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RESEARCH ARTICLE

STUDY CHEMICAL COMPOSITION OF BORAGO OFFICINALIS L. EXTRACTION AND ANTIBACTERIAL ACTIVITY AGAINST GASTROINTESTINAL INFECTIONS.

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Abstract

Borage (Borago officinalis L.), is an annual herbaceous plant and native to Europe, North Africa, and Asia Minor. It is an important vegetable crop which cultivated in some countries including Iraq. Also it is a medicinally important plant, many health effects have been attributed to the borage (Borago officinalis L.) plant, such as: antispasmodic, antihypertensive, antipyretic, aphrodisiac, demulcent, and diuretic properties. It is also considered useful to treat asthma, bronchitis, cramps, diarrhea, palpitations, and kidney ailments. The aim of study to evaluate antibacterial activity of extracts of Borage leaves against two species of bacterial (Escherichia coli and Staphylococcus aureus) that were isolated in general samawah hospital labs from feces of patients with gastrointestinal infections. qualitative analysis was carried out to detect some active chemical compounds in extracts of borage, It was found that it contains phenols , flavonoids , tannins , proteins and carbohydrate . Extracts in concentration 150 mg/ml show inhibition activity in varying diameter against two bacteria (Escherichia coli and Staphylococcus aureus) in patients with gastrointestinal infections. The study show that the aqueous extract of Borage leaves in hot temperature showed the highest inhibitory effect against the two species of bacteria (Escherichia coli and Staphylococcus aureus) 13 mm and 22 mm a diameter, respectively.

INTRODUCTION

Borage (Borago officinalis L.), is an annual herbaceous plant and native to Europe, North Africa, and Asia Minor. It is an important vegetable crop which cultivated in some countries including Iraq. Also it is a medicinally important plant which has more than 20% gamma linolenic acid (GLA) in the seed oil. The leaves of borage are reportedly used as diuretic, demulcent, emollient, expectorant, etc. (1). Many health effects have been attributed to the borage (Borago officinalis L.) plant, such as: antispasmodic, antihypertensive, antipyretic, aphrodisiac, demulcent, and diuretic properties. It is also considered useful to treat asthma, bronchitis, cramps, diarrhea, palpitations, and kidney ailments (2). Borage is also known as star flower. The flowers are, indeed, a star shape with 5 pointed purple or pink petals (flowers are often pink upon opening, and then turn purplish blue). The star shape is enhanced by five green sepals that appear at each spot where two petals meet, around the center of the flower. The plant reaches a height of 30 to 60 cm. Its stem is covered with hairs that secrete an intense smell resembling the aroma of fresh cucumbers while grinding (3). In Middle east traditional medicine , Borage are reportedly used for treatment of a variety of ailments. Therefore, identification/determination chemical composition of aerial parts of this plant can be suggested the medicinal value of borage.

Procedure :

Preparation of borage leaves extracts :

1- Preparation of aqueous extract in normal temperature :

20 gm of borage leaves was added to 300 ml of Distilled water and placed in conical flask for 24 hour in room temperature ,then the extract was filtered by using Whatman No.1 filter paper in Buchner Funnel , and by using rotary vacuum evaporator It was obtained extract as a powder form without solvent , then kept in Sterile plastic bottle, opaque and closed in the refrigerator 5 ° C until use (4)

2- Preparation of aqueous extract in hot temperature :

20 gm of of borage leaves was added to 300 ml of Distilled water and placed in Reflex for 24 hours in 70 ° C .then the extract was filtered by using Whatman No.1 filter paper in Buchner Funnel in room temperature , and by using rotary vacuum evaporator It was obtained extract as a powder without solvent , then kept in Sterile plastic bottle, opaque and closed in the refrigerator 5 ° C until use (5)

3- Preparation of alcoholic extract (ethanol 70 %)in normal temperature

Preparation of alcoholic extract (ethanol 70 %) in normal temperature according to the method described above in Preparation of aqueous extract in normal temperature and Distilled water was replacement with ethanol 70% , then the alcoholic extract 70% as a powder kept in Sterile plastic bottle, opaque and closed in the refrigerator 5 ° C until use (4).

4- Preparation of alcoholic extract (ethanol 70 %)in hot temperature

Preparation of alcoholic extract (ethanol 70 %) in hot temperature according to the method described above in Preparation of aqueous extract in hot temperature and Distilled water was replacement with ethanol 70% , then the alcoholic extract 70% in hot temperature as a powder form kept in Sterile plastic bottle, opaque and closed in the refrigerator 5 ° C until use (5).

