The Study of Antibacterial Activity of Plantago Major and Ceratonia Siliqua

Basma Monjd Abd Razik , Hiba Ali Hasan , Muna Khalil Murtadha

ABSTRACT:

BACKGROUND:

Antibiotics are generally beneficial in treating susceptible microbial infections but such beneficial effects are counter balanced by rampant irrational use. In fact, the misuse of antibiotics leads to the emergence of antibiotic-resistant bacteria. On the other hand, the consumption of foods contaminated with some pathogenic microorganisms represents a serious health risk to humans.

OBJECTIVE:

Therefore the present study describes the comparison of antibacterial activity between two plants which are known traditionally by their biological activities and used in Iraqi folk medicine as antidiarrheal.

METHODS:

Agar – well diffusion method tested antibacterial activities of methanolic crud extracts with different concentrations of the two plants on six types of bacteria strains which are:-:- Lactobacillus sp., Staphylococcus aureus, Proteus sp. , Pseudomonas aeruginosa, Escherichia coli ,and Enterococcus sp.

RESULTS:

Two plants extract had antibacterial activity ,methanolic extract of *Plantago major* was more active than methanolic extract of *Ceratonia siliqua* against the same tested bacteria. The biological activity was determined by measuring the inhibition zone in millimeters, results were expressed as means ± standard deviation of triplicate experiments.

CONCLUSION:

These results suggest that extract of two plants possesses antimicrobial and properties, and therefore they can be used as a potential source of active ingredients for food, pharmaceutical industry or preservatives.

KEY WORDS: plantago major, ceratonia siliqua , methanolic extract, antibacterial.

INTRODUCTION:

It has been well known since ancient times that some plants and spices have antimicrobial activity. There has been a considerable interest to use plants and spices for the elimination of microorganisms because of increasing antibiotic resistance of microorganisms [(1).]

Plantago major (greater plantain, common plantain) is a perennial medicinal herb that belongs to the highly diverse genus Plantago of the Plantaginaceae family. It has been used as a traditional medicinal plant for centuries, and is reported to cure numerous diseases from cold to hepatitis, skin diseases, infectious diseases, problems concerning the digestive organs, respiratory organs, reproduction, the circulation, and for reducing fever [(2)].

Arrange of biological activities of *P. major* leaves have been identified including anti-inflammatory, antiviral, analgesic, antioxidant , anticarcinogenic, antitumor, antinociceptive (reducing the sensitivity to painfulstimuli),weakly antibiotic, immune modulating, anti-ulcerogenic, antileukemic and antihypertensive effects[(3,4,5,6,7)]. *P. major* has also been used to neutralize poisons internally and externally [(2)].

Greek physicians described the use of *P. major* in wound healing already in the first century A.D. [(8)]. Either whole or crushed leaves, or juice from leaves of *P. major* have been used to treat burns, to stop bleeding and to treat all kinds of wounds to enhance the healing process [(9)]. In the Scandinavian countries, *P. major* has a strong reputation for its wound healing properties, which is also reflected in its common name (in Swedish and Norwegian: ‘groblad’) [(2)].

Carob is the fruit of an evergreen (*Ceratonia siliqua*) cultivated in the Mediterranean area [(10)].Recently, this species has attracted much attention and became economically important.

Department of Clinical Laboratory Science,College of Pharmacy,Al-Mustansiriya Unaversity..

Pods and seeds are used as row material in food, pharmaceutical and cosmetic industries [11]. The use of the whole fruit, however, in human consumption is limited, due to a high level of tannins causing excess astringency [10].

Many recent activities are reported for carob pods such as antihyperglycemic, antioxidant, immunomodulating and antiproliferative on mouse hepatocellular carcinoma cell line [12]. Its bark and leaves are used in Turkish folk medicine as an antidiarrheal and diuretic. The fruits of this plant are traditionally used as an antitussive and against warts [13].

The nutritive value of the carob pods was attributed to its high levels of carbohydrates (45%), appreciable amounts of protein (3%), and low levels of fat (0.6%) [12].

The objective of the present study was to assess the antibacterial activity of *C. siliqua* and *Plantago major* extracts against various pathogenic bacteria. .To our best knowledge and literary survey, there is no report available on antibacterial activities of these plants against a wide range of other microorganisms.

MATERIALS AND METHODS:

Plant material

C. siliqua fruits and *P. major* leaves were collected from herbs market and were separately dried in shade, pulverized by a mechanical grinder and stored in airtight glass containers in dark until extraction.

