

CORRELATION BETWEEN PHYTOCHEMICAL SCREENING, ANTIBACTERIAL AND ANTHELMINTIC ACTIVITIES OF *ELAEOCARPUS SERRATUS*

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ABSTRACT

The petroleum ether, benzene, chloroform and acetone extracts of *Elaeocarpus serratus* were prepared by following Soxhlet method of extraction. Subsequently, phytochemical screening of the extracts was performed by following standard methods. While from the chloroform extract, alkaloids and anthraquinone glycosides were found, flavonoids and anthraquinone glycosides were present in the acetone extract. In addition, from petroleum ether extract, flavonoids were detected. On the other hand, anthraquinone glycosides were found in the benzene extract. None of the extracts showed any antibacterial and anthelmintic activities.

KEYWORDS: *Elaeocarpus serratus*, phytochemical screening, antibacterial activity, anthelmintic activity.

INTRODUCTION

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind¹. With estimated 2,50,000 - 5,00,000 plant species only a small percentage has been investigated phytochemically which indicates the vast potential of higher plants as a source for new drugs². Medicinal plants are of great value to mankind and society in general³. A wide range of medicinal plant parts like root, stem, flower, fruit, twigs exudates and modified plant organs is used for extract as raw drugs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries⁴. Plants synthesize many compounds with complex molecular structures by a secondary metabolism. Some of these compounds and their derivatives are found to provide a rich source of botanicals, anthelmintics, antibacterials and insecticides^{5, 6}. Knowledge of the chemical constituents of plants is very important, not only for the discovery of drugs and other therapeutic agents, but also in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances⁷. Since many infectious microorganisms are resistant to synthetic drug, an alternative therapy is very much needed to attract the attention of many researchers all over the world⁸. Random screening as tool in discovering new biologically active molecules from medicinal plants has been most productive in the area of antibiotics and may give a new source of antimicrobial agents with possibly novel mechanisms of action^{2, 9 -12}. Moreover, antimicrobials of plant origin are not associated with many side effects¹³. Similarly, the importance of some plant extracts having anthelmintic activity cannot be ignored. The active components of herbal remedies can be combined with many inactive substances, increasing the safety and efficiency of the plant than that of its isolated and pure active components¹⁴. The phytoconstituents such as alkaloids, flavonoids, isoflavonoids, tannins, coumarins, glycosides, terpenes and phenolic compounds are responsible for antibacterial activity⁵. Similarly some investigators have mentioned the importance of some phytochemicals like alkaloids, flavonoids, tannins, terpenoids and glycosides in imparting anthelmintic activity^{6, 15-16}. Although hundreds of plant species have been tested for antimicrobial and anthelmintic properties, the vast majority of them have not been adequately explored¹⁷. Considering the vast potentiality of plants as sources for antimicrobial and anthelmintic

agents, a systematic investigation was undertaken to screen the plant *Elaeocarpus serratus* (*E. serratus*). *E. serratus* Linn. (*Elaeocarpaceae*) is locally known as Singhali Jolpai. It is a medium to big sized tree with simple leaves, small flowers in axillaries and one seeded drupes. It is used for its edible fruits and timber. Commonly called Ceylon-olive *E. serratus* is widely distributed in the Chittagong region and many other areas of Bangladesh, India, Srilanka, Pakistan, Thailand, and Madagascar. The plant is also found in East Africa as well as the subtropical and tropical Asia and tropical Australia. It is used as diuretic and as a cardiovascular stimulant. The leaves are used in the treatment of rheumatism and as antidote to poison, while the fruits are locally prescribed for the treatment of diarrhea and dysentery. The fruit juice of *E. serratus* is given for stimulating secretions from taste buds thus increasing appetite in patients. Leaves contain myrceicitrin, myrecetin, mearnsenin and ellagic acid. Fruits contain tannin and large amount of plant acids¹⁸⁻²⁰. Although an extensive literature survey does not reveal the phytochemical studies or antimicrobial or anthelmintic activities of *E. serratus*, the present study was undertaken to investigate the preliminary phytochemical studies, antimicrobial and subsequently the anthelmintic activities. This was done because some of the phytoconstituents are found to contain antibacterial and anthelmintic activities.

MATERIALS AND METHODS

Plant Material

The leaves of the plant *E. serratus* were collected from Chhend, Rourkela during Nov-Dec 2010. The plant was identified properly by consulting Dr. Prativa Sahoo, Head of the department (Botany), Government Autonomous College, Rourkela, affiliated to Sambalpur University. Leaves were shed dried and powdered to 40-mesh size. Powdered leaf material was successively extracted by taking non polar to polar solvents like Petroleum ether, Benzene, Chloroform, Acetone in Soxhlet apparatus and was subjected for identification of various plant constituents.

