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Research Article

PRELIMINARY PHYTOCHEMICAL ANALYSIS AND *IN VITRO* INVESTIGATION OF ANTIMICROBIAL ACTIVITY OF *EICHHORNIA CRASSIPES* (MART.) SOLMS. AGAINST POULTRY PATHOGENS

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ABSTRACT
Eichhornia crassipes (Mart.) Solms (Waterhyacinth) is an aquatic macrophyte, monocotyledon of the family pontederiaceae. It is listed as one of the most productive plant on earth and is considered the world's worst aquatic weed. The preliminary phytochemical analysis of leaf extracts of *Eichhornia crassipes* (Mart.) Solms. reveals the presence of alkaloids, amino acids, carbohydrate, fats, flavonoids, glycosides, phenols, protein, saponins, sterols, tannins and terpenoids. The antimicrobial efficacy of acetone, distilled water, ethanol, ethyl acetate, methanol and n-Butyl alcohol extracts of *E. crassipes* were analyzed by well agar method. The leaf extracts of *E. crassipes* (Mart.) Solms., standard antibiotics streptomycin and fluconazole exhibited variable degrees of antimicrobial activity. Among the six extracts tested, n-Butyl alcohol extract showed significant antimicrobial activity against the poultry pathogens.

Keywords: *Eichhornia Crassipes, Leaf Extracts, Antimicrobial Activity, Poultry Pathogens*

INTRODUCTION
The world is rich with natural and unique medicinal plants. Medicinal plants are now getting more attention than ever because they have potential of myriad benefits to society or indeed to all mankind, especially in the line of medicinal and pharmacological aspect. Traditional use of the medicinal plants have fewer side effect over allopathic medicine such promising fact leads the development of herbal derive medicines whole over the world (Pal and Shukla, 2003). Plants contains flavonoids, alkaloids, Tannins, phenols etc., which have biological significance in terms of medicine development and extracts of aqueous, methanol and ethanol are good source of antiviral, antitumor and antibacterial agents (Cowan, 1999).
Eichhornia crassipes (Mart.) Solms. commonly known as Water hyacinth is a warm water aquatic plant belonging to the family Pontideriaceae. It 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, form dense rafts in the water and mud (Mane *et al*., 2011). 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 (Jayanthi *et al*., 2011).
It is listed as one of the most productive plant on earth and is considered the world worst aquatic weed (Grodowitz, 1998).
The 'beautiful blue devil', water hyacinth is recognised by its lavender flowers and shining bright leaves which spread at an alarming rate. Its habitat ranges from tropical desert to subtropical or warm temperate rain forest zones, and tolerates a temperature range of 21.1 to 27.2ºC (Lata and Venapani, 2010). It can be used as compost, paper, fuel, and animal feed and water purification (Kristie, 2012). It is also an excellent source of biomass; and use to make furniture, hand bags and ropes in East Africa. Its flowers are used as a medication on skin of horses and a tonic (Wikipedia, 2012).
Waterhyacinth possesses phytochemicals (Lata and Dubey, 2010; Jayanthi *et al*., 2011; Jayanthi and Lalitha, 2011) which are of medicinal importance (Sayeed Ahmad, 2010). The methanol extract of leaves of this plant aids in wound healing process (Ali *et al*., 2010) and has tumour inhibition potential (Ali *et al*., 2009). In addition, the extracts of this plant showed antimicrobial activity (Shanab *et al*., 2010).

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. Hence the present investigation reports the phytochemical and antimicrobial activity of *Eichhornia crassipes* against poultry pathogens.

MATERIALS AND METHODS

Collection of Plant Material

Fresh leaves of *E. crassipes* (Mart.) Solms. free from disease were collected at local pond in Nagapattinam District (Figure 1). The leaves were washed thoroughly 2-3 times with running water and once with sterile distilled water, leaf material was then air-dried on sterile blotter under shade.

