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Phytochemical constituents and antibacterial activity of some green leafy vegetables

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PEER REVIEW

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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