World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Bio-autography guided screening of antimicrobial compounds produce by *Enicostemma littorale* Blume

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Received: 22-02-2015 / Revised: 17-03-2015 / Accepted: 28-03-2015

ABSTRACT

The present study is attempt to evaluate the antimicrobial activity and to screen out the antimicrobial component produced by *Enicostemma littorale* Blume. Ethyl acetate used for the extraction. Disk diffusion method was used for antimicrobial activity and bio-autography was carried out for the detection of antimicrobial component. Bio-autography is a technique that combines thin layer chromatography with bioassay *in situ*. Extract of root and stem show the zone of inhibition against *Escherichia coli*, *Pseudomonas auroginos*, *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus* due to the presence of flavonoid and flavones component determine by bio-autography.

KEYWORDS: *Enicostemma littorale,* Screening, Antimicrobial activity, Bio-autography.

INTRODUCTION

The taxonomic position of *Enicostemma littorale* Blume is as follows,

Subdivision : Angiosperm
Class : Dicotyledonae
Subclass : Gamopetalae
Sires : Bicarpellatae
Order : Gentianales
Genus : *Enicostemma*
Species : *littorale*

Enicostemma littorale Blume belongs to family Gentianaceae. It is also called as Vellarugu in Tamil, Chota chirayata in Hindi, Mamejavo in Gujarati and Nagajivha in Bengal. It is a glabrous perennial herb attaining height of 15-20 inch with sessile lanceolate leaves and is found throughout India up to a height of 1500ft. The plant is used in folk medicine to treat diabetes mellitus, rheumatism, abdominal ulcers, hernia, swelling, itching and insect poisoning. It's anti-inflammatory, hypoglycaemic, and anticancer activity have been reported. The plant has number of phytochemicals which includes alkaloids, catechins, saponins, sterols, triterpenoids, phenolic acid, flavonoids and xanthones. It also contains minerals like iron, potassium, calcium, silica, phosphate, chloride sulphate[1]. Plant is pungent and very bitter, anthelmentic, cures fever. Plant is very bitter and is used in Madras as stomachic. It is also a tonic and laxative. The plant is crushed and applied locally in snack-bite. It is used as stomachic and Vata[2]. The plant is acrid, thermogenic, digestive, carminative, stomachic, laxative, anti-inflammatory, livertonic, urinary astringent, depurative, revulsive and anti-periodic and useful in dyspepsia, colic, flatulence, helminthiasis, abdominal ulcers, hernia, constipation, dropsy, swellings, hepathopathy, glycosuria, leprosy, skin diseases, prutitus, intermittent, fever and malaise. Powder is given with honey as a blood purifier and in dropsy. The leaves are used in diabetes[3-9].

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MATERIAL AND METHODS

Plant material: The plant material of *Enicostemma littorale* Blume (Family - Gentianaceae) was collected from kamli, Mahesana District, Gujarat, India. The taxonomic identification of the plant was done with the help of Dr. Minoo Parabia at Department of Biotechnology, Veer Narmad South Gujarat University, Surat, (Gujarat) India.

Extraction preparation: The plant material of *E. littorale viz.* leaf, stem, root were collected, shade dried and pulverized to powder in a mechanical grinder. 10gm of each powdered plant material were extracted with 150ml of Ethyl acetate by soxhlet apparatus[10]. The organic solvent was removed by evaporation using heating mantle at 20ºC. Get do solid yield and concentrated by DMSO. These stock solutions were stored at 4ºC in air tight bottle for further studied.

Bacterial species: The five bacterial species which used in this study were, the gram negative species: *Escherichia coli*, *salmonella typhi*, *pseudomonas aeruginosa*, the gram positive species: *Staphylococcus aureus*, *Bacillus subtilis*. They were collected from Department of biotechnology, Veer Narmad South Gujarat University, Surat (Gujrat, India) in pure form.

Determination of antibacterial activity: Antibacterial activity of each extract of plant sample (1000mg/ml) was evaluated by the paper disc diffusion method. Active culture of test bacteria was grown in nutrient broth medium at 37ºC for 24 hours. A lawn culture then prepared on Muller Hinton agar. Sterile filter paper discs (5mm in diameter) impregnated with each extract was placed on the culture plates and incubated at 37ºC. Take DMSO solvent as negative control and streptomycin as positive control. After 24 hours of incubation, the antibacterial activity was assessed by measuring the inhibition zone[10,11].

