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Antimicrobial activity of solvent extracts of *Eichhornia crassipes* (Mart.) Solms

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ABSTRACT

Eichhornia crassipes (Mart.) Solms, Waterhyacinth , once looked upon nonchalantly, has received supreme interest in recent years owing to its constructiveness in various fields. The solvent extracts of waterhaycinth was tested against a bacteria Staphylococcus albus by disc diffusion method and a fungal stains Mucor sp by streak plate method. Acetone extract evidenced better activity against the microbes among other extracts. The result adds to the value of the plant in pharmaceutical field as an antimicrobial agent.

Keywords: *Eichhornia crassipes*, hyacinth, extract, antibacterial, *Staphylococcus albus*, antifungal, *Mucor* sp

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INTRODUCTION

Antimicrobials have long been synthesized with the advent of the first synthetic antibiotic as these were single molecules which are competent against countless organisms. Development of antibiotic resistance has led to the structural alteration of the existing drugs which in many case has been proved to be effective thus extending the life span of the drugs. These synthetic drugs again warrant modification when the organisms start resisting them. Rational drug design does not always give way to effectual antimicrobials. In the past, owing to the complex matter of drug uptake by cells, synthesize of potent enzyme inhibitors with diffident antibacterial activity was only carried out. An alternative approach for the development of novel drugs is represented by the wide empirical screening of chemical entities for antimicrobial activity [1].

Renewal in the plant derived phytochemicals has again bloomed as the structural modification cannot be continuously made to produce an effective drug targeting other sites than that already targeted. Curative nature of plants has been exploited with scientific significance in past few decades. Perhaps many researchers have started exploiting the plant sources for the lead compounds which can be active against such organisms. Natural products have been a largely rich source of anti-infective agents [1]. Many plants have been studied for their antibacterial activity [2-6] in past few years indicating the surge of the researchers to identify a plant with good antimicrobial activity.

Researchers mainly focus on the medicinal plants rather than on the common weeds which are also the source of many phytochemicals. In this present study, *Eichhornia crassipes* (Mart.) Solms an aquatic weed generally called waterhyacinth [7] was tested for its antibacterial activity against *Staphylococcus albus* and antifungal activity against *Mucor* sp. Waterhyacinth have been demonstrated to be a good antimicrobial agent [8-14]. In the present study, the antibacterial activity against *Staphylococcus albus (Staphylococcus epidermis)*, and antifungal activity against *Mucor* sp. have been tested with various solvent extracts of waterhyacinth, as there are no other reports on the activity with the extracts against these strains. These strains are reported to cause various pathological conditions in humans.

Staphyloccous sp. are predominant among the organisms responsible for infective complications following surgical vascular grafts or the implantation of prosthetic devices. *Staphyloccous albus* is the chief organism accountable for infections of prosthetic heart valves, artificial joints and cerebrospinal fluid shunts [15]. *Mucor* species can cause catastrophic infections in the immunocompromised patient. The incursion of the fungus into the blood vessels causes thrombosis, infarction, and necrosis of the infected tissue. Classically, *Mucor* species infections have been classified as rhinocerebral, pulmonary, gastrointestinal and cutaneous [20]. *Mucor* species causes prosthetic mitral valve mucormycosis [21].

Hence this plant has been tested against these bacteria and fungi due to its pharmaceutical properties. This plant also shows good antioxidant activity [22-24], wound healing activity [25], larvicidal activity [7]. In this study, the chloroform, ethanol and methanol extracts were tested for the presence of phytochemicals. The petroleum ether, ethyl acetate, chloroform, ethanol, methanol, acetone extract, methanol fractionate, ethanol fractionate of aqueous extract and the compound isolated from the ethyl acetate extract of waterhyacinth (mixture of stigmasterol and β-sitosterol) were tested for their antibacterial and antifungal activity against the foresaid organisms by disc diffusion method and steak plate method respectively.

MATERIALS AND METHODS

Plant collection

Waterhyacinth was collected from Singanallur boat house, Coimbatore, Tamil Nadu in the month of March, 2010. The plant sample was identified by Dr.G.V.S.Murthy, Scientist F & Head of Office, Botanical Survey of India, Southern Regional Centre, Coimbatore- 641 002 with the number BSI/SRC/5/23/2011-12/Tech.

