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Research Article

In vitro Antimicrobial Assessment of *Lepidium Sativum L.* and *Capsicumannuum L* Extracts

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Abstract

Medicinal plants play a major role in all the traditional system of medicine and contain the rich source of natural products. Most of which have been used for human welfare especially to cure disease caused by pathogenic microorganisms without any side effects. The antimicrobial effect of ethanol extracts of *Lepidiumsativum L. (LS)* and *Capsicumannuum L (CL)* on pathogenic bacteria namely, *Pseudomonas aeroginosa*, *Shigellashinga, Klebsiellapneumoniae, Salmonella typhi, Proteus mirabilis, Serratiamarcescens, Bacilluscereus, Enterobacter cloacae, Staphylococcus saprophyticus* and *Staphylococcus aureus* were determined using broth microdiluation method. Our results demonstrate possible antibacterial effects of same components in LS and CL on gram-positive and gram- negative bacteria specially *Serratiam arcescens.*

Keywords: Antimicrobial activity; *Lepidium sativum*; *Capsicum L*; Minimum Inhibitory (MIC)

Introduction

In recent years, there has been a great interest in herbal remedies for the treatmentof a number of ailments. Medicinal plants are promising source of antidiarrheal drugs [1]. The medicinal plants are being used for treatment of infections is an age-old practice especially in developing countries [2]. Plants still remain a major source for drug discovery in spite of the great development of synthetic molecules. Consequently, the uses of traditional plant extract in the treatment of various diseases have been flourished [3]. *Lepidium sativum*, known as pepper cress or Elrashad, belongs to the family *Brassicaceae* (cruciferae) and it is an erect, annual herb grows up to 50 cm height. The seeds and leaves of the plant contain volatile oils [25]. The plant is eaten and seed oils are used in treating dysentery and diarrhea [3] and migraine [14]. The plant was found to contain glucosinolate and glucotropaeolin [23]. *Lepidiumsativum L*(LS).is largely recommended by traditional herbal healers for hypertension, diabetes control, renal disease and phytotherapy [7].The seeds and leaves of the plant contain volatile oils [25]. The plant is eaten and seed oils are used in treating *Lepidiumsativum L* (LS). is largely recommended by traditional herbal healers for hypertension, diabetes control, renal disease and phytotherapy[7]. The seeds are consumed in salad and as spice [9]. The plant is also reported to possess haemagglutinating, hypoglycemic, antihypertensive, diuretic and fracture healing property [10]. Previous studies have been demonstrated the protective action of LS against carcinogenic compounds [12] and growth inhibition of *Pseudomonas aeruginosa*, a bacteria strain with a potent antibiotic resistance. Bell peppers (*Capsicum L.*)(CL) are the mostimportant vegetable spice grown in the tropical andsub-tropical regions of the world because of theircolor, taste, pungency, flavor and aroma [15,17,18]. Pepper (Capsicum) is a tropical and an important agricultural crop andone of the popular vegetables, not only because ofits economic value, but also for the combination ofcolor, taste and nutritional values of its fruit [4,8]. Fruit and vegetables are important sources ofbioactive compounds (such as phenoliccompounds, terpenoids, steroids and alkaloids) known for their health-promoting effect againstdegenerative diseases [13,15]. The present study was carried out to determine the in vitro potential antibacterial agent of *Lepidiumsativum L.* and *Capsicumannuum L* against ten bacteria which are known to cause pneumonia or wound infection (*Klebsiella pneumonia*, *Proteus mirabilis*), hemoragic diarrhea (*Shigellashinga*), typhoid fever or food borne illness (*Salmonella typhi, Bacillus cereus*) and urinary or respiratory tract infections (*Pseudomonas aeroginosa, Serratiamarcescens, Enterobacter cloacae, Staphylococcus aureus, Staphylococcus saprophyticus*) in humans.

Materials and Methods

Bacterial Strains and Culture Conditions:

Bacterial strains were obtained from standard laboratory. Evaluate the antibacterial activity of the plant extracts we reinvestigated using strain of gram-negative bacteria *[Pseudomonas aeroginosa* (ATCC9027), *Shigellashinga* (ATCC1013), *Klebsiella pneumonia* (ATCC13183), *Salmonella typhi* (ATCC1006), *Proteus mirabilis* (ATCC49565), *Serratiamarcescens* (ATCC21074)] and strain of gram-positive bacteria [*Bacillus cereus* (ATCC4010), *Enterobacter cloacae* (ATCC13047), *Staphylococcus saprophyticus* (ATCC15305) and *Staphylococcus aureus* (ATCC6538p)]. Thetyped cultures of bacteria was sub-cultured on Nutrient agar (Oxoid) and stored at 4oC untilrequired for study. The typed cultures of bacteria was sub-cultured on Nutrient agar (Oxoid) and stored at 4oC until required for study.

Plant Materials:

The seed *Lepidium sativum* (LS) and Fruit *Capsicum annuum L* (CL)were collection in the region of Iran (Zahedan and Kerman, south-eastern, Iran) and plant in kermanazad university herbarium received approval and dried at room temperature. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

Preparation of Extracts:

Plants were properly dried and pulverized into a coarse powder as reported by Hanafy and Hatem [5]. Each of 20 g grinded powders was soaked in 60 ml ethanol95 %, separately for one day (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered (Whatman no. 1 filter paper) .Then the filtrates were evaporated using rotary evaporator. At last, 0.97 g of dried extracts were obtained and then stored at 40C in air tight screw-cap tube.