Bacterial strains

All bacterial strains used in the study are clinical strains, and kindly provided by microbiology laboratory in college of pharmacy in (April 2011). They are Gram positive:- *Lactobacillus sp.* and *Staphylococcus aureus,* and Gram negative: - *Proteus sp.,*

Pseudomonas aeruginosa, Escherichia coli, and Enterococcus sp..

Preparation of extraction

For extraction of *P. major* leaves and *C. siliqua* fruits, methanol was used as solvent, 30 grams of dried and powdered plant materials were extracted with 300 ml of methanol by using soxhlet apparatus for 10 h. at a temperature not exceeding the boiling point of the solvents [14], then the obtained extracts were filtered by using Whatman No.1 filter paper and the solvent was evaporated using rotary distillation apparatus.

In order to obtain a completing dry extract, the resultant extracts were transferred to glass dishes and were left in 40°C oven for 24 hrs. , then they were left at 4°C until assessments of their antibacterial activities.

Antibacterial activity:-

Antibacterial activities of the *P. major* leaves and *C. siliqua* fruits were evaluated by means of agar-well diffusion assay [15] with some modifications. Fifteen milliliters of the molten agar (45 °C) were poured into sterile petri dishes (Ø90 mm). Working cell suspensions were prepared and 100 µl was evenly spreaded onto the surface of the agar plates of Mueller-Hinton agar (HIMEDIA ,India). Once the plates had been aseptically dried, 6 mm wells were punched into the agar with a sterile Pasteur pipette. The residual extracts were dissolved in their extracting solvent to yield the final concentration: 1000,500,250 and 125 mg/ml and sterilized by filtration (filter pore size 0.45µm). Thus, 100µl were placed into the wells and the plates were incubated at 37 °C for 24 h .Solvents were used as negative control while antibiotic of streptomycin at the same concentration were used as positive control. .Antimicrobial activity was evaluated by measuring the diameter of circular inhibition zones around the well. Tests were performed in triplicate.

Statistical analysis:-

Experimental results concerning this study were expressed as means ± standard deviation of three parallel measurements.

RESULTS:

The *invitro* antimicrobial activity of the two plants against the tested bacteria was assessed by the presence or absence of inhibition zone diameters. We found that the activity of the two extracts depends on its concentration and the strain of tested bacteria.

The result showed that the methanol extract of *Plantago major* leaves and *Ceratonia siliqua* fruits showed an antibacterial activity against tested Gram-positive and negative bacteria. Different concentrations of methanol extract of *Plantago major* are shown in (table 1). The methanol extract of *Plantago major* produced inhibition zones against Gram positive bacteria:- *Lactobacillus sp.* it's sensitive to concentration ranges from 1000-250 (mg/ml) and *Staphylococcus aureus* sensitive to concentration ranges from 1000-125 (mg/ml). Also it's produced inhibition zones against Gram negative bacteria:-*Pseudomonas aeruginosa* sensitive to concentration ranges from 1000-125 (mg/ml), *Proteus sp.* and Escherichia *coli* sensitive to concentration ranges from 1000-250 (mg/ml), and *Enterococcus sp.* ranges from 1000-500 (mg/ml).

Table 1: Antibacterial activities of methanol extract of Plantago major leaves and methanol (negative control).

Tested bacteria	Inhibition zone diameter in (mm)* at different concentrations of methanol extract in (mg/ml)			
	1000	500	250	125
Gram positive				
Lactobacillus sp.	25±1.3	20±0.9	15±0.8	-
Staphylococcus aureus	20 ±0.9	15±0.7	13±1.0	10±0.6
Gram negative				
Proteus sp.	20±1.2	17±0.9	15±0.7	-
Pseudomonas aeruginosa	24±0.9	18±1.2	17±0.8	15±1.0
Escherichia coli	18±0.6	16±0.9	12±0.5	-
Enterococcus sp.	12±0.7	10±0.6	-	-
Methanol	-	-	-	-

*Diameter of inhibition zones including diameter of well 6 mm
Values are given as mean ± S.D. of triplicate experiment

Antibacterial activity of *C. siliqua* fruits at different concentrations of methanol extract were shown in (table 2). This extract produced inhibition zones against Gram positive bacteria:- *Lactobacillus sp.*and *Staphylococcus aureus* sensitive to concentration ranges from 1000-500 (mg/ml). Also it's produced inhibition zones against Gram negative bacteria:-*Proteus sp.* and *Pseudomonas aeruginosa* sensitive to concentration ranges from 1000-125 (mg/ml), *Escherichia coli* and *Enterococcus sp.* sensitive to concentration ranges from 1000-500 (mg/ml). Methanol extract of *C. siliqua* fruits less effective than *Plantago major* extract against the same tested bacteria. Solvent (negative control) used for preparation different concentrations showed no activity against any tested bacteria. Streptomycin (positive control) at concentration of 125-1000 (mg/ml) showed inhibition zone ranges from 40-23 mm against all tested bacteria (table 3).

