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Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of *Eichhornia crassipes* (Mart.)Solms

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ABSTRACT

The ethanol extract of leaves and shoot portion of the fresh Eichhornia crassipes were screened for the presence of various phytochemicals by standard procedures. The present study indicates that the fresh plant contain alkaloids, flavonoids, phenols, sterols, terpenoids, anthoquinones and protein. The aqueous, chloroform fractionates and the ethanol and ethyl acetate extracts were screened for antibacterial activity and antifungal activity against two bacteria Micrococcus luteus and Rhodospirillum rubrum and two fungi Monoscus ruber and Rhodospirillum rubrum by disk diffusion method. The extracts and fractionates of fresh Eichhornia crassipes showed a significant and remarkable activity against two bacteria and two fungal species when compared to standard. The present work shows the presence of significant secondary metabolites in the world's worst weed and its appreciable antimicrobial activity.

Keywords: Eichhornia crassipes, phytochemical screening, secondary metabolites, anti-microbial activity.

INTRODUCTION

Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years –such extensive dependence of human being on "Mother Nature" has invoked tremendous interest in the scientific world, which ultimately led to the isolation of a vast number of chemical agents with potentials for multipurpose uses [1]. Aquatic plants have economic and environmental uses, depending on the natural characteristics. Some are consumed in human diet, while other species have medicinal values and still other species are good resource of minerals and vitamins. Since aquatic weeds are known to differ widely in their chemical composition depending upon species, season and location, an insight into their chemical composition is essential if utilization prospects are to be considered [2].

Eichhornia crassipes (Mart.) Solms is native to Brazil. Plants are thought to have been first introduced into the United States at the 1884 Cotton States Exposition in New Orleans, LA. Waterhyacinth is a floating, flowering, perennial weed (requiring a wet habitat), form dense rafts in the water and mud [3]. It can quickly grow to very high densities (over 60kg m^{-2}); thereby completely clogging water bodies, which in turn may have negative effects on the environment, human health and economic development [4]. Work on waterhyacinth parameters would be useful in future, in a wide range of areas. The word "Phyto" is the Greed word for plant. Phytochemicals, which not only that they are nonnutritive plant chemicals that have protective or disease preventive properties but also protect human from a host of diseases [5]. Phytochemical studies have shown that plants with antimicrobial activity contain bioactive constituents such as tannins, flavonoids, alkaloids and saponins [6]. Alkaloids & flavinoids have been used

as antiviral, antibacterial, antiamoebial & anticancer agents. Phenolic and polyphenolic are the other group of secondary metabolites [7]. The uses of plant-derived products as disease control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance [8]. *Hence the present paper reports the phytochemical and antimicrobial activity of Eichhornia crassipes.*

MATERIALS AND METHODS

Eichhornia crassipes was collected from the lake near Ukkadam at Coimbatore. The plant sample was identified by Dr. J. Sudhakar, Botanical survey of India, Coimbatore. The root portion was cut off and the plant was washed thoroughly under running tap water to free from debris. The leaves and shoot portion of the fresh plant material was chopped into small pieces. The plant material was extracted with ethyl acetate, ethanol and water in heating mantle and after complete extraction the solvent was removed by distillation in rotary evaporator. The crude ethanol extract was fractioned using $CHCl_3$ and HCl. Fractionates and the extracts were stored in refrigerator for the following studies.

Phytochemical Screening
Ethanolic extract of fresh *Eichhornia crassipes* was subjected to preliminary phytochemical screening for the detection of various plant constituents [4, 9, and 10].

Test for Alkaloids
Wagner's test: A fraction of extract was treated with Wagner's test reagent [1.27 g of iodine and 2 g of potassium iodide in 100 ml of water] and observed for the formation of reddish brown colour precipitate.

Test for Flavonoids
NaOH test: A small amount of extract was treated with aqueous NaOH and HCl, observed for the formation of yellow orange colour.

H_2SO_4 test: A fraction of extract was treated with concentrated H_2SO_4 and observed for the formation of orange colour.
Lead acetate test: A small amount of extract was treated with lead acetate and observed for the formation of white precipitate.