The bio-autography[12]: The method was for detection of antimicrobial compounds. TLC plate was loaded by 5 to 6 drops of Ethyl acetate extract of stem and root. The solvent system was selected on the basis of different component present in plant extract[13]. Developed chromatogram was placing over the sterile surface of solid nutrient agar, which was seeded by different microorganism at appropriate temperature. The Petri plates were kept at 4°C for diffusion for 3 hours. After then plate kept in incubator at 37ºC for 24 hours.

RESULTS

The result of antimicrobial activity of plant extract was given in table 1. The ethyl acetate extract of root show highest activity (about 20 mm inhibition zone) against *Salmonella typhi*. The root and stem extract show antimicrobial activity against all pathogen microorganisms. The result of TLC analysis is given in table 2. The TLC analysis shows there are various phytochemicals are present in plant extract but the antimicrobial activity due to the flavanoid and flavones that determine by the bio-autography.

Table 1: Antimicrobial activity of crude extract of *E. littorale*

Extract	*Bacillus subtilis*	*Pseudomonas aeroginosa*	*Escherichia Coli*	*Staphylococcus aureus*	*Salmonella typhi*
EALE	-	-	-	-	-
EARE	10	18	10	14	20
EASE	10	8	6	12	10
DMSO	-	-	-	-	-
Streptomycin	26	25	31	27	29

EALE: ethyl acetate leaf extract; EARE: ethyl acetate root extract; EASE: ethyl acetate stem extract

Table 2: TLC protocol of *E. littorale*

Compound	**Mobile Phases**	**Ratio**	**Rf Value of Stem Extract**	**Rf Value of Root Extract**
Flavonoid	n-butanol : acetic acid : water	3 : 6 : 9	0.68	0.70
Flavonols and Flavones	Chloroform : acetic : water	5 : 10 : 15	0.56	0.57
Alkaloids	Ethyl acetate : Chloroform : water	5 : 3 : 1	0.56	0.66
Coumarin	Chloroform : Ethyl acetate :methanol	8 : 5 : 9	0.62	0.60
Phenolic compound	Methanol : Water	3:6	0.76	0.74