Minimum Inhibitory Concentration(MIC) and Minimum Bactericidal Concentration (MBC) of Plant Extracts

The broth micro dilution method was used to determine MIC and MBC according to Yu (6). All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 0.3mg/ml to 10.00mgml. To each well, 10µl of indicator solution (prepared by dissolving a 10-mg extract in 2ml of DMSO) and 10µl of Mueller Hinton Broth were added. Finally, 10µl of bacterial suspension (106 CFU/ml) was added to each well to achieve a concentration of 104 CFU/ml. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18–24 hours. The colour change was then assessed visually. The lowest concentration at which the colour change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC and MBC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity. The MBC was defined as the lowest concentration of the extract at which the incubated microorganism was completely killed.

Statistical analysis

The result were expressed as mean and ranked in order of importance as percent (%). The data were subjected to one-way analysis of variance (ANOVA), using the SPSS-17 software. A p-value less than 0.05 were regarded as significant.

Result

The antimicrobial activity of the extracts and their potency was quantitatively assessed by the presence or absence of inhibition. All plants extracts showed inhibitory activity against gram-positive and gram-negative bacteria with varying magnitudes and these effects were dose dependent manner. The least MIC value for gram- negative bacteria was observed by the *Capsicum annuum L* extract against *Serratiamarcescens* (0.62mg/ml). The least MIC value for gram-positive bacteria was observed by the Capsicum L extract against *Staphylococcus aureus* (1.25mg/ml). The highest MIC value for gram- positive bacteriawas observed by the *Lepidium sativum* extract against *Staphylococcus saprophyticus* (5mg/ml) and the least MIC value was observed by the *Lepidium sativum* extract against *Serratiamarcescens*(1.25mg/ml).

Discussion

Plants are important source of potentially useful structures for the development of new chemotherapeuticagents. The first step towards this goal is the in vitro antibacterial activity assay [22]. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants [5,20].In the present studythe antimicrobial effect of ethanol extracts of *Lepidium sativum* L (LS) and *Capsicumannuum L* (CL) on pathogenic bacteria namely, *Pseudomonas aeroginosa, Shigellashinga, Klebsiellapneumoniae, Salmonella typhi, Proteus mirabilis, Serratiamarcescens, Bacilluscereus, Enterobacter cloacae, Staphylococcus saprophyticus and Staphylococcus aureus* were determined. Our results demonstrate possible antibacterial effects of samecomponents in LSandCL on gram-positive and gram- negative bacteria specially Serratiamarcescens.The study hero result showe the minimum inhibitory concentration (MIC) of L. sativum extracts was determined and it was 3% for *Klebsiella pneumoniae* and *Proteus*, whereas other bacterial species were sensitive to all concentrations of the extracts. It is noted from the present result that the extracts of L. Sativum had maximum antibacterial activity, which is

identical with results obtained from other researchers [6,17]. *Lepidium sativum L*. seeds increase weight gain as they are found to contain 18-24% of fat. Thirty fourpercent of the total fatty acids are alpha linolenic acid; and the oil has alpha linoleic acid which could give it nutritional advantages. The primary fatty acids in *Lepidium sativum* oil were oleic (30.6 wt %) and linolenic acids (29.3 wt%) and was found to contain high concentrations of tocopherols. It contains good amount of lignans and antioxidants, which can stabilize the n-3 polyunsaturated fatty acids in its seed oil. The primary phytosterols in *Lepidium sativum* were sitosterol and campesterol, with avenasterol.The another study Lepidiumsativum showed highest activity against *Proteus mirabilis*. Previous studies have demonstrated the protective action of L. sativumagainst growth inhibition of *Pseudomonas aeruginosa,* a bacterial strain with a potent antibioticresistance [1]. Plants are natural factories of secondary metabolites. These secondary metabolites may be responsible for antimicrobial activities. In the future, specific antimicrobial agent can be successfully isolate from this species. The antimicrobial effects of extracts against the studied microbes suggest that different crude parts of this plant species hold notable therapeutic action that can prop up the traditional practice of this plant in the treatment of disease causes by bacteria and fungi, such as gastrointestinal infection, diarrhea, arespiratory and skin diseases. Moreover, this study gave more specific result; it showed that the fraction of the plant that could be screened for specific anti-bacterial agent against bacteria.

Table 1. Antibacterial effects of Plants Extracts against pathogenic bacteria

Standard bacterial	*Capsicum L* MIC/MBC	*L. sativum*L. MIC/MBC
S.aureus	1/25 .2/5	**2/5 . 5**
B .cereus	2/5 . 5	**2/5 . 5**
E .cloacae	2/5 . 5	**2/5 . 5**
S. saprophyticus	2/5 . 5	**5 . 10**
K. pneumonia	2/5 . 5	**2/5 . 5**
S .typhi	2/5 . 5	**2/5 . 5**
S .shinga	2/5 . 5	**5 . 10**
P. mirabilis	2/5 . 5	**5 . 10**
P. aeroginosa	1/25 . 2/5	**5 . 10**
S. marcescens	0/62 . 1/25	**1/25 . 2/5**

Table2: Antimicrobial activity of the plant crude extracts as mean of inhibition diameter zone against gram-positive and gram-negative pathogenic bacteria (mm)

Standard bacterial	*Capsicum L(mm)*	*L. sativum*L. (mm)
S.aureus	15mm	9mm
B .cereus	11.5mm	10mm
E .cloacae	10mm	11mm
S. saprophyticus	11.5mm	7mm
K. pneumonia	12mm	10mm
S .typhi	13mm	11.5mm
S .shinga	11mm	8mm
P. mirabilis	10mm	7.5mm
P. aeroginosa	14mm	7mm
S. marcescens	17mm	14mm

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