International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.2, pp 1241-1245, April-June 2010

Comparative *In vitro* Antimicrobial Activity Studies of *Sida rhombifolia* Linn Fruit Extracts

Rashmi Ranjan Sarangi*, Uma Shankar Mishra, Prasanta Kumar Choudhury

Royal College of Pharmacy and Health Sciences, Andhapasara Road, Berhampur, (District-Ganjam), Orissa, India-760 002

Corres.author: sarangi_ranjan@yahoo.co.in
Phone- +91 680 2260024,Fax- +91 680 2260025

ABSTRACT: The present research work is designed to investigate the *in vitro* anti-bacterial activity of fruit extracts of *Sida rhombifolia* L. The Petroleum ether (C_2H_5-O- C_2H_5), chloroform ($CHCl_3$) and methanol (CH_3OH) fruit extracts of *Sida rhombifolia* L. were evaluated for antibacterial activity against different species of bacterial strains by detecting minimum inhibitory concentration and zone of inhibition.. The minimum inhibitory concentration values were compared with control and zone of inhibitions were compared with standard ciprofloxacin. The test materials were found to have significant in vitro antibacterial activities, against most of the test bacteria. The minimum inhibitory concentration (MIC) value for Methanolic extract was found to be within100µg/ml. The phytochemical screening indicates the presence of alkaloids, flavonoids, and reducing sugars in methanolic extract of *Sida rhombifolia* L.
KEY WORDS: S*ida rhombifolia*, antibacterial activity, phytochemical screening.

INTRODUCTION

Indian climatic condition is rich in growing various plants having medicinal property. So Indian biodiversity has excellent research and development opportunity. The wider acceptability of herbal therapeutics by the society in relation to modern drugs in another driving force for rapidly ongoing research activities in the field of alternate medical systems. As a step in this direction we focused our attention on one of the selected medicinal plants i.e. *Sida rhombifolia.* Literature survey revealed that the plant *Sida rhombifolia* belonging to family *Malvaceae* possess insecticidal and antimicrobial[1] properties. Malvaceae is a cosmopolitan family of herbs, shrubs and trees. Modern research carried out on the Malvaceae plants revealed that most of the plants belonging to this family are medicinally important as they contain biologically active compounds. *Sida rohmbifolia* locally known as Dholabadianla or Nalobadianla in the dist Ganjam of Orissa, India belongs to the family Malvaceae[2]. It has considerable reputation for its medicinal value in traditional medicine.

The plant is much used for poulticing ulcers, boils, swellings, broken bones, cuts, herpes and styles and for a skin application in chicken pox[3]. The roots and stems are useful in fever, heart disease, piles and all kinds of inflammation[4]. An infusion of the root is given in dysentery [5]. It is applied to the abdomen for abdominal complaints[6]. The plant is also useful in tuberculosis[7,8]. Leaves are used as a diuretic[8] and also in treatment of skin rashes[9]. Stem is also employed as edemulescent and emollient[10]. Traditionally the plant was used for urinary tract infections and infected wounds[11]. Although *Sida rhombifolia* is locally used for the above conditions, no antimicrobial study of fruit extracts of this plant has previously been reported. As a part of continuing search for novel antimicrobial principles from medicinal plants of Orissa, India, *Sida rhombifolia* fruit has been selected for studies and herein the results of *in vitro* antimicrobial investigations reported.

MATERIALS AND METHODS

Plant material

The plant was identified by the Department of Pharmacognosy, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India (Authenticated identification No. BIT/ OS/ 1102/ 2008-09). After authentication, fresh fruits were collected in bulk from young matured plants at the rural belt of Ganjam district of Orissa in the month of august. The fruits were washed, shade dried and milled in to coarse powder by a mechanical grinder. The powder materials were passed through sieve number 40 and used for further studies.

Preparation of extract

The dried fruits powder were successively extracted in soxhlet apparatus by using different solvents (Petroleum Ether, Chloroform and Methanol) with increasing order of polarity in the ratio of drug to solvent (1:8) for 72 hours. . Each extracts were concentrated at reduced pressure using rotary evaporator and further subjected for antibacterial screening. The type and extractive yield of different extracts of *S. rhombifolia* fruits were observed and result of such observation are tabulated in Table 1.

