



Preliminary Phytochemical and Anti-Bacterial studies on Flowers of selected medicinal plants

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Abstract: The present study was aimed to evaluate the phytochemical constituents and antibacterial activity of the flower extracts of *Albizia lebbek*, *Cordia sebestena*, *Thunbergia grandiflora* and *Antigonon leptopus*. Preliminary phytochemical screening was done by the standard methods described by Harborne. Antibacterial study was carried out by disc diffusion method. The results of the phytochemical antibacterial screening revealed the presence of higher degree of chemical diversity and antibacterial activity in the methanolic extracts of *A. lebbek* and *A. leptopus*. Antibacterial activity is comparatively less in methanolic extracts of flowers from *T. grandiflora* which show the presence of only three groups of compounds (alkaloids, phenols and flavonoids). The methanolic extracts of *A. lebbek*, *A. leptopus* and *C. sebestena* show the presence of several bioactive compounds. The isolation of the pure compounds and screening of the bioactivity of individual compounds of the flower extracts will give much information on the medicinal values of these plants.

Keywords: Phytochemistry; Bio-efficacy; Anti-bacterial; Flower extracts.

Introduction

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide, becoming an important cause of morbidity and mortality in immune-compromised patients in developing countries (Ara et al. 2009). Although a large number of antimicrobial agents have been discovered, pathogenic microorganisms are constantly developing resistance to these agents (Al-Bari et al. 2006). Antibiotics are sometimes associated with side effects whereas there are some advantages of using antimicrobial compounds of medicinal plants, such as often fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature (Reddy and Jose 2010). Antibacterial constituents of medicinal plants and their use for the treatment of microbial infections as possible alternatives to synthetic drugs to which many infectious microorganisms have become resistant seem to very

much promising (Bari et al. 2010). Over the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body (Ahsan et al. 2009). In majority of the cases, the aerial vegetative parts particularly stem and leaves have been tested with very few attempts on matured fruits and seeds. Although flowers of several plants are medicinally important, the investigation on medicinal values of flowers is of rare incidence. As far as India is concerned, flowers play an important role in daily, seasonal, cultural activities including herbal medicinal practice. Recent study by Wongwattanasathien et al. (2010) shows that methanol extract of flowers from *Curcuma sessilis* and *Punica granatum* showed the highest antimutagenic activity. The nitrite treated methanol extract of *Nelumbo nucifera* exhibited the highest mutagenicity. The protective effects of these

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flower extracts might be due to the presence of antimutagenic components like flavonoids. Recently Kiruba et al. (2011) have made phytochemical analysis on flowers from an endemic tree species, *Rhododendron arboreum* Sm. ssp. *nilagiricum* (Zenker) Tagg (Ericaceae), from south India.

Albizia lebbek Benth is widely distributed in India and is also found in South Africa and Australia. Traditionally, the barks are used in toothache and diseases of the gum. Decoction of the leaves and barks are protective against bronchial asthma and other allergic disorders. Barks and seeds are astringent and are given in piles and diarrhea. Ethanolic and methanolic extracts of pods possess anti-protozoal, anti-fertility activity, hypoglycemic and anticancer properties (Watt and Breyer-Brandwijk 1962; Chadha 1985; Gupta et al. 2004; Gupta et al. 2005). The plant extract is reported to have antiseptic, anti-dysenteric, anti-ovulatory, nootropic, anti-inflammatory, antimicrobial activity and anti-tubercular activities (Chintawar et al. 2002; Pratihya et al. 2004; Rahul et al. 2010; Kapoor et al. 2007). The plant also contains saponins, macrocyclic alkaloids, anthraquinone glycosides, tannins, and flavonols (Rahul et al. 2010). The saponin constituents of *Albizia* so far described are echinocystic acid glycosides (Carpani et al. 1989; Orsini et al. 1991). The albiziasaponins A, B, and C have been isolated from the barks of *A. lebbek* (Pal et al. 1995). Phytochemical investigations of *A. lebbek* pod showed that they contain 3', 5 Dihydroxy 4', 7 dimethoxy flavone, and N- Benzoyl L phenyl alaninol (Rashid et al. 2003). The beans of the plant contain albigenic acid-a new triterpenoid sapogenin (Barua and Raman 1959). The tri-O-glycoside flavonols kaempferol and quercetin were identified from the leaves of *Albizzia lebbek* (El-Mousallamy 1998). Albizzia-hexoside a new hexaglycosylated saponin has been isolated from leaves of *A. lebbek* (Ueda et al. 2003). Misra et al. (1995) isolated N-demethyl budmunchiamines from *A. lebbek* seeds and Maa et al. (1997) confirmed the presence of tannin in *A. lebbek*.

