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# Antibacterial evaluation and phytochemical analysis of certain medicinal plants, Western Ghats, Coimbatore

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**ABSTRACT:**

The antibacterial effect of some selected Indian medicinal plants were evaluated on bacterial strains *Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Salmonella typhi*. The solvents used for the extraction of plants were petroleum ether, chloroform ethanol and methanol. The in-vitro antibacterial activity was performed by disc diffusion method. The most susceptible gram positive bacteria were *S. aureus* while the most susceptible gram negative bacteria were *E.coli*. The most active antibacterial plant was *P.maximum*. The significant antibacterial activity of active extracts were compared with the standard antimicrobials Ciprofloxacin (10mcg/ml). The results obtained in the present study suggest that *P.maximum* can be used in treating disease caused by the test organisms.

**Keywords:**
solvents, medicinal plants, microorganisms, antimicrobial activity, Phytochemicals.

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Journal of Research in Biology

## Introduction

Antimicrobials of plant origin have enormous therapeutic potential and have been used since time im-memorial. They have been proved effective in the treatment of infectious diseases simultaneously mitigating many of the side effects which are often associated with synthetic antibiotics. Positive response of plant based drugs (less/no side effects) might lies in the structure of the natural products which reacts with toxins and / or pathogens in such a way that less harm is done to other important molecules or physiology of the host. It is because of this reason that drug designing studies nowadays have come up, as new field of research (Sharma and Kumar, 2009).

***Chromolaena odorata*** (L.) King & H.E.Robins.

*C. odorata* is a big bushy herb with long rambling (but not twining) branches; stems terete, pubescent; leaves opposite, flaccid-membranous, velvety-pubescent, deltoid-ovate, acute, three-nerved, very coarsely toothed, each margin with 1-5 teeth, or entire in youngest leaves; base obtuse or subtruncate but shortly decurrent; petiole slender, 1-1.5cm long; blade mostly 5-12cm long, 3-6cm wide, capitula in sub-corymbose axillary and terminal clusters; peduncles 1-3cm long, bracteate; bracts slender, 10-12mm long; involucre of about 4-5 series of bracts, pale with green nerves, acute, the lowest ones about 2mm long, upper ones 8-9mm long, all acute, distally ciliate, flat, appressed except the extreme divergent tip; florets all alike (disc-florets), pale purple to dull off-white, the styles extending about 4mm beyond the apex of the involucre, spreading radiately; receptacle very narrow; florets about 20-30 or a few more, 10-12mm long; ovarian portion 4mm long; corolla slender trumpet form; pappus of dull white hairs 5mm long; achenes glabrous or nearly so. The seeds of Siam weed are small (3-5mm long, ~1mm wide, and weigh about 2.5mg seed-1. *C. odorata* had the antipyretic, antibacterial and anti-spasmodic properties.

***Panicum maximum*** Jacq.

A tufted perennial, often with a shortly creeping rhizome, variable 60-200 cm high, leaf-blades up to 35 mm wide tapering to fine point; panicle 12- 40 cm long, open spikelets 3-3.5 mm long, obtuse, mostly purple red, glumes unequal, the lower one being one-third to one-fourth as long as the spikelet, lower floret usually male. Upper floret (seed) distinctly transversely wrinkled. Ethanolic leaf extract of *P.maximum* showed anti-diabetic activity. It has antibacterial activity against clinically important microbial pathogens.

***Barleria lupulina*** Lindl.

Erect shrub; stems and leaves glabrous; spines 3 in lower axils, 2 deflexed ca 1-2 cm long, 1 shorter and upright. Leaves narrowly obovate, spine -tipped; lamina 3.5-9 cm long, 0.8-1.2 cm wide; petiole 2-3 mm long. Inflorescence a terminal spike with overlapping bracts; bracts broadly ovate, 15 mm long, shortly mucronate, green with purple upper half, very shortly pubescent all over, cup-shaped-glandular at base; bracteoles lanceolate, ca 5.5 mm long, sparsely glandular. Calyx segments spine-tipped, pubescent, lanceolate; outer 10 mm long, inner ca 8 mm long. Corolla yellow, finely eglandular pubescent outside; tube ca 3 cm long; lobes ca 1 cm long. Longer stamen filaments ca 2 cm long; shorter stamens fertile. Style ca 3 cm long, glabrous. Capsule not seen. Leaves are used to treat snake bites, dog bites, swelling due to fall or assault boils, bleeding wounds and rheumatism. The present study was undertaken to investigate the effects of aqueous and organic extracts of *Chromolaena odorata*, *Panicum maximum* and *Barleria lupulina.*

## MATERIALS AND METHODS

### Plant collection

Fresh plant parts of *Barleria lupulina*, *Panicum maximum* and *Chromolaena odorata* were collected from Western Ghats, Coimbatore, Tamilnadu, India. The taxonomic identities of plants were confirmed by Dr. V. Sampath Kumar, Scientist, Botanical Survey of India (Southern Circle), Coimbatore, Tamilnadu, India and the voucher specimen of the plants have been preserved at RVS College Microbiology Laboratory. The collected plants were washed with running tap water, air dried, homogenized to a fine powder and stored in air-tight bottles at 4°C.