Preparation of the tested organisms

The lyophilized forms of different strains of microorganisms like *Bacillus licheniformis* (MTCC 429), *Escherichia coli* (MTCC 40), *Proteus vulgaris* (MTCC 426), *Pseudomonas aeruginosa* (MTCC 424), *Shigella flexneri* (MTCC 1457), *Bacillus subtilis* (MTCC441), S*taphylococcus aureus* (MTCC 87), S*taphylococcus epidermidis* (MTCC 2639) were obtained from the Microbial Type Culture Collection and Gene bank (MTCC), Chandigarh, India. The bacterial cultures were maintained on Mueller-Hinton Agar (MHA) and were subcultured in the microbiology laboratory of the Royal college of Pharmacy and Health Sciences, Berhampur, Orissa, India. The average number of viable of organisms per ml of the stock suspensions was determined by means of the surface viable counting technique[12]. About (10^8-10^9) colony-forming units per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained

Inoculation:

One loopful of an overnight grown nutrient broth culture of each test organism served as the inoculums for such antimicrobial activity determination. The average size of inoculums was about 10^6 cells contained in 3mm diameter of standard loop.[13]

Determination of the minimum inhibitory concentration (MIC)

Nutrient agar medium (250ml) was prepared and sterilized. Exactly 29 ml of media was dispersed in each of the 8 conical flasks, plugged with cotton and autoclaved. A Stock solution of *Sida rhombifolia* extract of 9mg/ml in 1% di-methyl sulphoxide (DMSO) was prepared. Measured quantities of the stock solution of extract were poured to the molten nutrient agar media to prepare concentration of 50, 100, 200 and 300µg/ml and then poured in Petri dishes. The Petri dishes were marked accordingly. One sterile nutrient agar plate without extract but with equal volume of the solvent served as the control plate. These plates were refrigerated overnight for uniform diffusion of the extract throughout the media. The plates were dried at 37°C by keeping them in the incubator. One loopful (diameter-3mm) of an overnight grown peptone water culture of each test organism was placed in petridish marked by the checker board technique. The spot inoculated plate was incubated at 37ºC for 24 hours and the MIC value obtained[14, 15 & 16]. The experiment was repeated in triplicate and average values were disclosed in the Table 2, 3 and Table 4.

Determination of zone of inhibition

For the determination of zone of inhibition, pure ciprofloxacin was taken as a standard antibiotic for comparison of the results. Two sets of two dilutions (100 and 200 µg/ml) of *Sida rhombifolia* fruit extract and ciprofloxacin (100 and 200 µg/ml) were prepared in double distilled water in Mc Cartney bottles. Sterile nutrient agar plates were prepared and incubated at 37ºC for 24hrs to check any sort of contamination. Two sterile filter paper discs (Whatmann no.1) of 6mm diameter were soaked in two different dilutions of crude extract and placed in appropriate position on the surface of the flooded plate, marked as quadrants at the back of the Petri dishes. The Petri dishes were incubated at 37 ºC for 24 hrs and the diameter of the zone of inhibition were measured in mm. Similar procedure were adopted for the pure ciprofloxacin and the corresponding zone diameter were compared accordingly[17]. The experiment was repeated in triplicate and average values were written in the Table 5, 6& 7.

Phytochemical analysis

Phytochemical analysis ware carried out in all the evaporated solvent extracts by using standard procedure for detection of phytoconstituents[18, 19].

RESULTS AND DISCUSSION

The results regarding the antibacterial activity of the different solvent extracts prepared from fruits of *S. rhombifolia* are indicated in Table 2, 3 and 4. The minimum inhibitory concentration of methanolic extract against most of the test bacteria were within 100µg/ml. Of the different extracts, the methanol extract displayed the highest antibacterial activity, as was evidenced by it displaying the highest mean zone of inhibition against maximum of the test bacteria. The antibacterial activity found to increases in the different fruits extracts in order of petroleum ether extracts, chloroform extracts and methanol extracts.

. A review of the literature revealed that information on the antibacterial potential of *S. rhombifolia* fruit is lacking. None of the earlier reports have demonstrated or reported the antibacterial potential of *S. rhombifolia* fruits. This report therefore is the first to demonstrate the antibacterial activity of the fruits of this plant. Preliminary phytochemical analysis of the methanol extract of fruits of *S. rhombifolia* revealed the presence of tannins, phenolics and flavonoids. Notably, both tannin and phenolics have been reported to possess antibacterial activity[20, 21]. The above antibacterial activity of *S. rhombifolia* fruit may be due to the combined or individual effect of the present phytoconstituents, which can be further confirmed by the extensive studies.

Table 1 Types and % yield of different extracts of *S. rhombifolia* fruits

SI. No.	Plant Part	Solvent Used for extraction	Colour of extract	Physical Appearance of the extract	% yield (W/W)
1	Fruits	Petroleum Ether	Blackish	Oily sticky mass	2.02
2	Fruits	Chloroform	Greenish Black	Solid sticky mass	1.03
3	Fruits	Methanol	Reddish black	Solid sticky mass	6.13

Table 2 Determination of M.I.C of the Petroleum Ether Extract of *Sida Rhombifolia* L.Fruit

