Advances in Natural and Applied Sciences, 3(2): 241-246, 2009
ISSN 1995-0772

ORIGINAL ARTICLE

Phytochemical Screening and Antimicrobial Assessment of Leaf Extracts of *Excoecaria Agallocha* L.: A Mangal Species of Bhitarkanika, Orissa, India

[1]Jayanta Kumar Patra, [1]Tapan Kumar Panigrahi, [1]Sakti kanta Rath, [2]Nabin Kumar Dhal and [1]Hrudyanath Thatoi

[1]P.G. Department of Biotechnology, North Orissa University, Baripada, Orissa, India
[2]Institute of Minerals and Materials Technology, Bhubaneswar, Orissa, India

Jayanta Kumar Patra, Tapan Kumar Panigrahi, Sakti kanta Rath, Nabin Kumar Dhal and Hrudyanath Thatoi: Phytochemical Screening and Antimicrobial Assessment of Leaf Extracts of *Excoecaria Agallocha* L.: a Mangal Species of Bhitarkanika, Orissa, India: *Adv. in Nat. Appl. Sci., 3(2): 241-246, 2009.*

ABSTRACT

Organic extracts of leaf of *Excoecaria agallocha* L., a mangal species from Bhitarkanika, Orissa, India were evaluated for antimicrobial activity along with their phytochemical screening. All the four crude extracts (chloroform, ethanol, methanol and aqueous) tested, showed significant antimicrobial activity against both gram positive and gram negative microorganisms with zone of inhibitions ranging between 1-21 mm. *Staphylococcus epidermidis* was the most susceptible strain to all the four extracts. Further evaluation of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) showed values ranging between 5-7 mg/ml and 5->7 mg/ml respectively. Preliminary phytochemical screening revealed the presence of tannins, alkaloids, steroids and anthroquinone glycosides in almost all extracts along with other active ingredients with the exception of flavonoids that was present in the aqueous extract alone.

Key words: Mangrove; *Excoecaria agallocha*; antimicrobial activity; phytochemicals

Introduction

Natural products have been used as a major tool for discovery of drugs of pharmaceutical importance. In recent years, antimicrobials derived from the plants have been receiving increasing attention, as the synthetic antibiotics have shown ineffectiveness against several pathogenic organisms, due to increasing drug resistance. Several workers have reported the usefulness of mangrove plants in traditional medicine (Premnathan *et al.*, 1992; Premnathan *et al.*, 1996; Kokpal *et al.*, 1990). However, very little is known about the antibacterial and phytochemical constituents of mangrove plants. Bhitarkanika wildlife sanctuary in the state of Orissa is one of the richest mangrove forests of India exhibiting maximum plant diversity. *Excoecaria agallocha* L. is a typical mangrove species found widely distributed throughout the sanctuary. The plant has many commercial uses like firewood, timber etc. Apart from this, the plant also provides many non-timber products such as tannin, fish poison and medicines for epilepsy, ulcers, hand and feet swelling, tooth ache etc (Bandaranayake, 2002; Miles *et al.*, 1997; Kirtikar and Basu, 1999; Jayaweera, 1980; Karalai *et al.*, 1994). There has been report on antimicrobial activity of the fatty acid methyl ester extracts of *Excoecaria agallocha* (Agoramoorthy *et al.*, 2007). However, there has been no report on crude extracts of *E. agallocha*. Hence, the present study was aimed at evaluating the antimicrobial properties and the major phytochemical constituents of different solvent extracts (chloroform, ethanol, methanol and aqueous) of leaf of *Excoecaria agallocha* L. with a view to assess their pharmacological potential.

Corresponding Author: Dr. H.N.Thatoi, P.G. Dept. of Biotechnology, North Orissa University, Baripada-757003, India.
E-mail: hn_thatoi@rediffmail.com

Materials and methods

Collection of Sample:

The Leaves of *Excoecaria agallocha* L. were collected from the mangrove forests of Bhitarkanika wildlife sanctuary which extends from 20° 30'-20° 50' N and 86° 30'- 87° 6' E. The specimen was identified at Department of Natural Products, Institute of Minerals and Materials Technology, Bhubaneswar, Orissa, India and a voucher specimen (No 10, 002) was deposited in the herbarium of Institute of Minerals and Materials Technology.