Extraction of the plant material

The root portion was cut off, washed thoroughly to free it from debris and was shade dried for 20 days. The dried plant material was sliced and ground.

Water hyacinth (1.5kg) was defatted twice with petroleum ether (20L) for 6 h and then twice with ethanolic KOH (17L) for 6 h. The extract was concentrated under reduced pressure and the residue was extracted thrice with acetone under reflux for 1 h. The acetone extract (Ac) were pooled and concentrated.

Waterhyacinth (20g) was extracted thrice for one hour with solvents of increasing polarity *viz* ethyl acetate, ethanol, methanol and water successively yielding ethyl acetate (EA), ethanol (EE), methanol (ME) and water extracts (AQ) respectively. The dried plant was extracted with chloroform four times for 6h giving chloroform extract (CE). The aqueous extract was fractionated with ethanol and methanol to yield the ethanol fractionate (EFA) and methanol fractionate (MFA) respectively. The column chromatography of the ethyl acetate extract gave mixture of stigmasterol and β-sitosterol [26] when eluted with petroleum ether: ethyl acetate (90:10).

Phytochemical screening

Phytochemical screening was carried out for the chloroform, ethanol and methanol extracts of waterhyacinth by adopting the procedure of Jayanthi et al., 2011 [27].

Evaluation of antimicrobial activity

Extracts of waterhyacinth and its fractionates were tested for their antibacterial and antifungal activity against the bacteria- *Staphylococcus albus* and the fungi-*Mucor* sp.

Preparation of culture media [28]

Muller-Hinton Medium

Muller-Hinton Medium was prepared by suspending 38 g of the medium in one liter of purified water. The suspension was heated with frequent agitation and boiled till the medium completely dissolved, autoclaved at 121°C for 15 minutes and cooled to room temperature.

Sabouard Dextrose Agar medium

The SDA (Sabouard Dextrose Agar medium) was prepared by suspending 65.0g of SDA in 100ml distilled water and boiling to dissolve the medium.

Antimicrobial Testing

Antimicrobial testing was carried out by adopting the procedure of Arulpriya et al., 2010 [28].

Disc Method for Determination of Zone of Inhibition for Antibacterial activity

Paper discs (4 mm diameter) were sterilized in an autoclave and dried at 100°C in an oven. The discs were soaked with extracts and fractionates of waterhyacinth at the rate of 50 µg (dry weight) per disc for antibacterial analysis. A drop of bacterial suspension was taken in a sterile Petri dish (90 mm diameter) and 20 ml of sterilized and melted nutrient agar (~45°C) was poured into the plate, and then mixed thoroughly. The paper discs soaked with test substances were placed at the center of the inoculated pour plate. A control plate was maintained in every case with alcohol. The plates were initially retained at low temperature (4°C), incubated at (35 ± 2) °C for growth of test organisms after 4 h, and were observed at 24 h intervals for two days. The activity was expressed in terms of zone of inhibition in mm. Each experiment was repeated three times. The standard antibiotic Gentamycin were used as a positive control and compared with extracts, fractionates and compound of waterhyacinth under identical conditions.

Streak Plate Isolation Method for Determination of Zone of Inhibition for Antifungal Activity

The SDA medium was taken in a conical flask and was sterilized in autoclave (at 121 °C and 15 Psi) for 15 min. Liquefied SDA was dispensed into a Petri dish and was rotated gently for uniform distribution of the medium. The inoculating loop was held at a 60 °C angle in the hottest part of the Bunsen burner flame and was heated to redness. The loop was cooled for 15 to 20 seconds before it touched the culture. A small amount of the culture was taken using the sterilized inoculating loop and the microorganisms were streaked in a plate by quadrant streak method. Inoculation was done under aseptic condition and the spores were inoculated in the medium and incubated for 5 days. The diameter of the clear zone developed on SDA plate was measured as the zone of inhibition. The antimicrobial activities of the compounds were recorded by photographing the Petri dishes.

