



ANTICANDIDAL ACTIVITY OF PHYTOCHEMICALS EXTRACTED FROM MEDICINAL PLANT *ECLIPTA PROSTRATA*

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ABSTRACT

Eclipta prostrata is a widely used plant in traditional medicine for the treatment of several human diseases like hepatitis, asthma and skin infections. The antimicrobial spectrum of *Eclipta prostrata* was initially checked against fungus *Aspergillus niger*, *Candida albicans* and bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. Phytochemicals extracted in organic solvents ethanol (70%), methanol (80%), chloroform (100%) and acetone (100%) were evaluated for their antimicrobial potential by agar well diffusion method. From the antibiogram analysis, it was observed that 70% Ethanolic leaves extract showed higher zone of inhibition against *Candida albicans* (22mm), *Aspergillus niger* (18.6mm) and bacterial strains *Pseudomonas aeruginosa* (20.6mm), *Escherichia coli* (19.9mm) and *Staphylococcus aureus* (21mm) compared to other organic solvents. Ethanolic leaves extract showing best results when added with Copper heavy metal augmented the anticandidal activity upto 2 fold and broad inhibition zones against *Candida albicans* (40mm) and *Pseudomonas aeruginosa* (25mm) were observed. It was thus concluded that *Eclipta prostrata* phytochemicals extracted in ethanol in combination with copper showed marked anticandidal activity.

KEYWORDS: *Anti-Candida, Phytochemicals, Eclipta prostrata*



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Received on: 21-07-2018

Revised and Accepted on: 31-08-2018

DOI: <http://dx.doi.org/10.22376/ijpbs.2018.9.4.p50-56>



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INTRODUCTION

Phytotherapy is practiced by a large population as a traditional system for the treatment of several physical, physiological and mental disorders. Phytochemicals have come up as effective and novel antifungal agents with less undesirable side effects, toxicity and drug-drug interaction. To promote the proper uses of herbal medicine and determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folkloric reputation. *Eclipta prostrata* (family Asteraceae) has been used in the treatment of infective hepatitis in India¹ and as an antidote snake venom poisoning in Brazil.² It has been reported that the leaves of this herb are used in the case of gastritis and respiratory disorders like cough and asthma.³ In addition, the crude form of the herb is reported to have anti-inflammatory, anti-fungal and anti-hepatotoxic properties.⁴ It is also used in catarrhal jaundice, for skin diseases and is a potential source of alkaloid.⁵ The water extract of *Eclipta prostrata* (whole plant) exhibit potent inhibitory activity against HIV-1 integrase (HIV-1 IN).⁶ The methanolic extract from the leaves, barks, and roots of *E. prostrata* have been shown to possess significant anti-bacterial and antifungal activity.⁷ Candidal infections in immunocompromised patients have significantly increased in recent years, which intensify the need for new drug discoveries. *Candida albicans* is the fourth major cause of chronic fungal infections which creates infection in the mucosa and deep tissue by the formation of biofilm. The ability of *Candida* to form biofilms makes it able to create a long lasting infection in humans and animals. Fighting and treating microorganisms which can form biofilms has become a serious problem in the domain of health. Crude extracts of many plants viz. *Clausena anisata*, *Sclerocariya birrea*, *Turraea holstii*, *Sterculia africana*, *Acacia robusta* sub sp.

Usambarensis, *Cyphosterna hildebrandti*, *Elaeodendron buchannanii*, *A. nilotica*, *Jatropha multifida*, and *Pteridium aquilinum* have been studied for their efficient anticandidal activity against *C. neoformans*, *C. krusei*, *C. tropicalis* and *C. parapsilosis*.⁸ Plant extracts have also been used as reducing and stabilizing agents in the synthesis of nanoparticles.^{9,10} Several studies have shown that biomolecules like amino acids, organic acids, phenols, and flavonoids present in plants play a vital role in reducing the metal ions to their respective nanomaterials as well as aid in the capping of the metal NPs, which keeps them stable for a longer period.¹¹ Biosynthesized nanoparticles have gained potential in recent years as a cost-effective eco-friendly alternative to antimicrobial agent which can have wide application in medicine. Many recent reports demonstrate the anticandidal activity of phytochemicals. Rajakumar (2012)¹² has report the synthesis of AuNPs by reduction of gold ions using *E. prostrata* extract. One of the Nps applicable in industry and medicine is copper oxide Nps. Thus with an idea that plant extracts have an ability to synthesize nanoparticles of heavy metals which has strong antimicrobial activity, the present study was undertaken to evaluate the anti-candidal activity of *E. prostrata* extract in presence of different heavy metals.

