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Evaluation of antibacterial activity of Ocimum tenuiflorum, chrysanthemum indicum and tabernaemontana divaricata flower extract

[*] Saroj Kumar Sahoo, P Venkateswara Rao, I Mounika, S Prasad, K Prasanthi, A Priyanka, K Tarangini, M Varalaxmi

Department of Pharmaceutical Chemistry, Sri Sivani College of Pharmacy, Chilakapalm Jn., Etcherla, Srikakulam, Andhra Pradesh, India

Abstract
The Ethanol and Chloroform mixture extracts of the flowers of Ocimum tenuiflorum, Chrysanthemum indicum and Tabernaemontana divericata were investigated for their antibacterial activity against gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, gram negative bacteria *Staphylococcus aureus*, *Proteus vulgaris*. And two concentrations (100,200 mg/ml) of each extract were studied in activity by agar cup plate method. The results were compared with standard streptomycin (100µg/ml).All studied extracts have shown antibacterial activity. The result of zone of inhibition study revealed concentration dependent nature of the extracts with broad spectrum activity against bacteria.

Keywords: antibacterial activity, streptomycin, zone of inhibition

Introduction
Ocimum tenuiflorum, also known as *Ocimum sanctum*, holy basil, or *tulasi* (also spelled *thulasi*), is an aromatic plant in the family Lamiaceae which is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics. It is an erect, many-branched subshrub, 30–60 cm (12–24 in) tall with hairy stems and simple phyllo taxic green or purple leaves that are strongly scented.

In Ayurveda, this plant has been well documented for its therapeutic potentials and described as DashemaniShwasaharni (antiasthmatic) and antikaphic drugs. Anticancer [1, 2], radioprotective, anticarcinogenic, antioxidant, chemopreventive, immunotherapeutic, antimicrobial, anti-inflammatory, analgesic, antipyretic, antispermatogenic and antistress activities of this plant have also been reported.

Chrysanthemum indicum- Chrysanthemum is a genus of about 30species of perennial flowering plants in the family Asteraceae, native to Asia and northeastern Europe. Amongst florists and in the floral industry, they are commonly referred to as "mums".

The whole plant is antiphlogistic, blood tonic, depurative, febrifuge and vulnerary. The plant is used in China to treat eye ailments. In conjunction with black pepper it is used in the treatment of gonorrhea. The leaves are depurative. They are used in China in the treatment of migraine. The flowers are aperient, bitter, hypotensive [3, 4], stomachic and vasodilator. They contain the glycoside chrysanthemin that yields glucose and cyanidin on hydrolysis, together with stachydrine and an essential oil.

Materials and Methods
Collection and authentication
The fresh flowers were collected from local village area of Srikakulam, Andhra Pradesh, India. The flower were identified by Taxonomist Dr. N.S.N. Swami, Department of Botany, Government Degree Arts College, Srikakulam. All the solvent are analytical grade.

Extraction Procedure
Plant active components were extracted using the cold extraction method [5]. Ethanol and Chloroform mixture in the ratio 10:1 were used for the extraction. To 110ml mixture of solvents were added 50g portions of the flower in a beaker and allowed to soak at room temperature for 48 hours. Occasional shaking was carried out to improve extraction of phytochemicals. The filtrate was obtained by means of a vacuum filter pump. The filtrate was evaporated in a weighed flask, with a water bath set at 40 °C. A small proportion of dry extracts was stored for phyto-chemical analysis. Remaining portion of the extracts was used for antibacterial assay. Extracts were reconstituted by re-dissolving in DMSO. The final filtrates were filter-sterilized by using syringe filter with a pore size of 0.45µm. Sterile extracts obtained were stored separately in labelled, sterile capped bottles, in a refrigerator at 4 °C before use during the antibacterial sensitivity tests.

Results and Discussion
Agar Cup Plate Method [6]
Anti-bacterial activity was determined by cup plate agar diffusion method. This method is based on the diffusion of active constituent form a cavity through the solidified agar layer of a Petri dish used for study. Growth of inoculated microorganisms is inhibited entirely in a circular area (zone around a cavity containing a solution of anti-microbial active constituent.

