Antimicrobial Activity Of *Plectranthus Asirensis* Extract From Jazan Region

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Abestract: In the present study, an antimicrobial activity of *Plectravthus asirensis* extract from Jazan region, Saudi Arabia was assessed using both well diffusion and microdilution method in multi-well micro-titer plates. *Plectravthus asirensis* extract investigated for its antibacterial activity against seven selected pathogenic bacteria: *Bacillus fastidiosus*, *Staphylococcus aureus*, *Proteus mirabilis, Proteus vulgaris, Salmonella choleraesuis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Serratia odorifera*. *Plectravthus asirensis* extract was active against all tested bacteria and the highest inhibitory effect was observed against S. mutans using the well diffusion method. Antibacterial activity of Aqueous extracts of selected commonly used *Plectravthus asirensis* were screened against multi drug resistant bacteria.
[Bakri Marwah M. **Antimicrobial Activity Of *Plectranthus Asirensis* Extract From Jazan Region**. *Life Sci J* 2013;10(4):1880-1883]. (ISSN:1097-8135). http://www.lifesciencesite.com. 248

Keywords: *Plectravthus asirensis*, Antimicrobial Activity, Jazan Region.

1. Introduction

Antibiotics provide the main basis for the therapy of microbial infection. Since the discovery of these antibiotics and their uses as chemotherapeutic agents, there was a belief in the medical fraternity that this would lead to the eventual eradication of infection diseases (Rosina et al, 2009). However, overuse of antibiotics has become the major factor for the emergence and dissemination of multidrug resistant strains of several groups of micro-organisms (Jagessa RC and Nazrana Mohamed, 2010). In the light of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agent is of paramount importance. However, the past record of rapid, wide spread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (Coates et al, 2002). A wide variety of antibiotics are commonly used for the treatment of serious infections caused by aerobic Gram -ve bacteria (Tumah, 2005). The increased use of antibiotics has resulted in the development of resistant bacteria (Jayaraman, *et. al.,* 2011). In recent years, misuse of antibiotics resulting in multi-drug resistance among bacteria has accelerated the search for drugs and dietary supplements effective against such multidrug resistant bacteria. It has been reported that in 1996, sales of botanical medicines increased by 37% over 1995 (Thongson et al, 2004). The genus *Plectranthus* (Family: Labiatae) has about 16 species (Jagessa RC and Nazrana Mohamed, 2010; Reddy, et al., 2012). This genus has distributed in the tropical and subtropical regions (Santosh and Ashwani, 2012). In Jazan region, Saudi Arabia, this genus comprising two species, *P. asirensis* J.R.I. Wood and *P. barbatus* Andr. In this connection, different parts of plants, herbs and spices have been used for many years for prevention of infections. These are easily available and can be used in domestic setting for self-medication. The present report gives an account of the antibacterial effect of different parts of *Plectravthus asirensis* plant.

2. Material and Methods

2.1. Plant material

Plectravthus asirensis leaves were obtained commercially from a local garden in Jazan Region, Saudi Arabia and identified by a botanical taxonomist at Botanical Survey, Department of Biology, College of Science Jazan University. The leaves were washed first under running tap water, followed by sterilized distilled water and dried at room temperature in dark then grinded to powder using an electrical blender.

2.2. Preparation of Extracts

The leaves of the plants were air dried at room temperature for 3 weeks and grounded to coarse powder .15g of the powder was placed in 100ml of distilled water (cold water extract) in conical flask and The crude preparation was left overnight in the shaker at 35oC and then centrifuged at 2500rpm for 10 mins. The supernatant containing the plant extract was then transferred to a preweighed beaker and the extract was concentrated by evaporating the solvent at 60°C. The crude extract was weighed and dissolved in a known volume of dimethyl sulphoxide, to obtain a final concentration of 200mg / ml. and sterilized by filtration through (0.45 µm) millipore filters. The Aqueous extracts were stored in sample bottles at 4oC prior to use (De and Ifeoma, 2002).