Extraction of Plant Leaf material

Coarsely powdered dried leaves were successively extracted with the above mentioned solvents for 48 h each using Soxhlet apparatus. The extracts obtained were later kept for evaporation to remove the excessive solvents. Those extracts were filtered and dried in an oven at 40-50°C and were stored in a cool dry place for the analysis of preliminary phytochemicals. The extracts were then dissolved in their respective solvents for the phytochemical studies. For the determination of antibacterial and anthelmintic activities, the extracts were dissolved in Dimethyl Sulphoxide (DMSO)²¹⁻²².

Phytochemical Screening

Following tests were performed to detect the presence of different chemical groups in the extracts.

Test for alkaloids

Mayer's test: Two ml solution of the extract and 0.2ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Mayer's reagent was added. Yellow color precipitate indicated the presence of alkaloids.

Test for flavonoids

To 2ml of the test plant extract 2 drops of sodium hydroxide solution was added. A Golden reddish color indicated the presence of flavonoids.

Test for tannins

To 2ml of the test plant extract 2 drops of ferric chloride solution was added. A wooly brownish precipitate confirmed the presence of tannins.

Test for terpenoids

Five ml of each extract was mixed in 2ml of chloroform, and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids.

Test for Saponins

Frothing Test: Two ml of extract was shaken vigorously to observe the reaction if the frothing persist the presence of saponins was confirmed.

Test for Cardiac Glycosides

Keller-Kiliani test: Five ml of each extracts was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1ml of concentrated sulphuric acid. A brown ring of the interface indicated a deoxysugar characteristic of cardenolides.

Test for Anthraquinone Glycosides

Borntragers test: To 2ml of extract, 10ml of Benzene and 5ml of 10% ammonia solution were added. A reddish color confirmed the presence of the anthraquinone glycosides.

Test for Fixed oils and Fats

Spot test: Extract was pressed between two filter papers; the appearance of stains after drying indicated the presence of fixed oils and fats.

Test for Carbohydrates

Molisch's test: Two ml of the extract was treated with Molisch's reagent and concentrated sulphuric acid, the appearance of violet ring indicated the presence of carbohydrates.

Test for Proteins

Two ml of extract was first treated with concentrated nitric acid which resulted in white precipitate. The solution was then cooled and treated with 40% sodium hydroxide; appearance of orange precipitate confirmed the presence of proteins²³⁻²⁴.

Antibacterial Activity

The screening of the petroleum ether, benzene, chloroform, acetone extracts of the plant for *in vitro* antibacterial activity was performed using agar-well diffusion method as described by Schillinger et al²⁵. Three different concentrations (50, 100 and 200µg/ml) of each plant extract were prepared by dissolving the dried plant extracts in 1% DMSO. A volume of 10ml of sterile nutrient agar medium was inoculated with 0.1ml of fresh overnight culture of the indicator strains such as *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* (approximately 10⁷CFU/ml) and poured into Petri dishes. Wells of 6mm in diameter were punched in the agar medium of a Petri dish. Three wells were filled with 50µl of each of the three different concentrations of Petroleum ether extract. In other wells of the same plate, 50µl of tetracycline (30µg) as experimental positive control and DMSO (negative control) were delivered. The same process was repeated for benzene, chloroform and acetone extracts

respectively. In those plates both positive and negative controls were also used in addition to the extracts. After holding all the plates at room temperature for 2 h to allow diffusion of the extract into the agar, the plates were incubated at 37°C for 24 h. Then they were examined for inhibition of the bacterial lawn and the diameters of the inhibition zones were measured.

Anthelmintic Activity

The anthelmintic activity was evaluated on adult Indian earthworm, *Phaeritima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings²⁶. The extracts were first dissolved in 1ml of DMSO. Subsequently using the normal saline containing 1% acacia, three different concentrations (50mg/ml, 25mg/ml and 12.5mg/ml) of each extract were prepared. Solution of 15mg/ml concentration of the standard anthelmintic drug like Piperazine citrate as positive control was also prepared in normal saline containing 1% acacia. In our study DMSO and normal saline containing 1% acacia were used as negative controls. One milliliter of each concentration of all the extracts and Piperazine citrate was diluted to 10ml separately with normal saline containing 1% acacia and poured into Petri dishes. Moreover, 10ml each of DMSO and normal saline containing 1% acacia were taken in two different petridishes. Five groups of approximately equal size of earthworms, consisting of six in number in each group, were released into each petridish. The anthelmintic activity was evaluated by adopting the standard method of Manjunath et al (2006)²⁷. Observations were made for the paralysis time (PT) and subsequently for death time (DT). Paralysis was said to occur when the worms did not revive even after the application of normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors.

