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In vitro Antibacterial Activity Assessment of the crude Phenolic, Alkaloid and Terpenoid compounds extracts of *Lepidium sativum* L. on Human Pathogenic Bacteria

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Abstract : Objective: To reveal the effect of the crude phenolic, alkaloid and terpenoid compounds extracts of *Lepidium sativum* L on some Human Pathogenic Bacteria.
Methods: Antibacterial activities of the crude Phenolic, Alkaloid and Terpenoid of medicinal plant were determined *in vitro* by agar well diffusion-method against some human pathogenic bacteria.
Results: obtained results showed that active compounds of *Lepidium sativum* L had wide spectrum antibacterial activity against gram-positive and gram-negative bacteria.
Conclusion: This study demonstrates that we can conclude that the effect of active compounds in same plant has different effect on different pathogenic organisms in different concentration.
Keywords: Antibacterial Activity; *Lepidium sativum* L; Pathogenic Bacteria.

Introduction

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind. Neanderthals living 60,000 years ago in present day Iraq used plants such as hollyback, these plants are still widely used in ethnomedicine around the world [1, 2].

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 leaves are variously lobed and entire, flowers are white small and found in racemes and Fruits are obovate pods, about 5 mm long, with two seeds per pods. The seeds and leaves of the plant contain volatile oils [3]. The plant is eaten and seed oils are used in treating dysentery and diarrhea [4] and migraine [5]. The plant was found to contain glucosinolate and glucotropaeolin[6]. Its seeds are rich source of proteins, dietary fiber, omega-3 fatty acids, iron, other essential nutrients and phytochemicals. Pepper cress is widely used in folk medicine for the treatment of hyperactive airways disorders, such as asthma, bronchitis and cough. Seeds are considered to be galactogogue, emmenagogue and recommenced in inflammation, bronchitis, muscular pain and rheumatism[7].Antibiotic resistance has become a global concern [8].There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientists to search for new antimicrobial substances from various sources like the medicinal plants. Search for new antibacterial agents should be continued by screening many plant families [9].This study aimed to assess the *in vitro* thepossible effects of antibacterial activity of active compounds of *Lepidium sativum* L. upon Human Pathogenic Bacteria.

Materials and Methods

Collection of Plant Material: *Lepidium sativum* were purchased from local market, Hilla, Iraq in May, 2012. The plant was identified by the taxonomist, Professor Dr.Abdull-Alkareem AL-Bermani, at the College of science for women, Babylon University. The leaves were washed thoroughly 2-3 times with running water and once with sterile distilled water, leaf material was then air-dried on sterile blotter under shade.

Solvent Extraction: Twenty five grams of shade-dried powder was filled in the thimble and extracted successively with methanol solvent in Soxhlet extractor for 24h. The solvent extracts were concentrated under reduced pressure and preserved at 5°C in airtight bottle until further use. One gram of each concentrated solvent extracts were dissolved in 9 ml of distilled water and used for antibacterial assays.

Phenolic Extraction: The Phenolic compounds were extracted according to [10].

Alkaloid Extraction: The Alkaloid compounds were extracted according to [11].

Terpenoid Extraction: The Terpenoid compounds were extracted according to [12].

Preparation of Inoculum: The gram positive and gram negative bacteria were pre-cultured in nutrient broth overnight at 37°C,

Anti-bacterial Activity: The organism to be tested was inoculated into sterile nutrient agar. After incubation period of 24 h at 37°C, a loop of inoculum was transferred into 5 ml of nutrient broth and incubated for 2 h at 37°C which served as fresh suspension inoculum. Five wells (5 mm diameter) were made in sterile nutrient agar plate by using Cork borer (one in the center and four wells at the corner) and inoculum containing 106 CFU/ml of test bacteria were spread on solid plates with the help of sterile swab moistened with the bacterial suspension. Then 50 µl of extract of all the leaves were placed in the wells made in inoculated plates. The treatment also includes 50 µl of sterilized distilled water as control. All the plates were incubated for 24 h at 37°C and zone of inhibition if any around the well were measured in millimetre (mm). For each treatment three replicates were maintained.

Results

The antibacterial activity of Terpenoid, Alkaloid and Phenolic compounds extracts of selected plants against human pathogenic bacteria both Gram-positive and Gram-negative bacteria are presented in Table (1).

Table 1: Antibacterial Activity of the crude phenolic, alkaloid and terpenoid *Lepidium sativum* L against some human pathogenic bacteria

Pathogenic bacteria	Phenolic compounds		Alkaloid compounds		Terpenoid compounds	
	Concentrations					
	50 mg/ml	100 mg/ml	50 mg/ml	100 mg/ml	50 mg/ml	100 mg/ml
	Inhibition zone/ mm/ diameter					
Staphylococcus aureus	R	R	R	R	R	R
Staphylococcus epidermedis	R	R	R	15	R	R
Staphylococcus saprophyticus	R	R	12	15	R	10
Klebsilla	R	R	R	R	R	R
Serratia	R	R	12	18	R	R
Proteus	R	R	12	13	11	15
Escherichia coli	R	R	R	15	R	10
Pseudomonas	R	R	R	10	R	10
Provedenatia	R	R	R	R	R	R

- R= Resistant

Activity was analyzed at (50 & 100) mg/ ml. the results revealed thatboth gram-positive and gram-negative bacteria species under study were resistant to phenolic compounds, While *Staphylococcus aureus*, *Klebsilla*and*Provedenatia*resistant to all active compounds presence in*Lepidiumsativum*.The results also revealed that active compounds of *Lepidiumsativum*L like Alkaloid and Terpenoid had wide spectrum antibacterial activity against gram-positive and gram-negative bacteria

Discussion

Medicinal plants are considered new resources for producing agents that could act as alternatives to antibiotics in the treatment of antibiotic-resistant bacteria [13]. On the basis of the result obtained in this present investigation, we conclude that the effect of active compounds in same plant have different effect on different pathogenic organisms in different concentration. This implied that the gram-positive bacteria were more susceptible to the active compounds extract than the gram-negative bacteria.Possibly because of the presence of outer membrane that serves as an effective barrier in gram-negative species [14]. In addition, the results showed that gram-positive bacteria the most susceptible bacteria, an observation that may be attributed to the presence of single membrane of the organism which makes it more accessible to permeation by active principles of the extract of active compounds [15], [16], [17]. The Results of this study demonstrated that active compounds in *Lepidiumsativum* exhibited antimicrobial activity against the Gram negative bacteria In addition, to gram positive bacteria; this may be attributed to the presence of active compounds effect on cell wall, proteins and DNA synthesis [18], [19].Alternatively, an important characteristic of plant extracts and their components is their hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death [20].The obtained results may provide a support to use of the plant in traditional medicine. Based on this, further chemical and pharmacological investigations to isolate and identify minor chemical constituents in *Lepidiumsativum* and to screen other potential bioactivities may be recommended.

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