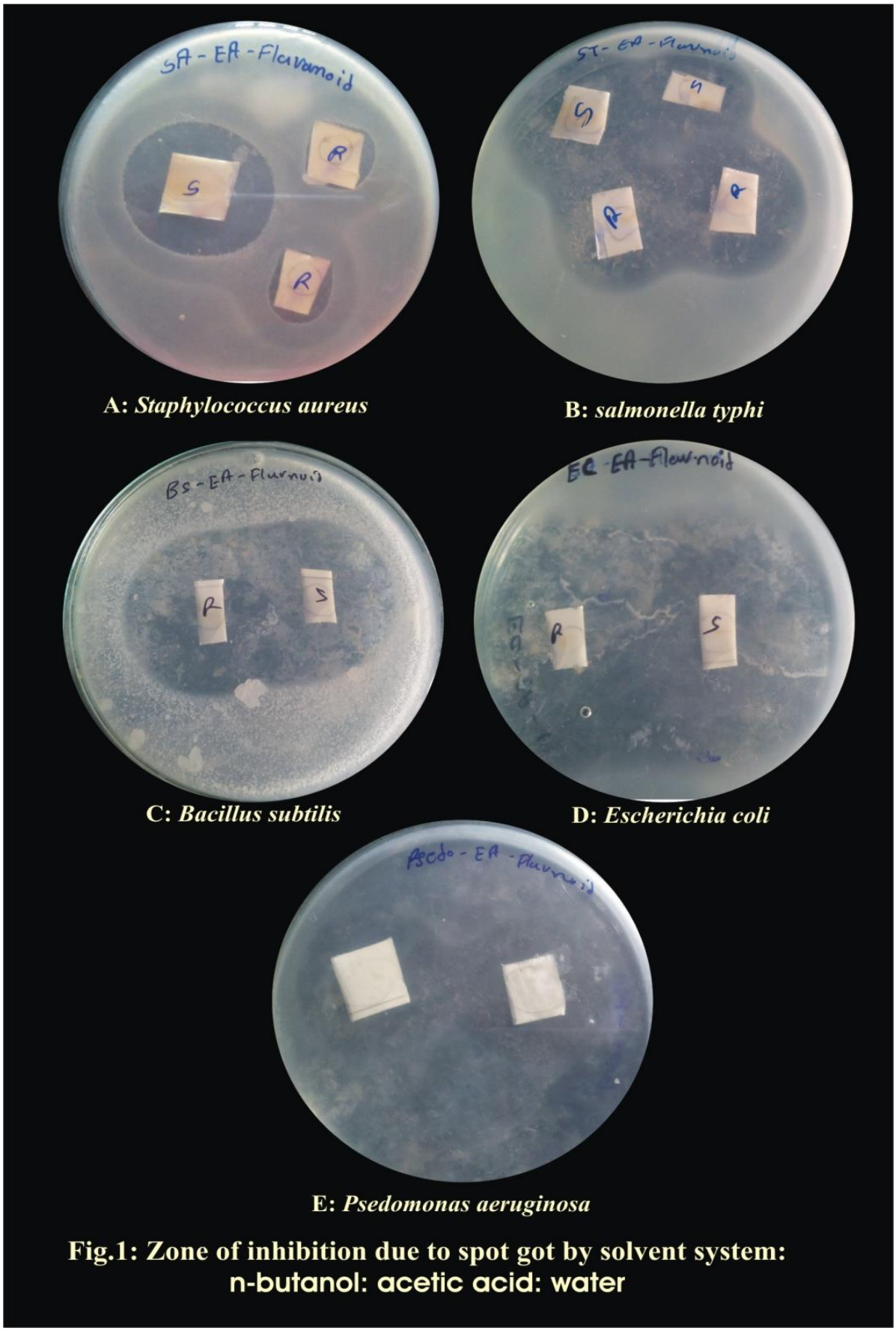

Fig.1: Zone of inhibition due to spot got by solvent system: n-butanol: acetic acid: water

