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PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIBACTERIAL ACTIVITY OF THE METHANOLIC LEAF EXTRACT: *SEBASTIANIA CHAMAELEA* MUELL. ARG

K. S. SHANTHI SREE*, N. YASODAMMA AND CH. PARAMAGEETHAM[1]
Department of Botany, Sri Venkateswara University, Tirupati - 517 502, A.P., INDIA
[1]Department of Microbiology, S. V. University P. G. Centre, Kavali - 524 201, A.P., INDIA
E-mail: npalli_yasoda@yahoo.co.in

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***Corresponding author**

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, Steriods and Glycosides and qualitative analysis of Phenolic and Flavonoid compounds, resulted about 15 Phenolic compounds of which Caffeic acid, Melilotic 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 *Esherichia coli* and *Pseudomonas aruginosa* (gram negative) bacteria showed concentration dependent inhibition of *B. subtilis*, *S. aureus, P. aruginosa* and *E. coli.*

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, herbs 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 undergrowth. Leaves alternate, penninerved, 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-glucouronide, quercetin-3-rhamnoside and quercetin-3-rutinoside were isolated while in a recent study, a pentacyclic triterpene betulin, the steroid â-cytosterol and number of fatty acids. All the members of this family contain skin irritating and tumour promoting diterpenoids (Evans and Taylor, 1983). Some species are used to cure diarrhoea, migrains, intestinal parasites and warts (Singla and Pathak, 1990). In addition several pentacyclic triterpenes have also 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 (E_d 50) ranging from 39 to 169 μg/mL (Kott *et al.*, 1998).

Flavonoids 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 metagraphytes* and *T. rubrum*. After fractionation, four flavonoids as Quercetin, Kaempferol, Isorhamnetin, Isoquercetrin 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

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 follwed.

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 pippeted 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 at37ºC 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*

Types of Secondary Metabolites	Tests	Presence of Secondary Metabolites
Alkaloids	Mayer's Test	-
	Wagner's Test	-
Flavonoids	Ferric chloride test	+ +
	Shinoda's test	+ +
	Lead acetate test	+ +
Phenols	Phenol test	+ +
	Ellagic acid test	+ +
Glycosides	Killar Kilani test	+
Tannins	Ferric chloride test	+ +
	Gelatin Test	+ +
	Lead acetate test	+ +
Steroids	Salkowski test	+ +
	Leiberman Burchard test	+ +
Lignins	Lignin test	-
Saponis		+
Fixed oils		-

S.chamaelea consists 15 phenolic acids such as caffeic acid, melilotic acid, aesculetin, p-hydroxy benzoic acid, coumarin, cinnamic acid, salicylic acid and scopoletin were identified along with five flavonoids like myrecetin, quercetin, 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 diffution 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

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

Name of the bacteria	Diameter of the zone of inhibition (mm)Methanol I / 5mg	II/10mg	III/ 15mg	IV / 20mg
Bacillus sabtilis	16.6 ± 0.94	18.0 ± 0.0	20.0 ± 1.63	23.3 ± 0.94
Esherichia coli	17.3 ± 0.94	19.3 ± 1.88	20.6 ± 0.94	23.3 ± 0.94
Staphylococcus aures	16.6 ± 0.94	18.0 ± 0.0	18.0 ± 0.0	19.3 ± 0.94
Pseudomonas aruginosa	18.6 ± 2.4	18.0 ± 1.6	18.6 ± 0.94	19.3 ± 1.8

Values are the mean of triplicates, ± SD; I : 5mg/disk, II : 10mg/disk, III: 15mg/disk, IV: 20mg/disk

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), cnnamic acid with antifungal, antihelmintic, natural protection against infections by pathogenic micro organisms (Champbel *et al*., 1999). Melilotic 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 graviodes, Soymida fabrifuga and Apium graviolens* also. Besides from the related species *S. adenophora* Macias-Rubilcava *et al*., (2007) *have isolated* 3-epi-beta-amyrin, beta-amyrinone, 3-epi-lupeol, 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.

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