Test for Tannins
Braymer's test: Few ml of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

Test for Saponins
Foam test: A small amount of extract was shaken with water and observed for the formation of persistent foam.

Test for Carbohydrates
Molisch's test: Few drops of Molisch's reagent was added to each of the portion dissolved in distilled water, this was then followed by addition of 1 ml of conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet colour at the interphase of the two layers was a positive test.

Fehling's test: About 0.5 g of each extract was dissolved in distilled water and filtered. The filtrate was heated with 5 ml of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars.

Test for Quinones: A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

Test for Terpenoids
Liebermann – Burchard test: Extract (1ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark green colour.

Test for Sterols
Liebermann – Burchard test: Extract (1ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark pink or red colour.

H_2SO_4 test: The fraction of extract was treated with ethanol and H_2SO_4 and observed for the formation of violet blue or green colour.

Test for Phenols
Ferric chloride test: The fraction of extract was treated with 5 % ferric chloride and observed for formation of deep blue or black colour.

Liebermann's test: The extract was heated with sodium nitrite, added H_2SO_4 solution diluted with water and excess of dilute NaOH was added and observed for the formation of deep red or green or blue colour.

Test for Anthraquinones
Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia; pink or deep red colourations of aqueous layer indicate the presence of anthraquinone.

Test for Anthocyanin
NaOH test: A small amount of extract was treated with 2M NaOH and observed for the formation of blue green colour.

Test for Proteins
Ninhydrin test (aqueous): The extract was treated with aqueous ninhydrin, purple colour indicates the presence of protein.

Ninhydrin (acetone): Ninhydrin was dissolved in acetone; the extract was treated with ninhydrin and observed for the formation of purple colour.

Evaluation of antimicrobial activity
Antibacterial activity
The potential of the crude extracts and fractionates of fresh *Eichhornia crassipes* to inhibit the growth of bacteria was determined by antibacterial activity [11]. The aqueous, chloroform fractionates and the ethanol and ethyl acetate extracts were used for the study. The test microorganism used in this study (*Micrococcus luteus* and *Rhodospirillum rubrum*) were obtained from KMCH Laboratory, Coimbatore.

Preparation of inoculums: The inoculums for the experiment were prepared in fresh Nutrient broth from preserved slant culture. The inoculums were standardized by adjusting the turbidity of the culture to that of McFarland standards by further incubating it.

Preparation of sterile swabs: Cotton wool swab on plastics were prepared and sterilized by autoclaving by packing the swabs in culture tubes.

Sterilization of forceps: The forceps were sterilized by dipping in alcohol and burning off the alcohol.

Experiment: The standardized inoculums were inoculated in the plates prepared earlier (aseptically) by dipping a sterile in the inoculums removing the excess of inoculums by passing by pressing and by rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60^oC after each application. Finally the swab was passed round the edge of the agar surface. The inoculums were left to dry at room temperature with the lid closed.

Each petri dish was divided into 5 parts, of which 4 parts extract sample disc (100µg), (discs were soaked overnight in extract solution) and another part for STD Ciprofloxacin 10µg, were placed with the help of sterile forceps. Then

petri dishes were placed in the refrigerator at 4^{o}C for diffusion. It was then incubated at 37^{o}C for 24 h. The zone of inhibition produced was measured and recorded.

Anti fungal activity

The potential of the crude extracts and fractionates of fresh *Eichhornia crassipes* to inhibit the growth of fungi was determined by suitable antifungal activity [11]. The aqueous, chloroform fractionates and the ethanol and ethyl acetate extracts were used for the study. The test fungi used in this study (*Monoscus ruber* and *Rhodospirillum rubrum*) was obtained from KMCH Laboratory, Coimbatore.

Preparation of inoculums: The inoculums for the experiment were prepared in fresh Sabouraud's broth from preserved slant culture. The inoculum was standardized by adjusting the turbidity of the culture to that of McFarland standards by further incubating it.

