



Investigation of the Phytochemical Constituents and Antimicrobial Potential of the Fruit and Flower of *Pistacia Atlantica* Desf. as a Herbal Medicine Growing in North-west of Iran

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Abstract

Investigation of the compounds of rich plants growing in all over the world is of great importance. The performed survey was accomplished to analyze the phytochemical constituents and antimicrobial potential of the fruit and flower of *Pistacia atlantica* subsp. *kurdica* Rech. f. which has been traditionally used for various therapeutic goals including tonic, aphrodisiac, and antiseptic aspects. Water, ethanol, acetone, and ethyl acetate solvents were utilized to obtain the extracts of *P. atlantica* which were screened to evaluate the existence of various phytochemicals including alkaloids, carbohydrates, glycosides, flavonoids, resins, tannins, triterpenoids, steroids, starch, tannins, inorganic and organic acids, amino acids, coumarins, proteins, phenolic compounds, phlobatannins, saponins, anthraquinones, oil and fat, flavonols, flavones, and chalcones. Moreover, the in vitro evaluation of the antimicrobial activity of the extracts were performed against five strains of bacteria including *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Klebsiella pneumoniae* and a fungus named *Candida kefyr* by agar plate well diffusion method. The findings of the survey suggest that the fruit and flower of *P. atlantica* are good sources of some phyto constituents such as carbohydrates, flavonoids, resins, inorganic acids, and phenolic compounds and have antibacterial effects against both gram-negative and -positive bacteria. These results prove the existence of many therapeutic chemical compounds in the fruit and flower of *P. atlantica* and its high potential of antimicrobial activity. This research supports the local use of this valuable plant as a herbal medicine, food additive, and jam.

Keywords: *Pistacia atlantica*, Phytochemicals, Antibacterial activity, Plant extracts, Herbal medicine

Introduction

Analyzing the phytochemical constituents and antibacterial activities of medicinal plants growing around the world is of great importance to identify their medicinal value which is because of the presence of special bioactive and phytochemical compounds. In addition, obtaining information about the phytochemical constituents of plants helps to reveal the reason of their traditional usages as folkloric remedies [1]. Herbal remedies extracted from different plants provide a popular alternative for preventing and treating various illnesses because of containing components having therapeutic values [2]. As a matter of fact, using herbal and safe remedies having less side effects is going to be a

widespread trend instead of synthetic drugs which have some adverse side effects [3]. This fact is the reason for the growing attention towards herbal remedies in many regions of the world. Medicinal herb is a phytochemical factory which produces valuable chemical compounds like resins, saponins, and oils which are rarely possible to be synthesized easily in the laboratory [4]. These plants produce phytochemical compounds to protect themselves against various threats. Also it has been established that these chemicals are able to protect humans and animals against illnesses, too [5]. These effects include cancer prevention, hormonal action, enzyme stimulation, antioxidative, and antibacterial aspects [6]. According to an evaluation, around 120 plant based drugs have been developed for usage in all the world which are derived

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from 95 plant species [7]. Also world health organization (WHO) estimates that 60-80% of people trust medicinal plants for health care [8]. Moreover, it has been mentioned that 25% of the prescribed medication in Canada and the USA are derived or modeled from medicinal plants [9]. Iran is also one of the countries that has a traditional culture to use herbs in order to prevent and cure diseases due to its widespread rich natural resources.

Natural antimicrobials are derived from various plants and microorganisms which can be safe and helpful [10]. Also phytochemical constituents can be detected and isolated from different plants for their biological, phytochemical, and medicinal usages [11]. Therefore developing the efficient, rich, easily accessible, and inexpensive medicinal plants in order to expand herbal medicines is of great importance for human health care in the world.