MATERIAL AND METHOD

Collection of plant

Plants of *E. prostrata* were collected from the campus of Banaras Hindu University, Varanasi, during the months of June-July. Plants were collected from different locations to make a composite sample. The plant was authenticated at Botanical Survey of India (BSI), Allahabad. Collection number BHU-171 and voucher number-91926 was given by BSI to the plant flora.



Figure1
Picture of *Eclipta prostrata* L. plant

Phytochemical Extraction

Plants were washed thoroughly for dust removal under running tap water. The plant material was oven dried at 40-45 °C for 24 h. The dried leaves were then powdered by using blender to get very fine powder and kept in fridge in a sealed shut glass compartment until further use. 5 grams of plant leaf powder was macerated in

methanol (80%), ethanol (70%), acetone (100%) and chloroform (100%) each with a volume of 100 ml. Extracts were filtered using a whatman filter paper No.1 in a pre-weighed bowl. The bowls were carefully covered with aluminum foil and pin holes were made into it so as to allow evaporation to dryness under reduced pressure of the solvents at the room temp.

When the solvents evaporated and the extracts dried, bowls were weighed again. Difference in weights gave the amount of dried extract.

Screening for antimicrobial activity

Kirby bauer agar well diffusion assay was used for assessing the antimicrobial activity of *Eclipta prostrata* in different organic solvent extract against diverse bacteria and fungi. The microbes selected to determine the antimicrobial activity of the leaves extract of *Eclipta prostrata* were gram-negative bacteria (*Pseudomonas aeruginosa*; *Escherichia coli* DH5 α), gram-positive bacteria (*Staphylococcus aureus*), *Aspergillus* sp. fungi and human pathogenic *Candida albicans*. Nutrient broth and nutrient agar media were used throughout the experiments for bacteria. For *Candida albicans*, Sabouraud Dextrose broth and Sabouraud Dextrose agar (SDA) was used during the experiments. Amphotericin B for *Candida albicans* and streptomycin for bacteria were used as positive control. Stock solutions were prepared by dissolving the 1mg of dried leaf extract in DMSO. From these stock solutions, 50 μ g/ml of working concentration was prepared. 30 μ l (10^9 CFUml $^{-1}$) of test microorganism were spread on nutrient agar plates. Four wells (8 mm in diameter) were cut into the agar media with a sterilized cork borer, 50 μ l of plant extract of each solvent containing 50 μ g/ml was poured into respective well. 50 μ l of antibiotic (50 μ g/ml) and DMSO (50 μ l per well) were also poured into one well each per plate as a positive and negative control, respectively. Inoculated plates were then incubated in

BOD incubator at 28 $^{\circ}$ C for 24 hours and zones of inhibition were measured in mm. The zone of inhibition was also observed by adding 1% metal salt ZnSO $_4$, FeSO $_4$ and CuSO $_4$ to the crude extract obtained in different solvents. All the tests were performed thrice to check the accuracy of antimicrobial activity.

RESULTS

Screening for antimicrobial activity of *Eclipta prostrata* in different organic solvents extract was done by Kirby bauer Agar Well diffusion method. The zone of clearance around the well indicates the positive antimicrobial activity given by test samples as shown in figure 2 and figure 4. The zone of inhibition measured (mm) for the test microorganisms are presented in Table 1. 70% ethanol and 100% acetone derived leaves crude extract of *Eclipta prostrata* showed best results against *Candida albicans* and *Pseudomonas aeruginosa*. The inhibitory action was observed in term of diameter of inhibition zone formed around agar well by diffusion of antimicrobial substances. The diameter of inhibition zone for each of the sample against every tested microorganism was found to be equal or greater than that of the standard antifungal antibiotic Amphotericin B (22mm) and antibacterial antibiotic streptomycin (20.2mm) used in the assay. 70% ethanol leaf extract showed average 22 mm zone of clearance around the well against *C.albicans* and 20.6mm against *Pseudomonas aeruginosa*.