Preparation of Plant Extracts:

The leaves of the plant were shade dried for 15 days and then pulverized into fine powder using pestle and mortar. Twenty five gram of fine powder was added to a soxhlet apparatus along with a solvent (chloroform/ ethanol/ methanol and aqueous) for extraction of chemicals. The liquid extracts were evaporated to dryness by vacuum distillation and stored at 4°C for further analysis (Patra *et al.*, 2008). Percentage yield was calculated from the dry extract powder.

Antimicrobial Activity:
Microbial Strains:

The microorganisms used in the study include: – *Staphylococcus aureus (*MTCC 1144), *Shigella flexneri* (Lab isolate), *Bacillus licheniformis* (MTCC 7425), *Bacillus brevis* (MTCC 7404), *Vibrio cholerae* (MTCC 3904), *Pseudomonas aeruginosa* (MTCC 1034), *Streptococcus aureus* (Lab isolate), *Staphylococcus epidermidis* (MTCC 3615), *Bacillus subtilis* (MTCC 7164) and *E. coli* (MTCC 1089). The bacterial strains with MTCC number were obtained from Institute of Microbial Technology, Chandigarh and others were lab isolates. The organisms were maintained on nutrient agar (Hi Media, India) slopes at 4^0C and subcultured before use. Active cultures for experiments were prepared by transferring a loopfull of cells from stock cultures to test tubes of Muller-Hinton broth (MHB) that were incubated without agitation for 24 hours at 37^0C.

Antimicrobial Assay:

Agar cup plate method (Khalid *et al.*, 1999) was carried out to establish the antibacterial activity of all the four solvent extracts against the test pathogens. Nutrient agar (NA) plates were prepared as per manufacturer instructions. Overnight nutrient broth culture of the test organisms was seeded over the NA plates using sterile cotton swab so as to make lawn culture. Wells of 6 mm diameter were punched over the agar plates using sterile gel puncher (cork borer). The bottom of the well was sealed by pouring 10 – 20 µl (1 – 2 drops) of molten NA into the scooped well by the sterile micropipette. 200 µl (50 mg/ml and 25 mg/ml) of extract were poured into the wells. The plates were incubated at 37°C for 24 h. The zone of the clearance around each well after the incubation period, confirms the antimicrobial activity of the respective extract. Each experiment was carried out in triplicates. The clear zones formed around each well were measured and average diameter of the inhibition zone (excluding inhibition zone by DMSO (Dimethyl sulphoxide) was expressed in millimeter. It was used to determine the antibacterial activity of extracts. Minimal inhibitory concentration (MIC) was determined by tube dilution method (Doughari, 2006). Minimal inhibitory concentration and Minimal bacteriocidal concentration (MBC) was seen on those bacterial strains which showed zones of inhibition against the plant extracts. Ampicillin (10 µg/disc), Erythromycin (15 µg/disc), Kanamycin (30 µg/disc) and Gentamycin (10 µg/disc) were used as standards. The following formula was used for comparison of the antimicrobial activity of the sample with that of the standards (antimicrobial index).

$$\text{Antimicrobial index} = \frac{\text{Inhibition zone of sample}}{\text{Inhibition zone of the standard}} \times 100$$

Preliminary Phytochemical Analysis:

A qualitative phytochemical test to detect the presence of alkaloid, tannin, saponin, flavonoid, glycoside and phenol were carried out using standard procedures (Sofowara, 1993; Edeoga *et al.*, 2005; Kumar *et al.*, 2007).

Results and discussion

The antimicrobial activity of the leaf extracts (chloroform, ethanol, methanol and aqueous) of *Excoecaria agallocha* was assayed *in vitro* by agar well diffusion method against ten bacterial strains (Table-1). The aqueous extract was more effective against *Streptococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *E. coli* whereas it showed less antimicrobial activity against, *Bacillus brevis*, *Shigella flexneri*, *Bacillus licheniformis* and *Staphylococcus aureus* (Table –1). On the other hand ethanol extract and chloroform extract showed antimicrobial activity against all the strains except *Vibrio cholerae.* The methanol extract was more effective against, *Staphylococcus aureus* and *E. coli* (Table-1). MIC was determined by tube dilution method. MIC values ranged from 5 to 7 mg/ml and MBC values varied from 5.5 mg/ml to values greater than 7 mg/ml (Table -2) so as to exhibit their growth to know the efficacy of the plant extracts. The significant antimicrobial activity of the active plant extracts was compared with standard antibiotics; Gentamycin (10 µg/disc), Ampicillin (10 µg/disc), Kanamycin (30 µg/disc), Erythromycin (15 µg/disc) (Table–3) and their corresponding antimicrobial index were calculated (Table-4).