Nutrient agar was used as medium for bacterial growth. The nutrient agar medium was sterilised by autoclave and Petri dishes were prepared in laminar air flow. Mother culture of each organism was step up 24 hr before the assay in order to reach stationary phase of growth. 20 ml of nutrient agar were distributed in to sterile Petri dishes. The test microorganisms

were sprayed on the surface of Petri dish by spread plate technique from the mother culture. By using flame sterilised cork borer no. 4, four cups were prepared in each Petri dish. Alternate cups were filled with 0.1 ml of standard and test extracts using micro pipette to labelled cavity of plates. The plates were kept in refrigerator for proper diffusion of solutions at 4^{o}c for 2 hrs. The plates were incubated in incubator at 37 ± 2^{o}c for 24 hrs. Two replicates were carried out for each extract against each of test organisms. Controlled experiments were carried out under the similar condition by using streptomycin (100µg/ml). Negative control experiments were prepared by taking only medium and solvents instead of extracts or standard antibiotic.

Determination of zone of inhibition [7-9]

The zone of inhibition was determined by measuring the diameter of zone of inhibition surrounding bacterial growth, averaged and the mean values were tabulated (table no 2) for the extracts of flowers of Ocimum tenuiflorum, Chrysanthemum indicum and Tabernaemontana divaricata the concentrations used were 100mg/ml and 200mg/ml. Streptomycin(100µg/ml) were used as reference standards.

Table 1: Extraction of flowers from different solvents

S. No.	Name of flower	Weight of flower(gm)	Amount of solvent(ml)	% yield
1	Ocimum tenuiflorum	50	110	4.22
2	Chrysanthemum indicum	75	160	4.64
3	Tabernaemontana divaricata	75	160	3.86

Table 2: Zone of inhibition (mm) for the extracts of Ocimum tenuiflorum, Chrysanthemum indicum and Tabernaemontana divaricata

Micro Organisms	Zone of Inhibition(mm) [a]						
	Elower Extracs(mg/ml)						Standards [b]
	F1		F2		F3		
	100	200	100	200	100	200	
Gram positive bacteria							
Staphylococcus aureus	5	7	3	5	4	7	26
Bacillus subtilis	4	6	2	3	2	3	19
Gram negative bacteria							
Klebsiellapneumoni	4	7	4	6	3	5	22
Proteus vulgaris	6	10	5	8	4	6	32

All Values are mean of two readings (n=2)
Standards: Antibacterial studies with Streptomycin(100µg/ml).
F1: Ocimum tenuiflorum
F2: Chrysanthemum indicum
F3: Tabernaemontana divaricata

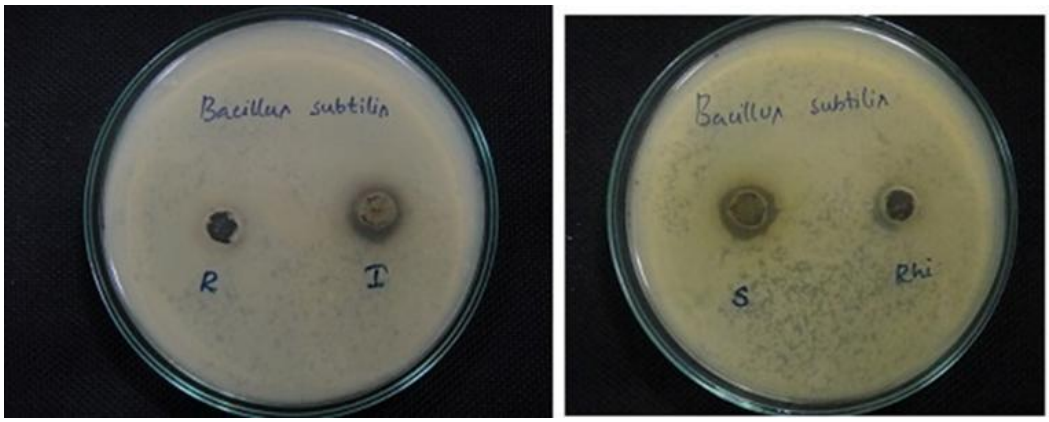
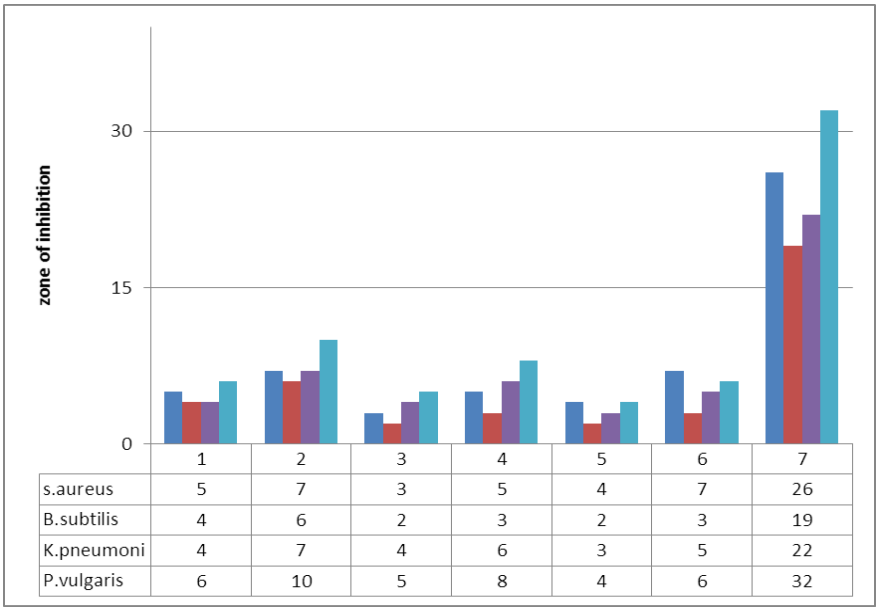


