ISSN:1991-8178

Australian Journal of Basic and Applied Sciences

Journal home page: www.ajbasweb.com

Antibacterial Activity of *Anastatica hierochuntica* L. Extracts Against Different Groups of Bacterial Pathogens: An *in- vitro* test

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ARTICLE INFO

Article history:
Received 10 November 2015
Accepted 30 December 2015
Available online 18 January 2016

Keywords:
Anastatica hierochuntica L.; antibacterial activity; Saudi Arabia

ABSTRACT

The antibacterial activity of *Anastatica hierochuntica* was studied using agar well diffusion method in ethanol, methanol and aqueous extracts against bacterial pathogens. All the extracts showed substantial antibacterial activity against the selected pathogens. The highest inhibition zone was shown by ethanol extract followed by methanol and aqueous extract. Among the bacterial pathogens *Bacillus* sp. and *E. coli* were more susceptible towards ethanol and methanol extract. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were less sensitive towards the extracts. However, aqueous extract was effective against the tested pathogens, when compared with the other extracts. Minimum inhibitory concentrations (MIC) of the extract were tested using tube dilution method. The MIC ranged from 10-30 mg/mL to inhibit the growth of the selected pathogens. Phytochemical analysis of the extracts showed presence of high contents in alkaloids, phenolic contents and reducing sugars. Carbohydrates, reducing sugars, proteins were found moderate in aqueous extracts. The results show that the plant contains bioactive components which can inhibit the bacterial pathogens which can be further purified and used against the multi drug resistant pathogens.

To Cite This Article: Sanad M. Al Sobeai, Abdullah A. Al-Ghanayem, Mohammed S. Al-Hussaini, Babu Joseph, Abdulmoneim M. Saadabi., Antibacterial Activity of *Anastatica hierochuntica* L. Extracts Against Different Groups of Bacterial Pathogens: *An in- vitro* test. ***Aust. J. Basic & Appl. Sci.,*** *9(36): 27-30, 2015*

INTRODUCTION

The use of traditional medicine has been gaining attention due to its importance in public health and management of infections. The severity and frequency of infectious disease has become a major problem in developing countries. Development of new multidrug resistant strains and uncommon infections has increased the concern of these diseases (Rahmoun *et al*., 2014). One of the strategies to overcome the current problem is the use of traditional herbs used in folk medicine. The use of medicinal plants for the treatment of bacterial infections has been practiced from olden days (Mahady, 2005). *Anastatica hierochuntica* L. belongs to Brassicaceae family which is also known as 'the true rose of Jericho' and widely found in desert regions. The small grey herb grows few centimeters high not more than 15 cms, with minute white colored sessile flowers and oblong dentate leaves (Law *et al*., 2009). In folk medicine the plant is widely used for uterine diseases and hemorrhage (Khalifa and Ahmad, 1980). It is also reported to treat other diseases such as asthma and related respiratory diseases, headaches, heart diseases, dysentery, typhoid, diabetes etc., (Eman *et al*., 2011). *A. hierochuntica* has been reported for its antimicrobial, antioxidant and hypoglycemic activity (Saleh and Machado, 2012). Moreover, it has been also reported for the popular treatment for the management of female reproductive disorders. Methanolic extracts of *A. hierochuntica* was found to have hepatoprotective effect in mouse hepatocytes with cytotoxicity induced by D galactosamine (Yoshikawa *et al.*, 2003). For the treatment of microbial infections, antimicrobials of plant origin are considered as effective due to less side effects (Parekh *et al.*, 2005). The polyphenolic compounds present in the plant inhibits the hydrolytic enzymes (proteases) and interferes with microbial adhesions inhibits the cell transport systems and some nonspecific carbohydrate inhibitions are also some of the mechanisms to exhibit antimicrobial activity (Cowan, 1999). Furthermore, the plant has been reported to have flavanoids: kaempferol 3 rhamnoglucoside, isovitexin, luteolin-7-glucoside, kaempferol 7 – glucoside, quercetin and lucitin. It has glucosinolates such as glucoiberin and glucoheirolin (Nakashima *et al.*, 2010). The aim of the present study was to determine the antibacterial

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activity of *Anastatica hierochuntica* against different clinical pathogenic bacterial isolates and its phytochemical analysis using different types of plant extracts.

MATERIALS AND METHODS

Plant material and preparation of extracts:

Anastatica hierochuntica L. of whole plant used in the study were obtained as fresh samples and were identified and authenticated by King Saud University Herbarium., and voucher specimens were deposited at the departmental Herbarium.