Table 1
Antimicrobial activity assessed through zone of inhibition formed by *Eclipta prostrata* leaves extract obtained in different solvents

Microorganism	Zone of inhibition (mm)				
	Methanol extract	Ethanol extract	Acetone extract	Chloroform extract	Positive Control
<i>Candida albicans</i>	12.5	22	17.5	13.1	22
<i>Aspergillus niger</i>	13.8	18.6	13.5	16.5	19.1
<i>Escherichia coli</i>	14.2	19.9	18.6	16.2	19.3
<i>Pseudomonas aeruginosa</i>	14.6	20.6	18.8	15.8	20.2
<i>Staphylococcus aureus</i>	13.5	21	25	15.2	20.1

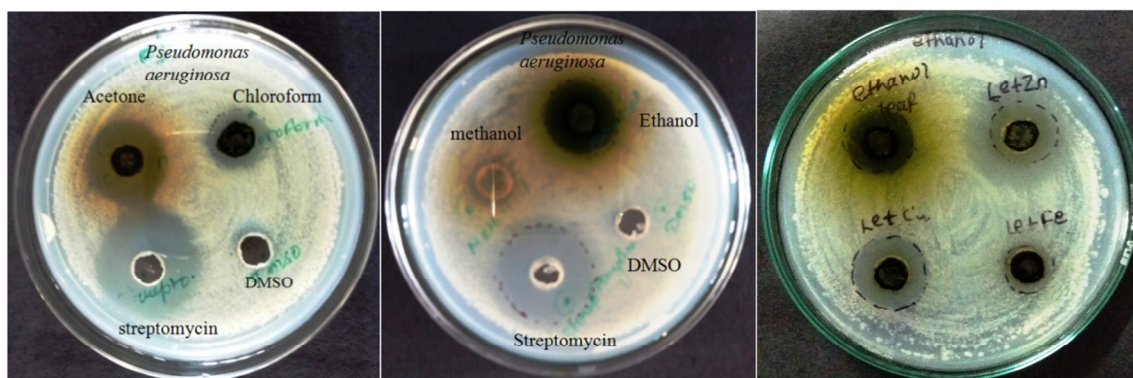


Figure 2
Zone of inhibition of leaves extract against *P. aeruginosa*

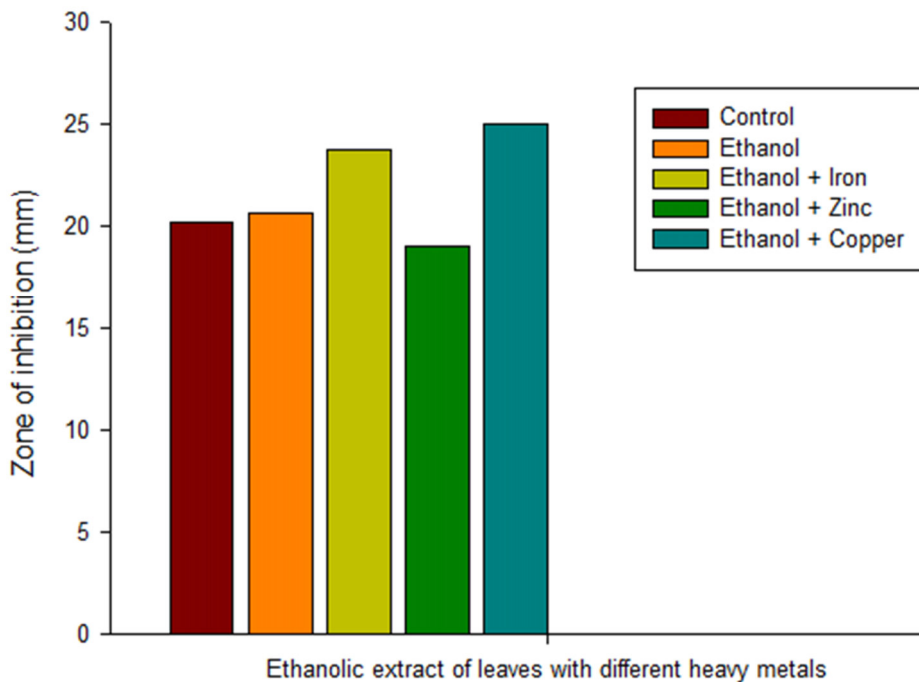


Figure 3
Antibiogram analysis of ethanol leaves extract with different metal ion solution against *P. aeruginosa*

Efficiency of antifungal compound was enhanced by addition of metal ion solution. Copper metal ion solution enhanced the anticandidal activity by 82% than that of ethanol leaves extract. The average zone of clearance of leaves extract with metal ion solution was 40 mm. The results showed that addition of Cu^{2+} metal ion solution to 70% ethanol extract magnified the

antimicrobial effect to almost 82% against *Candida albicans*, 26% against *Aspergillus niger*, 20% against *E. coli*, 24% against *Pseudomonas aeruginosa*, 18% against *Staphylococcus aureus* and a broad spectrum of inhibition zones against *Candida albicans* (40mm) and *Pseudomonas aeruginosa* (25mm) was observed.