Solvent Extraction

Acetone, ethanol, ethyl acetate, methanol and n-Butyl alcohol extracts of *E. crassipes* (Mart.) Solms. were prepared according to the methodology of Indian Pharmacopoeia (Anonymous, 1966). The aqueous extraction achieved through the percolation method. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50ºC). The extracts were put in air tight containers stored in a refrigerator.

Preliminary Phytochemical Analysis

Preliminary phytochemical screening of the leaf extracts of *E. crassipes* (Mart.) Solms. was carried out as per standard procedure (Kokate *et al*., 2005; Harborne, 2005).

Screening of Antimicrobial Activity

The antimicrobial activity of *E. crassipes* (Mart.) Solms. was evaluated by well agar method (Perez *et al*., 1990).

Selection of Microorganisms

Totally ten chicken pathogenic microorganisms namely five bacterial strains such as, *Escherichia coli, Proteus vulgaris, Salmonella pullorum, Staphylococcus aureus* and *Streptococcus pyogenes* and five fungal strains such as, *Aspergillus flavus, A. fumigatus, A. ochraceus, Candida albicans* and *Trichophyton megnini* were selected for the present investigation. The chicken pathogenic bacteria and fungi were originally obtained from Microbial Germ Plasm Culture Collection Unit (MGPCCU), Sri Gowri Biotech Research Academy, Thanjavur and used for the present investigation.

Antimicrobial Activity

The antimicrobial activity of ethanol, acetone, ethyl acetate, methanol, n-Butyl alcohol and distilled water extracts of *E. crassipes* (Mart.) Solms. were tested against the selected bacterial and fungal strains. The sterilized nutrient agar and potato dextrose agar medium was poured into each sterile petriplates and allowed to solidify. By using a sterile cotton swabs, a fresh bacterial and fungal cultures were spread over the plates by following spread plate technique. One well of 5mm size made into the agar plates with the help of sterile cork borer, the wells were loaded with 200µl of ethanol, acetone, ethyl acetate, methanol, n-Butyl alcohol and distilled water extracts of *E. crassipes* were loaded in to separated wells. The plates were incubated for 24 hours at 37^{o}C for bacteria and 28^{o}C for 48 - 72 hours for fungi. After the incubation period, the results were observed and the diameter of the inhibition zone was measured around the wells. The antibiotic sensitivity test was investigated using standard antibiotics Streptomycin (10 µg/ disc) for bacteria and Fluconazole (10 µg/ disc) for fungi.

RESULTS AND DISCUSSION

Phytochemicals are responsible for medicinal activity of plants (Savithramma *et al.,* 2011). In the present study, the preliminary phytochemical screening of the leaf extracts of *E. crassipes* (Mart.) Solms. revealed the presence of alkaloids, amino acids, carbohydrate, fats, flavonoids, glycosides, phenols, protein, saponins, sterols, tannins and terpenoids (Table 1). These results of phytochemical screening of study plant were in concurrence with other reports (Thamaraiselvi *et al*., 2012; Jayanthi and Lalitha, 2013; Aravind *et al*., 2013). There were a similarity between their results and the obtained results in this study.

In the present study, acetone, distilled water, ethanol, ethyl acetate, methanol and n-Butyl alcohol extracts of *E. crassipes* (Mart.) Solms. and standard antibiotics streptomycin and fluconazole exhibited variable degrees of antimicrobial activity. The results were given in Table 2, 3, 4 & 5 and Plate 1, 2, 3 & 4. The inhibition activity of the plant extracts were compared with standard antibiotics streptomycin and fluconazole. The maximum antibacterial activity was exhibited by n-Butyl alcohol extract against *Streptococcus pyogenes*. The acetone extract showed maximum zone of inhibition against *Aspergillus ochraceus*. These results of antimicrobial activity of different extracts of *E. crassipes* (Mart.) Solms. were in coincidence with results of Mahavir and Sandeep (2013) who reported that, the leaves of *E. crassipes* exhibited significant antibacterial activity. Similar works were done by Thamaraiselvi *et al.,* (2012) and Jayanthi and Lalitha (2013) who demonstrated that leaf extracts of *E. crassipes* exhibited considerable antibacterial and antifungal activity.