Traditionally, *Antigonon leptopus* Hook. & Arn. have been used to treat diabetes, asthma, liver and spleen disorders, cough and throat

constriction (Cheryl A Lans 2006; Idu and Onyibe 2007; Mitchell and Ahmad 2006). The methanolic extract of aerial parts of *Antigonon leptopus* possesses significant antidiabetic activity (Angothu et al. 2010). The ethanol and chloroform flower extracts of *A. leptopus* showed the anti-bacterial activity against the dental pathogens viz., *M. albus*, *S. aureus*, *P. vulgaris* and *P. aerogenosa* (Gupta et al. 2011) and *B. subtilis*, *B. peritolis* *S. typhi*, *E. coli*, *K. pneumoniae*, *P. aerogenosa* and *S. aureus* (Bolla and Bhogavalli 2010; Udayaprakash et al. 2011). The methanolic extract of the plant possess significant analgesic and anti-inflammatory properties, anticoagulant (Mitchell and Ahmad 2006; Mamidipalli et al. 2008). Eight compounds have been identified by Gas Chromatography – Mass Spectrometry (GC-MS) analysis in ethanolic extracts of flower of *Cordia sebestena*. Of which methyl salicylate (81.87 %) and 9, 12-octadecadienoic acid (Z, Z) - (5.78%) were the major constituents of ethanolic extract (Kumaresan et al. 2011).

In recent years several studies have been conducted on the medicinal plants viz., *A. lebbek*, *C. sebestena*, *T. grandiflora* and *A. leptopus* to prove bioactive compounds with antimicrobial efficacy. But except few, all the studies are on the major vegetative parts like leaves and stems. However, there is still an urgent need to identify novel active substances from other uninvestigated parts, like flowers to identify more active compounds against the pathogens with higher resistance. In view of this fact the present study was aimed to evaluate the phytochemical constituents and antibacterial activity of the flower extracts of the medicinal plants *A. lebbek*, *C. sebestena*, *T. grandiflora* and *A. leptopus*.

Materials and methods

Flowers of *Albizia lebbek* (Lebbek Tree - Fabaceae), *Cordia sebestena* (Geiger Tree - Boraginaceae), *Thunbergia grandiflora* (Clockvine - Acanthaceae) and *Antigonon leptopus* (Coral vine - Polygonaceae) were collected in the month of from the London Mission Puthalam Church Higher Secondary School (LMPC) campus, Puthalam Village, Kanyaku-

mari district, Tamilnadu, India. The fresh flowers were shade dried and extracted (cold extraction) by using methanol for phytochemical and antibacterial studies. Preliminary phytochemical screening was done by the standard methods described by Harborne (1973). Antibacterial study was carried out by disc diffusion method (Bauer et al. 1966) against the clinical isolates *E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. aureus*, *B. cereus*, *S. typhi*, *P. mirabilis*, *E. faecalis* and *S. pyogenes*.

Results

The results of the phytochemical screening revealed the presence of phenols, flavonoids, saponins, aminoacids, steroids, phytosterols, triterpenoids, tannins in the methanolic extracts

of *A. lebbeck*, *A. leptopus* and *C. sebestena* (Table 1). Maximum degree of chemical diversity (metabolites presence in the methanolic extracts) is seen in *A. leptopus* in which ten out of twelve different chemical-tests gave positive results. *C. sebestena* and *A. lebbeck* gave positive results for nine and eight different chemical-tests respectively (Table 1). *T. grandiflora* showed minimum degree of chemical diversity with the positive results for only three different chemical tests i.e for alkaloids, phenols and flavonoids (Table 1). Phenols and flavonoids are present in flowers of all the four species studied. In contrast quinines are absent in all the cases. Alkaloids are present only in flowers of *T. grandiflora*. Flowers of *A. leptopus* and *C. sebestena* are phytochemically similar except in the presence and absence of xanthoprotein.

Table 1: Phytochemical evaluation of the methanolic flower extracts.

