Research Article

Evaluation of Antibacterial Activity of Aqueous and Methanol Extract of Iraqi *Althaea officinalis* L. flowers on Gastrointestinal Key Pathogens

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ABSTRACT

In many countries, Plant therapy has become a corner stone in the scientific researches and the use of herbal medicine is highly growing. With increasing awareness of the side effects and high levels of resistance of synthetic antibiotics, the demand for natural herbal drugs has increased. The present study aims to investigate the antibacterial effect of aqueous and methanol extracts of Althaea officinalis L. on gastrointestinal pathogens (*Staphylococcus aureus, E.coli, Salmonella typhimirium, Klebsiella pneumonie, Proteus vulgaris* and *Shigella dysenteriae*) in addition to the qualitative phytochemical analysis of different metabolites using specific chemical tests. Phytochemical investigation of both extracts indicated the presence of various secondary metabolites like alkaloids, flavonoids, saponins, Glycosides and Tannins. The results obtained from the present study reveal that *Althaea officinalis* L.flowers extract of aqueous and methanol extracts exhibits valuable antibacterial activity against gastrointestinal pathogens.

Keywords: Antibacterial, *Althaea officinalis*, Pathogens, Phytochemistry.

INTRODUCTION

Plants are the basic source of life of all the living organisms in the world. They produce a wide range of secondary metabolites like alkaloids, fatty acids, flavonoids, phenols, tannins, sterols and terpenes that can be used to treat different chronic and infectious diseases[1]. Antibiotic abuse leads to increased drug resistance of bacterial species against different antibiotics[2]. This issue has been one reason for the growing use of plants as natural, safe, accessible, and affordable materials compared to synthetic antibiotics to treat bacterial infections. These medicines are more acceptable for the patients to use [3]. These are the reasons for increasing the new wave of international studies, and the introduction of anti-bacterial effects of different plants in recent years [4, 5]. *Althaea officinalis* Linn. (Malvaceae) is commonly known as Khatmi in Hindi and Marshmallow in English. It is native of British Isles and found in temperate regions, currently it is distributed throughout Europe, Asia and some parts of America [6]. *A. officinalis* is a perennial herb 60-120 cm high. Stem is erect, woody and unbranched. Leaves are short-petioled with an ovate and acute leaf blade. The reddish-white flowers are usually in axillary or terminal cluster. Compressed dark brown kidney-shaped seeds are glabrous [7]. It is used to improve of dry cough that combined with oral, pharyngeal mucosa irritation, mild gastritis, skin burns, insect bites, catarrh of the mouth and throat, gastrointestinal tract and urinary tract infection, ulcers, abscesses, burns, constipation and diarrhoea. Seeds are used as diuretic and febrifuge [8]. It has been utilized to aid in promoting coughing up of phlegm and respiratory difficulties. It is indicated to relive dryness and irritation of chest and throat that happened by colds and persistent coughing [9] due to high contents of polysaccharides. The antibacterial activity of *A. officinalis* roots were tested against anaerobic and facultative aerobic periodontal bacteria [10]. Many researches showed that *A. officinalis* possessed antimicrobial, anti-inflammatory, immunomodulatory, demulcent and soothing, antittusive and many other pharmacological effects [11, 12]. In present study the antibacterial screening of *A. officinalis* aqueous and methanol extracts has been aimed against selected gastrointestinal diseases microorganisms.

MATERIALS AND METHODS

Plant Material

Flowers were collected from Sulaymaniyah province. The plant was authenticated by the National Herbarium at Baghdad; the plant material was dried in the shade for few days and then and then powdered and stored at room temperature.

Extracts preparation

Methanol Extract

100 g of powdered plant materials were extracted by 250 ml of 80 % methanol using soxhlet apparatus until exhaustion for 12 hours. Plant extract was filtered through whatman No.1 filter paper and crude extract obtained by removing solvent in vacuum evaporator at 40°C. Residues were stored at 4°C for further analysis.

Aqueous Extract (Decoction)

50 g of plant materials was boiled in 500 ml water for 45 minutes, then cooled and filtered through whatman No.1 filter paper. The crude extract obtained by removing solvent in vacuum evaporator.