RESULTS

While from the chloroform extract, alkaloids and anthraquinone glycosides were found, flavonoids and anthraquinone glycosides were present in the acetone extract. In addition, from petroleum ether extract flavonoids were detected. On the other hand, anthraquinone glycosides were found in the benzene extract (Table1).

All the extracts were devoid of any antibacterial activity. But in case of positive control antibacterial activity was found (Table 2). Similarly, the extracts did not possess any anthelmintic activity. However, the positive control showed anthelmintic activity (Table3). Although several phytochemicals were present in some of the extracts, all the extracts did not show any antibacterial and anthelmintic activities.

DISCUSSION

From our study, it may be concluded that the extracts do not have any antibacterial and anthelmintic activities. However, different parts of the plant can be taken as another alternative for the study of antibacterial and anthelmintic activities. Studies of different extracts and some more tests can also be performed to evaluate their exact role as antibacterial or anthelmintic agents.

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REFERENCES

1. Thomson WAR. Medicines from the Earth. Maidenhead (UK): McGraw-Hill Book Co; 1978.
2. Mahesh B, Satish S. Antimicrobial Activity of some Important Medicinal plant against Plant and Human Pathogens. World J Agricul Sci 2008; 4(S): 839-843.
3. Hill AF. Economic Botany: A Textbook of Useful Plants and Plant Products. 2nd ed. New York: McGraw-Hill Book Company Inc; 1952.
4. Uniyal SK, Singh KN, Jamwal P, Lal B. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal. Western Himalaya J Ethnobiol Ethnomed 2006; 2: 1-14.

5. Simoes CMO, Schenckel EP, Gosman G, Mello JCP, Mentz LA, Perovick, PR. Farmacognosia: da planta ao medicamento. Santa Catarina: UFSC e UFRG; 1999.
6. Acharya S, Dash GK, Brahma DK, Chhetre RR. Preliminary phytochemical investigation and anthelmintic activity of *Acacia suma* (Roxb) barks. Int Res J Pharmacy 2011; 2(1):136-141.
7. Mojab F, Kamalinejad M, Ghaderi N, Vahidipour H. Phytochemical Screening of Some Iranian Plants. Iranian J Pharma Res 2003; 77-82.
8. Mohanan PV, Rao JM, Kutti MAS, Devi KS. Cytotoxicity of extracts of *Solanum trilobatum* and anticarcinogenic activity of sobatum. Biomedicine 1998; 18: 106-111.
9. Barbour EK, Al Sharif M, Sagherian VK, Habre AN, Talhouk RS, Talhouk SN. Screening of selected indigenous plants of Lebanon for antimicrobial activity. J Ethnopharmacol 2004; 93: 1-7.
10. Hamil FA, Apio S, Mubiru NK, Bukenya-Ziraba R, Mosango M, Maganyi OW, Soejarto DD. Traditional herbal drugs of Southern Uganda II: literature analysis and antimicrobial assays. J Ethnopharmacol 2003; 84: 57-78.
11. Machado TB, Pinto AV, Pinto MCFR, Leal ICR, Silva MG, Amaral ACF, Kuster RM, NettodosSantos KR. *In vitro* activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analogues, against methicillin-resistant *Staphylococcus aureus*. Int J Antimicrob 2003; 21: 279-284.
12. Motsei ML, Lindsey KL, Van Staden J, Jaeger AK. Screening of traditionally used South African plants for antifungal activity against *Candida albicans*. J Ethnopharmacol 2003; 86(2-3): 235-241.
13. Iwu MW, Duncan AR, Okunji CO. New antimicrobials of plant origin. In: Janick J, editor. Perspectives on New crops and New Uses. Alexandria: ASHS Press; 1999. p. 457-462.
14. Kamba AS, Hassan LG. Phytochemical screening and antimicrobial activities of *Euphorbia balsamifera* leaves stems and roots against some pathogenic microorganisms. African J Phar Pharmacol 2010; 4(9): 645-652.
15. Dash S, Das C, Sahoo DC. Phytochemical and anthelmintic screening of crude bark extracts of *Adenanthera pavonina*. Int J Comprehensive Pharmacy 2010; 2(10): 1-14.
16. Mali RG, Mahale NB. Evaluation of *Rhynchosia minima* (Linn) DC leaves for anthelmintic activity. Int J Pharm Sci Nanotechnol 2008; 1(2):191-194.
17. Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. Natural plant chemicals: Sources of Industrial and Medicinal materials. Science 1985; 228(4704):1154-60.
18. Sharker MDS, Shahid IJ. Assessment of antibacterial and cytotoxic activity of some locally used medicinal plants in Sundarban mangrove forest region. African J Pharm Pharmacol 2010; 4(2): 066-069.
19. Chopra RN, Chopra IC, Hunda KI, Kumar LD. Chopra's Indigenous Drug of India. India: Academic Publishers; 1982.
20. Most NP, Sarwar S, Chowdhury SA, Zakaria HM, Huda NH. *In-vitro* Cytotoxicity and Antioxidant Studies of *Elaeocarpus serratus*. Stamford J Pharm Sci 2009; 2(2): 86-90.
21. Singh B, Chopra A, Ishar MPS, Sharma A, Raj T. Pharmacognostic and antifungal investigations of *Elaeocarpus ganitrus* (Rudrakasha). Indian J Pharm Sci 2010; 72(2): 261-265.
22. Olotu NP, Ibrahim H, Iliyas N, Ajima U, Olotu AI. Phytochemical Screening and Analgesic studies of the Root Bark of *Hymenocardia acida*, Tul (Euphorbiaceae). Int J Drug Dev Res 2011; 3(1): 219-223.
23. Kadiri AB, Ajay GO. Phyto-anatomical characteristics of the West African {Umbrella tree} *Musanga cercropioides* M.Smithii R. Br. (Moraceae). Indian J Sci Technol 2009; 2:7.
24. Raaman N. Phytochemical Technique. New Delhi: New India Publishing Agency; 2008.
25. Kaoutar B, Tarik B, Driss M, Abdelaziz R, Abdelaziz S. Antibacterial activities of the crude ethanol extracts of medicinal plants against *Listeria monocytogenes* and some other pathogenic strains. African J Biotechnol 2010; 9 (27): 4251-4258.
26. Vidyarthi R.D. A Text book of Zoology. 14th. New Delhi : S.Chand and Co; 1977
27. Manjunath KP, Shivakumar H, Prakash T, Patil KS, Veeranagouda Rao R. Anthelmintic activity of roots of *Swertia chirata*. Ind J Nat Prod 2006; 22(1):8-10.