The plant *Pistacia* is a member of Anacardiaceae, having over 600 species and 70 genera. *Pistacia*'s species are either deciduous shrubs or evergreen trees which grow 8-10 m tall [12]. *P. atlantica* Desf., *P. lentiscus* L., *P. vera* L., *P. terebinthus* L., and *P. khinjuk* Stocks have been shared from the Mediterranean region to central Asia [13]. Iran is considered as one of the most important countries that grows and exports *P. vera* nuts [14]. In customary Iranian medicine sciences, various *Pistacia* species have been used as helpful remedies for many sicknesses. As some examples, *P. vera*'s fruit has been used as a stomach, hepatic, brain, and cardiac tonic. The fruits of *P. terebinthus*, *P. khinjuk*, and *P. atlantica* have been used for treatment of respiratory system, heart, kidney, and liver disorders and also for aphrodisiac activities. Moreover, the gum resin resulting from, *P. terebinthus*, *P. lentiscus*, *P. atlantica*, and *P. khinjuk*, have been used as a remedy for gastrointestinal and brain disorders and also due to their wound healing activity [15,16].

Also, the recent findings from different analysis have proved extensive pharmacological activities of the mentioned species including antihyperlipidemic, antidiabetic, anti-inflammatory, antiviral, antioxidant, antitumor, hepatoprotective, anticholinesterase, and antiatherosclerotic effects and their helpful consequences in solving gastrointestinal disorders [16]. The species of *P. vera*, *P. khinjuk*, and *P. atlantica* naturally grow in Iran. *P. atlantica* includes three subspecies named as *cabulica*, *kurdica*, and *mutica* [17]. Most of the medical resin usages of *P. atlantica* are focused on the treatment of kidney, hepatic, and digestive diseases [15]. Our present research is trying to describe a view of *P. atlantica* subsp. *kurdica*'s phytochemical constituents and its antibacterial activities against both Gram-positive and -negative bacteria.

Material and Methods

Plant Material

P. atlantica subsp. *kurdica* Rech. f. was obtained from the hillsides of Sahand Mountain which is located around Tabriz, Iran. The plant was authenticated by a botanist in the department of pharmacy, University of Tabriz. The plant was dried completely and subsequently powdered using an electrical grinder.

Preparation of the Plant Extracts

Soxhlet extraction method was performed for the extraction of the phytochemicals of the plant and flower of the studied plant. Soxhlet extraction is a simple and effective method for the extraction of the analytes that are sufficiently thermally stable. This process has been used for a wide range of samples like soils, sediments, and animal and plant tissues. A wide variety of solvents like dichloromethane, ethanol, acetone, etc. can be used in this procedure. The experimental Soxhlet extraction apparatus consists of a distillation flask, sample holder, siphon, and a condenser. First, the sample material is packed in a filter paper and placed in the thimble. Next, vapors of a fresh solvent, produced in a distillation flask, pass through the thimble containing the material to be extracted and are liquefied in the condenser. When the liquid reaches the overflow level in the thimble, a siphon aspirates the solution, and the liquid falls back into the distillation flask, carrying the extracted solutes into the bulk liquid. The separation of solute from solvent takes place in the distillation flask. Then solute is left in the flask and fresh solvent vapors pass back into the solid bed of sample material. The operation is repeated until complete extraction is achieved.

Four organic solvents were utilized to extract the phytochemical compounds from the plant. The fruit and flower powder (10g) of *P. atlantica* subsp. *kurdica* were successfully extracted with water, ethanol, acetone, and ethyl acetate solvents (50 mL of each) by means of a Soxhlet apparatus. The obtained extracts were evaporated until the volume of 25 mL by using a vacuum rotary evaporator (Heidolph VV 2000). Subsequently, the obtained extracts of the fruit and flower of the plant were analyzed by qualitative tests for the identification of various phytochemical constituents. Moreover, for the accomplishment of the antimicrobial survey, all the extracts were dried and subjected to antimicrobial potential analysis.

Phytochemical Tests Procedures

Phytochemical analysis was performed on the achieved fruit and flower extracts of the plant by using the standard procedures for the identification of the components mentioned below [18-21]. The outcome of the

phytochemical analysis procedures are summarized in Table 1.

Alkaloids Analysis

Dragendorff's procedure

Potassium bismuth iodide solution as Dragendorff's reagent was added to 0.5 mL of each extract. The presence of alkaloids shows a reddish brown precipitate.