Preliminary phytochemical screening of the leaf extracts showed that the solvent extracts contain most of the phytochemicals (Table-5) like alkaloids, steroids, tannins, saponins, ascorbic acid etc.

Table 1: Inhibition zone in mm (excluding the zone of inhibition by DMSO) of solvent extracts of *E. agallocha* against pathogenic microorganisms

Strains	Chloroform (50 mg/ml)	Ethanol (50 mg/ml)	Methanol (25 mg/ml)	Aqueous (50 mg/ml)
Staphylococcus aureus	11±0	11±0	10±1	9±0
Shigella flexneri	15±1.1	18±0.44	15±0.44	11±0.21
Bacillus licheniformis	19±0.44	21±0.33	4±0	ND
Bacillus brevis	10±0.44	7±1	3±0	2±0.04
Vibrio cholerae	ND	ND	ND	15±0.44
Pseudomonas aeruginosa	8±1	12±0.13	8±0.33	5±0.1
Streptococcus aureus	16±0.34	17±0.44	ND	17±0.33
Staphylococcus epidermidis	17±0.44	19±0.33	14±0.54	14±0.33
Bacillus subtilis	16±0.44	16±0.44	15±0.25	14±0.15
Escherichia coli	10±0	16±1	20±1.11	15±0.24

(Key: "ND"- Not detected, All data are expressed in MEAN±SD)

Table 2: Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) in mg/ml.

Strains	Chloroform extract		Ethanol extract		Methanol extract		Aqueous extract	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus	6	6.5	6.5	7	7	>7	7	>7
Shigella flexneri	7	>7	7	>7	7	>7	7	>7
Bacillus licheniformis	7	>7	7	>7	6	6.5	7	>7
Bacillus brevis	7	>7	7	>7	6.5	7	7	>7
Vibrio cholerae	-	-	-	-	-	-	7	>7
Pseudomonas aeruginosa	7	>7	7	>7	5	5.5	7	>7
Streptococcus aureus	6.5	7	7	>7	5	5.5	5	5.5
Staphylococcus epidermidis	6	6.5	7	>7	7	>7	7	>7
Bacillus subtilis	7	>7	7	>7	7	>7	6	6.5
Escherichia coli	7	>7	7	>7	7	>7	7	>7

(Key: "**-**" - Not detected, ">'- value is greater than)

Table 3: Inhibition zone in mm by standard antibiotics.

Strains	Ampicillin (10 µg/disc)	Erythromycin (15 µg /disc)	Kanamycin (30 µg /disc)	Gentamycin (10 µg /disc)
Staphylococcus aureus	25±0.44	24±0.44	-	26±0.33
Shigella flexneri	39±1.11	-	-	23±0.44
Bacillus licheniformis	40±1.21	-	-	20±0
Bacillus brevis	39±1.11	-	11±0.25	22±1
Vibrio cholerae	39±1.11	-	-	17±0.33
Pseudomonas aeruginosa	-	-	-	18±0.44
Streptococcus aureus	-	-	-	-
Staphylococcus epidermidis	ND	ND	7±0	20±0.33
Bacillus subtilis	10±0.15	18±0.44	20±0	19±0.44
Escherichia coli	8±0.05	14±0.33	18±0.44	18±1

(Key: "ND" - Not detected, '-' No inhibition zone, All data are expressed in MEAN±SD)

Table 4: Antimicrobial index of extracts of *E. agallocha* against standard antibiotics

	Ampicillin (10 µg/disc)				Erythromycin (15 µg /disc)			
Strains	Chloroform (50 mg/ml)	Ethanol (50 mg/ml)	Methanol (25 mg/ml)	Aqueous (50 mg/ml)	Chloroform (50 mg/ml)	Ethanol (50 mg/ml)	Methanol (25 mg/ml)	Aqueous (50 mg/ml)
Staphylococcus aureus	44	44	40	36	45.83	45.83	41.67	37.5
Shigella flexneri	38.46	46.15	38.46	28.2	100	100	100	100
Bacillus licheniformis	47.5	52.50	10	ND	100	100	100	ND
Bacillus brevis	25.64	17.95	57.69	5.13	100	100	100	100
Vibrio cholerae	ND	ND	ND	38.46	ND	ND	ND	100
Pseudomonas aeruginosa	100	100	100	100	100	100	100	100
Streptococcus aureus	100	100	ND	100	100	100	ND	100
Staphylococcus epidermidis	ND	ND	ND	ND	ND	ND	ND	ND
Bacillus subtilis	160	160	150	140	88.88	88.88	83.33	77.77
E. coli	125	200	250	187.5	71.43	114.29	142.857	107.14

