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Phytochemical constituents and antibacterial activity of some green leafy vegetables

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PEER REVIEW

Peer reviewer

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Comments

This is a good and applicable study in which the author tested the efficacy of 5 different green leafy plants as antibacterial extracts. The obtained results are promising showing the possibility of using these plants to treat different bacterial infections through their antioxidant, detoxification and antimicrobial effects.
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ABSTRACT

Objective: To investigate the antibacterial activity and photochemicals of five green leafy vegetables against a panel of five bacteria strains.
Methods: Disc diffusion method was used to determine the antibacterial activity, while kanamycin was used as a reference antibiotic. The phytochemical screening of the extracts was performed using standard methods.
Results: All methanol extracts were found active against all the test bacterial strains. Overall maximum extracts shows antibacterial activity which range from 6 to 15 mm. Proteins and carbohydrates was found in all the green leaves, whereas alkaloid, steroids, saponins, flavonoids, tannins were found in most of the test samples.
Conclusions: The obtain result suggests that green leafy vegetables have moderate antibacterial activity and contain various pharmacologically active compounds and thus provide the scientific basis for the traditional uses of the studied vegetables in the treatment of bacterial infections.

KEYWORDS
Green leafy vegetables, Antibacterial activity, Phytochemical screening.

1. Introduction

Green leafy vegetables have been used as medicine since ancient times and have been playing a very important role in our diet and nutrition. They are the most readily available sources of carbohydrates, fats, important proteins, vitamins, minerals, essential amino acids, and fibers[1]. Their bioactive substances have a wide range of biological functions, including antioxidant and antimicrobial activities[2-5] and can be helpful in management of oxidative stress and age related human aliments[6]. They are rich source of carotene, ascorbic acid, riboflavin, folic acids and minerals like calcium, iron and phosphorus[7]. Being a photosynthetic tissue, leafy vegetables have higher levels of vitamin K when compared with other fruits and vegetables due to direct involvement of vitamin K (phylloquinone) in photosynthesis process. Vegetables as medicinal plants contain none or less toxic effects[8,9], and have the ability to synthesize several secondary metabolites of relatively complex structures possessing antimicrobial activities[10-12]. Green leafy vegetables are also rich in compounds having antidiabetic[13], anti-histaminic[14], anti-carcinogenic[15]

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and hypolipidemic[16] properties and possess preventive or curative properties against cardiovascular disease, ageing, obesity, hypertension, insomnia and ageing[17-19]. Leafy vegetables are natural source of antioxidants and rich in phytochemicals[20,21]. The present work was therefore designed to investigate the antibacterial effects of five leafy vegetables namely *Coriandrum sativum* (*C. sativum*), *Lactuca sativa* (*L. sativa*), *Mentha piperita* (*M. piperita*), *Portulaca oleracea* (*P. oleracea*) and *Raphanus sativus* (*R. sativus*) against some bacteria strains and their phytochemical screening.

2. Materials and methods

2.1. Collection of plant material

Fresh leaves of *C. sativum*, *L. sativa*, *M. piperita*, *P.oleracea* and *R. sativus* free from disease were purchased from local farms in Al-Qassim. Samples were labeled and stored at 4 °C in polythene bags till they were processed. Collected materials were washed thoroughly in running tap water, rinsed in distilled water and shade dried for one week in open air, and then crushed using mortar and pestle, reduced to powder using Waring laboratory blender (MX-7011G) for 5 min at high speed and then stored in airtight closed bottles for two days before used for analysis. Fifty grams of all the fresh samples were stored for juice preparation.

2.2. Microorganisms

Bacteria cultures of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Bacilllus subtillis*, and *Pseudomonas aeruginosa* (clinical isolates) were obtained from Botany Department of King Saud University. The strains were maintained on agar slant at 4 °C and activated at 37 °C for 24 h on nutrient agar (Sigma-Aldrich, Germany) before any susceptibility test.

2.3. Preparation of leaf extract

2.3.1. Juice preparation

Fifty grams of raw leave samples after washing with water were crushed by grinder without adding any solvent. The residue was removed by filtering through 8 layers of muslin cloth. The filtrate was collected in clean airtight bottle and stored at 4 °C until use for antibacterial activity test.

2.3.2. Aqueous extraction

Ten grams of dry powder of samples were dissolved in 30 mL of 0.01 mol/L HCl containing 0.15 mol/L NaCl. (sample:extract solution, 1:3 w/v). The residue was then removed by filtering through cheese cloth. The filtrate was then centrifuged at 8 100×*g*, for 5 min. These leaves and extract were subjected to antibacterial activity experiments and protein determination[22].

2.3.3. Methanol extraction

Ten grams of powdered sample was dissolved in 100 mL of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 r/min for 24 h. The supernatant was collected slowly and evaporated in wide mouthed evaporating bowls at room temperature for 2-3 d till the final volume was reduced to one fourth of the original volume of the solvent used, giving the concentration of 400 mg/mL[23] and stored at 4 °C in airtight bottles.