Qualitative tests

Some qualitative anaylsis tests was carried out to detect some of chemical active compounds in extracts of borage leaves .

1- **Molish's test** : test used for the general detection of carbohydrates. 5 drops of alpha naphthol 1% was added to 2 ml of sample in test tube ,then concentration H_2SO_4 poured to the test tube . a red-violet layer at the interface between the acid (bottom) and aqueous (upper) layers is a positive test for carbohydrates (6) .

2- **Ninhydrine's test** : test using to detect amino acids and protein. 3 drops of Ninhydrine reagent 1% was added to 2 ml of sample solution in test tube and then was boiled to 2-3 minutes , blue color develops if the test is positive , proline and hydroxyl proline give yellow color (6) .

3- **Flavonoid's test** : test using to detect Flavonoid compounds . Ethanolic KOH in 5 N was added to 1 ml of sample solution , Yellow precipitate develops in positive test (7) .

4- **Tannin's test** : using to detect tannins compounds . 1 ml of lead acetate (1%) was added to 1 ml of sample solution in test tube , white precipitate develops in positive result (8)

5- **Phenol's test** : using to detect phenols compound . 1-2 drops of Ferric chloride solution $FeCl_3$(%1) was added to 1 ml of sample solution (1%) in test tube , blue or green color develops in positive result (9) .

The bacterial isolation :

Two species of bacteria :
Escherichia coli
staphylococcus aureus
they were isolated in general samawah hospital labs from feces of patients with gastrointestinal infections (10).

The result:
As show in table (1) It was obtained dry extracts from the borage leaves in different percentages :

	Type of extract	Weight of borage leaves in gm	Weight of extract in gm	Percentage of extraction (%)
1-	Aqueous extract in normal temperature	*20 gm*	*3.6 gm*	*18%*
2-	Aqueous extract in hot temperature	*20 gm*	*5.6 gm*	*28%*
3-	Alcoholic extract (ethanol 70 %)in normal temperature	*20 gm*	*2.8 gm*	*14%*
4-	Alcoholic extract (ethanol 70 %)in hot temperature	*20 gm*	*4.8 gm*	*24%*

Table (1) Explain the percentages of aqueous and alcoholic extraction in normal and hot temperature

Qualitative tests

Table (2) explain the qualitative analysis results which show that aqueous and alcoholic extracts in in normal and hot temperature contain many of active chemical compounds such as phenols , tannins and Flavonoid compounds

Name of test	*Type of compound*	*Aqueous extracts*		*Alcoholic extracts 70%*	
		In normal temperature	*In hot temperature*	*In normal temperature*	*In hot temperature*
Molisht	*Carbohydrate*	+++	+++	+++	+++
Ninhydrinet	*Proteins and amino acids*	–	+	–	–
$FeCl_3$(1%)	*phenols*	++	+++	+	++
Ethanolic KOH (5N)	Flavonoids	++	++	++	++
Lead acetate (1%)	*Tannins*	++	+++	+++	+++

-/ negative result +/positive result in low average
++/ positive result in normal average +++/positive result in high average
Table(2) : Qualitative analysis results of aqueous and alcoholic extracts in normal and hot temperature

The result of antibacterial activity :

The result show there is anti bacterial activity against two types of bacterial E.Coli and Staphylococcus aureus in different diameter inhibition activities as show in table (3):

Type of bacteria	*Diameter of antibacterial activity of Aqueous extracts*		*Diameter of antibacterial activity of Alcoholic extracts 70%*	
	In normal temperature	*In hot temperature*	*In normal temperature*	*In hot temperature*
E.Coli	*9 mm*	*13 mm*	-	-
Staphylococcus Aureus	*16 mm*	*22 mm*	*6 mm*	*8 mm*

Table (3) Show diameter of antibacterial activity of equeous and alcololic extracts (70%) in normal and hot temperature in concentration 150 mg/ml against E.Coli and Staphylococcus aureus

Antibacterial activity of aqueous extract in normal temperature:
as show in table (3) there are normal inhibition activity against E.Coli (9 mm) and high inhibition activity against Staphylococcus Aureus (16 mm).

Antibacterial activity of aqueous extract in hot temperature :
As show in table (3) there are high inhibition activity against E.Coli
(13 mm) and high inhibition activtivity against Staphylococcus Aureus (22 mm).

