INTRODUCTION

Diseases that remain most challenging for today’s health care system tend to be more complex than could be treated by current combination therapies. However plant based drugs contain a mixture of multiple components which serve the effective control of disease (Karnath, 2002). S. chamaelea is a perennial herb belonging to the family Euphorbiaceae. The family contains 300 genera and 7500 species belongs to various habitats as trees, shrubs and climber sout of which 150 species are of medicinal value in the Asia-pacific region. S. chamaelea is a weed of cultivated lands and forest underground. Leaves alternate, pinnerved, flowers minute, monoecious in slender racemes. Decoction of the plant in ghee is given as tonic and applied to the head in vertigo. The juice of the plant is astringent and is used as a remedy for syphilis and diarrhoea. (Thammanna and Narayana Rao, 1990).

Various classes of chemical constituents have been isolated from different species of Euphorbiaceae. From Euphorbia retusa flavonol glycosides like quercetin-3-glucoside, quercetin-3-glucuronide, quercetin-3-rhamnoside and quercetin-3-rutinoside were isolated while in a recent study, a pentacyclic triterpene betulin, the steroid â-cytosterol and flavonol glycosides like quercetin-3-glucoside, retusa kaempferol, luteolin and apigenin. The antibacterial activity of the methanolic leaf extract of S.chamaelea against pathogenic bacteria like Bacillus subtilis and Staphylococcus aureus (gram positive) and Escherichia coli and Pseudomonas aeruginosa (gram negative) bacteria showed concentration dependent inhibition of B. subtilis, S. aureus, P. aeruginosa and E. coli.

been isolated from the leaves of S. adenophora which were found to be selectively bioactive (Macias-Rubalcava et al., 2007). Methanolic extracts of S. shottiana roots contain analgesic compounds which justify the popular use of this plant for the treatment of urinary problems. The extracts of S. brasiliensis show the activity against gram positive and gram negative bacteria and fungi. Aqueous extract of S. brasiliensis and S. klotzschiana showed in vitro antihepatic activity with 50% effective dose (E50) ranging from 39 to 169 µg/mL (Kott et al., 1998).

Phenoloids are known to be synthesized by plants in response to microbial infection. (Dixon et al., 1983). Phenolic compounds have important pharmaceutical applications (Fairvairn, 1959) and as possible agents in the development of disease resistance in plants (Pridham, 1960). 50% ethanolic extract of S. commersoniana, a south American medicinal plant locally used as external antiseptic,which shows antifungal activity against Dermatophytes microsporumgypseum, Trichophyton metagaphytes and T. rubrum. After fractionation, four flavonoids as Quercetin, Kaempferol, Isorhamnetin, Isoquercetin and also four phenolic derivatives viz. Gallicin, Gallic, Syringic and Caffeic acids and Coumarin (Scopoletin) have been reported in S.commersoniana (Okasana Hnatyszyn et al., 2007).

These results support the popularity of the genus Sebastiania in traditional medicine for the treatment of diarrhoea, vertigo and syphilis. It was aimed to investigate further the phytochemical constituents, analytical identification of phenolic and flavonoid compounds and their antimicrobial

KEY WORDS
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Phytochemical screening
Antibacterial activity
Phenolic compounds
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ABSTRACT
Sebastiania chamaelea, a herbal medicinal plant belongs to the family Euphorbiaceae. Leaves contain a number of medicinally important phytochemical compounds. The preliminary phytochemical screening revealed the presence of 6 groups of secondary metabolites such as Phenols, Flavonoids, Tannins, Saponins, Steroids and Glycosides and qualitative analysis of Phenolic and Flavonoid compounds, resulted about 15 Phenolic compounds of which Caffeic acid, Mellilotic acid, Aesculetin, P-Hydroxy benzoic acid, Coumarin, Cinnamic acid, Salicylic acid and Scopoletin have been identified along with 5, Flavonoid compounds like Myrecetin, Quercetin, Kaempferol, Luteolin and Apigenin. The antibacterial activity of the methanolic leaf extract of S.chamaelea against pathogenic bacteria like Bacillus subtilis and Staphylococcus aureus (gram positive) and Escherichia coli and Pseudomonas aeruginosa (gram negative) bacteria showed concentration dependent inhibition of B. subtilis, S. aureus, P. aeruginosa and E. coli.