Sl.No.	Name of the Bacteria	Concentration of extract in nutrient agar (µg/ml)				
		0	50	100	200	300
1	*Bacillus licheniformis*(MTCC 429)	+	+	+	+	_
2	*E.coli* (MTCC 40)	+	+	+	+	_
3	*Proteus vulgaris*(MTCC 426)	+	+	+	+	_
4	*Pseudomonas aeruginosa*(MTCC 424)	+	+	+	_	_
5	*Shigella flexneri*(MTCC 1457)	+	+	+	_	_
6	*Bacillus subtilis*(MTCC441)	+	+	+	+	_
7	*Staphylococcus aureus*(MTCC 87)	+	+	+	+	_
8	*Staphylococcus epidermidis*(MTCC 2639)	+	+	+	+	_

'O' = Control, '+' = Growth, '_' = No Growth

Table 3 Determination of M.I.C of the Chloroform Extract of *Sida Rhombifolia* L. Fruit

Sl.No.	Name of the Bacteria	Conc. of extract in nutrient agar (µg/ml)				
		0	50	100	200	300
1	*Bacillus licheniformis*(MTCC 429)	+	+	+	_	_
2	*E.coli* (MTCC 40)	+	+	+	_	_
3	*Proteus vulgaris*(MTCC 426)	+	+	+	_	_
4	*Pseudomonas aeruginosa*(MTCC 424)	+	_	_	_	_
5	*Shigella flexneri*(MTCC 1457)	+	+	+	_	_
6	*Bacillus subtilis*(MTCC441)	+	+	+	_	_
7	*Staphylococcus aureus*(MTCC 87)	+	+	+	_	_
8	*Staphylococcus epidermidis*(MTCC 2639)	+	+	_	_	_

'O' = Control, '+' = Growth, '_' = No Growth

Table 4 Determination of M.I.C of the Methanolic Extract of *Sida Rhombifolia* L. Fruit

Sl.No.	Name of the Bacteria	Conc. of extract in nutrient agar (µg/ml)				
		0	50	100	200	300
1	*Bacillus licheniformis*(MTCC 429)	+	+	_	_	_
2	*E.coli* (MTCC 40)	+	+	+	_	_
3	*Proteus vulgaris*(MTCC 426)	+	+	_	_	_
4	*Pseudomonas aeruginosa*(MTCC 424)	+	+	_	_	_
5	*Shigella flexneri*(MTCC 1457)	+	+	+	_	_
6	*Bacillus subtilis*(MTCC441)	+	+	+	_	_
7	*Staphylococcus aureus*(MTCC 87)	+	+	_	_	_
8	*Staphylococcus epidermidis*(MTCC 2639)	+	+	_	_	_

'O' = Control, '+' = Growth, '_' = No Growth

Table 5. Determinations of zone of inhibition of petroleum ether extract of *sida rhombofolia* L. fruit.

Sl no.	Name of the Bacteria	petroleum ether extract (µg/ml))		Ciprofloxacin (µg/ml)	
		100	200	100	200
1	*Bacillus licheniformis*(MTCC 429)	08	10	19	24
2	*E.coli* (MTCC 40)	09	11	21	24
3	*Proteus vulgaris*(MTCC 426)	11	12	19	23
4	*Pseudomonas aeruginosa*(MTCC 424)	13	18	18	20
5	*Shigella flexneri*(MTCC 1457)	12	22	20	24
6	*Bacillus subtilis*(MTCC441)	10	12	19	23
7	*Staphylococcus aureus*(MTCC 87)	11	14	20	24
8	*Staphylococcus epidermidis*(MTCC 2639)	09	13	19	22

Values are average of triplicates in mm.

Table 6. Determination of zone of inhibition of Chloroform extracts of *sida rhombofolia* L. fruit.

Sl no.	Name of the Bacteria	Chloroform extract µg/ml))		Ciprofloxacin (µg/ml)	
		100	200	100	200
1	*Bacillus licheniformis*(MTCC 429)	10	21	19	24
2	*E.coli* (MTCC 40)	09	21	21	24
3	*Proteus vulgaris*(MTCC 426)	11	20	19	23
4	*Pseudomonas aeruginosa*(MTCC 424)	16	18	18	20
5	*Shigella flexneri*(MTCC 1457)	12	21	20	24
6	*Bacillus subtilis*(MTCC441)	10	20	19	23
7	*Staphylococcus aureus*(MTCC 87)	11	21	20	24
8	*Staphylococcus epidermidis* (MTCC 2639)	17	19	19	22

Values are average of triplicates in mm.

Table 7. Determination of zone of inhibition of Methanolic extracts of *sida rhombofolia* L. fruit.