2.4. Media preparation

Twenty three grams of nutrient agar (Sigma-Aldrich, Germany) were dissolved in 1 000 mL of distilled water and bring to boil. Agar was then autoclaved for 15 min at 121 °C and left to cool at room temperature. Once the LB medium was cooled (about 45 °C), it was poured into Petri dishes. Each Petri dish was left on the flat surface for 30-40 min until completely set.

2.5. Antibacterial activity

Antibacterial activity was assayed by disc diffusion method. For all bacteria strains, overnight culture grown in broth was adjusted to an inoculum's density of 100 μL: 0.1A600 culture containing 3.2×10^8 colony forming unit. Further, 20 μL was spread onto 20 mL of sterile agar plates by using a sterile cotton swab. The surface of the medium was allowed to dry for about 3 min. Sterile filter paper discs (5 mm in diameter) impregnated with different test extracts (100 μL disc) were then placed on the surface of inoculated agar plates. Kanamycin (30 μg/disc) was used as positive control. The plates were then incubated at 37 °C for 24 h after which microbial growth was determined by measuring the diameter of the inhibition zone (mm) using a transparent scale. Each extract was analyzed in triplicate, the mean values are presented. Kanamycin disc (30 μg/disc) was used for comparing the bioassay.

2.6. Phytochemical analysis

2.6.1. Molisch's test for Carbohydrates

At first 0.5 g of each powder was dissolved separately in 5 mL of distilled water and filtered. Few drops of Molisch's

reagent were added to each solution, this was then followed by addition of 1 mL of concentrated H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for 2 min and then diluted with 5 mL of distilled water. Formation of a red or dull violet colour at the interphase of the two layers was taken as positive test[24].

2.6.2. Test for alkaloids

A given weight of 0.1 g of each powder was dissolved in 5 mL of methanol separately and then filtered. A volume of 2 mL of each filtrate from each sample were stirred with 5 mL of 1% aqueous HCl on water bath and then filtered. Of the filtrate, 1 mL was taken individually into 2 test tubes. To the first portion (1 mL), few drops of Dragendorff's reagent were added. Occurrence of orange-red precipitate was taken as positive. To the second 1 mL, Mayer's reagent was added and appearance of buff-colored precipitate was taken as positive test for the presence of alkaloids[24].

2.6.3. Liebermann-Burchard test for steroids

At the beginning 0.2 g of crude powder of each sample was dissolved in 2 mL of acetic acid separately. The solutions were cooled well in ice followed by the addition of concentrated H_2SO_4 carefully. Color development from violet to blue or bluish-green indicated the presence of a steroidal ring[24].

2.6.4. Test for saponins

One gram of crude powder of each sample was boiled with 5 mL of distilled water separately and then filtered. To each filtrate, about 3 mL of distilled water was further added and shaken vigorously for about 5 min. Frothing which persisted on warming was taken as an evidence for the presence of saponins[24].

2.6.5. Shinoda's test for flavonoids

About 0.5 g of each powder was dissolved in 5 mL of ethanol separately, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of concentrated HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids[25].

2.6.6. Test for tannins

About 0.5 g of each portion of crude powder was stirred with about 10 mL of distilled water separately and then filtered. Few drops of 1% ferric chloride solution were added to 2 mL of each filtrate occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins[25].

2.7. Statistical analysis

The results were analyzed by using standard deviation (SD) statistical methods[26].

3. Results

3.1. Antibacterial activity of the vegetable extracts

The antibacterial activity of five green vegetables extract was assayed *in vitro* by agar disc diffusion against five bacterial species. The data in Table 1 show the antibacterial activities of the tested extracts on a panel of five Gram positive or Gram negative bacteria. All methanol extracts were found active against all the test bacterial strains. All the extracts of *C. sativum* showed maximum inhibitory activity against the entire test bacterial strains while all extracts from *M. piperita* were less active against test organism under study. The best activity was obtained with methanol extract from *C. sativum* against *E. coli*.

Table 1
Antibacterial activity of various extracts of test samples against bacterial species tested by disc diffusion assay.

