

Original Research Article

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Antibacterial Activity on Medicinal Herbs

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ABSTRACT

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Medicinal plants are gaining much interest recently, because they used to treat respiratory tract infections. The study on medicinal plants started with extraction procedures. Aqueous, Methanolic and Ethanolic extracts of the eight most popular plants, *Alapina calcarata*, *Andrographis paniculate*, *Justic adhatoda*, *Solanum trilobatum*, *Zingiber officinalis*, *Pipper longum*, *Solanum xanthocarpum*, *Piper nigrum*. The extracts of those plants were concentrated by rotary evaporator, and these concentrated samples were subjected to invivo antibacterial assay against three human pathogenic *Staphylococcus aureus*, *Streptococcus sps*, and *Pseudomonas sps*. The efficacy of the samples was tested by bio assay method using the disc diffusion test. Zone of inhibition of the extracts were compared with the standard Ampicillin. The results showed that, the remarkable inhibition of the bacterial growth was shown against the tested organisms in methanolic extraction mainly *Alapina calcarata* and *Andrographis paniculate*.

Introduction

Herbs are natural remedies for the infectious disease with higher safety profile and efficacy. Herbs used as a traditional medicine, because it contain wide range of substances that can be used to treat chronic disease (Duraipandiyan *et al.*, 2006). Various medicinal plants have been used for years to treat disease all over the world. So herbs have been used as a source of medicine (Farombi, 2003). Now a days there are many drugs which was used in clinical were discovered from the plants, were used in traditional communities. (Van Wyk *et al.*, 2002). Medicinal plants are widely used by all sections of the population either directly or indirectly in the preparation of modern

pharmaceuticals (Karthikeyan, 2000; Dubey *et al.*, 2004). Traditional medicine recently gives the fastest growth in medicinal field with herbal therapies. This mode of treatment is widely used because it is considered as more acceptable, accessible, histolic and low cost and it also proven to be safe (Gessler, 1995).

The older generation is slowly dying who has the knowledge of medicinal plants which contain bioactive agent, because the knowledge of medicinal plants and the methods of processing of the crude drugs are only available in the rural communities and only perpetuated by word of mouth and

within families and small communities. Younger people on the other hand who now have taken up formal education rarely get interested in traditional lifestyles. By the advantage of the active components which present in herbal remedies used to combined with many other substances that appear to be inactive. These complementary components give the safety and efficiency to the whole plant and much superior to that of its isolated and pure active components (Shariff, 2001).

The effects of plant extracts on bacteria by antibacterial method have been studied by a number of researchers in the world (Reddy *et al.*, 2001; Urul, 2002; Ateb and Urul, 2003). Antibacterial properties of plants has resulted in current problems which is associated with the use of antibiotics (Shiota *et al.*, 2004; Abu-Shanab *et al.*, 2004). The research on the medicinal plants should be extended with the identification of the active principles in the plants. Scientific examination of the herbal remedies could leads to the standardization and quality control of the products to ensure their safety.

Medicinal plants (*Alpina calcarata*, *Andrographis paniculate*, *Justic adhatoda*, *Solanum trilobatum*, *Zingiber officinalis*, *Pipper longum*, *Solanum xanthocarpum*, *Piper nigrum*) are collected either randomly in geographical areas where the plants are found. Initial screening of plants for possible antibacterial activities typically begins by using various organic extraction methods. The aromatic or saturated organic compounds, which present in the plants are active against micro organism, they are often obtained through initial ethanol or methanol extraction (Vilegs *et al.*, 1997). Those plants were evaluated for the preliminary antibacterial activity. The different extracts of plants were screened for antibacterial activity against some medically important bacterial strains which cause

infections in respiratory tract. Drugs which is derived from natural sources are play a significant role in the prevention and treatment of human diseases (Farnsworth., 1993, Houghton., 1995). The screening of plant extracts for antibacterial activity has shown that the higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003). Numerous studies have identified the compounds which present in the herbal plants are effective antibiotics (Basile *et al.*, 2000).

Materials and Methods

The plant material was collected from geographical area where the plant present. The leaves of the plants was withdrawn and dried carefully under shade and then homogenized to fine powder and stored in airtight bottles.

Aqueous Extracts

Approximately 50 g of dried and ground plant materials was successively extracted by maceration with water. These extracts were concentrated by freeze dried and stored at 4°C until use. (Virgilio Bocanegra-García, 2009).

Organic solvent extraction

The 50 g portions of each dried powdered plant part material was soaked separately in 250 ml ethanol and methanol. The extraction was carried out by maceration for 7 days in each solvent at room temperature (25±2°C). The solvents extracted material was filtered in separate flaks (Rawlins and Tindall, 1977). All extracts were then dried in a vacuum rotary evaporator, weighed and stored at 4°C until further analysis.

Preparation of dilution

The dried aqueous, methanol, and ethanol extracts were dissolved in their respective

solvents. For aqueous extracts distilled water and for organic extracts, DMSO was used as a solvent in a proportion of 100 mg/ml. The antibiotic Ampicillin was used at the concentration of 100 µg/ml (Virgilio Bocanegra-García *et al.*, 2009; Muhammad Altaf Hussain *et al.*, 2011).

Microorganisms

In the present study gram positive bacteria namely *Staphylococcus aureus*, *Streptococcus sps* whereas gram negative bacteria like *Pseudomonas sps*, were used to evaluate the antibacterial potential of different extracts of the selected plant parts.