2.3. Microbial Cultures

Nine strains of bacteria were used as test microorganisms. All microorganisms were clinical

isolates, obtained from the Microbiological Laboratory of King Fahad Hospital, Jazan, Saudi Arabia.

2.4. Standardization of Inoculum

Exactly 0.2ml of 24/hours old culture of each organism was dispensed into 20ml of sterile nutrient broth and was incubated for 3-5/hours to standardize the culture to 106cfu/ml. Antibacterial Testing: This was done using the agar wells diffusion method(s) of (Odeyemi and Fagbohun, 2005). 0.5ml of overnight broth culture of each clinical isolates containing 106 cfu/ml was ascetically transferred to the solidified nutrient agar and spread evenly on the agar surface using a sterile glass spreader. Four 6mm wells were bored unto the agar and filled with the Aqueous extracts (cold water extract) while the distill water serves as the control. The Petri dishes were incubated at 370C for 18-24/hr and the inhibition zones were measured (mm).

2.5. Minimum Inhibition Concentration (MIC) of the Extract

The (MIC) was defined as the lowest concentration that completely incubated the growth of microorganisms for 24 hours (Thongson et al, 2004). The MIC of the extracts was also done using the agar well diffusion technique. Two fold dilution series was prepared to achieve a decreasing concentration range of 200 to 12.5% (V/V). A 0.5ml volume of each solution was added ascetically into the wells of Mueller Hinton agar plates that were already seeded with standardized inoculum (106 cfu/ml) of the bacterial isolates. The plates were incubated at 37°C for 24/hr. The lowest concentration of the extracts showing a clear zone of inhibition was considered as the (MIC).

3. Results

Table 2 showed the susceptibility pattern of the Aqueous extract of *Plectravthus asirensis* against the bacterial isolates. The extract of *Plectravthus asirensis* was the most effective extract showing the most antibacterial activity against all the isolates tested *Bacillus fastidiosus*, *Staphylococcus aureus*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella choleraesuis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, with inhibition zones (mm) of 24, 18, 15, 15, 15, 14,13,8 respectively, The extract was effective on all the test isolates except *Serratia odorifera*.

Table 1 The highest inhibitory effect was observed against Bacillus fastidiosus (zone of inhibition: 24 mm) while the weakest activity was demonstrated against Staphylococcus aureus, Proteus mirabilis, Proteus vulgaris, Salmonella choleraesuis, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae (zone of inhibition: 18, 15, 15, 15, 14, 13 and 8 mm) respectively. In view of the results obtained by the well diffusion method, the minimal inhibitory concentration (MIC) of *Plectravthus asirensis* extract was determined by broth microdilution assay (Table 2). The highest (MIC) value (8, 16, 32, 32 and 32 µg/ml) was observed against Bacillus fastidiosus, Staphylococcus aureus, Proteus mirabilis, Proteus vulgaris and Salmonella choleraesuis respectively, while Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Serratia odorifera ranked next (MIC 64, 128, 256 and 512 µg /ml) respectively. The standard drug Tetracycline was active against all reference bacteria (zone of inhibition range: 9– 18 mm; MIC range: 32–256 µg/ml), Chloramphenicol was active against all reference bacteria (zone of inhibition range: 7– 20 mm; MIC range: 1–256 µg/ml), Ampicillin was active against all reference bacteria (zone of inhibition range: 0– 8 mm; MIC range: 64–512 µg/ml) and Rifampin was active against all reference bacteria (zone of inhibition range: 8– 12 mm; MIC range: 128–512 µg/ml).