Preparation of sterile swabs: Cotton wool swab on plastics were prepared and sterilized by autoclaving by packing the swabs in culture tubes.

Sterilization of forceps: The forceps were sterilized by dipping in alcohol and burning off the alcohol.

Experiment: The standardized inoculums were inoculated in the plates prepared earlier (aseptically) by dipping a sterile in the inoculums removing the excess of inoculums by passing, by pressing and by rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60^{o}C after each application. The swab was passed round the edge of the agar surface. The inoculums were left to dry at room temperature with the lid closed.

Each petri dish was divided into 5 parts, in 4 part extract sample disc (100mcg), (discs are soaked overnight in extract solution) and another part for STD Co-Trimaxozole 25µg, are placed with the help of sterile forceps. Then Petri dishes were placed in the refrigerator at 4^{o}C for 1 h for diffusion. It was then incubated at room temperature for 24– 48h. The zone of inhibition produced was measured and recorded.

RESULTS AND DISCUSSION

Phytochemical screening suggests that ethanolic extract contain various constituents which are given in the table 1. The preliminary phytochemical tests result indicates the presence of alkaloids, flavonoids, phenols, sterols, terpenoids, anthoquinones and protein and the absence of quinines, anthocyanin, saponin, carbohydrates and tannins. The presence of wide range of phytochemical constituent indicates that the plant could be used in a multitude of ways which may be beneficiary to the population [4]. An important part of natural products from plants, biomolecules and secondary metabolites usually exhibits some kind of biological activities. They are widely used in the human therapy, veterinary, agriculture, scientific research and in countless other areas [12]. The usefulness of plant materials medicinally is due to the presence of bioactive constituents such as alkaloids, tannins, flavonoids and phenolic compounds [13].

Alkaloids play some metabolic role and control development in living system. They are also involved in protective function in animals and are used as medicine especially the steroidal alkaloids. Tannins are known to inhibit pathogenic fungi. Flavonoids are known to inhibit the initiation, promotion and progression of tumours [13]. The flavonoids and phenolic compounds in plants have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic etc [14]. Anthraquinones are considered to be one of the most active agents in metastatic breast cancer. The antimicrobial activity of the extracts could be explained by the presence of tannins. The mechanism of action of tannins is based on their ability to bind proteins thereby inhibiting cell protein synthesis [15]. Triterpenoids are a large class of natural isoprenoids present in higher plants, which exhibit a wide range of biological activities. Steroids (anabolic steroids) have been observed to promote nitrogen retention in osteoporosis and in animals with wasting illness [4].

Jayanthi *et al* [4, 16] reported the presence of alkaloids, flavonoids, sterols, terpenoids, anthroquinone, proteins, phenols and anthocyanins in ethanol fractionate of dry waterhyacinth where as in this study it was found that fresh plant also contains metabolites as in dry plant except anthocyanins. Lata and Dubey [13] demonstrated the presence of alkaloid, flavonoids, steroid, tannins, phenolic contents, quinone and anthraquinone in aqueous extract of dry

Eichhornia crassipes. Kandukuri *et al* [12] reported the presence of alkaloid, phenol, steroid, tannin and saponin in methanol extract of dry *Eichhornia crassipes* where as reported the absence of flavonoids. Ndubuisi *et al* [17] revealed the presence of saponin, glycoside and anthraquinone but absence of alkaloid in chloroform extract of dry waterhyacinth. Presence of flavonoids in this plant was reported by Nyananyo *et al* [18]. All the above study was done in dry *Eichhornia crassipes* but the present study was carried out in fresh *Eichhornia crassipes*. This study was carried out to compare the potential of fresh plant with dry plant.

Ahmed *et al* [19] revealed the presence of phenol and tannin-like compounds in aqueous extract of fresh waterhyacinth. The presence of high phenol content was due to the presence of specialized phenol cells. Tannin-like compounds were found to be released from the plant detritus and decayed materials after cutting or herbicide application.