Sl no.	Name of the Bacteria	Methanol extract (µg/ml))		Ciprofloxacin (µg/ml)	
		100	200	100	200
1	*Bacillus licheniformis*(MTCC 429)	17	23	19	24
2	*E.coli* (MTCC 40)	14	21	21	24
3	*Proteus vulgaris*(MTCC 426)	17	21	19	23
4	*Pseudomonas aeruginosa*(MTCC 424)	16	18	18	20
5	*Shigella flexneri*(MTCC 1457)	12	21	20	24
6	*Bacillus subtilis*(MTCC441)	10	21	19	23
7	*Staphylococcus aureus*(MTCC 87)	19	21	20	24
8	*Staphylococcus epidermidis*(MTCC 2639)	17	21	19	22

Values are average of triplicates in mm.

ACKNOWLEDGMENTS

The authors extend gratitude to the management and Head of the institution Prof. (Dr.) P.N. Murthy, Royal college of Pharmacy and Health Sciences, Berhampur, Orissa for providing necessary facilities and setups for the study. The authors also would like thank the Microbial Type Culture Collection and Gene bank, Chandigarh, India for supply of test organisms. We are highly thankful to the Department of Pharmacognosy, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India for Authentication and identification of the plant.

REFERENCES

1. Akendengue B., Medicinal plants used by the fang traditional healers in Equatorial Guinea, J Ethnopharmacol, 1992, 37(2), 165-173
2. Kirtikar K. R. and Basu B. T., Indian Medicinal Plants,Vol. 1, India, 312
3. Lentz D.L., Medicinal and other economic plants of the paya of Honduras, Econ Bot, 1993, 47(4), 358-370.
4. Deka L., Majumdar R., Dutta A. M., Some ayurvedic important plants from district Kamrup (Assam), Ancient Sci Life, 1983, 3(2), 108-115
5. Karkar I., Holdsworth D., Phytomedicine of the Madang Province, Papua New Guinea Part I, Int J Crude Drug Res, 1984, 22(3), 111-119.
6. Holdsworth D., Pilokos B., Lambes P., Traditional medicinal plants of New Ireland, Papua New Guinea, Int J Crude Drug Res, 1983, 21(4), 161-168.
7. Hedberg i., Hedbrerg O., Madati P. J., Mshigeni K. E., Mshiu E. N., Samuelsson G., Inventory of plants used in traditional medicine in Tanzania, Plants of the families Dilleniaceae-Opiliaceae, J Ethnopharmacol, 1983, 9(1), 105-127.
8. Amazonian ethnobotanical dictionary. Duke,ja: book (1994) pp. 181pp-. Book USA
9. Coee F. G., Anderson G. J., Ethnobotany of the garifuna of Eastern Nicaragua, Econ Bot, 1996, 50(1), 71-107.
10. Rivera D., Obon C., The ethnopharmacology of Madeira and Porto Santo Islands, A Review, J Ethnopharmacol, 1995, 46(2), 73-93.
11. Muanza D. N., Kim B. W., Euler K. L., Williams L., Antibacterial and antifungal activities of nine medicinal plants from Zaire, Int J Pharmacog, 1994, 32(4), 337-345.
12. Miles A.A. and Misra S.S., Estimation of bacterial power of blood, J.Hyg., 1938**,** 38, 732.
13. Anon, Pharmacopoeia of India, Government of India, New Delhi, Ministry of Health and Family welfare, 1985.
14. Mishra U.S., Behera S.R., Murthy P.N., Kumar Manish, Kumar D., Antibacterial and analgesic effects of the leaves of *Dichrostachys cinerea*, International Journal Of Pharmacy And Pharmaceutical Sciences, 2009, 1(2),Oct-Dec.
15. Panda B.R., Mohanta S. R., Mishra U. S., Kar S., Panda B. K. and Chakrabarty P., Antibacterial activity of the leaves of *Cocculus hirsutus*, Indian Drugs, 2007, 44(2), 108-110.
16. Mishra U. S., Dutta N. K., Majumdar K., Mahapatro S. K., Chakraborty P. and Dastidar S. G., Anti salmonella activity of flavone from *Butea froundosa* bark in mice, Opem, 2008, 4, 339-348.
17. Mishra U. S., Kumari R., Mishra A., Murty P.N. and Das P., Antibacterial effect of stem bark of *Azadirachta Indica*, J.T.R.Chem., 2007, 14(2), 16-19
18. Kokate C.K., Practical Pharmacognosy, Vallabh Prakashan, 2001, 107-111.
19. Harborne J.B., Phytochemical methods: A guide to modern techniques of plant analysis. 3rd edition, 1998, Chapman and Hall Pub., London, UK.
20. Javanmardi J., Khalighi A., Bais H. P., Vivanco J. M., Chemical charecterization of Basil (Osmium basilium L.) found in local accession and used in traditional medicine, Iran. J. Agri. Food.Chem., 2002, 50, 5878-5883
21. Leven M., Vanden Berghe D. A., Mertens F., Victinck A., Lammens E., Screening of higher plants for biological activities/antimicrobial activity, Plant. Med., 1979, 36, 311-321.

*****