Antibacterial assay

A 24 h old culture of each bacterium was used as an inoculum for the test. The slants were prepared in test tube. The nutrient agar medium was used for bacterial growth. A disc diffusion method was used to assess the presence of antibacterial activities in aqueous and organic extracts of the different plants. Agar well diffusion method is done to detect the antibacterial activity (Tagg and Mengiven, 1971; Stainer *et al.*, 1986). About 15-20ml of Muller-Hinton agar medium was poured in the sterilized petridish and allowed for solidifying. One drop of bacterial cultures like *Staphylococcus aureus*, *Streptococcus sps*, *Pseudomonas sps* was inoculated by swabbing with sterile swab over the MHA plates. Then wells were prepared to load different concentration like 25, 50, 75, 100µl of medicinal plants extract. DMSO and the standard antibiotic ampicillin served as a control. The plates were allowed to stand at room temperature for 30 minutes and incubated in upright position at 37°C for 24hours. The diameters of the zone of inhibition were measured with a ruler. The measurement is recorded (Quinto *et al.*, 2005 and Perez *et al.*, 1990 and Akinyemi *et al.*, 2005).

Results and Discussion

Plant extracts have been studied against bacteria extensively in the last three decades. Plants have provided a source of inspiration for novel drug components towards human health. A lot of antibacterial screening evaluations published for traditional use in China, Africa and Asia (Nishibe *et al.*, 1982; Vlietinck *et al.*, 1995; Perez and Anesini, 1994; Macfoy and Cline, 1990; Maikere *et al.*, 1989; Forestiere *et al.*, 1988). Unani and Ayurvedic system of medicines are the base of natural blueprint for the development of drugs (Didry *et al.*, 1988).

Subsequent isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. In olden days water was used as a primary solvent (Jigna parekh, and Sumitra Chanda, 2007). In this study antibacterial activity on methanol extracts of *Alapina calacarata* and *Andrographis paniculate*, shows higher efficiency than alcohol and water extracts, to fight against bacterial strains which cause respiratory tract infections especially *Streptococcus sps*, when compared to *Justic adhatoda*, *Solanum trilobatum*, *Zingiber officinalis*, *Pipper longum*, *Solanum xanthocarpum* and *Piper nigrum*.

The antibacterial activity of methanolic extract of different medicinal herbs namely *Alapina calcarata*, *Andrographis paniculate*, *Justic adhatoda*, *Solanum trilobatum*, *Zingiber officinalis*, *Pipper longum*, *Solanum xanthocarpum*, *Piper nigrum* were tested against respiratory tract infections causing pathogens like *Staphylococcus aureus*, *Streptococcus sps*, *Pseudomonas sps*.

Table.1 Antibacterial Activity of Medicinal herbs in methanol extract
Table-1 (Zone of inhibition in mm)

Plant	<i>Staphylococcus aureus</i>	<i>Streptococcus sps</i>	<i>Pseudomonas.sps</i>
<i>Alpina calcarata</i>	17	18	16
<i>Andrographispaniculata</i>	15	17	15
<i>Justica adhatoda</i>	12	12	10
<i>Solanum trilobatum</i>	15	15	14
<i>Zingiber officinale</i>	15	15	16
<i>Piper longum</i>	15	12	13
<i>Solanum xanthocarpum</i>	10	15	12
<i>Piper nigrum</i>	13	15	13
<i>Ampicillin</i>	25	27	20

Table.2 Antibacterial Activity of Medicinal herbs in alcohol extract
Table-2 (Zone of inhibition in mm)

Plant	<i>Staphylococcus aureus</i>	<i>Streptococcus sps</i>	<i>Pseudomonas sps</i>
<i>Alapina calcarata</i>	7	9	7
<i>Andrographispaniculata</i>	7	8	7
<i>Justica adhatoda</i>	6	7	5
<i>Solanum trilobatum</i>	5	7	4
<i>Zingiber officinale</i>	4	7	2
<i>Piper longum</i>	6	7	3
<i>Solanum xanthocarpum</i>	3	5	2
<i>Piper nigrum</i>	5	7	5
<i>Ampicillin</i>	25	27	20

Table.3 Antibacterial Activity of Medicinal herbs in aqueous extract
Table-3 (Zone of inhibition in mm)

Plant	<i>Staphylococcus aureus</i>	<i>Streptococcus sps</i>	<i>Pseudomonas sps</i>
<i>Alapina calcarata</i>	8	9	7
<i>Andrographispaniculata</i>	8	9	8
<i>Justica adhatoda</i>	5	7	5
<i>Solanum trilobatum</i>	3	3	7
<i>Zingiber officinale</i>	7	3	3
<i>Piper longum</i>	9	11	9
<i>Solanum xanthocarpum</i>	4	6	4
<i>Piper nigrum</i>	4	6	3
<i>Ampicillin</i>	25	27	20

In this *Alpina calcarata* and *Andrographis paniculata* gives the high zone of inhibition against *Streptococcus* sps. (Table.1)

The antibacterial activity of alcoholic extract of different medicinal herbs namely *Alapina calcarata*, *Andrographis paniculate*, *Justic adhatoda*, *Solanum trilobatum*, *Zingiber officinalis*, *Pipper longum*, *Solanum xanthocarpum*, *Piper nigrum* were tested against respiratory tract infections causing pathogens like *Staphylococcus aureus*, *Streptococcus* sps, *Pseudomonas* sps. In this *Alpina calcarata* and *Andrographis paniculata* gives the high zone of inhibition against *Streptococcus* sps. (Table.2)

The antibacterial activity of aqueous extract of different medicinal herbs namely *Alapina calcarata*, *Andrographis paniculate*, *Justic adhatoda*, *Solanum trilobatum*, *Zingiber officinalis*, *Pipper longum*, *Solanum xanthocarpum*, *Piper nigrum* were tested against respiratory tract infections causing pathogens like *Staphylococcus aureus*, *Streptococcus* sps, *Pseudomonas* sps. In this *Alpina calcarata* and *Andrographis paniculata* gives the high zone of inhibition against *Streptococcus* sps. (Table.3).

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