Fig 1: Zone of inhibition of Bacillus Subtilis



	1	2	3	4	5	6	7
s.aureus	5	7	3	5	4	7	26
B.subtilis	4	6	2	3	2	3	19
K.pneumoni	4	7	4	6	3	5	22
P.vulgaris	6	10	5	8	4	6	32

Fig 2: Zone of inhibition (mm) for the extracts of Ocimum tenuiflorum, Chrysanthemum indicum and Tabernaemontana divaricata

Conclusion

The antimicrobial activity for the flowers of Ocimum tenuiflorum, Chrysanthemum indicum and Tabernaemontana divaricata. The results of zone of inhibition studies revealed that the extracts posses significant antimicrobial activity in a concentration dependent manner against the test organisms and were comparable with the standard drugs.

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References

1. Sirkar NN. Pharmacological Basis of Ayurvedic Therapeutics. In: C. K. Atal & B. M. Kapoor (Eds.), Cultivation and utilization of medicinal plants. New Delhi: PID, CSIR. 1989.
2. Kathiresan K, Guanasekan P, Rammurthy N, Govidswami S. Anticancer activity of Ocimum sanctum. Pharmaceutical Biology, 1999; 37:285-290.
3. Devi PU. Radioprotective, anticarcinogenic andantioxidant properties of the Indian holy basil, Ocimumsanctum (Tulasi). Indian Journal of Experimental Biology, 2001; 39:185-190.
4. Prashar R, Kumar A, Banerjee S, Rao AR. Chemopreventive action by an extract from Ocimumsanctum on mouse skin papillomagenesis and itsenhancement of skin glutathione-S-transferase activity andacid soluble sulfhydryl level. Anticancer Drugs, 1994; 5:567-572.
5. Farnsworth NR. Screening plants for new medicines. Wilson Education Press. Washington, D.C. National Academy. 1988, 83-97.
6. Kavanagh F. Analytical microbiology, F. Kavanagh (ED). academic press, Newyork and London, 1972; 11:11.
7. Devi PU. Radioprotective, anticarcinogenic andantioxidant properties of the Indian holy basil, Ocimumsanctum (Tulasi). Indian Journal of Experimental Biology, 2001; 39:185-190.
8. Singh S, Malhotra M, Majumdar DK. Antibacterialactivity of Ocimum sanctum L. fixed oil. Indian Journal of Experimental Biology, 2005; 43:835-837.
9. Godhwani S, Godhwani JL, Vyas DS. Ocimumsanctum: an experimental study evaluating itsantiinflammatory, analgesic and antipyretic activity inanimals. Journal of Ethnopharmacology. 1987; 21:153-163.