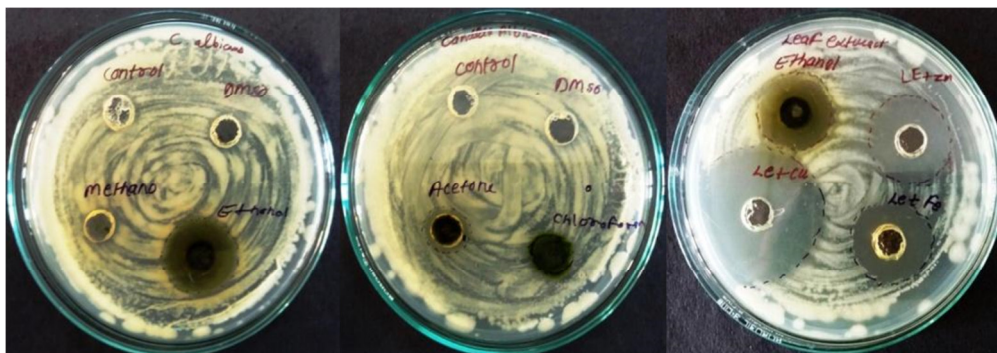


Figure 4
Antibiogram analysis of leaves extract with different solvent against *C. albicans*

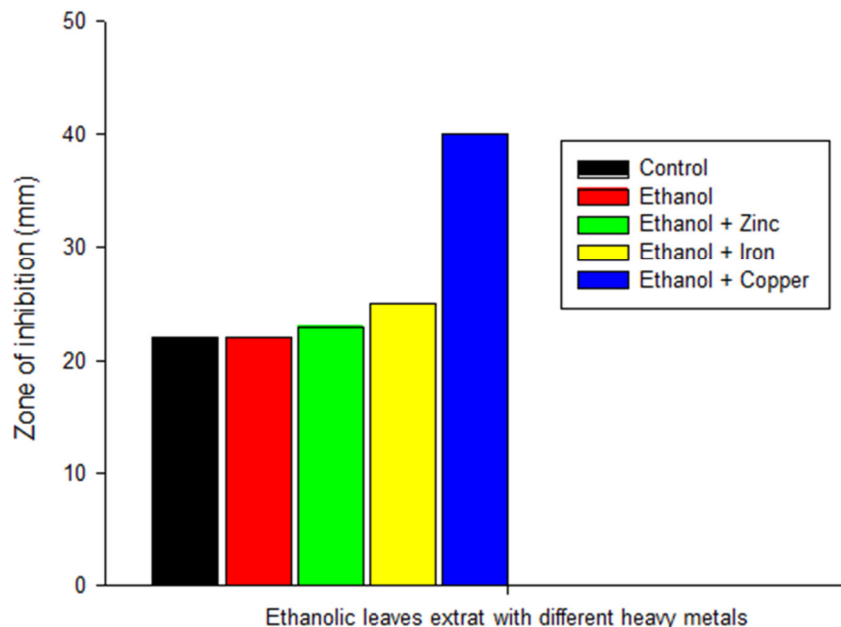


Figure 5
Antibiogram analysis of ethanol leaves extract with metal ion solution against *Candida albicans*