Hager's Procedure

A few drops of picric acid solution as Hager's reagent were poured into 0.5 mL of each extract. The existence of alkaloids is proved by observing the yellow precipitates.

Wagner's Procedure

Wagner's reagent (the solution of iodine in potassium iodide) was poured into 0.5 mL of each extract. The alkaloids give reddish brown precipitate in this test.

Mayer's Procedure

Mayer's reagent (solution of potassium mercuric iodide) was added into 0.5 mL of each extract. The alkaloids give cream color precipitate.

Carbohydrates Analysis

Anthrone Procedure

0.5 mL of each extract was mixed with 2.5 mL of water, shaken well, and filtered. 0.5 mL of Anthrone reagent solution was poured into the obtained filtrate which was concentrated. Forming a blue or green color proves the existence of carbohydrates.

Benedict's Procedure

2.5 mL of water was poured into 0.5 mL of each extract, shaken and filtrated. Subsequently, 1.25 mL of Benedict's solution was added to the prior solution. The existence of carbohydrates is proved by observing a red color precipitate after boiling the solution for 5 minutes.

Fehling's Procedure (Free Reducing sugars)

Initially, equal volume of Fehling's A reagent (copper sulfate in distilled water) was mixed carefully with Fehling's B (potassium tartrate and sodium hydroxide in distilled water) reagent. Then 3-4 drops of the obtained extracts were poured in the mixture and boiled. According to the presence of free reducing sugars in the studied samples a brick red precipitate of cuprous oxide is formed.

Molisch's Procedure

0.5 mL of each extract was mixed with few drops of alcoholic α -naphthol. Subsequently, 0.2 mL of concentrated sulfuric acid was added to the solution along the test tube sides. Appearing the violet or purple color ring illustrates the existence of carbohydrates.

Barfoed's Procedure

About 0.5 mL of each extract was poured into 2 mL of distilled water, shaken, and filtrated. 1 mL of the obtained filtrate was heated on a water bath for 2 minutes after mixing with 1 mL of Barfoed's reagent. The existence of carbohydrates is proved by observing reddish precipitate of cuprous oxide.

Fehling's Procedure (Combined Reducing sugars)

5 mL of diluted hydrochloric acid was added to 0.5 mL of each extract. This solution was hydrolyzed by boiling. Subsequently, sodium hydroxide solution was used to neutralize it. Few drops of Fehling's solution were poured into the prior solution and then put in a water bath for 2 minutes. The existence of carbohydrates are proved by observing a reddish-brown precipitate.

Flavonoids Analysis

Method I

Shinoda's Procedure

To 0.5 mL of each extract few pieces of magnesium ribbon were added. Then concentrated hydrochloric acid was poured into the solution. Appearing red, pink, or sometimes green to blue color shows the positive result.

Ferric Chloride Test

Few drops of ferric chloride solution was added to 0.5 mL of each extract. Forming a green color shows the existence of flavonoids in the extract.

Lead Acetate Test

0.5 mL of each extract was treated with few drops of 10% lead acetate solution. Observing a yellow precipitate proves the existence of flavonoids.

Alkaline Reagent Test

Few drops of sodium hydroxide solution were poured into 0.5 mL of each extract. Yellow color is formed. Adding few drops of diluted acetic acid makes it become colorless.

Method II

In order to remove the lipid layer, 0.5 mL of the extracts were mixed and shaken with petroleum ether. The obtained solution was poured into 20 mL of 80% ethanol. The obtained solution was utilized for accomplishment of the tests below:

- a) 4 mL of aluminum chloride in methanol was mixed with 3 mL of the obtained solution. The existence of flavonols, flavones and chalcones are established by formation of yellow color.
- b) 4 mL of 1% potassium hydroxide solution was mixed with 3 mL of the obtained solution. Formation of a dark-yellow color indicates the existence of flavonoids.

c) 5 mL of dilute ammonia solution was poured into the obtained solution of each plant extract following by the addition of concentrated sulfuric acid. Yellow color indicates the existence of flavonoids.