### Preparation of crude extracts

About 100 g of dried plant material was extracted with 200 ml of Petroleum ether kept on a rotary shaker for 24 h. There after, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume $1/5^{th}$ of the original volume (Sasikumar *et al*., 2005). It was stored at 4°C in airtight bottles for further studies. Same conditions were applied for chloroform, methanol and ethanol extracts. It was stored at 4°C in airtight bottles for further studies.

Journal of Research in Biology

**Phytochemical Components**

This was carried out according to the methods described by Trease and Evans (1997). Quatification phytochemical analysis of the crude powder of three plants for the identification of phytochemicals like as a tannins, alkaloid, steroid, phenols and terpenoid, flavonoid etc.

**Bacterial Strains**

Microorganisms were obtained from the Microbial Type Culture Collection Centre (MTCC), Chandigarh, India. Amongst seven microorganisms were investigated, two Gram-positive bacteria are *Staphylococcus aureus,*? while five Gram-negative bacteria are *Proteus mirabilis* MTCC 425, *Escherichia coli* MTCC 2961, *Pseudomonas aeruginosa* MTCC 4676, *Klebsiella pneumoniae* MTCC 432 and *Salmonella typhi* MTCC 733. All the microorganisms were maintained at $4^0$C on nutrient agar slants.

**Antibacterial Activity**

The antimicrobial assay was performed by agar disc diffusion method for solvent extract (Bauer *et al*., 1996). The molten Mueller Hinton agar was inoculated with 100 µl of the inoculum (1 x $10^6$ CFU/ml) and poured into the Petri plate (Hi-media). For agar disc diffusion method, the disc (0.7 cm) (Hi-Media) was saturated with 100 µl of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated overnight at $37^0$C. Microbial growth was determined by measuring the diameter of the zone of inhibition. For each bacterial strain, controls were maintained where pure solvents are used instead of the extract. The result was obtained by measuring the zone diameter. The experiment was done three times and the mean values are presented.

**Minimum Inhibitory Concentration (MIC)**

For determination of MIC, 1 ml of broth medium was taken into 10 test tubes for each bacteria (Muhamed Mubarack *et al*., 2011). Different concentrations of plant extracts ranging from 0.125 - 8 mg/ $ml^{-1}$concentration were incorporated into the broth and the tubes were then inoculated with 0.1 ml of inoculums of respective bacteria ($10^5$ CFU $ml^{-1}$) and kept at 37°C for 24 h. The test tube containing the lowest concentration of extract showing reduction in turbidity when compared with control was regarded as MIC of that extract.

## RESULTS AND DISCUSSION

Different solvents and water extracts tested at 100 mg/ml concentrations against six important microbial pathogens are presented in **Table 1**. Among five solvents (Petrolum ether, benzene, chloroform, methanol and water) tested against six microbial pathogens, benzene, methanol and water extracts recorded significant antibacterial activity against all the test pathogens. Antibacterial activity was not observed in petroleum ether and chloroform extracts against all the pathogens. Among benzene, methanol and water extracts, methanol extracts recorded significant antibacterial activity followed by water and benzene. *Staphylococcus aureus* found highly susceptible to methanol extract, where as *Klebsilla pneumonia* and *Pseudomonas aeruginosa* are less susceptible to methanol extract. Methanol extract exhibited similar antibacterial activity against, *E.coli*, *Salmonella typhi* and *Proteus mirabilis*. Antibacterial activity of water extract varied greatly among the different test pathogenic bacteria. Highest antibacterial activity was observed against *Staphylococcus aureus* followed by *E.coli* even though antibacterial activity was observed against other pathogens, also it was not found significant. Of the three candidate plants in this study *Panicum maximum* showed significant antibacterial activity against all the tested bacteria and the remaining plants showed moderate activity after alcoholic extraction.

**Table 1. Antibacterial activity of *Barleria lupulina***

| Microorganisms | Conc. ($mg/ml^{-1}$) | Extracts | | | | | Synthetic drug |
|---|---|---|---|---|---|---|---|
| | | P.ether | Ben | Chl | Met | Water | |
| *S.aureus* | 100 | - | - | - | 16 | - | 21 |
| *E.coli* | | - | - | - | 12 | - | 15 |
| *S.typhi* | | - | - | - | - | - | 20 |
| *P.mirabilis* | | - | - | - | - | - | 23 |
| *K. pneumonia* | | - | - | - | - | - | 16 |
| *P. aeruginosa* | | - | - | - | - | - | 14 |

Journal of Research in Biology

**Table 2. Antibacterial activity of *Chromolaena odorata***

| Microorganisms | Conc. ($mg/ml^{-1}$) | Extracts | | | | | Synthetic drug |
|---|---|---|---|---|---|---|---|
| | | P.ether | Ben | Chl | Met | Water | |
| *S.aureus* | 100 | - | | - | 15 | - | 21 |
| *E.coli* | | - | - | - | 10 | - | 15 |
| *S.typhi* | | - | - | - | 11 | - | 20 |
| *P.mirabilis* | | - | - | - | 9 | - | 23 |
| *K. pneumonia* | | - | - | - | 10 | - | 16 |
| *P. aeruginosa* | | - | 13 | - | - | - | 14 |