Determination of the activity index [29]

The activity index of the crude plant extract was calculated as

$$\text{Activity index (AI)} = \frac{\text{Zone of inhibition of the extract}}{\text{Zone of inhibition obtained for standard antibiotic drug}}$$

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening tests carried out for the extracts of waterhyacinth shows that the plant possesses many phytochemicals which have therapeutic applications. The results of the phytochemical screening tests for the extracts of waterhyacinth are given in table 1. The chloroform extract tested positive for anthocyanins, phenolics, proteins and carbohydrates and the ethanol extract indicated the presence of metabolites such as flavonoids, anthroquinones, phenolics and carbohydrates. Anthroquinones, proteins and carbohydrates were found to be present in the methanol extract of waterhyacinth. Previous reports demonstrated the presence of alkaloids, phenol, steroid, tannin and saponins in the methanol extract of waterhyacinth [30]. The ethanol extract of fresh waterhyacinth was reported to possess alkaloids, flavonoids, sterols, terpenoids, anthroquinones, proteins and phenols [31]. Aneke et al.,[32] detailed the presence of presence of saponins, alkaloids, glycosides and anthraquinones in the chloroform extract of waterhyacinth.

The results of the antimicrobial activity of the solvent extracts, fractionates and compound of waterhyacinth against *Staphylococcus albus*, *Mucor* sp are given in table 2,3 and figure 1.

Antibacterial activity

Hydrophobicity is an important characteristic of plant extracts and their components and it enables them to partition the lipid of the bacterial cell membrane and mitochondria, upsetting the cell structures and rendering them more permeable. Death occurs when there is extensive leakage from bacterial cells or the exit of critical molecules and ions. The discrepancy in the effectiveness of the extract against different microorganisms depends upon the chemical composition of the extracts and membrane permeability of the microbes for the chemicals and their metabolism [33].

The antibacterial activity of extracts, fractionates and compound of *Eichhornia crassipes* against *Staphylococcus albus* was carried out by disc diffusion method with ethanol as solvent. 50µg/ml solutions of extracts were evaluated against the standard drug gentamycin. A clear zone of growth inhibition was noted around the disc due to diffusion of drug. The diameter of the inhibition zone denotes the relative susceptibility of the test microorganisms to a particular anti microbe [28].

Table 1. Phytochemical screening of the extracts of waterhyacinth

S.No	Phytochemicals	CE	EE	ME
1	Alkaloids			
a	Meyers test	-	-	-
b	Wagner's test	-	-	-
c	Hager's test	-	-	-
2	Flavonoids			
a	NaOH Test	-	+	-
b	H_2SO_4 test	-	+	-
3	Sterols			
a	Liebermann-Burchard test	-	-	-
4	Terpenoids			
a	Liebermann-Burchard test	-	-	-
5	Anthroquinone			
a	Borntrager's test	-	+	+
6	Anthocyanins			
a	NaOH test	+	-	-
7	Proteins			
a	Ninhydrin test	+	+	+
8	Phenolics			
a	Ferric chloride test	+	+	-
b	Libermann's test	+	+	-
9	Quinones			
a	HCl test	-	-	-
10	Carbohydrates			
a	Molisch test	+	+	+
b	Fehling's test	-	+	+

Zone of Inhibition(mm)	Type of antimicrobe
> 13	Highly sensitive or susceptible
8-13	Moderately sensitive or intermediate
< 8	Resistant

It is obvious from table 2 that methanol extract is highly active against *Staphyloccus albus* compared to all other extracts. The organisms were resistant to CE, AQ, MFA and EFA. The higher activity of the extract against *Staphyloccus albus* may be due to the presence of phytochemicals like alkaloids, sterols, terpenoids, anthocyanins and anthroquinones [27].

The EA and Ac extract showed higher activity against *Mucor* sp but was half that of the activity exhibited by the standard. The CE and AQ extracts, MFA and EFA fractionates of aqueous extract did not exhibit any activity against the two fungi tested.