Glycosides Analysis

Borntrager's procedure (Anthraquinone Glycosides)

For the separation of the organic layer, 0.5 mL of each extract was shaken well with benzene. Subsequently, 0.25 mL of 10% ammonia solution was poured into the plant extracts. Indication of a violet, pink, or red color proves the existence of anthraquinone glycosides.

Keller Killaini's Procedure (Cardiac glycosides)

To 0.5 mL of each extract, 0.4 mL of glacial acetic acid containing a trace amount of ferric chloride was added. Then 0.5 mL of concentrated sulfuric acid was added along the test tube sides. Blue color appears in the acetic acid layer which shows the existence of cardiac glycosides.

Resins Analysis

Acetone was utilized for dissolving 0.5 mL of each extract. Then the solution was added to distilled water. The turbidity of the solution proves the presence of resins.

Saponins Analysis

Froth Procedure

Creation of foam after adding small amount of the powdered studied plant in 3 mL of distilled water and shaking the suspension proves the existence of saponins.

Steroids and Triterpenoids Analysis

Liebermann-Burchard Procedure

Few drops of acetic anhydride were poured into 0.5 mL of each extract. The solution was boiled and cooled. Then few drops of concentrated sulfuric acid were poured along the test tube. The appearance of a brown ring and green color proves the existence of steroids. Also observing a deep red color shows the existence of triterpenoids.

Salkowski Procedure

The solution of chloroform containing few drops of concentrated sulfuric acid was mixed with 0.5 mL of each extract. Then was shaken well and let stand for a while. Red color illustrates the existence of steroids. Observation of yellow color shows the presence of triterpenoids.

Tannins Analysis

Lead Acetate Test

0.5 mL of each extract was treated with a few drops of 10% lead acetate solution. Formation of a precipitate proves the existence of tannins.

Ferric Chloride Test

Few drops of ferric chloride reagent were added to 0.5 mL of each extract. Formation of a green, blue, black, or purple color proves the existence of tannins.

Starch Analysis

Iodine was added to 0.5 mL of each extract. Observation of blue color that disappears when heating and reappears when cooling proves the existence of starch.

Inorganic Acids analysis

Sulfate Test

Lead acetate reagent was added to 0.5 mL of each extract. Observation of a white color precipitate that is soluble in sodium hydroxide proves the positive test.

Carbonate Test

Diluted hydrogen chloride solution was added to 0.5 mL of each extract. Liberation of CO₂ gas from the test tube shows the existence of carbonates.

Organic Acids Analysis

Malic Acid Test

Few drops of 1% potassium permanganate and dilute sulfuric acid were added to 0.5 mL of each extract. Vanishing the color shows the presence of organic acids.

Oxalic Acid Test

To 0.5 mL of each extract, few drops of 40% ferric chloride solution were added. The appearance of yellow color indicates the existence of organic acids.

Ascorbic Acid Analysis

2 mL of water, 0.1g of sodium bicarbonate, and about 20 mg of ferrous sulfate were added to 2 mL of each extract. The result was shaken well. Violet color is generated which disappears by adding 5 mL of 1M sulfuric acid which proves the existence of ascorbic acid.

Phenolic Compounds Analysis

Lead Acetate Test

Generation of a white precipitate when adding 3-4 drops of 10% lead acetate solution to 0.5 mL of each extract illustrates the existence of phenolic compounds.

Ferric Chloride Test

Few drops of 5% ferric chloride solution were added to 0.5 mL of each extract. Observation of a green color illustrates the existence of phenolic compounds in the studied sample.

Amino Acids Analysis

Millons Test

0.5 mL of each extract was treated with 2 mL of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid). Appearance of a white precipitate that changes to red when heating shows the positive test result.

Ninhydrin Test

Few drops of 5% ninhydrin solution were added to 0.5 mL of each extract. Then the solution was boiled. Observing violet color shows the existence of amino acids.

Proteins Analysis

Biuret Procedure

4% hydroxide sodium solution and few drops of 1% copper sulfate solution were added to 0.5 mL of each extract. Appearance of violet color shows the existence of proteins.

Millon's Procedure

2 mL of Millon's reagent was added to 0.5 mL of each extract. Observation of white precipitate that changes into red when heating shows the positive test.