(Key: "ND"- Not detected, all data are expressed in percentage)

Table 4: Continue

	Kanamycin (30 µg /disc)				Gentamycin (10 µg /disc)			
Strains	Chloroform (50 mg/ml)	Ethanol (50 mg/ml)	Methanol (25 mg/ml)	Aqueous (50 mg/ml)	Chloroform (50 mg/ml)	Ethanol (50 mg/ml)	Methanol (25 mg/ml)	Aqueous (50 mg/ml)
Staphylococcus aureus	100	100	100	100	42.31	42.31	38.46	34.62
Shigella flexneri	100	100	100	100	65.22	78.36	65.22	47.83
Bacillus licheniformis	100	100	100	ND	95	105	20	ND
Bacillus brevis	90.90	63.63	27.27	18.18	45.46	31.81	13.64	9.09
Vibrio cholerae	ND	ND	ND	100	ND	ND	ND	88.24
Pseudomonas aeruginosa	100	100	100	100	44.44	66.66	44.44	27.77
Streptococcus aureus	100	100	ND	100	100	100	ND	100
Staphylococcus epidermidis	242.86	271.43	200	200	85	95	70	70
Bacillus subtilis	80	80	75	70	84.21	84.21	78.95	73.65
E. coli	55.55	88.88	111.11	83.33	55.55	88.88	111.11	83.33

(Key: "ND"- Not detected, all data are expressed in percentage)

Table 5: Phytochemical analysis of leaf extracts of *Exocoecaria agallocha.*

Test group	Chloroform extract	Ethanol extract	Methanol extract	Aqueous extract
Alkaloid	+	+	+	+
Protein and amino acids	+	-	+	+
Carbohydrates	+	+	+	+
Cardiac glycosides	+	+	+	+
Anthroquinone glycosides	+	+	+	+
Tannin and Phenolic compound	+	+	+	-
Steroids and sterols	+	-	-	+
Saponins	-	-	-	+
Flavonoids	-	-	-	+

(Key: '+' = Present, '-' = Absent).

Discussion:

The studied plant was most active against gram-negative bacteria than gram-positive bacteria. Incase of solution with low activity, a large concentration or volume is needed. In general gram-positive bacteria are considered more sensitive than gram-negative bacteria towards different antimicrobial compounds because of the difference in the structure of their cell walls (Scherrer and Gerhardt, 1971) but our result showed that the extracts are effective against both gram-positive and gram-negative bacteria. Earlier report suggests (Agoramoorthy *et al.,* 2007) significant antimicrobial activity of the fatty acid methyl ester extracts of the leaves of *E. agallocha*, which corroborates to our findings. Antimicrobial properties of substances are desirable tools in the control of undesirable microorganisms especially in the treatment of infections and in food spoilage. The active constituents of plants usually interfere with growth and metabolism of microorganisms in a negative manner (Aboaba *et al.,* 2006).

The organic solvent extracts showed presence of many phytochemicals which is summarized in Table -5. The presence of such phytochemicals may be correlated with the fact that solvent extracts showed maximum activity against the bacterial strains. Several phenolic compounds like tannins found in plant cells are potent inhibitors of hydrolytic enzymes used by plant pathogens. Other compounds like saponins also have antifungal properties. Many plants release phenolic compounds that are toxic to microbial pathogens (Aboaba *et al.,* 2006). Flavonoids on the other hand are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity (Okwu, 2004). Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics include formation of foams in aqueous

solution, haemolytic activity, cholesterol binding properties and bitterness (Okwu, 2004). Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for analgesic, antispasmodic and antibacterial effect (Okwu, 2004). It has also been shown that saponins are active antifungal agents (Sodipo *et al.*, 1991). Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable to them (Sodipo *et al.*, 1991). Steroids and phlobatannins were found to be present in all the plants. It has been found that the investigated plant contained steroidal compounds. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones (Okwu, 2001). Hence the compound detected may be responsible for antimicrobial activity of plant extracts.

Conclusion:

The potential for developing antimicrobial agents from mangrove species appears rewarding, as it may lead to development of a phytomedicine to act against pathogenic microbes. The result of the study shows that *Excoecaria agallocha* posses a broad spectrum of activity against a panel of microorganisms responsible for most common microbial diseases. Continued further exploration of plant-derived antimicrobials is needed today. Further research is necessary to determine the antibacterial compounds from *Excoecaria agallocha*, which will give a platform for further phytochemical and pharmaceutical applications.