Table 1: Phytochemical screening of the extracts of *E. serratus* leaves.

Name of the test	Procedure	Observation	PE	B	C	A
Alkaloids	Extract+Mayer's reagent	Dark yellowish ppt	-	-	+	-
Flavonoids	Extract+ dil NaOH	Golden reddish ppt	+	-	-	+
Tannins	Extract+FeCl ₃	Wooly brownish ppt	-	-	-	-
Terpenoids	Extract+ chloroform +conc H ₂ SO ₄	A reddish brown coloration of the interface was formed.	-	-	-	-
Saponins	Extract+water+shaking	Formation of honey comb like froth.	-	-	-	-
Cardiac glycosides	Extract+Glacial acetic acid +FeCl ₃ +concn H ₂ SO ₄ .	A brown ring of the interface indicates a deoxysugar characteristic of cardenolides.	-	-	-	-
Anthraquinone glycosides	Extract+benzene+10%NH ₃ .	Reddish colour	-	+	+	+
Fixed oils and fats	Extract was pressed between two filter papers.	Appearance of oil stains on paper.	-	-	-	-
Carbohydr-ate	Extract+Molisch 's reagent+conc. H ₂ SO ₄	Appearance of violet ring.	-	-	-	-
Proteins	Extract+conc HNO ₃ =Whiteppt+heated+cooled +40% NaOH	Appearance of Orange ppt.	-	-	-	-

“+” = Present; “-” = absent; PE = Petroleum ether, B= Benzene, C= Chloroform, A= Acetone

Table 2: Antibacterial activity of the extracts of *E. serratus* leaves.

Test sample	Concentration µg/ml	Average zone of inhibition (in mm)		
		<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>
Tetracycline	30	35	33	32
DMSO	-	-	-	-
Petroleum ether	50	-	-	-
	100	-	-	-
	200	-	-	-
Benzene	50	-	-	-
	100	-	-	-
	200	-	-	-
Chloroform	50	-	-	-
	100	-	-	-
	200	-	-	-
Acetone	50	-	-	-
	100	-	-	-
	200	-	-	-

“-” = No zone of inhibition

Table 3: Anthelmintic activity of the extracts of *E. serratus* leaves.

Treatment	Concentration(mg/ml)	Time taken for paralysis(min)	Time taken for death(min)
Vehicle	-	-	-
DMSO	-	-	-
Piperazine Citrate	15	3.24	5.36
Petroleum Ether	50	-	-
	25	-	-
	12.5	-	-
Vehicle	-	-	-
DMSO	-	-	-
Piperazine Citrate	15	3.24	5.36
Benzene	50	-	-
	25	-	-
	12.5	-	-
Vehicle	-	-	-
DMSO	-	-	-
Piperazine Citrate	15	3.24	5.36
Chloroform	50	-	-
	25	-	-
	12.5	-	-
Vehicle	-	-	-
DMSO	-	-	-
Piperazine Citrate	15	3.24	5.36
Acetone	50	-	-
	25	-	-
	12.5	-	-

“-” = No paralysis/No death

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