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activity to enable effective inhibition of *S. chamaelea* against microorganisms.

**MATERIALS AND METHODS**

**Collection and identification of plant material**

The leaves of *S. chamaelea* were collected from agricultural fields of S.V. Veterinary College, Tirupati, Andhra Pradesh, India, during the month of July - November, 2008. The taxonomic identity of the plant is confirmed by Prof. N. Yasodamma, Department of Botany, Sri Venkateswara university, Tirupati and the voucher specimen numbers of the plants were preserved. Fresh leaves were used for the extraction of phenolic compounds and shade dried leaf powder is used for extraction of flavonoids and antimicrobial activity.

**Preparation of plant extracts**

Fresh leaf diethylether extract for phenolic compounds (Ibrahim and Towers, 1960) and for flavonoids dry powder methanolic extract (Markham, 1982) was used. 100g of powder dissolved with 500mL of methanol and stored in darkness for 72hrs, then crude extract was filtered through Whatmann 3 filter paper and the filtrate was evaporated to dryness on water bath. A portion of the residue was used to test for plant constituents while the rest was used for bacterial susceptibility test.

**Preliminary phytochemical analysis**

To detect the different classes of secondary metabolites with the methanolic extract, standard methods of Harborne (1973) and Kokate (2003) were followed.

**Test organisms**

Pure cultures of *Esherichia coli*, *Staphylococcus aureus, Pseudomonas aruginosa* and *Bacillus subtilis* were procured from the department of microbiology, S.V. University and Sri Venkateswara Institute of Medical Sciences, Tirupati. These were further maintained on nutrient agar slants at 4ºC until further use.

**Media preparation and antimicrobial activity**

The sensitivity testing of the plant extracts were determined by using disc diffusion method (Bauer et al., 1996). 18 hrs old bacterial broth cultures were used as inoculums after adjusting their population to 10 CFU/ mL. (colony forming units) using 0.9% (w/v) sterile saline by the method as described by Forbes et al., (1990). 0.5 mL of standard inoculums were pipetted into a sterile Petri plate, 20mL of melted agar medium is then added in each plate and mixed well by gently swirling on the table top. The seeded plates are allowed to solidify.

Sterile paper discs previously soaked in a known concentration of extract (50,100,150,200 mg/mL) were carefully placed on the labeled seeded plates. The plates were later incubated at 37ºC for 24 hrs after which they were observed for zone of inhibition. The microbial growth was determined by measuring the diameter of zone of inhibition. The inhibition zones were measured with a ruler and compared with control containing standard antibiotic Gentamycin at a concentration of 10 mcg/ disc. For each bacterial strain, controls were maintained where pure solvent was 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.

**RESULTS AND DISCUSSION**

Preliminary phytochemical screening of the methanolic extract of *S. chamaelea* showed many types of phytochemical constituents mainly flavonoids, phenols, tannins and steroids and some glycosides and saponins, whereas alkaloids, lignins and fixed oils were almost absent (Table 1).

**Table 1: Results of preliminary phytochemical screening with methanolic extract of ** *S. chamaelea*

<table>
<thead>
<tr>
<th>Types of Metabolites</th>
<th>Tests</th>
<th>Presence of Secondary Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer's Test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Wagner's Test</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Ferric chloride test</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Shinoda's test</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Lead acetate test</td>
<td>++</td>
</tr>
<tr>
<td>Phenols</td>
<td>Phenol test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Ellagic acid test</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Killar Kilani test</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride test</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Gelatin Test</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>Lead acetate test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Salkowski test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Leiberman Burchard test</td>
<td>+</td>
</tr>
<tr>
<td>Lignins</td>
<td>Lignin test</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Fixed oils</td>
<td>-</td>
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</table>

*S.chamaelea* consists 15 phenolic acids such as caffeic acid, melioliotic acid, aesculetin, p-hydroxy benzoic acid, coumarin, cinnamic acid, salicylic acid and scopoletin were identified along with five flavonoids like myrecetin, quercretin, kaempferol, luteolin and apigenin.