Antibacterial activity of alcoholic extract (ethanol 70 %)in normal temperature
As show in table (3) no inhibition activity against E.Coli and there are normal inhibition activity against Staphylococcus Aureus (6 mm) .

Antibacterial activity of alcoholic extract (ethanol 70 %) in hot temperature
As show in table (3) there are no inhabition activity against E.Coli and normal inhibition activity against Staphylococcus Aureus (8 mm)

Staphylococcus aureus *Escherichia coli*

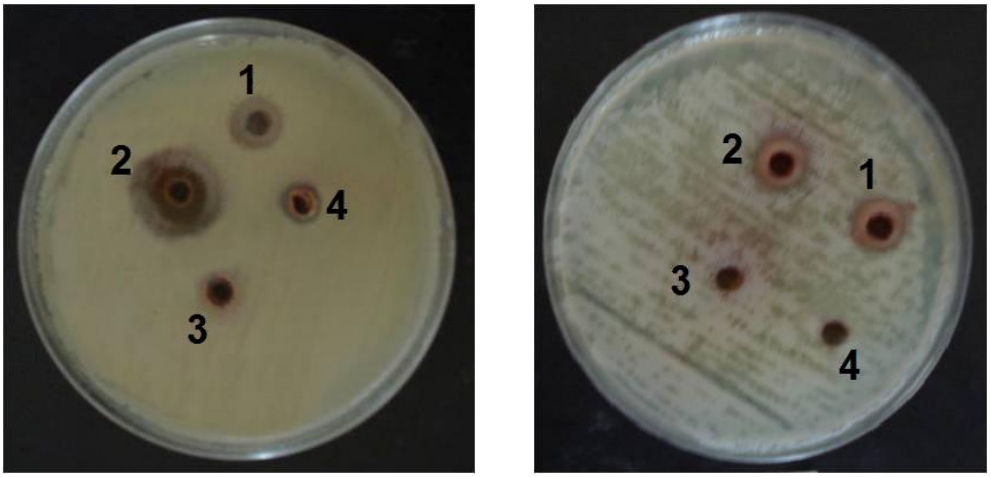

Figure (1) : Inhibition activity of : (1) Aqueous extract in normal temperature (2)Aqueous extract in hot temperature (3)Alcoholic extract (70%) in normal temperature (4)Alcoholic extract (70%) in hot temperature.
Extracts in conc. 150 mg/ml against *E. coli* and *Staphylococcus aureus*

Discussion *:*

Aqueous and alcoholic (70%) extracts of* borage leaves in normal and hot temperature*:
Table (1) show that aqueous extracts of borage leaves in hot temperature has the highest percentage extraction may be because the water consider suitable solvent for many chemical compounds such as phenol and Flavonoid which contain hydroxyl group as polar active group (11).

Qualitative tests :
The purpose of using qualitative analysis to determine some important active compound in the extracts that can play important role to inhibit bacteria , all extract that have been prepared containing in different percentage phenols , Flavonoid , tannins and carbohydrate .

Antibacterial activity against E.Coli and *Staphylococcus aureus :*

as show in table (3) , the extracts which prepared from borage leaves show different antibacterial activity in varying diameters , aqueous extract in hot temperature show highest antibacterial activity against E.Coli and *Staphylococcus aureus that isolated from* feces of patients with gastrointestinal infections ,compared to other extracts from borage leaves ,the reason may be due to polarity of water which can play important role in extraction and precipitation more of active chemical compound which can inhibit bacteria **(12).** In addition, inhibition activity of aqueous extract in hot temperature show higher activity against positive gram bacteria (*Staphylococcus aureus) than negative gram bacteria (E.Coli.),* the reason may be due to that gram-negative cell walls are much more complex than gram-positive walls. In E. coli it is about 2 nm thick and contains only one or two layers or sheets of peptidoglycan, In addition E. coli 20 to 100 nm areas of contact between the two membranes are seen in plasmolyzed cells. Adhesion sites may be regions of direct contact or possibly true membrane fusions. It has been proposed that substances can move into the cell through these adhesion sites rather than traveling through the periplasm (13).

Conclusion

borage (Borago officinalis L.) leaves have antibacterial activity against E.Coli and Staphylococcus Aureus that couse and accumulate in gastrointestinal infections therefore borage leaves in hot water can inhibit bacteria and lead to improve in Digestive tracts

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