replications using a completely randomised design (CRD), with two or three factors tested by least significant difference (LSD) at a probability of 1% ($P \leq 0.01$) [19].

3. Results

3.1. Phytochemical screening

Table 1. The extract weight (20gm in 200ml)

Plant type	Methanolic extract weight(g)
Punica granatum Peels	0.35
Cordia myxa fruits	1.04
Citrullus colosynthis Fruits	0.4

Table 2. Preliminary phytochemical screening of extracts

Plant / Reagents		Punica granatum peels	Cordia myxa Fruits	Citrullus olosynthis Fruits
Ferric Chloride	Phenol	+	+	++
lead acetate		+	+	+
Dragendroff reagents	Alkaloid	++	++	++
Mayer's reagent		++	++	++
Glycoside		++	+	+
Flavonides		++	++	+
Saponins		-	++	+
Tannins		++	+	+

Coumarins	+	+	+

(+) presence compound and (-) for absence.

The antioxidant activity of the extraction of plant species parts by methanol against free radicals in DPPH was examined at different concentrations of the methanolic extract, as well as in a control group using DPPH only. The results in **Table 3** showed strong antioxidant activity at high concentrations of 100 mg/ml, 0.75 mg/ml, 0.50 mg/ml and 0.25 mg/ml, and showed significant differences among all concentrations compared with the control group for different types. The same table also investigated significant differences among other plant types, with the highest antioxidant activity found in Cordia myxa, followed by other plant types, but no significant differences were found in *Citrullus colosynthis* and *Punica granatum*. The interaction in **Table 3** also showed that there were significant differences among extract concentrations and plant types when Soxhlet methanol part extraction was used. The percentage of free radical scavenging was 84.989% at a concentration of 100 mg/ml with the *C. myxa* plant and the lowest percentage, 57.745%, was found at a concentration of 0.25 mg/ml with the *C. colosynthis* plant, with an LSD of 4.699.

Table 3. shows the antioxidant effects of extracts at different concentrations and their interaction (percentage of free radical scavenging).

Extract (100%)	**Plant Type**			**Mean of Extract**
	***Punica granatum* peels**	***Cordia myxa* Fruits**	***Citrullus colosynthis Fruits***	
100mg/m	78.461	84.989	79.156	80.868
0.75 mg/ml	71.600	80.531	75.988	76.039
0.50mg/ml	67.361	74.796	61.880	68.012
0.25mg/ml	60.210	71.919	57.745	63.291
Mean of Plant	69.408	78.058	68.692	
	L.S.D. 0.05 Extract =3.435	, Plant =2.523	, Interaction = 4.699	

3.1.1. Antibacterial activity of methanol plant extracts

After drilling into the culture medium and adding the plant extracts, the results showed inhibitory activity for each extract. The effectiveness of each extract was determined by measuring the inhibition zone area in millimetres. The results showed clear inhibitory activity of the plant extracts against the bacteria used in the study. After 24 hours of incubation at 37°C, a variation in the effect of the extract concentrations on the inhibition of the three types of bacteria was noted. The inhibitory effect of pomegranate peel extract ranged between 14 and 16 mm. The *C. myxa* plant had no effect on *Staphylococcus aureus* but had an inhibitory effect on *Pseudomonas aeruginosa* at 16 mm and *E. coli* at 17 mm. The bitter melon extract had an inhibitory effect on bacteria of 10–12 mm. Plant extract *Staphylococcus* (+) *Pseudomonas aeruginosa* (-) Pomegranate peels (100 mg/ml): 16 Pomegranate peels (250 mg/ml): 16 Pomegranate peels (500 mg/ml): 15 *Cordia myxa* peels (250 mg/ml): 0 *Cordia myxa* peels (500 mg/ml): 0 *Citrullus colosynthis* (250 mg/ml): 10 *Citrullus colosynthis*: 10(500 mg/ml) 12 .

Table 4. The activity of pomegranate, *Cordia* and *Citrullus* against *Staphylococcus* and *E. coli*. and *Pseudomonas*

Plants extract	**Conc.**	***Staph***	***P aeruginosa***	***E. coli***
Punica granatum peels	100mg/ml	16	16	11
	250mg/ml	16	16	15
	500 mg/ml	15	14	15
Cordia myxa fruits	100 mg/ml	Zero	13	12
	250 mg/ml	Zero	17	16
	500 mg/ml	Zero	17	17

Plants extract	Conc.	*Staph*	*P aeruginosa*	*E. coli*
Citrullus olosynthis Fruits	100 mg/ml	5	6	13
	250 mg/ml	10	10	16
	500mg/ml	14	19	21

4. Discussion

The results indicated the presence of phenolic compounds, alkaloids, glycosides, flavonoids, saponins, tannins and coumarins. A similar study conducted by [20] reported the presence of secondary metabolic products in all plant parts. The concentration of compounds may vary depending on the concentration of the compounds , the presence or absence of phytoconstituents in plant extracts mainly depends on solvent polarity extraction efficiency increases when different solvents are used to dissolve the various phytochemical compounds present in different plant parts [14]. The number of hydroxyl groups found in phenols is related to their toxicity towards microorganisms, and there is evidence that increased hydroxylation is directly proportional to toxicity [21]. It has also been reported that highly oxidized phenols exhibit greater inhibitory activity ,Phenols have also been reported to be effective hydrogen donors, making them potent antioxidants [1].. Antimicrobial and antifungal activities, and are found in the molecular structures of various plant metabolites which have been reported to be strong antibacterial agents. Plants produce many active compounds containing these active groups (secondary metabolites). Certainly, other chemical components of the extracts could also contribute, although a lack of chemical profiling has never been reported in this regard. It is possible that these compounds are mainly responsible for the antifungal activities observed in this study [22]. A similar study conducted by [1] and [16] reported the presence of secondary metabolic products in all plant parts.

The peels of Pomegranate is a gorgeous reservoir of antioxidants, polyphenols, dietary fiber, and vitamins, also organic acids, the extraction of bioactive compounds from pomegranate peel requires careful selection of techniques to maximize the yield and quality[5].

[23] showed that many factors can affect the ability to scavenge, such as the varied modes of action of natural antioxidants, which could involve multiple mechanisms of action. Phenols act as primary antioxidants. The antioxidant activity of a natural source is generally related to one of these activities or to acting as a synergist. [24] reported synergism between various antioxidants and established that coriander antioxidant activity may be affected by other factors, including production procedures and climatic changes. For example, average precipitation, harvesting time, altitude and storage conditions can significantly influence the composition of phytochemicals in plants, which may not be concentrated enough to produce the expected effect. The study of [25] reported the medicinl plant stimulate the cells to increased immune matters on other hand the bacteria may be development its resistance mechanism .

5. Conclusion

In conclusion, water extraction at 100 °C for at least three is suitable for extracting active ingredients from *Citrullus colocynthis*. The alcoholic extract of *Citrullus colocynthis* under these conditions was a very effective inhibitor of *E. coli*, *Staphylococcus aureus* and *Streptococcus spp*., while the water extract was effective against *E. coli* and *Klebsiella pneumoniae*. This extract was inexpensive, simple and effective for treatment purposes.

Conflict of interest

The authors declare no conflict of interest

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