Extracts were prepared according to the methodology of Ahmed and Beg (2008) with slight modification. Each 20g of powdered plant material was extracted by maceration with 200ml methanol, ethanol or distilled water separately for overnight at room temperature before filtration. After filtration solvents and water was evaporated under reduced pressure until dryness. Approximately 0.1 g of the crude extract was dissolved in dimethyl sulphoxide (DMSO) to a final stock concentration of 50mg/mL or 100mg/mL. All crude extract was then kept at -20°C till use.

Bacterial strains:

Bacterial strains used in the present study were *Escherichia coli, Pseudomonas aeruginosa Bacillus* sp. and *Staphylococcus aureus.* These bacterial strains were isolated from clinical samples and identified by morphological and biochemical characteristics. Pure cultures were maintained and stored at 4°C until use.

Antibacterial bioassay:

For determination of antimicrobial activity agar well-diffusion plate method was followed. Sterile Muller Hinton Agar plates were prepared and swabbed with 8 hour old cultures of the four bacterial strains. With sterile cork borer (5mm) wells were made 2.5 cm apart and the extracts of the plant were dispensed into the wells by using a micropipette. Stock solutions of ethanol, methanol and aqueous extracts (1, 10 and 20 mg/mL) were made using DMSO. All of the plates were incubated at 37°C for 18-14 h. The diameters of the zone inhibition (mm) was then measured.

Minimum Inhibitory Concentration (MIC):

Minimum inhibitory concentration (MIC) is the lowest concentration of the plant extract which inhibits the visible bacterial growth on solid media. It was determined by tube dilution method (Chitwood, 1969). Various concentrations of extracts were incorporated into liquid media and incubated for 18 hours. The dilutions were determined for bacterial growth by streaking into nutrient agar plates. The lowest concentration which inhibits bacterial growth is determined.

Antibiotic sensitivity test:

In an attempt to compare efficacy of the plant extract with synthetic antibiotics, antibiotic sensitivity tests was done using standard disc diffusion method of Kirby – Bauer (Bauer *et al.*, 1966). The antibiotics used were Tetracycline (30 µg), Erythromycin (15 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg) and Gentamicin (10 µg).

Phytochemical analysis:

The Aqueous extracts of *Anastatica hierochuntica* L. were subjected to phytochemical analysis according to the methodology of Crombie *et al.* (1990).

RESULTS AND DISCUSSION

The plant extracts that tested for antibacterial activity with clinical isolates representing Gram negative and Gram positive bacteria. In the present study ethanol extract at 10 mg/mL showed maximum inhibitory zone against *Bacillus* sp.(15mm). Similarly, methanol extract also inhibited the growth of *Bacillus* sp. (Table 1). On the other hand, the extracts showed less activity against Gram positive bacteria (i.e., 6-11 mm) as compared to ethanol. Aqueous extracts showed less activity towards the Gram positive bacteria in comparison with Gram negative bacteria (Table 2). *E. coli* showed more sensitivity in higher concentration (10 and 20 mg/mL) of the ethanol and methanol extracts. Aqueous extracts also showed less effect in *E. coli*, when compared with the other two extracts tested. Higher concentration (20 mg/mL) of ethanol and methanol extracts had inhibitory effect on *Pseudomonas aeruginosa*. The aqueous extract had less antibacterial activity against *Pseudomonas aeruginosa* as compared to *E. coli.* This may be due to the presence of active components in ethanol and methanol extract as compared to aqueous extract. Ethanol and methanol solvents are widely used to detect bioactive components from plant materials (Ahmad *et al*., 1998). Similar to our experiments many studies have been reported for *A. hierochuntica* methanol and aqueous extracts with Gram negative and Gram positive bacteria (Mohamed *et al*., 2010). The antibacterial activity against both Gram positive and Gram negative bacteria reveals that the plant extract contains wide spectrum activity against various bacterial pathogens due to the presence of antimicrobial components and metabolic toxins (Moniharapon and Hashinaga, 2004). In a previous study n –hexane extract of *A. hierochuntica* leaves showed antibacterial effect against both Gram positive (*Staphylococcus aureus* and *Streptococcus pyogens*) and Gram negative bacteria (*E. coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*) (Mohamed *et al.*, 2010).

