IJPSR (2021), Volume 12, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 05 February 2020; received in revised form, 28 April 2020; accepted, 28 August 2020; published 01 February 2021

EVALUATION OF ANTIMICROBIAL ACTIVITY OF *SESUVIUM PORTULACASTRUM* L. AND *EXCOECARIA AGALLOCHA* L.

K. Samuel Kolli [*1], Sai Kumar Ella [1], J. Suneetha [2] and P. K. Ratna Kumar [3]

Department of Botany, Adikavi Nannaya University, Student, L. B P. G College, Visakhapatnam - 530013, Andhra Pradesh, India.
Department of Botany, Government College, Rajahmundry - 533103, Andhra Pradesh, India.
Department of Botany, Andhra University. Visakhapatnam - 530003, Andhra Pradesh, India.

Keywords:

Sesuvium portulacastrum, Excoecaria agallocha, Medicinal plants, Hexane, Ethyl acetate

Correspondence to Author:
Dr. K. Samuel Kolli

Assistant Professor,
Department of Botany,
Adikavi Nannaya University
Rajamahendravaram - 533296,
Andhra Pradesh, India.

E-mail: kskbotanyrjy@gmail.com

ABSTRACT: Background: *Sesuvium portulacastrum* L. and *Excoecaria agallocha* (L.) are two important medicinal plants inhabited in mangrove regions. Early researches focused on antimicrobial activity of leaves of concerned plants with various solvents among which ethanol, chloroform, and methanol were the best **Objective:** In the present study the extracts of two therapeutic plants were researched to measure the activity against 2 Gram-positive bacteria, 2 Gram-negative bacteria and 3 fungal strains. **Materials and Methods:** Hexane, Ethyl acetate, and Methanol were utilized for extraction. Bacterial Strains *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa*, and fungal strains *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* were used to test the antimicrobial activity. **Results:** The activity increased in dose dependent manner thus hexane concentrate of *Sesuvium portulacastrum* display most noteworthy action against *P.aeruginosa* and *E. coli*and *Aspergillus flavus* demonstrated maximum. *Excoecaria agallocha* show the highest zone against *S. aureus*, and *P. aeruginosa* demonstrated the least movement. Hexane was demonstrated best dissolvable for *Sesuvium portulacastrum* and *Excoecaria agallocha*. Ethyl acetate was finest for the fungal activity of both plants. **Conclusion:** These outcomes bolster the thought that therapeutic plants may have a job as pharmaceuticals and additives.

INTRODUCTION: Mangroves are a distinctive group of vascular plants that occur in saline coastal habitats (Coringa estuary) and are known to tolerate extreme environmental conditions. Mangrove plants have primary and secondary metabolites such as proteins, carbohydrates, carotenoids, hydrocarbons, aliphatic alcohols, polyunsaturated fatty acids, lipids, pheromones, phorbol esters, phenolics, steroids, terpenes, tannins and glycosides *etc.* [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(2).904-09

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(2).904-09

These metabolites were described for bioactive substances as bactericidal, fungicidal, pharmaceutical agents for animal and human beings [2]. Application of chemo-the rapeutants has created problems *via* toxicity, resistance, residue leftover, and possibly some public health and environmental consequences.

Therefore, new drugs have to be found in order to combat such consequences, and it is essential to find new compounds that have antimicrobial properties. Among the mangrove associated plants, *Sesuvium portulacastrum* (L.) L. is known as "Sea purslane" belongs to the family Aizoaceae and grows in the Mediterranean coast and sub-tropical areas around the world. In traditional medicine, *Sesuvium* has been used for the treatment of conjunctivitis, leprosy, dermatitis, and toothache [3].