Acknowledgements

Authors are grateful to the Vice Chancellor of North Orissa University, Baripada, for providing laboratory facilities. The help and cooperation of field staff and DFO, Rajnagar is thankfully acknowledged.

References

Aboaba, O.O., S.I. Smith and F.O. Olude, 2006. Antimicrobial effect of edible plant extract on *Escherichia coli* 0157: H7. Pakistan Journal of Nutrition., 5(4): 325-327.

Agoramoorthy, G., M. Chandrasekaran, V. Venkatesalu and M.J. Hsu, 2007. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India, Brazilian Journal of Microbiology, 38: 739-742.

Bandaranayake, W.M., 2002. Bioactivities, bioactive compounds and chemical constituent of mangrove plant. Wetland Ecology and Management, 10(32): 421-452.

Doughari, J.H., 2006. Antimicrobial activity of *Tamarindus indica* Linn. Tropical Journal of Pharmaceutical Research, 5(2): 597-603.

Edeoga, H.O., D.E. Okwa and B.O. Mbaebie, 2005. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology, 4(7): 685-688.

Jayaweera, D.M.A., 1980. Medicinal plants used in Ceylon. Journal of the Natural Science Council of Srilanka, 2: 214-215.

Karalai, C., P. Wiriyachitra, H.J. Opferkuch and E. Hecker, 1994. Cryptic and free skin irritants of the daphnane and tigliane types in latex of *Excoecaria agallocha.* Planta Medica, 60: 351-355.

Khalid, F., R. Siddiqi and N. Mojgani, 1999. Detection and characterization of a heat stable bacteriocin (Lactocin LC-09) produced by a clinical isolate of *Lactobacilli*. Medical Journal of Islamic Academy of Sciences, 12(3): 67-71.

Kirtikar, K.R. and B.D. Basu, 1999. Indian medicinal plants. Lalit Mohan Basu Publishers, Allahabad, India.

Kokpal, V., D.H. Miles, A.M. Payne and V. Chittarwong, 1990. Chemical constituents and bioactive compounds from mangrove plants. Studies in Natural products Chemistry, 7: 175-199.

Kumar, A.R., K.M. Subburathinam and G. Prabakar, 2007. Phytochemical screening of selected medicinal plants of Asclepiadaceae family. Asian Journal of Microbiology, Biotechnolology and Environmental Sciences, 9(1): 177-180.

Miles, D.H., U. Kokpol, V. Chittawong, S. Tip-Pyang, K. Tunsuwan and C. Nguyen, 1997. Mangrove Forests-The importance of conservation as a bioresource for ecosystem diversity and utilization as a source of chemical constituents with potential medicinal and agricultural values. Invited lecture presented at the International Conference on Biodiversity and Bioresources: Conservation and Utilization.

Okwu, D.E., 2001. Evaluation of the chemical composition of indigenous spices and flavouring agents. Global Journal of Pure and Applied Sciences, 7: 455-459.

Okwu, D.E., 2004. Phytochemicals and vitamin content of indigenous spices of south Eastern Nigeria. Journal of Sustainable Agriculture and the Environment, 6(1): 30- 37.

Patra, J.K., S.K. Rath, K. Jena, V.K. Rathod and H.N. Thatoi, 2008. Evaluation of antioxidant and antimicrobial activity of seaweed *(Sargassum sp.)* extract: A study on inhibition of Glutathione-S-transferase activity. Turkish Journal of Biology, 32: 119-125.

Premnathan, M., K. Chandra, S.K. Bajpai and K. Kathiresan, 1992. A survey of some Indian marine plants for antiviral activity. Botanica Marina., 35: 321-324.

Premnathan, M., H. Nakashima, K. Kathiresan, N. Rajendra and N. Yamamoto, 1996. In Vitro antihuman immunodeficiency virus activity of mangrove plants. Indian Journal of Medicinal Research, 103: 278-281.

Scherrer, R. and P. Gerhardt, 1971. Molecular sieving by the *Bacillus megatrium* cell wall and Protoplast. Journal of Bacteriology, 107: 718-735.

Sodipo, O.A., MA. Akanji, F.B. Kolawole and A.A. Odutuga, 1991. Saponin is the active antifungal principle in *Garcinia kola*, heckle seed. Bioscience Research Communications., 3: 171-175.

Sofowara, A.E., 1993. Medicinal Plants and traditional medicine in Africa. Spectrum books Limited, Ibadan, Nigeria, pp: 288-289.