Oils and Fats Analysis

Observing the oil on filter papers when pressing a pinch of the dried plant sample between the filter papers proves the existence of oils and fats in the studied sample.

Coumarins Analysis

To 0.5 mL of each extract, 10% solution of NaOH was added. Yellow color indicates the existence of coumarins in the studied sample extracts.

Phlobatannins Analysis

Few drops of 1% hydrogen chloride was added to 0.5 mL of each extract. Then the solution was heated in a boiling water bath. Deposition of red precipitate shows the existence of phlobatannins.

Anthraquinones Analysis

To 0.5 mL of each extract, 10 mL of benzene and 5 mL of 10% ammonia solution were added and shaken well. Presence of red, pink, or violet color indicates the presence of Anthraquinones.

Antimicrobial Potential Survey

Antimicrobial potential survey is of great importance in order to obtain comprehensive information around therapeutic activities of various materials such as plant extracts against some microbial infections. By performing this survey, we clarify that the studied plant parts not only have beneficial phytochemical constituents but also act as antimicrobial agents to preserve the host body from different attacks. During the determination of the zone of inhibition, two standard drugs were taken for comparison of the results among various extracts of the fruit and flower of the plant. The antimicrobial activities of the extracts of the plant were tested against a fungus and also Gram-positive and negative bacterial microorganisms. The zone of inhibition which is the area of a disc that contains no bacterial growth was obtained by agar well diffusion method. The more the zone of inhibition obtained, the more powerful antimicrobial agent is the sample.

Antimicrobial activity of the obtained fruit and flower extracts of the studied plant was appraised against some Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 14990, and *Bacillus subtilis* NCIB10106), and Gram-negative bacteria (*Klebsiella pneumoniae* ATCC 10031, and *Escherichia coli* C 600) and a fungus (*Candida kefyr* ATCC 38296). All the performed analysis were accomplished by utilizing a disc (6mm diameter) impregnated with 20 μ l of the utilized crude solvent extracts on the Muller Hinton Agar surface which were inoculated with 10 mL of MHA liquid medium with Gram-positive and Gram-negative bacteria, previously. The crude solvents which were empty from any plant extracts were assumed as the negative control. Two standard antibiotics including tetracycline and chloramphenicol were utilized as positive controls. The inhibition zones at the end of an incubation period of 24 hours at 37 °C were measured in millimeters. The obtained results are shown in Table 2. Moreover, figures 1-4 illustrate the antimicrobial activities of water, ethanol, acetone, and ethyl acetate extracts of the plant's fruit and flower, respectively.

Table 1 Preliminary phytochemical screening of flower and fruit extracts of *P. atlantica* subsp. *kurdica* Rech. f.