Table 2: Antibacterial activities of methanol extract of *C. siliqua* fruits and methanol (negative control).

Tested bacteria	Inhibition zone diameter in (mm)* at different concentrations of methanol extract in (mg/ml)			
	1000	500	250	125
Gram positive				
Lactobacillus sp.	13±1.0	9±0.8	-	-
Staphylococcus aureus	17±0.9	12±0.7	-	-
Gram negative				
Proteus sp.	17±0.5	15±1.0	12±	10±0.8
Pseudomonas aeruginosa	16±0.9	14±0.9	0.9	10±0.6
Escherichia coli	12±0.7	10±0.5	12±	-
Enterococcus sp.	14±0.8	10±0.6	0.7	-
			-	
			-	
Methanol (negative control)	-	-	-	-

*Diameter of inhibition zones including diameter of well 6 mm
Values are given as mean ± S.D. of triplicate experiment

Table 3: Anti bacterial activity of streptomycin (positive control) 125-1000 (mg/ml).

Tested bacteria	Inhibition zone diameter in (mm)* at different concentrations of streptomycin in (mg/ml)			
	1000	500	250	125
Gram positive				
Lactobacillus sp.	40±1.0	40±1.5	35±1.2	33±0.8
Staphylococcus aureus	40±0.9	40±2.0	35±1.4	30±1.6
Gram negative				
Proteus sp.	35±1.4	33±0.9	30±1.3	30±0.8
Pseudomonas aeruginosa	40±1.2	39±1.2	35±1.5	35±0.7
Escherichia coli	39±1.0	35±1.5	34±1.2	32±1.4
Enterococcus sp.	20±0.7	16±0.9	-	-

*****Diameter of inhibition zones including diameter of well 6 mm
Values are given as mean ± S.D. of triplicate experiment

DISCUSSION:

The presented study was designed to obtain preliminary information on the antibacterial activity of *Plantago major* leaves and *C. siliqua* fruits on pathogenic bacteria. The agar- well diffusion method was preferred to be used in this study. The results showed a remarkable antibacterial activity of the methanol extracts of these plants.

The strong antimicrobial activity of the *Ceratonia seliqua* against the tested microorganisms could be attributed to the presence of high percentage of hydrocarbon (51.06 %), monoterpene (0.9 %), and oxygenated monoterpene (1.19 %) appreciated for their antibacterial potentials (16,17%). These results are in agreement with those reported in literature for methanol extract of ceratonia seliqua showed strong action on *Enterococcus* ,*Escherichia coli* [18], and *Staphylococcus aureus* [13].

On the other hand *P. major* is used for different purposes in traditional medicine around the world; therefore, researchers have tested it for different types of biological activities. Most tests have been performed on crude extracts without examining the nature of the active compounds [2]. Presently it is not known exactly what phytochemicals in *P. major* are most important in mediating the beneficial effects. Both polysaccharides and polyphenols have been proposed to be bioactive, and the antiviral activity of *P. major* is reported to derive mainly from its phenolic compounds [19]. *P. major* leaves contain a mixture of different polyphenolic antioxidants that may contribute to its wound healing properties [20].

Also it seems to be some intermediately polar or non polar substances of relatively low molecular weight in *P. major* that have antibiotic activity against some gram negative and gram positive bacteria in addition to a weak antimycotic activity [2].These results are in agreement with those reported in literature for the antibacterial activity of methanolic extract of *P. major* against *S. aureus* and fungi [2].

The obtained results are of a great importance, particularly in the case of *S. aureus* which are well-known for their resistance to a number of phytochemical compounds and for the production of several types of enterotoxins that cause gastroenteritis [21].

Generally the Gram positive bacteria were more susceptible to the antimicrobial properties of two plants extract than Gram negative ones. These differences could be attributed in part to the great complexity of the double membrane-containing cell envelope in Gram negative bacteria compared to the single membrane structure of positive ones [22],or its related to lipopolysaccharides in their outer membrane [23].

CONCLUSION:

The results of this study revealed that the two plants possess some antibacterial properties as antibiotics , the diameters of inhibition zone of the antibacterial agents i.e. *ceratonia siliqua* ,*Plantago major*, and streptomycin were different, according to the kinds, concentrations and purity. This supports the fact that more have to be done on purification , identification and quantification of the active of extracts components with the view of using them for *invivo* studies.

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