Phyto-constituents	<i>Albizia lebbeck</i>	<i>Antigonon leptopus</i>	<i>Cordia sebestena</i>	<i>Thunbergia grandiflora</i>
Alkaloids	-	-	-	+
Phenol	+	+	+	+
Flavanoids	+	+	+	+
Saponins	+	+	+	-
Aminoacids	+	+	+	-
Quinones	-	-	-	-
Steroids, Phytosterols, Triterpenoidal sapogenins	+	+	+	-
Tannins	+	+	+	-
Xanthoproteins	-	+	-	-
Carboxylic acid	-	+	+	-
Coumarins	+	+	+	-
Carbohydrates	+	+	+	-
	08	10	09	03

(+) Sign indicates the presence of metabolites; (-) Sign indicates the absence of the metabolites in the extracts.

Antibacterial study on 12 different pathogenic bacteria shows that, nine bacterial strains viz. *E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. aureus*, *B. cereus*, *S. typhi*, *P. mirabilis*, *E. faecalis* and *S. pyogenes* were sensitive to methanolic extract of *A. leptopus* and *A. lebbeck*. Whereas six bacterial strains (*E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. aureus*, *B. cereus* and *S. pyogenes*) were sensitive to methanolic extract of *C. sebestena*. In general, *A. leptopus* methanolic extracts showed 9-16 mm mean zone of

inhibition followed by, *C. sebestena* 6-13mm. The range of inhibition zone observed for *A. lebbeck* is 8-12mm (Table 2). Although the methanolic extract of flower from *T. grandiflora* was active against seven different bacteria, it is with low and narrow range of inhibition zones (5-8mm). The methanolic flower extracts of *C. sebestena* showed the antibacterial activity against six different bacteria with the inhibition zone range from 6mm to 13mm.

Table 2: Antibacterial efficacy of the methanolic flower extracts against human pathogens (Mean zone of inhibition in mm± S.E.).

Bacterial Pathogens	<i>A. lebbeck</i>	<i>A. leptopus</i>	<i>C. sebestena</i>	<i>T. grandiflora</i>	Amikacin (30µg/ml)
<i>Escherichia coli</i>	11.1± 0.35	12.1 ± 0.36	7.1 ± 0.12	6.3 ± 0.42	17
<i>Klebsiella pneumoniae</i>	10.7 ± 0.26	14.4 ± 0.53	7.3 ± 0.24	7.6 ± 0.25	18
<i>Pseudomonas aeruginosa</i>	9.4 ± 0.34	13.4 ± 0.33	6.4 ± 0.34	6.4 ± 0.24	25
<i>Staphylococcus aureus</i>	12.3 ± 0.33	12.6 ± 0.21	8.3 ± 0.42	5.4 ± 0.36	17
<i>Bacillus cereus</i>	8.6 ± 0.36	16.4 ± 0.42	13.4 ± 0.12	8.2 ± 0.43	40
<i>Salmonella typhi</i>	8.4 ± 0.21	11.4 ± 0.26	-	-	20
<i>Serratia marsecens</i>	-	-	-	-	18
<i>Acinetobacter sp.</i>	-	-	-	-	-
<i>Enterobacter sp.</i>	-	-	-	-	15
<i>Proteus mirabilis</i>	11.4 ± 0.42	13.3 ± 0.24	-	6.4 ± 0.34	19
<i>Enterococcus faecalis</i>	-	10.3 ± 0.48	-	-	13
<i>Streptococcus pyogenes</i>	8.3 ± 0.41	10.4 ± 0.52	8.3 ± 0.35	7.1 ± 0.26	20
Total Activity (Max. 12)	8/12	9/12	6/12	7/12	11/12

Discussion

The medicinal plants are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, insecticides, steroids, related active metabolites, which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. Recently, a number of studies have been reported on the phytochemistry of medicinal plants, particularly on the vegetative parts like leaves and stems (Balakumar et al. 2011; Paulraj et al. 2011; Premkumar et al. 2011; Anpin Raja et al. 2011; Rajan et al. 2011; Kala et al. 2011). Interestingly, in the present investigation, flowers of four medicinal plants have been screened for the presence of various groups of bioactive compounds along with the *in vitro* screening for antibacterial activity. The results shows the presence of phenols, flavonoids, saponins, aminoacids, steroids, phytosterols, triterpenoids, tannins in the methanolic extracts of *A. lebbeck*, *A. leptopus* and *C. sebestena*.