Table 2. Antimicrobial activity (Zone of Inhibition-ZOI) and activity index (AI) of the extracts/fractionates of waterhyacinth

Code	Zone of inhibition and AI	*Staphylococcus albus*	*Mucor sp*
PE	ZOI	6	6
	AI	0.17	0.3
EA	ZOI	8	10
	AI	0.22	0.5
CE	ZOI	R	R
	AI	-	-
EE	ZOI	8	6
	AI	0.22	0.3
ME	ZOI	18	6
	AI	0.51	0.3
AQ	ZOI	R	R
	AI	-	-
AC	ZOI	12	10
	AI	0.34	0.5
MFA	ZOI	R	R
	AI	-	-
EFA	ZOI	R	R
	AI	-	-
E12	ZOI	10	6
	AI	0.28	0.3

The activity index of the test substance above 0.5 is considered as significant activity [34]. From the table 2, it is obvious that ME possess significant activity against *Staphylococcus albus* and EA and AC possess significant

activity against *Mucor* sp. ME, EA and Ac [27] contains anthraquinones which were reported to show good antimicrobial activity against *Staphylococcus albus* and *Mucor* sp [35, 36].

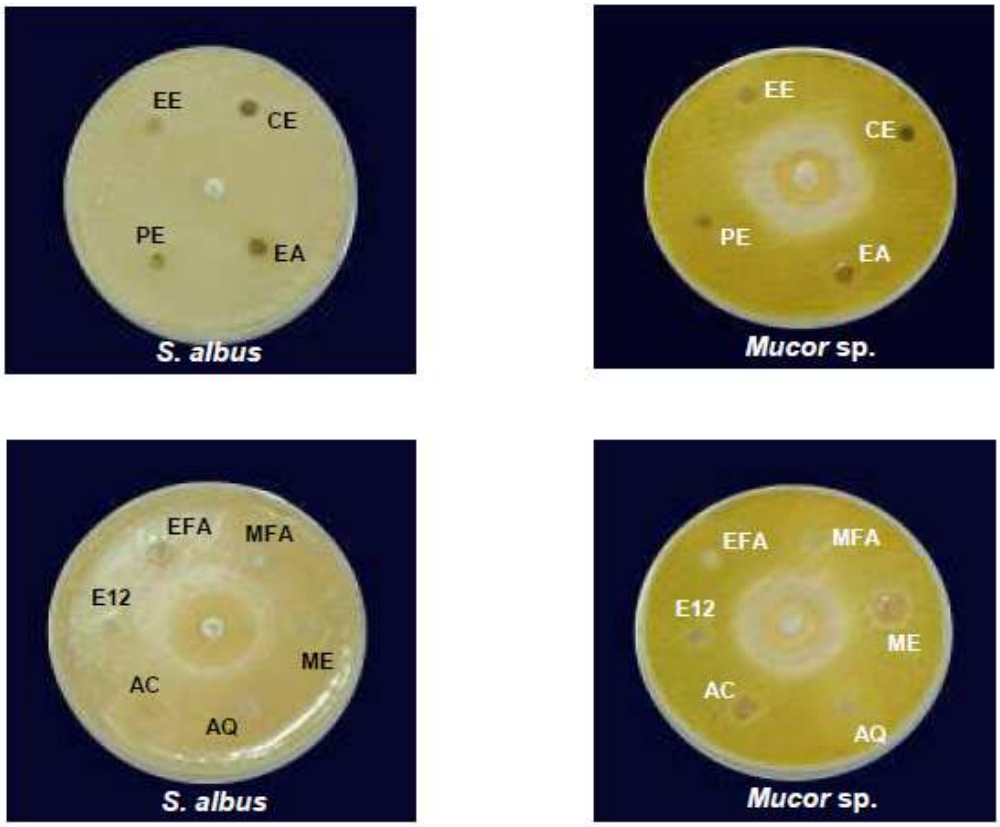

Figure 1. Plates depicting the antimicrobial activity of the extracts of *E.crassipes*

CONCLUSION

Waterhyacinth which is often considered a weed has in recent days been taken up for many researches concerning the phytochemical and pharmaceutical applications. The antimicrobial screening carried out for the extracts/fractionates and compound of waterhyacinth showed that this plant exhibits significant activity against the organisms tested. The acetone extract of waterhyacinth exhibits higher activity against all the test organisms indicating that the extract possess such compounds responsible for the activity. This study suggests that the plant can be productively used in the pharmaceutical area because of its possible activities reported.

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