Table 1: Qualitative screening of the phytochemicals in the ethanol extract of fresh plant *Eichhornia crassipes*

S.No	Photochemicals	Ethanol extract
1.	Alkaloids	
A.	Wagner's test	+
2.	Flavonoids	
A.	Lead acetate test	+
B.	H_2SO_4 test	+
3.	Sterols	
A.	H_2SO_4 test	+
B.	Liebermann-Burchard test	+
4.	Terpenoids	
A.	Chloroform test	+
B.	Liebermann-Burchard test	+
5.	Anthroquinone	
A.	Borntrager's test	+
6.	Anthocyanins	
A.	NaOH test	-
7.	Proteins	
A.	Ninhydrin (acetone) test	+
8.	Phenols	
A.	Ferric chloride test	+
B.	Libermann's test	+
9.	Quinones	
A.	HCl test	-
10.	Carbohydrates	
A.	Molisch test	-
B.	Fehling's test	-
11.	Saponin	-
12.	Tannin	-

The *Eichhornia crassipes* extracts such as aqueous, chloroform (from fractionation), ethanol and ethyl acetate were screened for their antibacterial activity against *Micrococcus luteus* and *Rhodospirillum rubrum* by disk diffusion method and the results were appreciable.

Table 2: Phytochemicals in waterhyacinth

Waterhyacinth	Extract	Reported by	Metabolites
Dry	Aqueous extract	Lata & Dubey [13]	alkaloid, flavonoids, steroid, tannins, phenolic contents, quinone and anthraquinone
Dry	Methanol extract	Kandukuri *et al* [12]	alkaloid, phenol, steroid, tannin and saponin
Dry	Chloroform	Ndubuisi *et al* [17]	saponin, glycoside and anthraquinone
Dry	Ethanol fractionate	Jayanthi *et al* [4, 16]	alkaloids, flavonoids, sterols, terpenoids, anthroquinone, proteins, phenols and anthocyanins
Fresh	Ethanol extract	Present study	alkaloids, flavonoids, sterols, terpenoids, anthroquinone, proteins, and phenols
Fresh	Aqueous extract	Ahmed *et al* [19]	phenol and tannin

In the disc diffusion method, concentration gradients of the drug in a nutrient medium was prepared and grow of the bacteria, seeded in the medium after an inoculation period was observed. The clear zone of growth inhibition was noted around the disc due to diffusion of drug and growth of bacteria.

The diameter of the zone denotes the relative susceptibility of the test microorganism to a particular antimicrobe. The term susceptible implies that an infection caused by strain tested may be expected to respond favorably to the indicated antimicrobial agent for that type of infection and pathogen. In the present study the four extracts were highly sensitive and moderately susceptible.

Table 3: Antibacterial activity of extracts of *Eichhornia crassipes*

S.No	Organisms Samples	Zone of inhibition (mm)	
		Micrococcus luteus	*Rhodospirillum rubrum*
1	Ciprofloxacin (10 µg/disc)	17	21
2	Ethanol	11	10
3	Ethyl acetate	10	12
4	Aqueous	9	10
5	Chloroform	8	14