Excoecaria agallocha L. (Euphorbiaceae) is a small mangrove tree found extensively in the tidal forests and swamps of the Krishna-Godavari area. This plant is also well-distributed in a number of other countries of temperate and tropical Asia. The bark oil has been found effective against rheumatism, leprosy, and paralysis. This plant also has been traditionally used to treat sores and stings from marine creatures, and ulcers, as a purgative and an emetic. However, the milky sap of this tree can cause temporary blindness if it enters the eyes. The sap can also cause skin blisters and irritation. Clinical trials carried out on this plant have shown its potential anti-HIV, anticancer, antibacterial, and antiviral properties. The aim of this study was to test two medicinal plant extracts against a diverse range of organisms comprising Gram-positive and Gram-negative bacteria and fungi. Therefore the present study deals with antimicrobial activity of Hexane, Ethyl acetate, and methanol extracts of these two medicinal plants against 4 bacteria and 3 fungal species.

MATERIALS AND METHODS:

Plants Extraction Preparation: Plant materials of two plant species included in this study were collected from coringa wildlife sanctuary and estuary situated in Andhra Pradesh, India. The collected plants were watery washed, disinfected, rinsed with distilled water, and finally dried in the shade. The dried plant material of each plant species was grounded into fine powder to pass 100 mm sieve. 50 g of the fine powder was subjected to Soxhlet extraction by using Hexane, Ethyl acetate and Methanol for 48 h resulting extracts in different solvents were evaporated and dried at 40 °C under reduced pressure using a rotatory vacuum evaporator. The extract yields were weighted, stored in small bottles in fridge at 5 °C and these crude extracts were tested for standard strains of microorganisms.

Antibacterial Activity of the Plant Extracts:

Bacterial Strains: The antibacterial potency of each plant extract was evaluated using five bacterial strains Two strains of Gram positive (*Staphylococcus aureus, Streptococcus pyogenes*) and two strains of Gram negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria. Three fungal strains (*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus).*

The bacterial and fungal strainswere provided from the microbial type culture (MTCC), Institute of Microbial echnology, Chandigarh, India (**Table 1**).

TABLE 1: DETAILS OF THE BACTERIAL AND FUNGAL STRAINS USED IN BIOASSAY

S. no.	Name of the Bacterial/ Fungal Strains	MMTC Catalogue No.
	Gram Positive Bacteria	
1	*Staphylococcus aureus*	MTCC 3160
2	*Streptococcus pyogenes*	MTCC 442
	Gram Negative Bacteria	
3	*Escherichia coli*	MTCC 443
4	*Pseudomonas aeruginosa*	MTCC 424
	Fungal Pathogens	
5	*Aspergillus niger*	MTCC 961
6	*Aspergillus flavus*	MTCC 3396
7	*Aspergillus fumigatus*	MTCC 2584

Assay for Antimicrobial Testing: Isolated test bacteria were grown overnight on nutrient agar plates, and fungi were grown on Sabouraud dextrose agar plates. Bacterial inoculums were prepared from overnight grown cultures (24 h) in liquid broth (Hi-Media, Mumbai, India) and the turbidity was adjusted equivalent to 0.5 McFarland units (approximately 10^8 CFU/ml for bacteria and fungi inoculums turbidity was equivalent at 10^5 or 10^6 CFU/ml). The microorganisms were inoculated into the liquid broth and incubated at 35 ± 2 °C for 4 h. The positive control was taken streptomycin (10 µg/ml) for antibacterial activity and Ketocanozole (10 µg/ml) for antifungal activity. The DMSO was taken as a negative control to determine the possible inhibitory activity of the dilutant of extract. The susceptibilities of the isolated pathogens were determined by the modified Olurinola.1996 [4] agar well diffusion method with Muller Hinton agar plates. Aliquots of inoculums were spread over the surface of agar plates with a sterile glass spreader. To test the antimicrobial activity, all extracts were dissolved in DMSO to make a final concentration of 25 and 50 µl. After culture medium poured in the Petri plates remained placed at room temperature for settling and then kept refrigerator for 30 min. After this procedure was done, took 3 number cup borer (6 mm) sterilized properly by flaming and then used to make uniform cups/wells in each Petri plate. Then cups/wells filled with different extracts and allowed to diffusing the extract into the medium for about 45 min. These plates were incubated for a period of 24 h at 37 °C in incubator for bacteria and

at 30 °C for 24-48 h in B. O. D incubator for fungi. Each experiment was done in triplicate and mean values were taken. Antimicrobial activity was measured in the diameter (mm) of the clear inhibitory zone formed around the well.