No.	Phyto constituents	Water		Ethanol		Acetone		Ethyl acetate	
		Fruit	Flower	Fruit	Flower	Fruit	Flower	Fruit	Flower
1	Alkaloids								
	a. Dragendorff's test	-	+	-	+	-	+	-	
	b. Hager's test	-	+	-	+	-	+	-	
	c. Wagner's test	-	-	-	+	-	+	-	
	d. Mayer's test	-	-	-	-	-	+	-	
2	Carbohydrates								
	a. Anthrone test	+	+	+	+	+	+	+	+
	b. Benedict's test	+	+	+	+	+	+	+	+
	c. Fehling's test (free reducing sugars)	+	-	+	-	+	-	+	-
	d. Molisch's test	+	-	+	-	+	-	+	-
	e. Barfoed test (monosaccharids)	-	-	-	-	+	+	+	+
	f. Fehling's test (combined reducing sugars)	-	+	+	-	-	-	-	-
3	Flavonoids								
	a. Shinoda test	+	+	-	+	+	-	+	-
	b. Ferric chloride test	+	-	+	+	+	-	-	-
	c. Lead acetate test	-	+	-	+	+	+	-	+
	d. Alkaline reagent test	-	+	-	+	+	+	+	-
	e. potassium hydroxide	+	+	-	+	+	+	+	-
	f. ammonia solution	+	+	-	+	+	+	-	-
4	flavonols, flavones and chalcones	-	-	-	-	-	-	-	-
5	Glycosides								
	a. Borntrager's test (Antraquinone glycosides)	-	+	-	-	-	-	-	-
	b. Keller killaini (Cardiac glycosides)	-	+	-	+	-	-	-	-
6	Resins	+	+	+	+	+	+	+	+
7	Steroids and triterpenoids								
	a. Liebermann-Burchard's test	+	-	+	-	+	-	+	-
	b. Salkowski test	+	-	+	-	+	-	+	-
8	Tannins								
	a. Lead acetate test	+	+	+	+	+	+	-	+
	b. Ferric chloride test	+	+	+	+	+	+	-	+
9	Starch	-	-	-	-	-	-	-	-
10	Inorganic acids								
	a. Sulphate test	+	+	+	+	+	+	-	-
	b. Carbonate test	+	+	+	+	+	+	-	-
11	Organic acids								
	a. Malic acid test	-	-	-	-	-	-	-	-
	b. Oxalic acid test	-	+	-	+	-	+	-	-
12	Ascorbic acid	+	-	+	-	+	-	+	-
13	Phenolic compounds								
	a. Lead acetate test	+	+	+	+	+	+	-	+
	b. Ferric chloride test	+	+	+	+	+	+	+	-
14	Amino acids								
	a. Millons test	-	-	-	-	-	-	-	-
	b. Ninhydrin test	-	+	-	+	-	+	-	-
15	Proteins								
	a. Biuret test	-	-	-	-	-	-	-	-
	b. Millons test	-	-	-	-	-	-	-	-
16	Coumarins	+	-	-	+	+	+	+	-
17	Phelobatannins	-	-	-	-	-	-	-	-
18	Antraquinones	-	-	-	-	-	-	-	-
19	Saponins	+	+	+	+	+	+	+	+
20	oils and fats	+	+	+	+	+	+	+	+

Key: + = present, and - = absent

Table 2 Antimicrobial activity of the flower and fruit of *P. atlantica* subsp. *kurdica* Rech. f.

Plant extracts	Concentration (µg/mL)	Zone of inhibition (mm)											
		<i>Escherichia coli</i>		<i>Klebsiella pneumoniae</i>		<i>Staphylococcus aureus</i>		<i>Staphylococcus epidermidis</i>		<i>Bacillus subtilis</i>		<i>Candida kefyr</i>	
		flower	fruit	flower	fruit	flower	fruit	flower	fruit	flower	fruit	flower	fruit
Water	10	6	5	5	5	6	7	7	6	7	5	7	6
	25	7	7	7	6	5	6	6	6	5	7	5	7
	50	7	6	7	8	7	5	7	7	6	8	6	7
	100	8	7	6	7	6	5	8	8	6	6	6	8
Ethanol	10	24	25	24	24	25	24	25	23	23	24	25	24
	25	26	26	25	27	26	26	27	25	25	27	27	26
	50	27	28	27	28	27	27	29	27	25	28	29	28
	100	28	29	28	29	30	28	30	29	28	29	30	29
acetone	10	7	6	7	8	5	6	6	7	6	7	7	8
	25	7	7	8	9	6	8	7	8	7	9	8	9
	50	8	8	9	9	8	9	9	10	8	11	9	10
	100	9	10	11	10	12	10	11	12	11	12	11	12
Ethyl acetate	10	9	10	8	9	8	9	10	12	11	10	12	13
	25	13	12	12	11	10	13	14	15	13	12	14	14
	50	16	15	14	13	12	15	16	16	15	17	18	15
	100	19	19	18	15	15	17	20	19	19	20	21	19
Chloramphenicol	10	9	9	8	9	9	9	9	9	9	10	9	8
Tetracycline	10	8	9	9	8	9	9	10	9	9	10	9	9

