International Journal of Microbiology and Immunology Research Vol. 1(5), pp. 053-056, July 2013
Available online at http://academeresearchjournals.org/journal/ijmir
ISSN 2327-7769 ©2013 Academe Research Journals

Full Length Research Paper

Antibacterial activity of some plant extracts against clinical pathogen

Marwan Khalil Qader[1], Nezek Salim khalid[1] and Arshad Mohammad Abdullah[2]*

[1]Faculty of Science, University of Duhok, Duhok, Iraq.
[2]Directorate of Higher Studies, University of Duhok, Duhok, Iraq.

Accepted 7 July, 2013

Seven plant extracts (Plantago mediastepposa, Quercusc infectoria, Punic granatum, Thymus lcotschyana, Ginger officeinals, Rhus angustifolia and Cinnamon) were collected from different regions of Kurdistan region of Iraq. These plants' extracts were dissolved in absolute ethanol and distillate water, after which they were assayed *in vitro* as an antibacterial activity against 2 Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and 3 Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsilla pneumonia*) using agar dilution methods. The minimum inhibition zone of the Punic granatum ethanol extracts was 0.2 mg/ml for all microorganisms tested. *Klebsilla pneumonia* was the most sensitive bacterial strain to Quercusc infectoria and Rhus angustifolia ethanol extracts. Among both Gram-positive and Gram-negative bacteria tested with MIC of 0.2 mg/ml, the minimum inhibition zone of Ginger officeinals D.W. extracts was 0.2 mg/mL against *Pseudomonas aeruginosa* and *Klebsilla pneumonia.* The most sensitive bacterial strain to Thymus lcotschyana and Plantago mediastepposa D.W. extracts was *S. aureus* and *E. coli*.

Key words: Antibacterial activity, pathogenic bacteria, plant extracts.

INTRODUCTION

Humans have frequently used plants to treat common infectious diseases, and some of these traditional medicines are still part of the habitual treatment of various maladies (Thomson, 1978; Zaika, 1988). The evolution of bacterial resistance to currently available antibiotics has necessitated the research for novel and effective antimicrobial compounds. It is known that local plants have medicinal properties and this has made traditional medicine cheaper than modern medicine (Mahesh and Satish, 2008). Globally, plant extracts are employed for their antimicrobial, antifungal and antiviral activities. In particular, the antimicrobial activity of plant extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, and alternative medicine (Prasannabalaji et al., 2012). In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines (Nakatani, 1994). Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and ascertain the parameters associated with it.

MATERIALS AND METHODS

Collection and preparation of extracts

Plant specimens were collected and identified from different locations of Kurdistan region of Iraq. Samples were dried at room temperature for 5 days, after which they were ground dried to powder plants and then dissolved by using solvents (ethanol and distilled water). The dried plant materials of 5 g each were dissolved in 25 ml (0.2 gm/ml). After the plant materials were successively extracted with ethanol and distilled water separately, the extract was filtered through Whatman filter paper and stored at -2°C until it was used.

*Corresponding author. E-mail: arshadzanko@yahoo.com. Tel: 009647504572331.

Table 1. Antibacterial activity of different ethanol plant extracts (0.2 gm/ml) against bacterial species tested by disc diffusion assay (zone of inhibition mm).

Extract	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*K. pneumonia*	*E. coli*
Plantago mediastepposa	12 mm	6 mm	12 mm	6 mm	25 mm
Quercusc infectoria	12 mm	17 mm	10 mm	32 mm	20 mm
Punic granatum	7 mm	8 mm	8 mm	8 mm	8 mm
Thymus lcotschyana	10 mm	20 mm	14 mm	17 mm	30 mm
Ginger officeinals	14 mm	12 mm	13 mm	10 mm	20 mm
Rhus angustifolia	17 mm	8 mm	11 mm	33 mm	17 mm
Cinnamon	5 mm	7 mm	7 mm	7 mm	8 mm
Mixture of all the plants used	10 mm	15 mm	15 mm	16 mm	20 mm
Tetracycline	10	5	R	R	12
Kanamycin	5	R	6	R	10

Preparation of test organisms

B. subtilis, *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumonia* were isolated from the clinical samples obtained from patients attending Government Hospital Azadi, Kurdistan region. The organisms were isolated in nutrient agar medium and selectively cultured at 37°C for 24 h. The bacterial strains were identified by biochemical and standard antibiogram tests as per the directions from Bergy's manual for determinative bacteriology.