RESULTS: The antimicrobial activity of *S. portulacastrum* and *E. agallocha* extracts are shown in **Table 2 and Table 3** respectively. The results showed that the antimicrobial activities of the crude extracts were increased with increasing concentration. Although the antimicrobial activity of the extracts tested is variable, two gram-positive bacteria (*Staphylococcus aureus, streptococcus pyogenes*) and two gram-negative bacteria (*E. coli, Pseudomonas aeruginosa*) and three strains of fungi (*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus*) were inhibited by the extracts.

TABLE 2: ANTI-MICROBIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *SESUVIUM PORTULACASTRUM* L.

Organism	Solvent	DMSO	25µl	50µl	Control
Streptomycin (10 µg/ml)					
Staphylococcus aureus	EA	1.6±0.01	0.63±0.04	0.8±0.04	ND
	ME	0.8±0.08	ND	ND	ND
	HE	1.6±0.08	ND	ND	ND
Streptococcus pyogenes	EA	1.6±0.01	ND	0.76±0.04	ND
	ME	1.6±0.04	ND	0.7±0.08	ND
	HE	1.5±0	1.1±0.12	1.5±0	0.7±0.08
Escherichia coli	EA	1.8±0.08	1.7±0.04	2.3±0.04	0.4±5.5
	ME	1.7±0	0.8±0.08	0.9±0.08	ND
	HE	1.83±0.04	1.7±0.08	2.1±0.12	0.7±0.08
Pseudomonas aeruginosa	EA	1.5±0.08	ND	ND	0.5±0.0
	ME	1.9±0.08	0.6±0.12	0.9±0.08	0.4±0.04
	HE	1.9±0.08	1.5±0.08	2.3±0.08	0.7±0.08
Ketocanozole (10 µg/ml)					
Aspergillus niger	EA	1.3±0.08	0.6±0.04	ND	ND
	ME	0.8±0.04	0.9±0.08	1.4±0.08	ND
	HE	0.7±0.08	1.3±0.16	2.1±0.08	ND
Aspergillus flavus	EA	1.3±0.08	ND	ND	0.8±0.08
	ME	ND	1.0±0.04	1.7±0.08	ND
	HE	0.7±0.04	1.16±0.12	1.36±0.04	ND
Aspergillus fumigatus	EA	1.8±0.09	1.3±0.04	ND	0.7±0.08
	ME	0.8±0.08	ND	1.0±0.09	ND
	HE	0.7±0.04	1.16±0.12	1.36±0.04	ND

Note: abbreviations EA = Ethyl acetate, ME = Methanol, HE = Hexane, DMSO = Negative control, ND = Not Detected,and Control = Positive control

Table: 2 shows the antimicrobial activity of *S. portulacastrum* where among three solvents used Hexane exhibit better activity followed by ethyl acetate and methanol. The highest zone of inhibition was observed in Hexane extract against *Pseudomonas aeruginosa* (2.3 ± 0.08 at 50µl concentration) followed by ethyl acetate extract against *E. coli* (2.3 ± 0.04 at 50 µl concentration). Lowest activity recorded in ethyl acetate extract against *Staphylococcus aureus* (0.8 ± 0.04 at 50 µl concentration). While *Pseudomonas aeruginosa, Staphylococcus aureus* strains appear to be resistant to the tested concentration in Ethyl acetate, Methanol, and Hexane solvents since no inhibition zone was observed. Coming to the fungal strains among tested solvents Hexane gives better activity followed by Methanol, Ethyl acetate. *Aspergillus niger* showed highest zone of inhibition (2.1 ± 0.08 at 50 µl concentration) in Hexane extract. Lowest recorded with *Aspergillus fumigatus* (1.0 ± 0.09 at 50µl concentration) in Methanol extract. While *Aspergillus flavus* strains appear to be resistant in Ethyl acetate extract since there is no inhibition zone was recorded.