Gram negative bacteria are known to be more resistant due to the thick murein layer in their outer membrane, which prevents the entry of inhibitor substances in the cell. This is the reason why studies involving test of efficacy of plant extract against bacteria show positive results mainly against gram positive bacteria (Suresh et al., 2008). The present in vitro agar disc diffusion antibacterial activity studies of leaf methanolic extracts against four bacterial strains shows concentration dependent inhibition more or less linearly (Table 2). However, *B. subtilis* and *E. coli* were found to be more susceptible in comparison to *P. aruginosa*. The inhibition by the extract was fairly equivalent to that of the Gentamycin at the rate of 10 mcg / disc. Similar inhibition against *S. epidermidis* also been reported (Parekh et al., 2005) through methanolic extract of *Euphorbia hirta* and *E. tirucalli* (Natarajan et al., 2005). The methanolic and acetone extracts of *E. fusiformis* also inhibited the growth of *P. aruginosa, Klebsiella pneumoniae, Proteus vulgaris* and *Salmonella typhii*. The efficacy of the species *S.chamaelea* is further supported by the comparison of the traditional practices with aqueous extract of *E. hirta* against dysentery, colic ulcers, asthma and chronic bronchial infections showing the inhibition of *E. coli, P. vulgaris, P. aruginosa* and *S. aureus* (Sudhakar et al., 2006).

Active compounds of *S. chamaelea* like phenolic acids, aesculatin, p-hydroxybenzoic acid and flavonoids like...
myricetin, kaempferol, luteolin and apigenin are known to be antimicrobial. Quercetin also possess an antimicrobial activity (Beschia et al., 2003). Some other phenolic acids like caffeic acid has anti inflammatory activity (Fernandez, 1998), inhibit zoospore germination (Timothy, 2006), cinnamic acid with antifungal, antihelmintic, natural protection against infections by pathogenic micro organisms (Champbel et al., 1999). Mellilotic acid shows antimicrobial property. Coumarin acts as an anticoagulant, salicylic acid which is keratolytic, fungistatic and antiseptic, scopoletin showed a direct antifungal activity against Ophiostoma ulmi spore germination. These flavonoids are commonly found in Azadirachta indica, Apium graveolens, Soymida labrifiuga and Apium graveolens also. Besides from the related species S. adenosperma Macias-Rubilcava et al., (2007) have isolated 3-epi-beta-amyrin, beta-amyrinone, 3-epi-lupeo, lupenone, taraxerol and taraxerone. These compounds are reported to play as allelochemicals. In recent years multi resistant bacterial strains have increased dramatically and thus the treatment of several infections has become very difficult, reducing the therapeutic options. Present study hints towards the fact that the therapeutic action of the plant may not be due to a single compound but due to synergistic action of a number of compounds. This synergistic impact could counteract the resistance of bacteria which are hard to kill by a single antibiotic, hence holds the key to cure the health disorders due to microbial infections.

REFERENCES


Table 2: Antimicrobial activity of methanolic extract of Sebastania chamaelea against different gram positive and gram negative bacterial strains

<table>
<thead>
<tr>
<th>Name of the bacteria</th>
<th>Diameter of the zone of inhibition (mm)</th>
<th>Methanol</th>
<th>Values are the mean of triplicates, ± SD; I : 5mg/disk, II : 10mg/disk, III: 15mg/disk, IV / 20mg/disk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>I/ 5mg 16.6±0.94 II/10mg 18.0±0.0 III/15mg 20.0±1.6 IV / 20mg 23.3±0.94</td>
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<tr>
<td>Esherichia coli</td>
<td>I/ 5mg 17.3±0.94 II/10mg 19.3±1.88 III/15mg 18.0±0.0 IV / 20mg 19.3±0.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aures</td>
<td>I/ 5mg 16.6±0.94 II/10mg 18.0±0.0 III/15mg 18.6±0.94 IV / 20mg 19.3±1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aruginosa</td>
<td>I/ 5mg 18.6±2.4 II/10mg 18.0±1.6 III/15mg 18.6±0.94 IV / 20mg 19.3±1.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>