Table 2. Diameter of zone of inhibition (mm) of Antimicrobial extracted from Mentha plant against Clinical Bacterial Isolates

Antibiotic Resistant Isolates	*P. asirensis* extract (mm)	Control			
		1	2	3	4
Bacillus fastidiosus	**24**	**13**	**12**	**8**	**11**
Staphylococcus aureus	**18**	**15**	**19**	**7**	**10**
Proteus mirabilis	**15**	**12**	**12**	**7**	**11**
Proteus vulgaris	**15**	**12**	**12**	**8**	**11**
Salmonella choleraesuis	**15**	**11**	**20**	**7**	**8**
Escherichia coli	**14**	**18**	**11**	**8**	**12**
Pseudomonas aeruginosa	**13**	**14**	**15**	**5**	**12**
Klebsiella pneumoniae	**8**	**10**	**9**	**5**	**8**
Serratia odorifera	**0**	**9**	**7**	**0**	**9**

1: Tetracycycline, 2: chloramphenicol, 3: Ampicillin, 4: Rifampin

Table 2. Minimum Inhibitory Concentration (MIC) of Antimicrobial extracted from *Mentha* plant against Clinical Bacterial Isolates

Antibiotic Resistant Isolates	*P. asirensis* extract (µg / ml)	Control			
		1	2	3	4
Bacillus fastidiosus	**8**	**128**	**256**	**128**	**256**
Staphylococcus aureus	**16**	**64**	**2**	**128**	**128**
Proteus mirabilis	**32**	**128**	**128**	**128**	**512**
Proteus vulgaris	**32**	**128**	**128**	**128**	**512**
Salmonella choleraesuis	**32**	**128**	**1**	**256**	**128**
Escherichia coli	**64**	**32**	**128**	**64**	**128**
Pseudomonas aeruginosa	**128**	**32**	**128**	**256**	**256**
Klebsiella pneumoniae	**256**	**256**	**256**	**512**	**256**
Serratia odorifera	**512**	**256**	**256**	**512**	**256**

1: Tetracycycline, 2: chloramphenicol, 3: Ampicillin, 4: Rifampin

4. Discussion

A variety of plant species are capable of synthesizing many substances with antibacterial activity. These properties have been described to extracts of many plants found in Jazan flora. However, to the plants analyzed in this work, there aren't previous studies evaluating this characteristic, except to *Mentha spicata*, *M. piperita* and *Plectranthus spp*. The extract of Plectravthus asirensis presented antimicrobial activity against fluorquinolone-resistant and macrolide-resistant Staphylococcus aureus strains.

Plants have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development (Sumathi, P., and Parvathi, 2011). In addition to these properties, it has also been used as appetite stimulant, a treatment for gastrointestinal infection and to lower blood sugar in diabetics. Its use for the treatment of certain types of cancer and viral infections has also been reported (Abascal et al, 2003). Its active constituents are 5-a-stigmasta-7, 25-dien-3-b-ol, elasterol and lanosterol which may be responsible for its antibacterial activity. Leaf extracts of M. charantia showed broad spectrum antimicrobial activity since various water, ethanol and methanol extracts of the leaves have exhibited antibacterial activities against E. coli, Staphylococcus, Pseudomonas, Salmonella, Streptobacillus. Besides, extract of the entire plant has shown antiprotozoal activity against Entamoeba histolytica and its fruit extract has demonstrated antibacterial properties against Helicobacter pylori, the bacteria causing stomach ulcer. It has been documented in the literature that *P. asirensis* is used internally as a tea, tincture, oil or extracts, and applied externally as a rub or liniment. Herbalists consider it as an astringent, antiseptic, antipuritic, antipasmodic, anticatarrhal, antimicrobial, rubefacient, stimulant and emmenagogue (Gislene et al, 2000). The varying degree of sensitivity of the bacterial strains may be due to the intrinsic tolerance of the bacterial and the nature and combinations of phytocompounds present in the extracts as observed by Suree and Pana (2005).

The plant extracts can be applied as an alternative to prevent and control outbreaks. Since these substances are natural, their hazardous potential is lower when compared with other products. The results show that analyzed plants presented a high potential as alternative therapy.

Acknowledgments

This project was supported by Jazan University, deanship of Scientific Research, College of Science, for their valuable criticism and advice.

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11/6/2013