In *E. agallocha* **Table 3,** all the extracts were found to possess various antimicrobial activity against gram-positive Bacterial, gram-negative Bacterial, and fungal strains. Among the tested extracts, Hexane exhibited the highest antibacterial potential of activity against all the tested bacterial species irrespective of their gram nature.

TABLE 3: ANTI-MICROBIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *EXCOECARIA AGALLOCHAL*

Organism	Solvent	DMSO	25µl	50µl	Control
Streptomycin (10 µg/ml)					
Staphylococcus aureus	EA	1.2±0.08	1.1±0.12	1.6±0.12	ND
	ME	1.7±0.08	1.9±0.12	2.0±0.12	1.0±0.12
	HE	1.6±0.04	1.3±0.08	2.2±0.12	ND
Streptococcus pyogenes	EA	1.2±0.04	0.7±0.08	1.2±0.09	ND
	ME	0.8±0.04	ND	ND	ND
	HE	1.3±0.04	1.6±0.12	2.1±0.08	0.7±0.08
Escherichia coli	EA	1.7±0.04	1.8±0.08	2.1±0.08	0.7±0.04
	ME	1.6±0.08	0.7±0.08	1.0±0.09	ND
	HE	1.4±0.04	1.1±0.12	1.5±0.09	0.4±0.08
Pseudomonas aeruginosa	EA	1.6±2.22	0.83±0.08	0.8±0.08	0.4±0.04
	ME	0.8±0.04	ND	0.3±0.08	ND
	HE	1.8±0.04	0.5±0.04	1.5±0.12	ND
Ketocanozole (10 µg/ml)					
Aspergillus niger	EA	1.4±2.22	0.83±0.12	**2.1±0.12**	ND
	ME	1.3±0.04	ND	0.8±0.04	ND
	HE	0.8±0.04	0.4±0.08	1.0±0	ND
Aspergillus flavus	EA	1.3±0.08	ND	ND	ND
	ME	0.7±0.08	ND	ND	ND
	HE	0.7±0.08	ND	ND	ND
Aspergillus fumigatus	EA	1.9±0.08	1.3±0.04	1.7±0	ND
	ME	0.9±0	ND	ND	ND
	HE	2.0±0.08	ND	1.1±0.12	ND

Note: abbreviations EA = Ethyl acetate, ME = Methanol, HE = Hexane, DMSO = Negative control, ND = Not Detected, and Control = Positive control

However, ethyl acetate was found to be active after hexane. Methanol exhibited a mixed responses. Further, the higher zone of inhibition recorded in hexane extract against *Staphylococcus aureus* (2.2 ± 0.12 at 50 µl concentration) followed by *streptococcus pyogenes* (2.1 ± 0.08 at 50 µl concentration). *E. coli* (2.1 ± 0.08 at 50 µl concentration) was exhibit good activity in Ethyl acetate extract. Minimum inhibition was noted in Methanol extract against *Pseudomonas aeruginosa* (0.3 ± 0.08 at 50 µl concentration). Antifungal activity of *E. agallocha* the results revealed that Ethyl acetate extract gives somehow better results than Hexane and Methanol. Methanol solvent proved weak solvent than remaining solvents. The highest zone of inhibition noted in Ethyl acetate extracts against *Aspergillus niger* (2.1 ± 0.12 at 50 µl concentration) and least also recorded from methanol extract against *Aspergillus niger* (0.8 ± 0.04 at 50 µl concentration), *Aspergillus flavus* in methanol. Hexane extracts were resistant and methanol of *Aspergillus fumigatus* also resistant because there were no zones of inhibition observed.

DISCUSSION: Mangroves are an inimitable assembly of vascular plants that happen in saline seashore front natural surroundings and are known to endure outrageous ecological conditions. The antimicrobial activity exhibited by the mangrove plant parts could be due to the presence of phytochemicals like alkaloids, tannins, flavonoids and sugars present in the plant extract. Primary and secondary metabolites are very important for the regular mechanism/survival of the species and also it can be used as therapeutic agents. Potential antimicrobial agents from mangrove species were due to the presence of phytoconstituents.