Antibacterial sensitivity testing using disc diffusion method

Circular disc of 6 mm diameter were made from the Whatman no 1 filter paper. Discs were impregnated with equal volume (50 µl) of each plant extracts concentration (0.2 g/ml). The discs were aseptically placed over plates of Muller Hinton agar seeded with each of the test pathogens, which were tested against Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative bacteria (*E. coli, P. aeruginosa* and *K. pneumonia*) (Haniyeh et al., 2010). The antibacterial assay plates were incubated at 37°C for 24 h, and the zone of inhibition was measured (in mm diameter). Inhibition zones with diameter less than 10 mm were considered as having low antibacterial activity. Diameters between 10 and 14 mm were considered moderately active, and these with >16 mm were considered highly active (Indu et al., 2006). The control experiment was carried out to compare the diameter zone of clearing from the extracts and already standardized antibiotics. The antibiotics used were Tetracycline and Kanamycin. The standard discs of the antibiotics tetracycline (10 µg per disc) and kanamycin (10 µg per disc) served as positive antibacterial control. The diameter of the zones of inhibition around each of the discs (disc diameter included) was taken as a measure of the antibacterial activity. The diameters of the zones of inhibition by the samples were then compared with the diameter of the zone of inhibition produced by the standard antibiotic disc used. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded.

RESULTS

The antibacterial activities of the ethanol extracts from the test samples in terms of minimum inhibitory concentrations (MIC) and diameters of inhibition zones are reported in Table 1. The plant extracts were found to be prominently active against the tested microorganisms (*B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumonia*) at the concentration of 0.2 mg/mL (MIC). Among the tested plant extracts, Quercusc infectoria and Rhus angustifolia ethanol extracts showed the highest activity (33 mm and 32 mm 0.2 g/ml respectively) of inhibition zone against *K. pneumonia*, whereas Punic granatum and Cinnamon ethanol extracts (5 mm and 6 mm 0.2 g/ml respectively) showed the minimum inhibition zone against *S. aureus*. Ethanol extract for Plantago mediastepposa, Quercusc infectoria, Thymus lcotschyana, Ginger officeinals, Rhus angustifolia and mixture of all plants tested displayed excellent activity against Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative bacteria (*E. coli, P. aeruginosa* and *K. pneumonia*). On the other hand, the antibacterial activities of the Distil water extract from the test samples (Table 2) also showed the activity with the tested plant extract Thymus lcotschyana and Plantago mediastepposa D.W. extract against both Gram positive (*S. aureus*) and Gram-negative bacteria (*E. coli*) measured between 25-26 and 27-28 mm 0.2 g/ml respectively. However, the minimum activity was observed with the tested plant extract (Ginger officeinals D.W. extract) which showed 6 mm 0.2 g/ml inhibition zone against *P. aeruginosa* and *K. pneumonia*.

Ethanol extract showed variation in the zone of inhibition from a minimum of 5 mm for ethanol extract Cinnamon against *S. aureus* to a maximum of 33 mm for ethanol extract Rhus angustifolia against *K. pneumonia*.

Table 2. Antibacterial activity of different D.W. plant extracts (0.2 gm/ml) against bacterial species tested by disc diffusion assay (zone of inhibition mm).

Extract	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*K. pneumonia*	*E. coli*
Plantago mediastepposa	25 mm	11 mm	9 mm	9 mm	26 mm
Quercusc infectoria	20 mm	17 mm	23 mm	17 mm	13 mm
Punic granatum	22 mm	16 mm	18 mm	18 mm	15 mm
Thymus lcotschyana	27 mm	11 mm	8 mm	8 mm	28 mm
Ginger officeinals	10 mm	12 mm	6 mm	6 mm	18 mm
Rhus angustifolia	12 mm	15 mm	9 mm	10 mm	17 mm
Cinnamon	10 mm	9 mm	15 mm	15 mm	16 mm
Mixture of all the plants used	13 mm	14 mm	17 mm	10 mm	10 mm
Tetracycline	10	5	R	R	12
Kanamycin	5	R	6	R	10

D.W. extract Thymus lcotschyana showed the highest activity against *E. coli* (28 mm), while D.W. extract Ginger officeinals showed the lowest activity against *K. pneumonia* and *P. aeruginosa* (6 mm). Also, the ethanol extract and D.W. extracts of all the plants used showed significant zone of inhibition for all microorganisms tested.