**Table 3. Antibacterial activity of *Panicum maximum***

| Microorganisms | Conc. ($mg/ml^{-1}$) | Extracts | | | | | Synthetic drug |
|---|---|---|---|---|---|---|---|
| | | P.ether | Ben | Chl | Met | Water | |
| *S.aureus* | 100 | - | - | - | 19 | 9 | 21 |
| *E.coli* | | - | - | - | 14 | 8 | 15 |
| *S.typhi* | | - | - | - | 13 | - | 20 |
| *P.mirabilis* | | - | 12 | - | 11 | - | 23 |
| *K. pneumonia* | | - | - | - | 10 | - | 16 |
| *P. aeruginosa* | | - | - | - | 10 | - | 14 |
| Synthetic drug | | | | | | | 21 |

Minimum Inhibitory Concentration (MIC) of the active extracts is shown in **Table 4**. *P.maximum* and *B. lupulina* showed the strongest antibacterial activity with MIC values of 0.125 mg/ $ml^{-1}$, followed by *C. odorata* (MIC of 0.250 mg/ $ml^{-1}$). Available literature results indicate a strong activity when MIC values are between 0.05-0.50 mg/ $ml^{-1}$, moderate activity in values between 0.6-1.50 mg $mL^{-1}$ and weak activity above 1.50 mg/ $ml^{-1}$ (Diaz *et al*., 2009). In conformity to the existing trend, *P.maximum* and *B. lupulina* showed strong activity, *C. odorata* displayed moderate activity.

Phytochemical analysis of all the plant extracts revealed that alkaloids are generally present in petroleum ether extracts. Tannins were found in Petroleum ether & Chloroform extract and steroids in methanol extract. Flavonoids were found in benzene and methanol extracts (**Table 5**). All plant parts synthesize some chemicals by themselves, to perform their physiological activities. In our present study, the investigated plants have exhibited different kinds of secondary metabolites. The medicinal value of these secondary metabolites are due to the presence of chemical substances that

**Table 4. Minimum Inhibitory Concentrations of *Barleria lupulina*, *Chromolaena odorata* and *Panicum maximum***

| Medicinal Plants | Extracts | Minimum Inhibitory Concentrations (mg/ml) | | | | | |
|---|---|---|---|---|---|---|---|
| | | *S.aureus* | *E.coli* | *S.typhi* | *P.mirabilis* | *K. pneumonia* | *P. aeruginosa* |
| *B. lupulina* | P.ether | - | - | - | - | - | - |
| | Ben | - | - | - | - | - | - |
| | Chl | - | - | - | - | - | - |
| | Met | 0.250 | 0.500 | - | - | - | - |
| | Water | - | - | - | - | - | - |
| *C. odorata* | P.ether | - | - | - | - | - | - |
| | Ben | - | - | - | - | - | 0.500 |
| | Chl | - | - | - | - | - | - |
| | Met | 0.250 | 1.0 | 1.0 | 4 | 1.0 | - |
| | Water | | | | | | |
| *P. maximum* | P.ether | - | - | - | - | - | - |
| | Ben | - | - | - | 0.500 | - | - |
| | Chl | - | - | - | - | - | - |
| | Met | 0.125 | 0.250 | 0.250 | 1.0 | 1.0 | 1.0 |
| | Water | 4 | 4 | - | - | - | - |

**Table 5. Phytochemical screening of *Barleria lupulina*, *Chromolaena odorata* and *Panicum maximum***

| Medicinal Plants | Phytoconstituents | Extracts | | | | |
|---|---|---|---|---|---|---|
| | | P.ether | Ben | Chl | Met | Water |
| *B. lupulina* | Alkaloids | + | - | + | - | - |
| | Tannins | + | - | - | - | - |
| | Steroids | - | - | - | + | - |
| | Saponins | - | - | + | - | - |
| | Flvonoids | - | + | - | + | - |
| *C. odorata* | Alkaloids | + | - | - | - | - |
| | Tannins | - | - | + | - | - |
| | Steroids | - | - | - | - | - |
| | Saponins | - | + | - | - | - |
| | Flvonoids | + | - | - | + | - |
| *P. maximum* | Alkaloids | + | + | - | - | - |
| | Tannins | - | + | - | - | - |
| | Steroids | - | - | - | + | - |
| | Saponins | - | - | - | - | - |
| | Flvonoids | - | - | - | - | - |

produce a definite physiological action on the human body. The most important of these substances include, alkaloids, glucosides, steroids, flavonoids, fatty oils, resins, mucilages, tannins, gums, phosphorus and calcium for cell growth, replacement, and body building (Kubmarawa *et al*., 2008).

Wynn (2001) describes today's traditional medicine, as undoubtedly the oldest form of medicine and probably had evolved simultaneously with the evolution of human beings. With the traditional knowledge in the background, potential plants can be prospected to reach the active fraction or molecule (s) which can be further formulated. Further studies may be necessary to elucidate the specific phytoactive compounds in the leaf extracts of the plant *P.maximum*.

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