Results and Discussion

The outcome of this study shows that the four extracts of the fruit of *P. atlantica* subsp. *Kurdica* indicate the presence of resins, tannins, carbohydrates, flavonoids, steroids and triterpenoids, inorganic acids, ascorbic acid, phenolic compounds, coumarins, saponins, and oil and fats. Moreover, the results of the tests proved that the flower of the plant is a rich source of phytochemicals which contains alkaloids, carbohydrates, flavonoids, resins, tannins, inorganic and organic acids, phenolic compounds, amino acids, coumarins, saponins, and oil and fat. Surprisingly, the ethyl acetate extract of the fruit tested negative for the presence of tannins where the other three extracts tested positive for it. Also, for coumarins analysis in the extracts of fruit samples, the ethanol extract tested negative unlike the three other extracts. The existence of these compounds in the studied plant proves its traditional usage as a herbal remedy. The presence of steroids, tannins, and saponins in the studied plant causes to have several curative applications against different pathogens [22]. Also, the presence of saponins in the extracts of the plant makes it a good food supplement for enhancing the sexual appeal due to its androgen increasing property [23]. The presence of flavonoids and tannins in the plant is of great importance due to the fact that these chemicals are

natural antioxidants and radical scavengers. So they play a significant role in the prevention of various illnesses such as cancer, diabetes, and cardiovascular diseases [24]. Flavonoids obstruct the action of several harmful enzymes including nitric oxide synthase and xanthine oxidase that cause the generation of free radicals that can be so detrimental for human health [25]. Also ascorbic acid detection in the fruit extracts illustrated its high antioxidant property [22]. The antimicrobial activity of the tested extracts of *P. atlantica* subsp. *Kurdica* was proved by observing the dose dependent antimicrobial activity according to the zones of inhibition for the analyzed bacteria. The zone of inhibition of the studied bacteria enhanced while increasing the concentration of the extracts. In the performed survey, the maximum zone of inhibition was observed to be 30 mm which was obtained in the ethanol extract of flower at 100 µg/mL against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Candida kefyr* which was followed by *klebsiella pneumoniae* and *Bacillus subtilis* (28mm). Similarly, maximum zone of inhibition for the fruit sample was obtained in ethanol extract at the concentration of 100 µg/mL which was observed to be 29 mm against *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Candida kefyr* which was followed by *Staphylococcus aureus* (28 mm).

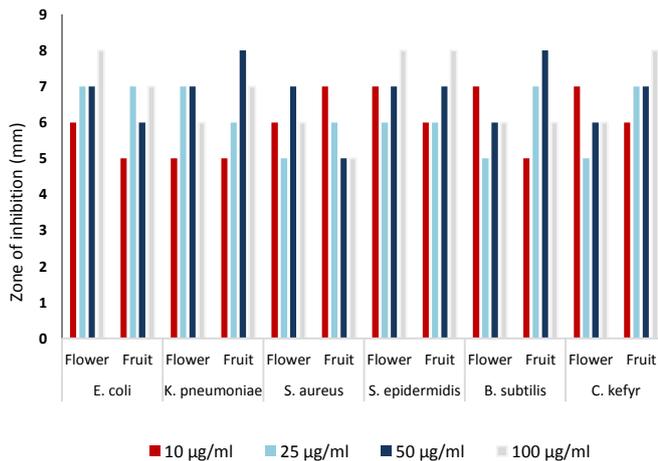


Fig. 1 Antimicrobial activity of the water extract of *P. atlantica* subsp. *Kurdica*'s fruit and flower

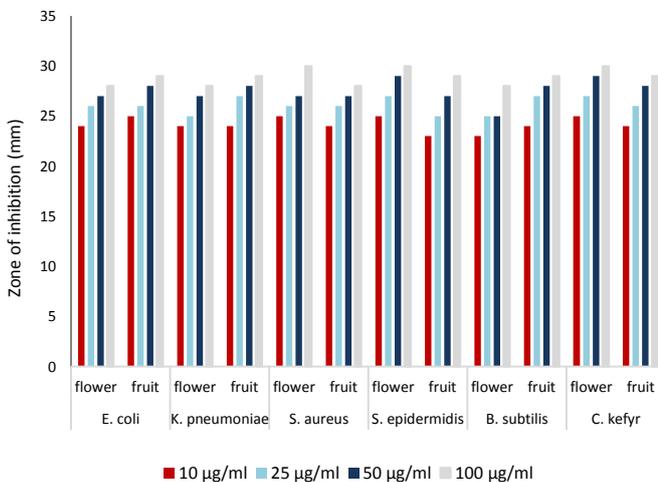


Fig. 2 Antimicrobial activity of the ethanol extract of *P. atlantica* subsp. *Kurdica*'s fruit and flower