DISCUSSION

Medicinal herb has traditionally been used worldwide for the treatment and cure of various types of human diseases for a long time¹³. The experimental result of *E. prostrata* leaf extract had immense potential of antimicrobial activity. Antimicrobial activity of *Eclipta* leaf extract obtained in ethanol and acetone were strong against *E. coli*, *S. aureus* and *Candida albicans* fungi. The Antibiogram analysis of *Eclipta prostrata* L. leaves extract showed strong anticandidal nature. Lopez et al¹⁴ reported that pinocembrin chalcone from *Piper lanceaefolium* inhibit the growth of *C. albicans*. Jantan et al¹⁵ reported that the oil of *B. pandurata* rhizomes was effective against dermatophytes, filamentous fungi and yeast like fungi including *Candida albicans* and *C. neoformans*. It has been reported that free radical scavenging and antioxidant properties of many medicinal plants are responsible for their therapeutic effect against cardiac-disorder, thrombosis, hepatotoxicity, anti-carcinogenic, anti-mutagenic, etc.^{16,17} The observed anti-candidal activity in the present study may be due to the presence of potent phytoconstituents in the plant extract that have a significant potential to inhibit the candidal activity which can also be used as a drug when isolated as a pure compound. It was found that the extract when obtained in few of the organic solvents like 70% ethanol, 80% methanol, absolute acetone and chloroform exhibited the zone of inhibition, which was comparable or higher than zone of inhibition formed using the standard antibiotic. Antimicrobial activity of *E. prostrata* extract was compared with the antibiotic amphotericin B and streptomycin. It was found that the extract in some cases exhibited a zone of inhibition which was equal or greater than zone of inhibition of antibiotic (positive control). These extracts are rich in radical scavengers, such as flavonoids also known as antioxidants. They have also been proved to be abundant source of

biologically active compounds, many of which have been used to develop new pharmaceuticals. The anticandidal activity of *E. prostrata* extract was enhanced two folds in presence of Copper. *E. prostrata* extracts has been reported to form silver nanoparticles.¹² Bioreduction of metal ions by extract of *E. prostrata* has been done readily at room temperature¹⁸ and the nanoparticles formed have been confirmed by UV-visible spectroscopy, X-ray diffraction, FTIR and SEM studies. The antibacterial activity of synthesized AgNPs showed effective inhibitory activity against human pathogens, including, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The plant extract rich in flavanoids¹⁷ and phenols act as reducing agents causing formation of nanoparticles which are efficient antimicrobial agents. In the present study the strong antimicrobial activity observed after addition of copper in ethanolic plant extract can be attributed to the formation of nanoparticles, as *Eclipta prostrata* has been reported to form silver nanoparticles.¹² SEM observations of *Candida* biofilms treated with silver nanoparticles showed overall loss of structure due to disruption of outer cell membrane/wall and inhibition of filamentation.¹⁹ Earlier studies have indicated that copper oxide Nps with an average size of 50 nm and crystalline nature have a significant effect on biofilm of pathogenic yeast *Candida albicans* in a way that 98% reduction in biofilm at the concentration of 54 μgml^{-1} has been observed.²⁰ Copper oxide Nps can produce active oxygen types such as hydrogen peroxide inside epithelial cells and thus prevent the formation of intracellular antioxidant defense mechanism. Copper oxide Nps result in oxidative damage in bacterial cells. Copper oxide Nps can produce divalent copper ions and thereby, by attaching cell DNA, result in cell death. Also, copper oxide Nps, through change in apoptotic gene expression result in planned cell death.²¹ Fabrication of silver nanoparticles and their antibacterial potency using stem broth of *Cananga odorata* has been well demonstrated in *Staphylococcus aureus* (gram positive

bacteria) and *Salmonella typhi*.²² Thus phytochemicals can have miscellaneous dimensions in testing antimicrobial activity adopting green chemistry.

CONCLUSION

Eclipta prostrata is a good source of herbal medicine to cure the potentially harmful diseases. Phytochemicals of *Eclipta prostrata* isolated in 70% ethanol demonstrated high antimicrobial activity against bacteria and fungi. Activity of *Eclipta prostrata* leaves extract can be enhanced by addition of different heavy metals. We conclude that the phytochemicals in association with copper can be a promising source inhibiting *Candida albicans* growth. Study of phytochemicals extracted in ethanol can be a significant contribution to the knowledge of compounds as potential sources of new drugs in pharmacological industry.

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AUTHOR CONTRIBUTION STATEMENT

RR and JKP conceived the idea and planned the experiments. JKP and SKG conducted the experiments. RR and JKP interpreted and analysed the results. RR, JKP and SKG had written the manuscript.

FUNDING ACKNOWLEDGMENTS

Authors are grateful to Department of Biochemical Engineering, IIT, Banaras Hindu University for providing the *Candida* strain (ATCC10231).

CONFLICT OF INTEREST

Conflict of interest declared none.

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