Table 1: Antibacterial activity of *Anastatica hierochuntica* extracts against Gram positive bacteria*

Isolate	*Bacillus sp.*			*Staphylococcus aureus*		
Extract Conc. (mg/mL)	1	10	20	1	10	20
Ethanol	9	15	10	10	7	6
Methanol	10	11	11	8	6	6
Aqueous	10	6	7	6	6	6

*values are means of 3 replicates (inhibition zone in mm)

Table 2: Antibacterial activity of *Anastatica hierochuntica* extracts against Gram negative bacteria*

Isolate	*E. coli*			*Pseudomonas aeruginosa*		
Extract Conc. (mg/mL)	1	10	20	1	10	20
Ethanol extract	6	10	12	6	7	11
Methanol Extract	6	10	10	7	8	10
Aqueous extract	7	8	10	6	6	8

*values are means of 3 replicates (inhibition zone in mm)

The minimum inhibitory concentration (MIC) of *A. hierochuntica* extracts against Gram positive and Gram negative bacteria has been evaluated (Table 3). A low concentration (10mg/mL) of ethanol extract inhibited *Bacillus* sp. growth whereas, 30mg/mL concentration of extract was needed for the growth inhibition of *Staphylococcus aureus, E. coli* and *Pseudomonas aeruginosa.* With methanol extract 20mg/mL was sufficient for inhibiting the growth of *Bacillus sp., Staphylococcus aureus* and *Pseudomonas aeruginosa.* Whereas, growth of *E. coli* was inhibited by a higher concentration of methanol extract reached 30mg/mL. As observed in antibacterial activity of higher concentration (30mg/mL) of aqueous extracts were needed to inhibit the growth of all test bacterial strain except *Bacillus* sp., which was inhibited by 20mg/mL concentration. The difference in inhibitory activities of different extract may be due the solubility of phytochemicals in different solvents. There are many reports on solubility of different bioactive components in different solvents according to their polarity (Marjorie, 2001). However, the inhibitory activity of the extracts may be due to complex mechanisms such as interference with cell wall and membrane synthesis, synthesis of protein and nucleic acids (Oyaizu *et al.*, 2003).

Table 3: Minimum inhibitory concentration (MIC) values (mg/mL) of *Anastatica hierochuntica* for bacterial isolates

Isolate / Extract type	Ethanol	Methanol	Aqueous
Bacillus sp.	10	20	20
Staphylococcus aureus	30	20	30
E. coli	30	30	30
Pseudomonas aeruginosa	30	20	30

The antibiotic sensitivity for the test pathogens was carried out and the results are tabulated in Table 4. Gram positive bacteria used in this study such as *Bacillus* sp. and *Staphylococcus aureus* showed sensitivity towards all of the tested antibiotics. Gram negative bacteria such as *E. coli* was resistant to Tetracycline (30 µg) and ciprofloxacin (5 µg). However, *Pseudomonas aeruginosa* was sensitive to all of the antibiotics used in the present study.

Table 4: Antibiotic sensitivity pattern shown by the clinical isolates

	Antibiotics*				
Test bacteria	Tetracycline 30	Erythromycin 15	Chloramphenicol 30	Ciprofloxacin 5	Gentamicin 10
Bacillus sp.	27	25	28	29	26
Staphylococcus aureus	20	22	25	30	20
E. coli	R	20	24	6	15
Pseudomonas aeruginosa	25	15	20	26	24

*Dose of antibiotics in µg; Values represent means of 3 replicates (Inhibition zone in mm)

Generally, antibacterial properties of a plant depend on the phytochemical components which interfere with the bacterial cells. The important phytochemical constituents involved in antibacterial activity are tannins, alkaloids, phenolic components and flavanoids (Shariff 2001). In the present study the phytochemical analysis showed the presence of alkaloids and phenolic components in high rates (Table 5). These components may be responsible for antimicrobial activity. However the extracts contain moderate amount of tannins. Medicinal plants consist of variety of phytochemicals which can be used for treatment of diseases. The use of plant-based antimicrobial agents will mitigate many of the side effects and decrease the trend of developing multidrug resistance associated with synthetic

antibiotics (Parekh *et al.*, 2005). The polyphenolic components of the plant may interfere with hydrolytic enzymes or other interactions to inactivate microbial adhesions, cell envelope, transport proteins and non-specific interactions with carbohydrates (Cowan, 1999).

Table 5: Phytochemical analysis of *Anastatica hierochuntica* aqueous extract samples.

Constituent	Level*
Alkaloids	+++
Flavanoids	++
Phenolic contents	+++
Carbohydrates	++
Reducing sugars	+++
Tannins	++
Aminoacids and proteins	++
Polysaccharides	+
Volatile oils	±

*:+ = low concentration, ++ = medium concentration, +++ = high concentration,
± = traces, - = not detectable.

To fight against these multidrug resistant pathogens there is a need to explore herbs that used in traditional medicine. The present study supports the use of *A. hierochuntica* as a medicinal plant which can be used as preventive agent for various diseases. However, the data presented here are preliminary and a detailed phytochemical study, separation of active components and pharmacological studies need to be conducted to explore the usage of *A. hierochuntica* for treatment of diseases.

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