Waterhyacinth which is often considered a weed has in recent days been taken up for many researches concerning the phytochemical and pharmaceutical applications. From the present study it can be concluded that the leaf extracts of Waterhyacinth showed significant antimicrobial activity against all the microorganisms tested.

Table 1: Qualitative phytochemical analysis of leaf extracts of *Eichhornia crassipes* (Mart.) Solms

S. No	Phytocompounds	Acetone	Distilled water	Ethanol	Ethyl acetate	Methanol	n-Butyl alcohol
1.	Alkaloids	+	+	-	-	+	+
2.	Amino acids	-	-	+	+	-	-
3.	Carbohydrates	-	+	+	+	-	-
4.	Fat	-	-	-	-	-	+
5.	Flavonoids	-	-	-	+	+	+
6.	Glycosides	-	+	-	-	+	-
7.	Phenols	+	-	+	-	-	+
8.	Protein	-	+	+	-	-	+
9.	Saponins	-	+	-	+	+	-
10.	Sterols	+	-	+	+	+	-
11.	Tannins	+	-	-	-	+	-
12.	Terpenoids	+	+	+	-	+	+

+: Presence - : Absence

Table 2: Antibacterial activity of *Eichhornia crassipes* (Mart.) Solms

S.No	Bacterial pathogens	Zone of inhibition (diameter in mm)					
		Acetone	Distilled water	Ethanol	Ethyl acetate	Methanol	n-Butyl alcohol
1.	*Escherichia coli*	10	8	9	-	9	10
2.	*Proteus vulgaris*	11	9	10	-	-	-
3.	*Salmonella pullorum*	8	-	-	-	8	10
4.	*Staphylococcus aureus*	-	-	10	-	10	15
5.	*Streptococcus pyogenes*	9	-	9	10	8	20

Table 3: Antifungal activity of *Eichhornia crassipes* (Mart.) Solms

S.No	Fungal pathogens	Zone of inhibition (diameter in mm)					
		Acetone	Distilled water	Ethanol	Ethyl acetate	Methanol	n-Butyl alcohol
1.	*Aspergillus flavus*	10	9	10	-	-	-
2.	*A. fumigates*	14	10	13	8	-	10
3.	*A. ochraceus*	15	9	12	-	-	10
4.	*Candida albicans*	10	9	8	-	-	11
5.	*Trichophyton megnini*	10	9	8	9	-	10

Table 4: Effect of standard antibiotics on bacterial pathogens

S.No	Bacterial pathogens	Zone of inhibition (diameter in mm) Streptomycin
1.	*Escherichia coli*	12
2.	*Proteus vulgaris*	10
3.	*Salmonella pullorum*	13
4.	*Staphylococcus aureus*	9
5.	*Streptococcus pyogenes*	19

Table 5: Effect of standard antibiotics on fungal pathogens

S.No	Bacterial pathogens	Zone of inhibition (diameter in mm) Fluconazole
1.	*Aspergillus flavus*	10
2.	*A. fumigatus*	19
3.	*A. ochraceus*	18
4.	*Candida albicans*	20
5.	*Trichophyton megnini*	9