Al-Azzawi. A *et al.*, 2012 [5] reported antimicrobial screening of *S. portulacastrum* in the United Arab Emirates; they have used leaves as their interesting part for activity and used Ethanol, Aqueous Dichloromethane for extraction. Among used solvents Ethanol was considered as best and given good activity against *staphylococcus aureus* and *E. coli*. Satish P *et al.*, 2016 [6] reported antibacterial activity of SAH marsh plant extracts, and they conclude that *S. portulacastrum* did not show any activity against three tested human pathogens. Lincy, M. P *et al*., 2013 [7] reported *in vitro* antibacterial activity of leaf of *S. portulacastrum*. In their investigation, they were used petroleum ether, Benzene, Ethyl acetate, Methanol, and Ethanol for extraction.

They concluded that Ethanol extract shows very good activity than the remaining tested solvents. Abirami. H and Rameshwari. R 2013 [8] reported antibacterial and antifungal screening of *S. portulacastrum* extracts against leather contaminating organisms. They used leaf, Methanol, Chloroform, Petroleum ether, and Ethyl acetate for extraction. They concluded form their findings that chloroform was found best and *Klebsiella pneumonia* showed the least activity in petroleum ether and *staphylococcus aureus, E. coli* recorded the highest activity in chloroform extract. They also concluded that *S. portulacastrum* inhibited the reproductive activity in *A. niger* and *A. flaus*. K. Feroz khan and G. Sankar reported antibacterial activity of salt marsh plants against Marine ornamental fish pathogens.

They used solvents like Aqueous, Methanol, and Diethyl ether for extraction. Among tested, Methanol extract showed more significant activity than remain tested.

In the present study, we were taking the whole plant of *S. portulacastrum* for antimicrobial activity. For extraction were used Ethyl acetate, Methanol, and Hexane solvents. From our findings, it was concluded that Hexane was proved better than the remaining tested solvents. *Pseudomonas aeruginosa* was exhibited the highest zone of inhibition in hexane extract. *Aspergillus niger* gives the highest inhibition zone in hexane extract. So this was the first result that hexane proved better.

Prakash and Sivakumar 2013 [9] reported antimicrobial activity of *Excoecaria agallocha*. For extraction, they used fresh and dried parts of leaves, stems, and roots. Ethanol was used as a solvent and they concluded that dried plant samples of the leaf having higher inhibitory activity against pathogenic bacteria compared than the fresh plant extracts. Vadlapudi *et al.,* 2009 [10] reported antimicrobial activity of *E. agallocha*, they took leaves as interesting parts, and Chloroform and methanol, and hexane were used for extraction. They finalize from their findings that chloroform and methanol were found to be effective, whereas hexane extracts were inactive. Ravikumar e*t al.,* 2010 [11] reported the antibacterial activity of the leaves of *E. agallocha* against selected fish pathogens. Sahoo *et al.,* 2012 [12] had been reported antibacterial activity of *E. agallocha* leaf extracts against human pathogens. Among tested solvent ethanol extract showed its activity in all the tested pathogens. Lalith. A. A and Najiah. M 2014 [13] reported antimicrobial activity of *E. agallocha* against selected pathogens. Leaves were obtained via extraction with methanol, and they concluded that methanol exhibited strong activity against tested fish pathogens. Parasuraman. P and Rameshwari.R. 2017 [14] reported antibacterial activity of *E. agallocha* leaf in chloroform and ethanol extracts. The chloroform extract was highly effective on *Bacillus cereus* strain. So by reviewing all the literature, in this present study, whole plant was used and Ethyl acetate, Methanol, and Hexane were utilized for extraction. Among tested solvents, Hexane gives better activity against *Staphylococcus aureus* (2.2 mm), and for fungi Ethyl acetate provides maximum activity against *A. niger* (2.1 mm). This is also the first record with hexane because no one gets better activity with hexane so far. Our finding also concluded that Ethyl acetate was good for the fungal activity of both plants.