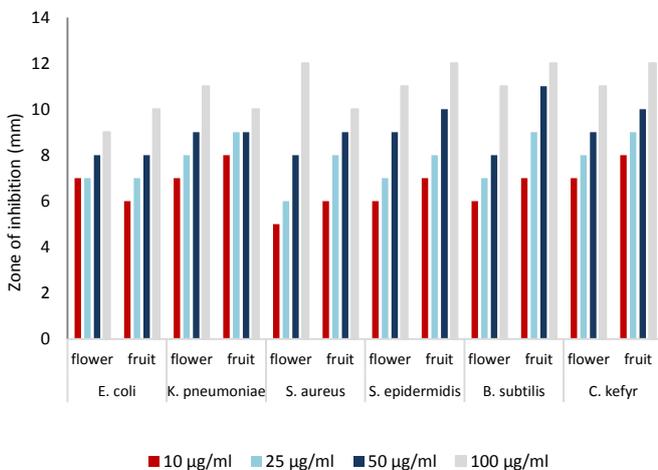


Fig. 3 Antimicrobial activity of the acetone extract of *P. atlantica* subsp. *Kurdica*'s fruit and flower

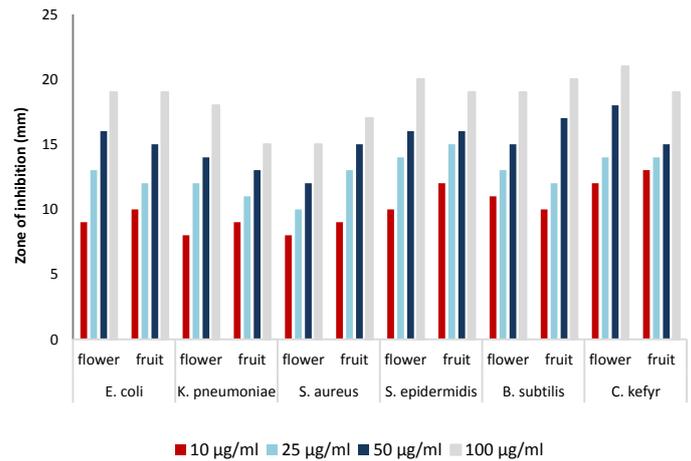


Fig. 4 Antimicrobial activity of the ethyl acetate extract of *P. atlantica* subsp. *Kurdica*'s fruit and flower

Moreover, Extracts of the flower and fruit at the other concentrations in the ethanol (10, 25, and 50 µg/mL) illustrated efficient activity (23–29 mm of zone of inhibition) against the analyzed bacteria. The ethyl acetate extract of flower and fruit was proved to have moderate antimicrobial activity against the tested organisms. Water and acetone extracts of the flower and fruit were less effective.

Conclusion

Phytochemical analysis of the four extracts (water, ethanol, acetone, and ethyl acetate) of the fruit and flower of *P. atlantica* subsp. *Kurdica* by means of a soxhlet extractor proved the presence of many beneficial phyto constituents such as carbohydrates, flavonoids, resins, inorganic acids, phenolic compounds, coumarins, saponins, and oil and fats in its fruit and flower. Also, the results of antimicrobial activity tests showed that the flower and the fruit extracts of *P. atlantica* subsp. *Kurdica* have good potential against many pathogens. The ethanol extract of both fruit and flower proved to have the maximum antimicrobial activity against the studied bacteria and fungus. Eventually, it is noted that the plant's fruit and flower are rich sources of phytochemicals and have good antimicrobial potential so can be used as a traditional and potential remedy for the related illnesses.

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