Plant extract		Zone of inhibition (mm)				
		S. aureus	*S. pyogenes*	*B. subtillis*	*E. coli*	*P. aeruginosa*
C. sativum	Fresh juice	8.50±0.57	7.00±0.10	8.00±0.33	10.00±0.77	8.50±0.00
	Aqueous	11.00±0.20	12.00±0.00	11.00±0.42	10.00±0.43	11.00±0.15
	Methanol	14.50±0.00	13.00±0.60	12.00±0.45	15.00±0.56	11.00±0.11
L. sativa	Fresh juice	–	–	6.00±0.44	9.00±0.45	–
	Aqueous	10.00±0.55	9.00±0.20	–	11.00±0.23	–
	Methanol	13.00±0.10	14.00±0.66	11.50±0.55	12.50±0.44	11.00±0.50
M. piperita	Fresh juice	7.00±0.50	8.00±0.56	6.50±0.00	7.50±0.76	–
	Aqueous	8.50±0.40	11.00±0.68	–	9.00±0.23	–
	Methanol	12.00±0.00	12.50±0.33	10.00±0.42	12.00±0.56	12.00±0.00
P. oleracea	Fresh juice	9.00±0.15	8.50±0.57	–	7.00±0.63	6.50±0.15
	Aqueous	12.00±0.33	12.00±0.34	–	12.00±0.71	–
	Methanol	14.00±0.66	13.00±0.40	11.50±0.33	14.00±0.55	10.00±0.33
R. sativus	Fresh juice	10.00±0.80	8.00±0.00	–	8.00±0.00	7.00±0.15
	Aqueous	11.50±0.43	10.00±0.20	10.00±0.32	9.00±0.31	9.00±0.35
	Methanol	15.00±0.12	12.00±0.33	13.00±0.10	11.00±0.45	13.00±0.25
Kanamycin (30 μg/disc)		26.50±0.33	28.00±0.57	21.00±0.15	20.00±0.33	25.00±0.10

Values are mean inhibition zone (mm)±SD of three replicates.

3.2. Chemical composition of the vegetable extracts

The results of the qualitative analysis showed that carbohydrates and proteins were found in all the leaves under study (Table 2). Flavonoids were present in all samples except in *M. piperita*. Only green leaves of *M. piperita* and *R. sativus* contain steroids while alkaloids were found in *L. sativa*, *P. oleracea* and *R. sativus*. Saponins were found in *M. piperita*, *P. oleracea* and *R. sativus*, and tannin was found in *C. sativum*, *M. piperita* and *P. oleracea*.

Table 2
Phytochemical composition of different salad leaves.

Pleants	Part used	Carbohydrates	Alkaloids	Steroids	Saponins	Flavonoids	Tannins	Proteins
C. sativum	Leaves	+	–	–	–	+	+	+
L. sativa	Leaves	+	+	–	–	+	–	+
M. piperita	Leaves	+	–	+	+	–	+	+
P. oleracea	Leaves	+	+	–	+	+	+	+
R. sativus	Leaves	+	+	+	+	+	–	+

+: Present; –: Absent.

4. Discussion

The various leave extracts showed varied antimicrobial activity to the test organism which was species dependent. Gram-positive bacterial strains were more susceptible to the extracts when compared to Gram negative bacteria. Gram negative bacteria are surrounded by the cell wall which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering. The absence of this barrier in Gram positive bacteria allows the direct contact of the essential oil's hydrophobic constituents with the phospholipids bilayer of the cell membrane, causing either an increase of ion permeability and leakage of vital intracellular constituents, or impairment of the bacterial enzyme systems[27]. Also two groups of bacteria differ in their structure of cell wall. Ability of tannin to disintegrate bacterial colonies is hinder with bacterial cell wall[28]. Medicinal plants which are rich in tannins are used to treat inflamed or ulcerated tissues[29].

Bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration varies according to the plant parts, seasons, climates and particular growth phases. Leaves are one of the highest sources of accumulation and are highly beneficial[30,31].

Most of the secondary metabolite identified in the test samples like flavonoids, saponins, tannin, steroids and alkaloids are phytoprotectants and are important for cell growth, replacement, and body building[32]. Their medicinal value is due to presence of some chemical substances that can produce a defined physiological action on human body with antioxidant, antibacterial, anti-inflammatory, antiviral, immune system stimulant and detoxification activities[33].

Green leafy vegetables contain various pharmacologically active compounds. On the whole the present investigation confirmed the traditional uses of the studied vegetables in the treatment of bacterial infections.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The authors gave a strong and well written background on the medicinal uses of green leafy vegetables, their antioxidant and antimicrobial efficacy. The cited references covered a period from 1998 until 2013 which reflect the awareness of the author with the presented topic.

Research frontiers

Green leafy vegetables have phyto-protectants that are important for cell growth, replacement, and body building. Their detoxification and antimicrobial effects increase their nutritional values.

Related reports

The antimicrobial effects of the five green leafy plants were ascertained in this study.

Innovations and breakthroughs

The present study foucuses on the possibility of the traditional use of green leafy plant extracts in treating bacterial infections.

Applications

The research results indicate that the five plants could be used in developing antimicrobials.

Peer review

This is a good and applicable study in which the author tested the efficacy of 5 different green leafy plants as antibacterial extracts. The obtained results are promising, showing the possibility of using these plants to treat different bacterial infections through their antioxidant, detoxification and antimicrobial effects.

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