Figure 1: Leaves of *Eichhornia crassipes* (Mart.) Solms

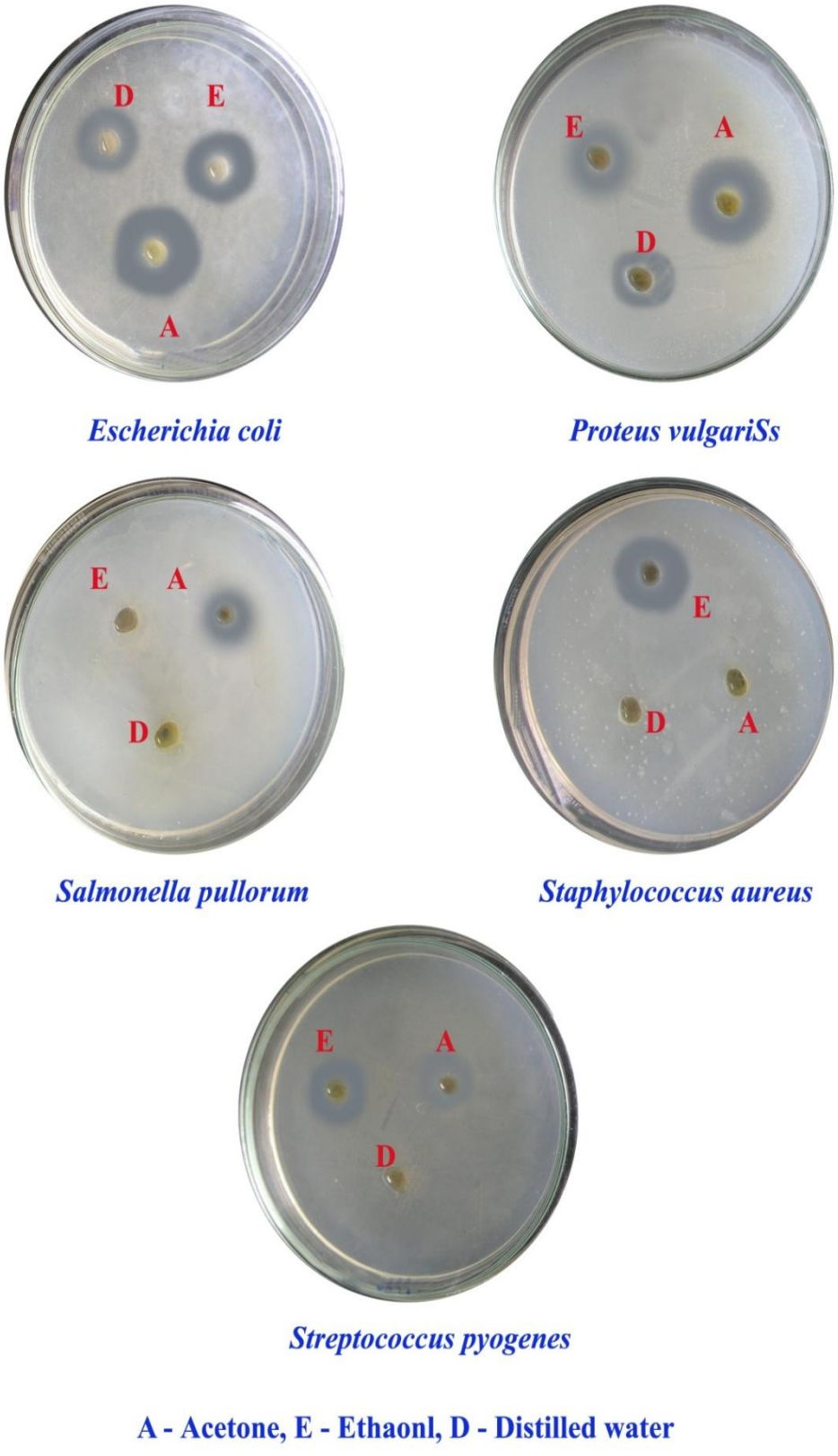

Plate 1: Antibacterial activity of *Eichhornia