In the present study, methanolic-flower extract of *A. leptopus* shows higher degree of chemical diversity and antibacterial activity. This is in accordance with the previous studies on other parts of this plant. Phenolic compound 2,3,4-trihydroxy benzaldehyde with selective COX-2 enzyme inhibitory activity has been reported from the aerial parts of *A. leptopus* (Vanisree et al. 2008, 2011). The crude extract fractions hexane, ethyl acetate and methanol of the plant *A. leptopus* found to contain triterpenoids, flavonoids and tannins (Battu and

Raju 2009). The extracts showed significant antibacterial and antifungal activity. Studies on the methanolic root extracts of *A. Leptopus* confirmed the presence of analgesic, anti-inflammatory (Mamidipalli et al. 2008) and anthelmintic (Raju and Rao 2011) activity. Chistokhodova (2002) also confirmed the antithrombin activity from methanol extract of *A. Leptopus*. The present study on antibacterial study in flowers of *A. leptopus* confirmed the observation of Bolla and Bhogavalli (2010) who stated that the flower of *A. leptopus* possess significant antibacterial activity against the gram positive pathogens. Bolla and Bhogavalli (2010) and Gupta et al. (2011) observed the antibacterial activity against *M. albus*, *S. aureus*, *P. vulgaris*, *P. aeruginosa*, *B. subtilis*, *E. coli*, *K. penumoniae*. The present study confirms the earlier observations and supplements by showing inhibition against the pathogens viz., *S. typhi*, *P. mirabilis*, *E. faecalis* and *S. pyogenes* (Table 2).

Rahul et al. (2010) studied the antibacterial activity of leaves extracts of *A. lebbeck* against *E. coli*, *P. aeruginosa*, *S. aureu* and *B. cereus*. In the present study antibacterial activity has been confirmed in the flower extracts of *A. lebbeck* against *E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. aureus*, *B. cereus*, *S. typhi*, *P. mirabilis* and *S. pyogenes*. The results of the present study authenticates and confirms the folkloric usage, traditional practices, ethnobotanical, anti-microbial and pharmacological values of the medicinally important plant *A. lebbeck*.

S. marsecens, *Acinetobacter* sp., and *Enterobacter* sp. were resistant to methanolic-flower extract of all the four selected medicinal plants that probably could be due to cell membrane permeability or due to other genetic factors and this result is supported by Motamedi et al. (2009) and Seyydnejad et al. (2010). However methanolic leaf extract of *A. leptopus* showed better activity against the bacterium, *E. coli* (Udayaprakash et al. 2011).

Flowers of *A. lebbeck* are light yellow in colour, and they are pale blue in *T. grandiflora*. While the remaining two species are with very bright coloured flowers: bright orange-red (*C. sebestina*) and bright rose or pink (*A. leptopus*). The principal flower colouring matters are the anthocyanins, anthoxanthins and carotenoids. Variation in colour depends upon the presence or absence of one or more of these substances, upon structural alterations in their molecules, changes in the pH of the cell sap, or quantitative changes affecting the amounts of pigment produced. Pigment production is genetically controlled, and in a number of cases complementary genes are involved. Variation in the amount of any pigment is also gene controlled (Lawrence and Price 1940). In the present study, the chemical diversity and antimicrobial activity of the bright coloured, odourless flowers of *A. leptopus* and pale yellow coloured, fragrant flowers of *A. lebbeck* are more when compared to the bright orange-red coloured, odourless flowers of *C. sebestina* and pale blue-coloured, odourless flowers of *T. grandiflora*. The present study shows that the brightness of the flowers is not an important factor to determine the chemical composition and bioactivity of the flowers. Because, the pollinators are attracted not only by the bright colour of the flowers, but also by the attractive sweet smell of the flowers due to the presence of variety of colourless aromatic chemical compounds. Honeybees usually collect nectar, pollen, or both from particular species of plants, which are called honey plants. Of the four species of the present study, only one species, *A. leptopus*, with maximum degree of chemical diversity and bioactivity, has been listed as honey plant (<http://www.nationmaster.com/encyclopedia/List-of-honey-plants>).

Pollen is a concentrated, energy and vitamin rich food those in contemporary times is not only consumed as a dietary component, but also is used in alternative medical treatments. Pollen has been used medically in prostatitis, bleeding stomach ulcers and some infectious diseases. Pollen may also be used for treatment and prevention of the high-altitude-sickness syndrome (Linskens and Jorde 1997). In the present study, the methanolic extracts of *A. lebbeck*, *A. leptopus* and *C. sebestina* show the presence of several bioactive compounds. The next step is to identify the particular part of the flower, such as sepals, petals, pollen, ovary etc. and to isolate the pure compounds from the particular part for successful use of antibacterial compounds.

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