Phytochemical Screening

The plant extracts were subjected to phytochemical examination for alkaloids, flavonoids, glycosides, steroids, saponins and tannins as described by using standard procedures [13].

Antibacterial Assay

The microbial strains causing gastrointestinal infections used in this study were *Staphylococcus aureous, E.coli, Salmonella typhimirium, Klebsiella pneumonia, Proteus vulgaris* and *Shigella dysenteriae* Microorganisms were procured from Baghdad teaching hospital, medical city.

Antibacterial testing

Antibacterial activity of aqueous and methanol extracts was determined by agar well-diffusion method [14]. In vitro antibacterial activity was screened by using Mueller-Hinton Agar (MHA) medium. Well diffusion assay was carried out and all plates were left to set and incubated at 37 ^{0}C for one day. Then, control is prepared which consist of sterile distal water with 0.1 % DMSO. Efficacy of extracts against bacteria was compared with broad spectrum antibiotics Gentamycin (10 mg) and Ciprofloxacine (5 mg) and cork borer (6 mm diameter) used to punch wells in medium and filled with extracts of 45 µl of (100, 200,300 and 400 mg ml^{-1}). The bacterial activity was expressed in term of average diameter of the clear zone around each well that called inhibition zone in (mm). Plates were then incubated at 37 °C for 24 h and diameters of the growth inhibition zones were measured.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening of *A. officinalis* extracts showed the presence of alkaloids, flavonoids, glycosides, saponins and tannins in aqueous and methanol extracts, Steroids test indicated positive result in methanol extract as shown in (Table 1). The plant has wide range of curative properties with lots of studied phytoconstituents including pectins 11%, starch 25-35%, mono-, and di-saccharide saccharose 10%, mucilage 5%, flavonoids (Hypolaetin-8-glucoside, isoquercitrin, kaempferol, caffeic, pcoumaric acid), coumarins, scopoletin, phytosterols, tannins, asparagines and many amino acids [15]. Two new phenolic compounds and one new acid ester characterized as 3,4- dihydroxy benzyl octadecane, 24β, 28β-dihydroxyocta tetracont-36-en-1-oic acid and 5β, 13β-dihydroxynacosanylgodoleate had been isolated from roots of *A. officinalis* along with known compounds n-triacotanic acid, n-tetracosane, n-pentatriacontane and althaealanostenoic acid glucoside [16]

Table 1: Phytochemical analysis of *A. officinalis* extracts

NO.	Extract	Phytoconstituents					
		Alkaloids	Flavonoids	Glycosides	Steroids	Saponins	Tannins
1	Aqueous	+	+	+	_	+	+
2	Methanol	+	+	+	+	+	+

Antibacterial activity

The present study showed that *A. officinalis* possess good antimicrobial activity against selected gastrointestinal tract pathogens compared with antibiotics used. The antibacterial efficacy of aqueous and methanol extracts was summarized in (Table 2).

Table 2: Antibacterial properties of *A. officinalis* extracts, and reference antibiotics

	Concentration	Diameters of the inhibition zone (mm)					
		E.coli	*S. aureous*	*Salmonella typhimirium*	*K. pneumonie*	*P.vulgaris*	*Shigella dysenteriae*
Aqueous (mg.ml^{-1})	100	N/E	N/E	7	N/E	N/E	11.65 ± 0.55
	200	N/E	13.66 ± 0.55	7.9 ± 0.66	N/E	N/E	14 ± 1
	300	11.7	18.3 ± 0.23	9.1 ± 0.17	8.2 ± 0.9	8.2	14.2 ± 0.2
	400	14.25 ± 0.76	18.7 ± 0.3	9.0	9.1 ± 0.2	9.6 ± 0.15	14.5
Methanol Ex. (mg.ml-1)	100	12.5 ± 0.65	14.77 ± 0.34	6.4	15.3	12.2 ± 16	16.4
	200	19.6 ± 0.22	16.77 ± 0.34	6.7 ± 0.55	17.9 ± 0.2	15.44 ± 22	18.2 ± 0.22
	300	21.6 ± 0.22	19.3 ± 0.4	10 ± 0.8	19.3	18.4 ± 16	18.7 ± 0.5
	400	23.5 ± 0.2	22.6 ± 0.24	12 ± 0.74	21.2 ± 0.3	19	19
Gentamycin (µg/disck)	10	30	22	18	14	20	22
Ciprofloxacine (µg/disck)	5	25	26	23	25	34	23
Data presented as: mean ± Standard deviation, N/E: No effect							