DISCUSSION

In recent years, although technology and medicine have developed extensively, some countries have made it obligatory to use natural products for many different purposes due to decrease in natural richness and draw backs (Leela and Satirapipathkul, 2011). Like in many other countries, the plants known by people with health benefits are picked up and used for the treatment of various diseases in Kurdistan region. In this study, the antimicrobial capacity of the extracts from 7 spices against both Gram positive and Gram negative bacteria was determined. Plant ethanolic extracts were more significant than plant D.W. extracts, which showed that the results of this study are similar to those of Avato et al. (1997) and Zavala et al. (1997). The use of some antibiotics is no longer recommended because of the potency of the widespread resistance to them (Zavala et al., 1997). Thus, these spices and herbs, like many other plants, can be used instead of antibiotics. The activity of some crude extracts used in the study against *S. aureus, B. subtilis, P. aeruginosa, K. pneumonia* and *E. coli* was more potent than the standard antibacterial Tetracycline and Kanamycin. The plants investigated are known to have healing powers, and are used for the treatment of various diseases among people (Omer, 2006).

The continuance of this study should include the isolation of the compounds responsible for the antimicrobial activity present in Plantago mediastepposa, Quercusc infectoria, Punic granatum, Thymus lcotschyana, Ginger officeinals, Rhus angustifolia and Cinnamon, which are the plants showing the largest inhibitory activity over the growth of the microorganisms tested. Results of this study demonstrated that the Gram positive bacteria were more susceptible to the extracts than the Gram-negative bacteria such as *P. aeruginosa* which exhibited more resistance than *S. aureus and B. subtilis* when tested with D.W. extract Ginger officeinals. The reason would be that lipopolysaccharide (LPS) layer of Gram-negative bacteria in the outer membrane has high hydrophobicity and acts as a strong barrier against hydrophobic molecules (Evans, 1996). However, it can pass through the cell wall of Gram-positive bacteria easily than the Gram-negative bacteria because the cell wall of the Gram positive bacteria contains peptidoglycan and lacks an outer membrane (Leach, 1986).

REFERENCES

Avato P, Vitali C, Mongelli P, Tava A (1997) .Antimicrobial activity of polyacetylenes from Bellis perennis and synthetic derivatives. J. Planta Med., 63: 503-507.

Evans WC (1996). Pharmacopoeial and related drugs of biological origin. In: Trease and Evan's Pharmacognosy. London: WB Saunders Co. Ltd., 17(11): 327-332.

Haniyeh K, Seyyed MS, Hussein M (2010). Preliminary study on the antibacterial activity of some medicinal plants of Khuzestan (Iran). Asian Pacific J. Trop. Med., 3(3): 180-184.

Indu MN, Hatha AM, Abirosh C, Harsha U, Vivekanandan G (2006). Antimicrobial Activity of Some of The South-Indian Spices Against Serotypes of Escherichia Coli, Salmonella, Listeria Monocytogenes and Aeromonas Hydrophila. Brazilian J. Microbiol., 37(2): 153-158.

Leach CK (1986). The phenolic contents of some British cynipid galls", Cecidology. J. Br. Plant Gall Soc., 1(1): 10-12.

Leela T, Satirapipathkul C (2011). Studies on the Antibacterial Activity of Quercus Infectoria Galls. International Conference on Bioscience, Biochemistry

and Bioinformatics, 5: 410-414.
Mahesh B, Satish S (2008). Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens. World J. Agric. Sci., 4 : 839-843.
Nakatani N (1994). Antioxidative and antimicrobial constituents of herbs and spices. In G. Charalambous, ed. Spices, Herbs, and Edible Fungi. Elsevier, Amsterdam, pp. 251–272.
Omer E (2006). Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants. Institute of Molecular Biology, Slovak Acad. Sci., 61(3): 275-278.
Prasannabalaji N, Muralitharan G, Sivanandan RN, Kumaran S Pugazhvendan SR (2012). Antibacterial activities of some Indian traditional plant extracts. Asian Pacific J. Trop. Dis., 9(12): 291-S295.
Thomson WAR (1978).Antimicrobials from plants and their use in therapeutics and drug discovery. J. Iioab, 1(3): 31-37.
Zaika LL (1988). Spices and herbs: their antimicrobial activity and its determination. J. Food Safety, 9: 97-118.
Zavala MA, Perez S, Perez RM (1997). Antimicrobial screening of some medicinal plants. Phytother. J. Biologia Bratislava, 11: 368–371.