CONCLUSION: *Sesuvium portulacastrum* L. and *Excoecaria agallocha* L. extracts had the ability to inhibit bacterial growth. In the present study, whole-plant were assessed to test antimicrobial activity against human pathogens. By the results, it was concluded that among the tested extracts Hexane proved better for *S. portulacastrum* and *Excoecaria agallocha* L. Among tested pathogens *P. aeruginosa* and *S. aureus* display the highest inhibitory activity for 2 plants, respectively. All these extracts were not effective than antibiotics to combat the pathogenic microorganisms studied. This indicated that these plants have potent antibacterial properties and could be used in the development of novel antibacterial agents.

ACKNOWLEDGEMENT: We acknowledge the Director of MS Projects, Rajamahendravaram Sri Manohar Dandu, for providing the necessary chemicals and laboratory for doing this work.

CONFLICTS OF INTEREST: None.

REFERENCES:

1. Bandaranayake WM: Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands ecology and management 2002; 10(6): 421-52.

2. Suryati E and Hala Y: Bioactive substances of mangrove *Excoecaria agallocha*, Hexogoniaacaloza as shrimp diseases inhibitor. Marin Chim Acta 2002; 21: 9-14.
3. Chandrasekaran M, Senthilkumar A and Venkatesalu V: Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum* L. European Review for Medical and Pharmacological Science 2011; 15(7): 775-780.
4. Olurinola PF: A laboratory manual of pharmaceutical microbiology. Idu Abuja Nigeria 1996; 69: 1-05.
5. Al-Azzawi A, Alguboori A, Hachim MY, Najat M, Al Shaimaa A and Sad M: Preliminary phytochemical and antibacterial screening of *Sesuvium portulacastrum* in the United Arab Emirates. Pharmacognosy Research 2012; 4(4): 219-24.
6. Sathish P, Jaswanth G, Gurudhathan KB, Gopinath J, Gayathri PK and Yuvaraj D: Phytochemical investigation and antibacterial activity of salt marsh plant extracts. Journal of Chemical and Pharmaceutical Sciences 2016; 9(1): 292-94.
7. Lincy MP, Paulpriya K and Mohan V: International J of Pharmaceu Research and Bio-Science 2013: 2(6): 140-55.
8. Abirami HH and Rameshwari RR: Antibacterial and Antifungal screening of *Sesuvium portulacastrum* extracts against leather contaminating organisms. International Journal of Current Research 2013; 5(6): 1376-77.
9. Prakash M and Sivakumar T: A study on antibacterial activity of mangrove plant *Excoecaria agallocha* L. Int J Curr Microbiol App Sci 2013; 2(8): 260-62.
10. Vadlapudi V, Bobbarala V, Penumajji S and Naidu KC: *Excoecaria agallocha* L. antimicrobial properties against important pathogenic microorganisms. International Journal of Pharm Tech Research 2009; 4(1): 865-7.
11. Ravikumar S, Muthuraja M, Sivaperumal P and Gnanadesigan M: Antibacterial Activity of the Mangrove Leaves" *Exoecaria agallocha*" Against Selected Fish Pathogens. Asian Journal of Medical Sciences 2010; 102(5); 211-13.
12. Sahoo G, Mulla NS, Ansari ZA and Mohandass C: Antibacterial activity of mangrove leaf extracts against human pathogens. Indian Journal of Pharmaceutical Sciences 2012; 74(4): 348-51.
13. Laith AA and Najiah M: Antimicrobial activities of blinding tree, *Excoecaria agallocha* against selected bacterial pathogens. J Microbiol Antimicrob 2016; 29-36.
14. Parasuraman P and Rameshwari R: Antibacterial activity of *Excoecaria agallocha* L. leaf in chloroform and ethanol extracts. (GC-MS Analysis) 2017; 7(2): 11796-802.

How to cite this article:
Kolli KS, Ella SK, Suneetha J and Kumar PKR: Evaluation of antimicrobial activity of *Sesuvium portulacastrum* L. and *Excoecaria agallocha* L. Int J Pharm Sci & Res 2021; 12(2): 904-09. doi: 10.13040/IJPSR.0975-8232.12(2).904-09.