crassipes* (Mart.) Solms

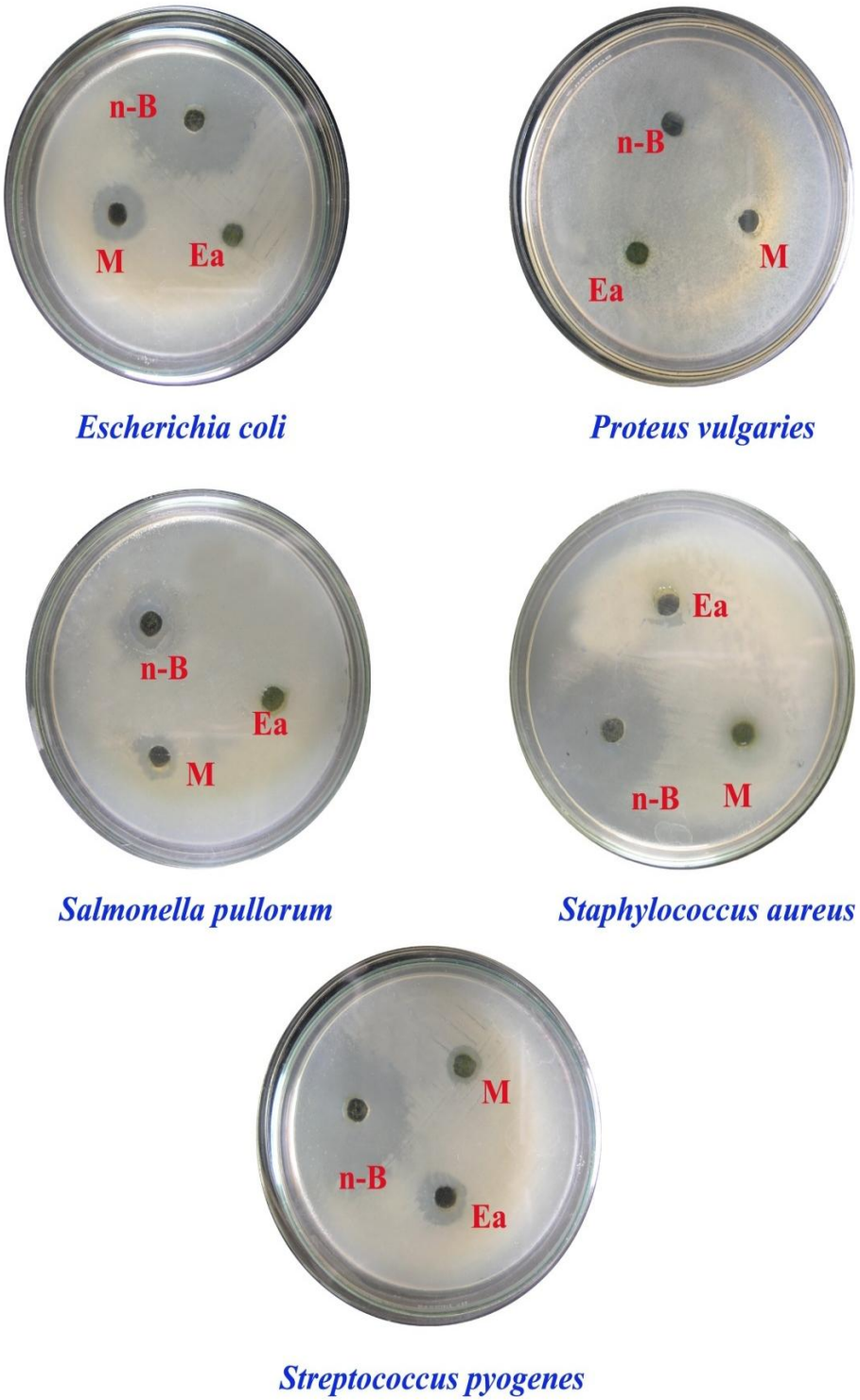

Plate 2: Antibacterial activity of *Eichhornia crassipes* (Mart.) Solms