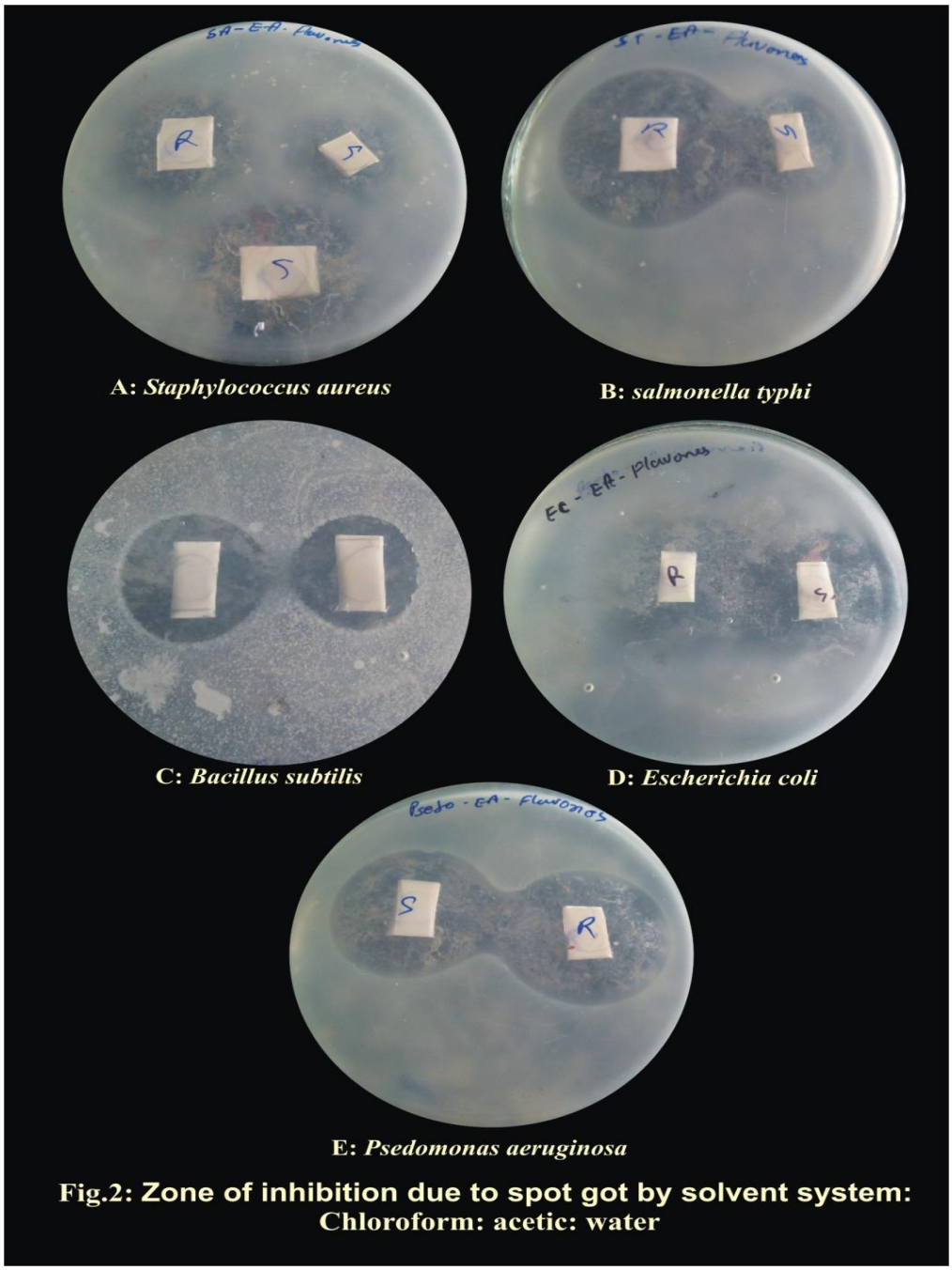

Fig.2: Zone of inhibition due to spot got by solvent system: Chloroform: acetic: water

DISCUSSION AND CONCLUSION

Antibiotic provide the main basis for the treatment of bacterial infection. The high genetic variability of microorganisms enables them to rapidly evade action of antibiotic by developing antibiotic resistance. Thus, there has been a continuing search for new and more antibiotic. In present invigation the ethyl acetate extract of stem and root of *Enicostemma littorale* were evaluated for its antibacterial activity against important pathogenic organisms. Ethyl acetate extract of *Enicostemma littorale* blume of various part give antimicrobial activity against pathogenic microorganisum. Both in the stem and root, ethyl acetate extract found to be more effective against all the microorganisms. The highest antimicrobial activity of root extract show against *salmonella typhi* (20 mm) and stem extract against *staphylococcus aureus* (12 mm) due to the various phytochemicals present in extract. The phytochemical analysis and TLC report revealed that the phytochemical responsible for antimicrobial activity. The Bio-autography method was use for detection of various active components. Specific solvent systems were used for specific components. This extract was undergone for TLC and spots were identified in the solvent systems i.e. n-butanol: Acetic acid: Water, Chloroform: Acetic acid: Water, Chloroform: Ethyl acetate for

Flavonoid molecules; Chloroform: Ethyl acetate: Methanol for Coumarin molecules; Ethyl acetate: chloroform: water for alkaloid molecules; Methanol: water for phenolic compounds. The spots got by the solvent system: n-butanol: Acetic acid: Water, Chloroform: Acetic acid: Water that give inhibition zone against all microorganisms and this solvent system used for flvonoid and flavones. So, the antibacterial components in extract were flavonoid and flavones.

ACKNOWLEDGEMENT

The authors thank the coordinator, Department of Biotechnology, VNSGU, Surat, India, for providing the necessary facilities to carry out the work and constant encouragement during study.

REFERENCE

[1] Abirami P, Gomathinayagam M. A Review on *Enicostemma littorale*, Pharmacologyonline 2011; 1: 75-83.
[2] Agharkar SP. Medicinal Plants of Bombay Presidency, Scientific Publication, Jodhpur, India, 1991.
[3] Annonymous. Indian Pharmacopoeia. vol. 2.3rd Ed. Govt. of India, Ministry of Health, Controller of Publications, New Delhi, India, 1986.
[4] Bhandari CR. Vanaushadhi Chandrodaya (Hindi), Bhanpura, Indore, Vol. II: 549, 1970.
[5] Chopra RN et al. Glossary of Indian Medicinal Plants, PID, CSIR, New Delhi, 1956.
[6] Matthew KM. The flora of Tamilnadu Carnatic, The Rapinet Herbarium St. Joseph's College, Tiruchirapalli-620002, India Vol. II, 1982, pp- 973.
[7] Theodore Cooke, Flora of Presidency of Bombay, Botanical Survey of India, Calcutta 1967.
[8] Vaidya BG. Some Controversial drugs of Indian Medicine, V. J. Res. Indian Med. 1965; 7(2):45.
[9] Vyas DS et al. Preliminary studies on anti-diabetic properties of. *E. littorale*, Jour.RS.Ind. Med.Yoga and Homeo. 1979; 14(3):63-65.
[10] Leelaprakash G, Mohandas S. Antimicrobial activity and Phyatochemical screening of Methanol extract of *enicostemma axillare*, International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4 (1):. 342-348.
[11] Irena M. Bioautography detection in thin-layer chromatography, Journal of Chromatography Anchroma 2012; 5(1): 708.
[12] Chakraborty DD et al. Phytochemical evaluation and TLC protocol of various extracts of *Bombax ceiba* Linn, International Journal of Pharmaceutical Sciences and Research 2010; 1(8): 66–73.
[13] Gopal TK et al. Chamundeeswari R.S., Saidulu A., *In vitro* antifungal activity of various extracts of *Enicostemma littorale*. J Biotech Biother 2011; 1:2.