Figure 1: Zone of inhibition - bacteria *Micrococcus luteus*

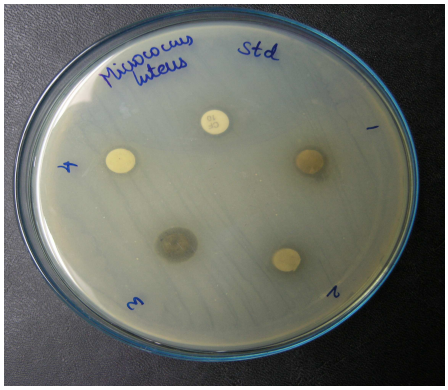


Figure 2: Zone of inhibition - bacteria *Rhodospirillum rubrum*

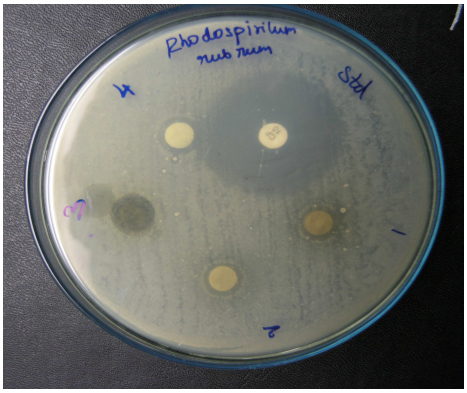


Figure 3: Zone of inhibition - fungi *Monoscus ruber*

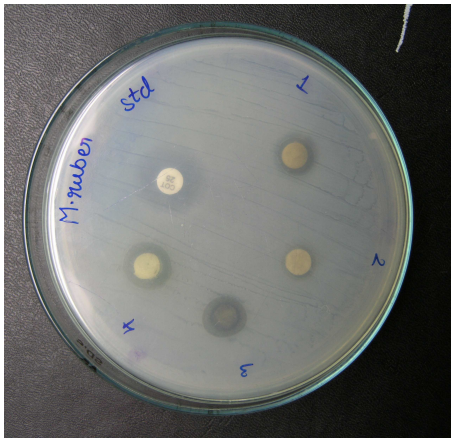
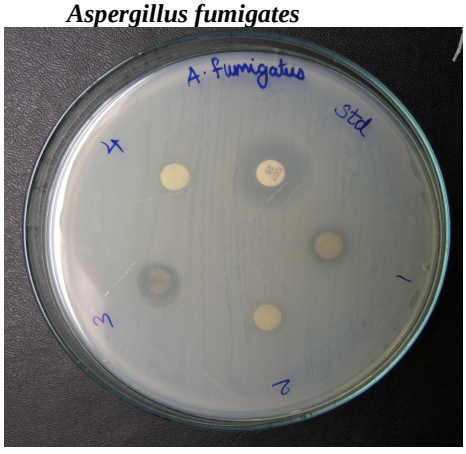


Figure 4: Zone of inhibition - fungi *Aspergillus fumigates*



It was obvious from the results that among the extracts; ethanol extract showed significant activity against *Micrococcus luteus* and chloroform extract showed significant activity against *Rhodospirillum rubrum*, whereas ethyl acetate and aqueous extracts were appreciable. The zone of inhibition of aqueous and chloroform fractionate, ethanol and ethyl acetate extracts against two bacteria *Micrococcus luteus* and *Rhodospirillum rubrum* are shown in the figure 1 and 2.

The *Eichhornia crassipes* extracts were screened for their antifungal activity against *Monoscus ruber* and *Aspergillus fumigatus* by disk diffusion method and the activity of extracts were appreciable.

Ethanol and chloroform extracts showed significant activity against *Monoscus ruber* whereas aqueous and ethanol extracts showed significant activity against *Aspergillus fumigates.*

Table 3 shows the efficiency of *Eichhornia crassipes* to inhibit the growth of fungi. The zone of inhibition of aqueous, chloroform, ethanol and ethyl acetate extracts against two fungi *Monoscus ruber* and *Aspergillus fumigatus* were shown in the figure 3 and 4.

Table 4: Antifungal activity of extracts of *Eichhornia crassipes*

S.No	Organisms Samples	Zone of inhibition (mm)	
		Monoscus ruber	***Aspergillus fumigates***
1	Co-Trimaxozole (25µg/disc)	18	16
2	Ethanol	12	11
3	Ethyl acetate	10	09
4	Aqueous	11	11
5	Chloroform	12	08

The antimicrobial studies show the good antibacterial potential of the ethanol extracts of waterhyacinth. The results of phytochemical screening indicate that the plant extracts contains alkaloid and flavonoids. The antimicrobial activity of the extracts might be attributed to the presence of the foresaid secondary metabolites in the extracts.

CONCLUSION

Phytochemical screening carried out with fresh waterhyacinth has revealed the presence of many metabolites which in turn contributes to its antimicrobial activity. Not much work has been reported with fresh waterhyacinth. The present study portrays that the phytochemicals in fresh waterhyacinth may contribute in many significant ways for various studies in a truthful manner to the pharmaceutical activity of the plant.

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