At 100 and 200 mg ml^{-1} methanol extract was produced higher zone of inhibition in all tested microorganisms than that produced from aqueous extract. Whereas, both extracts were produce comparable zone of inhibition against Salmonella typhimirium with p value <0.001 (figure 1). In addition, methanol extract showed superior activity in the mentioned concentrations against K. pneumonie than that of standard antibiotic gentamicin with zone of inhibition of 15.3 and 17.9 respectivily (Figure 2).

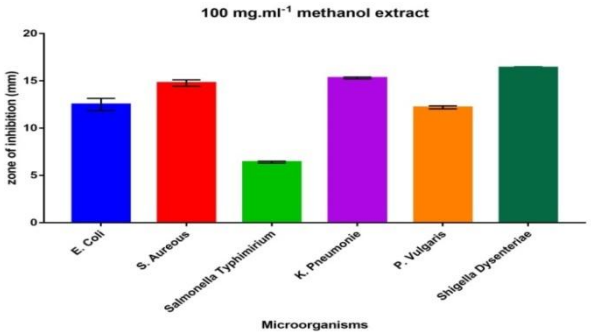
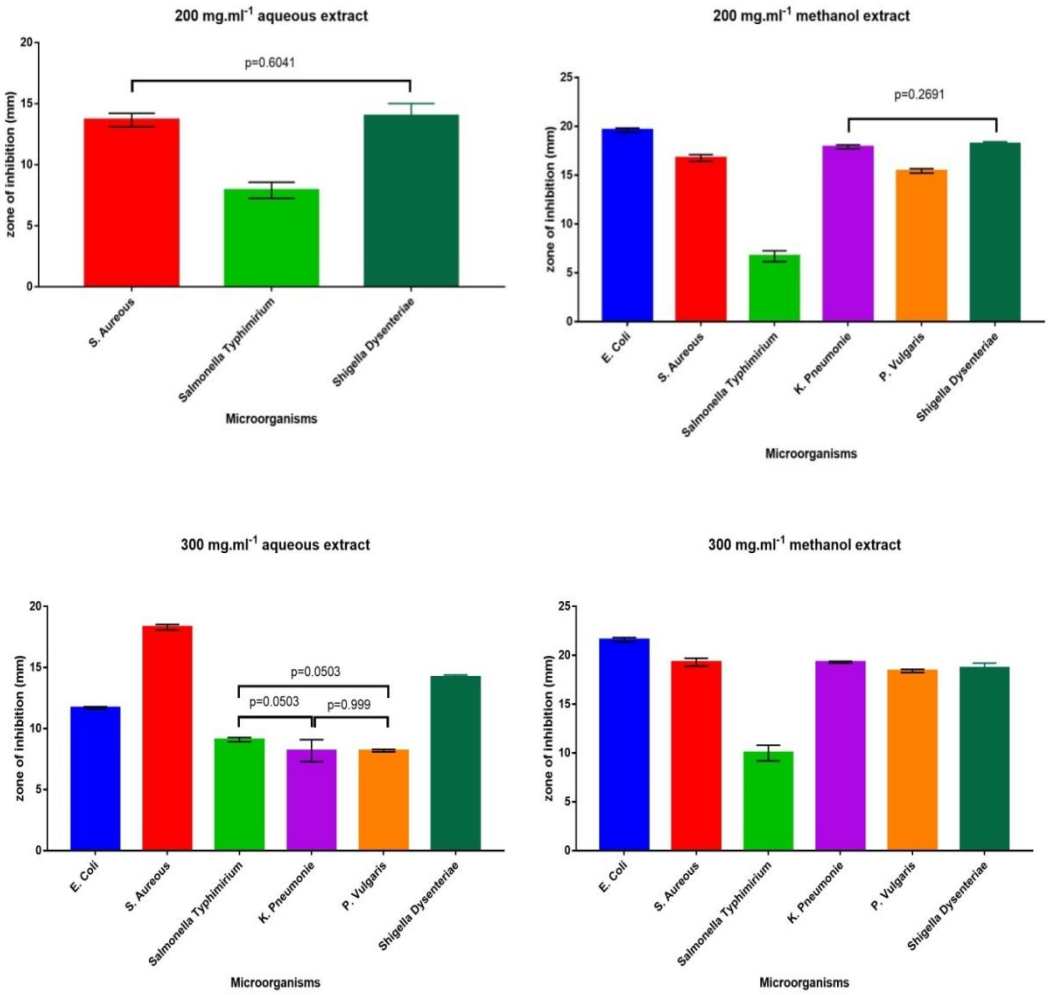