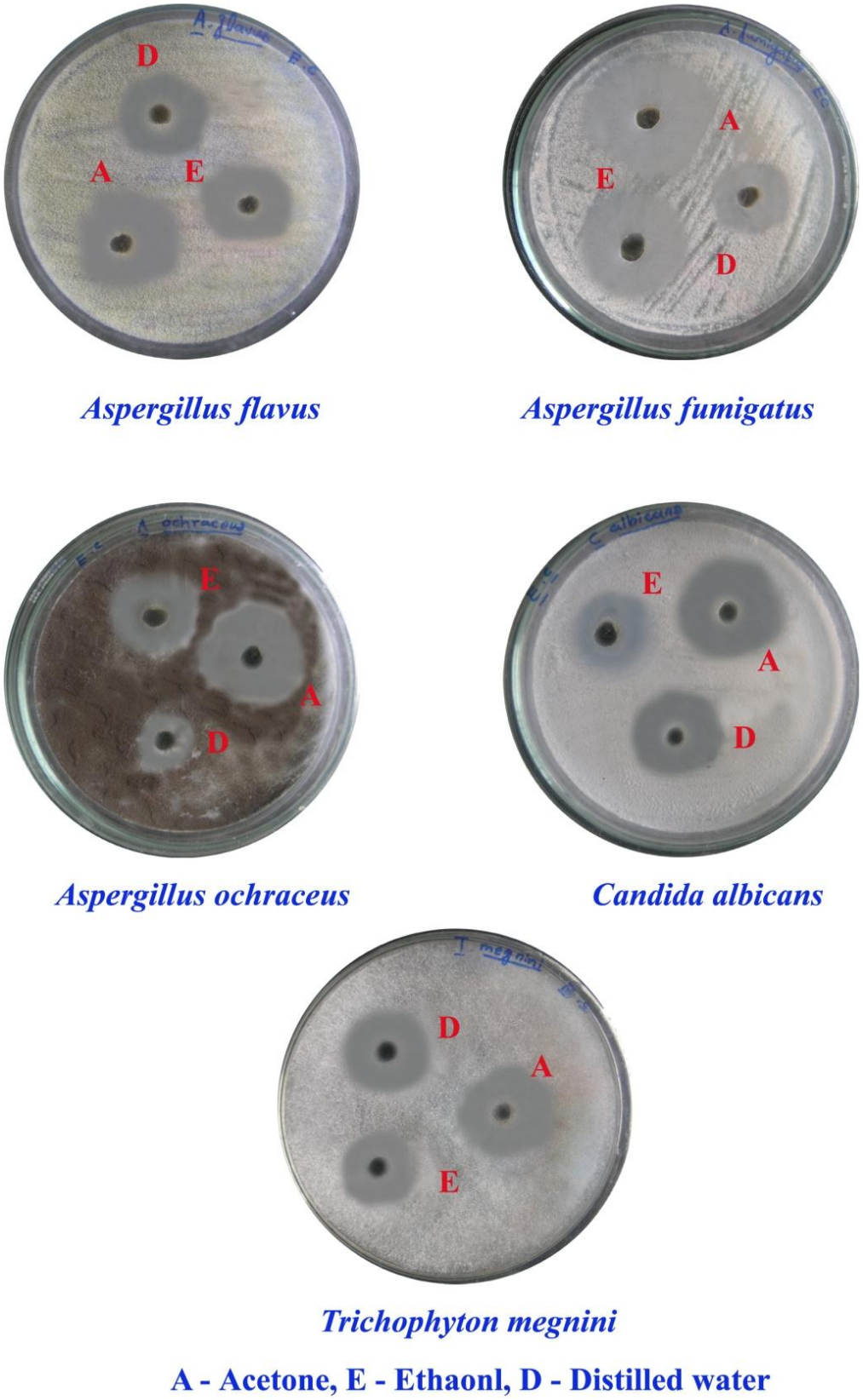

Plate 3: Antifungal activity of *Eichhornia crassipes* (Mart.) Solms

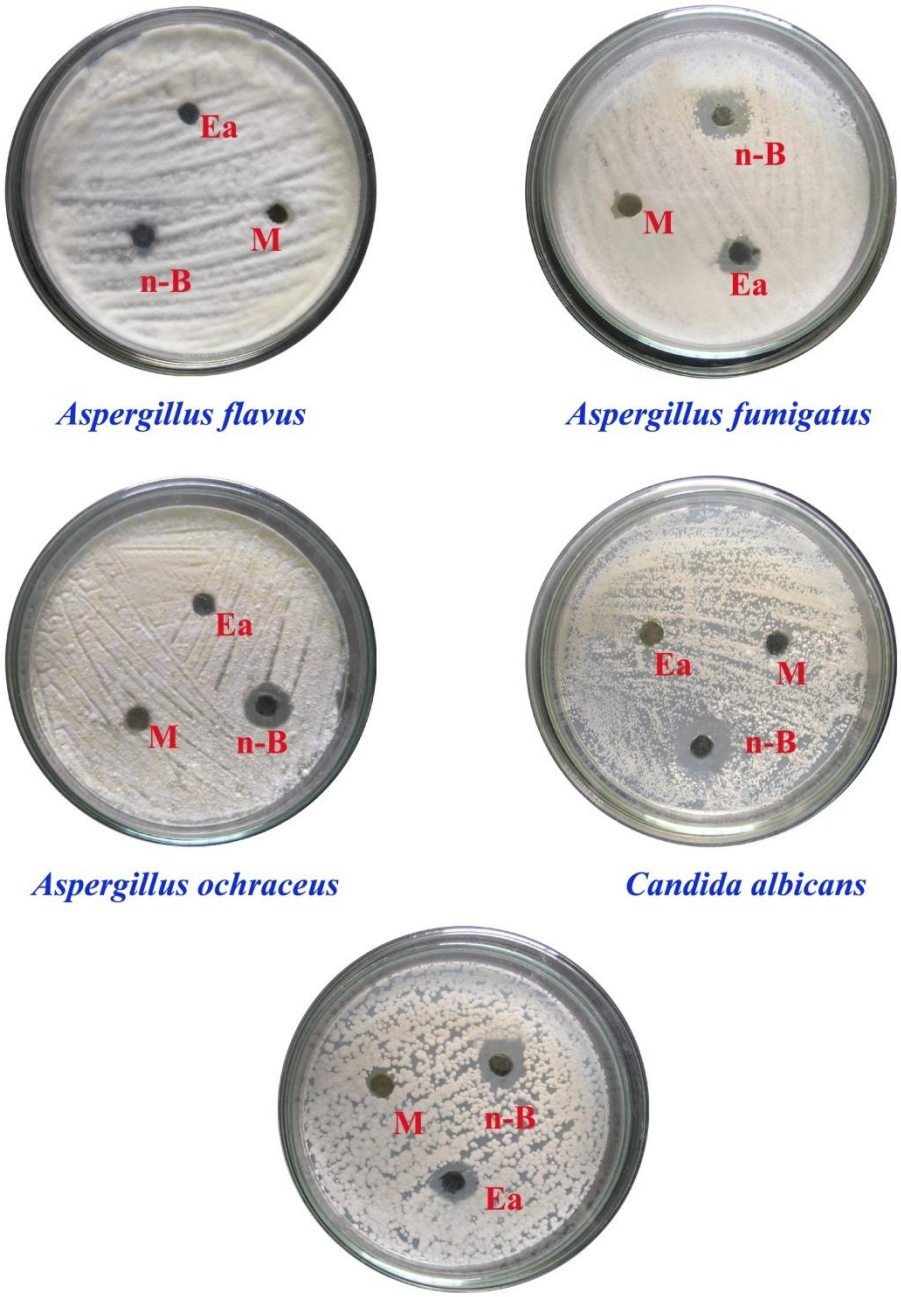

Plate 4: Antifungal activity of *Eichhornia crassipes* (Mart.) Solms

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