Figure 1: histogram of methanol extract at 100 mg/ml concentration

The tested plant extracts were found to possess an activity against tested microorganisms at 300 and 400 mg ml^{-1}. The results revealed that the methanol extract of Althaea officinalis was found to have more potent inhibition effects on the growth of bacteria species. These results confirmed the evidence reported in previous studies that methanol is the better solvent for more consistent extraction of antimicrobial substances from medical plants compared with water [14]. The most pronounced activity was shown by the methanol extract at 400 mg ml^{-1} against S. aureous, , P.vulgaris and Shigella dysenteriae when all bacteria showed the same sensitivity of gentmicine. Methanol extract have higher activity than gentamicin against K. pneumonie at all concentrations with inhibition zone ranged from (15.3 mm) to (21.2 ± 0.3 mm) mm. However, the inhibition zones were noted against E.coli (23.5 ± 0.2 mm), K. pneumonie (21.2 ± 0.3 mm) and Shigella dysenteriae (18.7 ± 0.5 mm) and all reading showed comparable effect when compared with the results that have been given by ciprofloxacin p value <0.001 (figure 2).



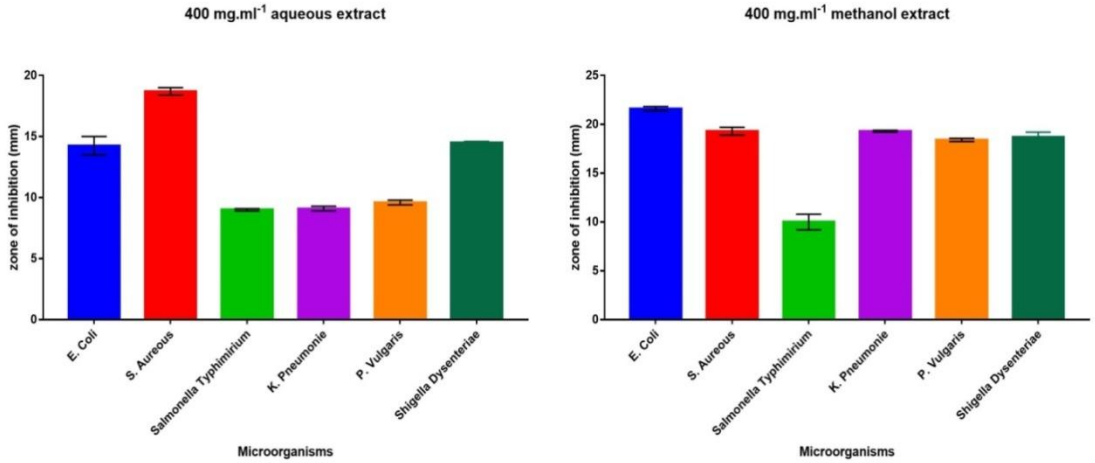


Figure 2: histogram of aqueous and methanol extracts at 200, 300 and 400 mg ml^{-1}concentration

CONCLUSION

In total, results in laboratory conditions showed that the aqueous and methanol extracts of Iraqi *A. officinalis* L. flowers consist of alkaloids, flavonoids, glycosides, saponins and tannins. It can be concluded that *A. officinalis* have good antimicrobial potential against tested microorganisms. However, further phytochemical studies are required to determine the types of compounds responsible for the antibacterial effects of these species.

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