ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF METHANOLIC EXTRACT OF ECLIPTA PROSTRATA

Sumathi R*, *Assistant Professor, Department of Biochemistry, Rabiammal Ahamed maideen College for Women, Thiruvarur.*

**Corresponding author's E-mail: sumathiraguraman@ymail.com*

ABSTRACT

Eclipta prostrata is an indigenous herb belonging to family Euphorbiaceae has been used in the Indian system of medicine. It was spread all over the India and in some other Asian countries. It contains some phytochemicals such as alkaloids, tannins, flavonoids, terpenes, and glycosides. They have been isolated for the study of pharmacological activities such antibacterial, antifungal and anti-inflammatory activities. In the present study we clearly investigate the in vitro activity of Eclipta prostrata against human pathogen Pseudomonas aeroginosa, Staphylococcus aureus (bacteria) and Aspergillus niger, candida albicans (fungi). Our research reveals that the methanolic extract shows maximum inhibitory effect against tested bacteria and fungi.

Keywords: *Eclipta prostrata*, Antibacterial and Antifungal activity, phytochemicals.

1. INTRODUCTION

Infectious diseases caused by fungi and bacteria are among the major causes of illness and death worldwide. Those consequences are mainly implicated by the dramatic rise of antimicrobial resistance of the current treatment regimens (CDC, Atlanta, GA, USA, 2019). Nowadays, infectious diseases are a major cause of human troubles around the world. New approaches have been proposed to fight microbial resistance, such as nanotechnology (Pelgrifta and Friedmanbc, 2013) to use natural products due to their healing potential. This idea dates back to ancient times and has been taken up and developed in recent years, especially against multi-drug resistant human pathogenic microorganisms (Savoia, 2012). Folk medicine provides an important and unexplored resource for the discovery and development of potential new medicines against microbial infections to decrease the emergence of resistance and adverse drug reactions. Furthermore, the use of medicinal plants provides opportunities for developing countries as they may be more affordable, accessible

and available (Ali NAA and Ju W, 2001). Plants have been reported showing strong antimicrobial properties (Surendra *et al.*, 2016). Investigation of plants originated antibiotic agents explored a new chapter in the field of pharmacology (Valsalam *et al.*, 2019). Since the advent of the miracle drug penicillin, in 1928 by Sir Alexander Fleming, antibiotics saved countless lives around the world (Lethek, 2020). However, parallel to the advent of lifesaving antibiotics, the life-threatening antibiotic resistance has emerged (Arshad *et al*.2018).

Eclipta prostrata commonly known as false daisy, yerba de tago, Karisalankanni, and bhringraj, is a species of plant in the sunflower family. It is widely distributed throughout India, Nepal, China, Thailand, and Brazil. The plant has traditional uses in Ayurveda. It is bitter, hot, sharp, and dry in taste. In India, it is known as bhangra or bhringaraj. It appears to have beneficial effect in reducing diarrhoea and symptoms arising from bacterial infections (Tiwari, R. K., 2014). The plant is claimed to possess immunological, antibacterial, anti-inflammatory, antithrombotic, hepatoprotective, hypoglycaemic and hypotensive properties the importance of search and exploitation of natural antimicrobials and antioxidants of plant origin has greatly increased in recent years (Ananthi J, Prakasam A *et al.*, 2003).

Despite those facts, however, the antibacterial and antifungal effects of *Eclipta prostrata* have not been explored yet. Hence, this preliminary study was aimed at investigating the antibacterial and antifungal effects of the leaf exudates of *Eclipta prostrata* against clinically isolated and standard bacterial and fungal strains by using the agar well diffusion technique.

2. MATERIALS AND METHODS

Plant collection

The mature and healthy plant leaves of *Eclipta prostrate* was collected from in and around area of Thanjavur districts of Tamilnadu, India.

Chemicals and instruments used

The chemicals were used from Hi-media- laboratory grade for highly precise study and completely sterilized glass wares were used in every step throughout the experiment.

Extraction

The collected leaves were washed with fresh water and dried for three days and grounded into a coarse powder with the help of a suitable mortar and pestle. As a final point,

the crushed powder is stored in a cool, dark, and dry place in an airtight container, and kept until analysis commenced. By using soxhlet's apparatus, 100 gm of dried plant leaf material were refluxed with methanol continuously for 24 hrs. The vapour flows through a coil where they condense back to a liquid which is then collected in the receiving vessel. The whole mixture was successively filtered through a piece of clean, white cotton material and Whatman no.1 filter paper. All the extracted samples were stored in a refrigerator at 4 °C.

Antibacterial Activity

Bacterial strains: *Pseudomonas aeroginosa, Staphylococcus aureus,*

Test samples were prepared by dissolving 80 mg of each of leaf powder in 2 ml of respective methanol. The resulting solution contained 0.8mg/50 µl. For the preparation of sample disc, paper discs of 5 mm diameter were made from Whatman filter paper by punch machine and then autoclaved at 121°C for 15 min. 50 µl of extract was applied to the paper discs under aseptic conditions. Blank discs were also prepared using solvents only. Tetracycline discs (30 µg/disc) were used as standards. For each of the test organisms, the pre-culture was taken from stock cultures and was grown in nutrient broth at 37°C for 24 h. using sterile forceps, both the sample and blanks discs were placed on the marked positions on the seeded petri dishes maintaining an aseptic condition. The standard discs were placed separately onto another set of seeded Petri dishes. The plates were kept at 4°C for 24 h to allow sufficient time for the test material to diffuse to a considerable area of the medium. Afterwards, they were incubated at 37°C for 24 h. The resulting clear zones were measured by a transparent scale (Meignanalakshmi *et al.,* 2013; Samy and Ignacimuthu, 2000).

Antifungal activity

Fungal strains: *Aspergillus niger, candida albicans,* were the fungal strains used for studying *in vitro* activity of *Eclipta prostrate.*

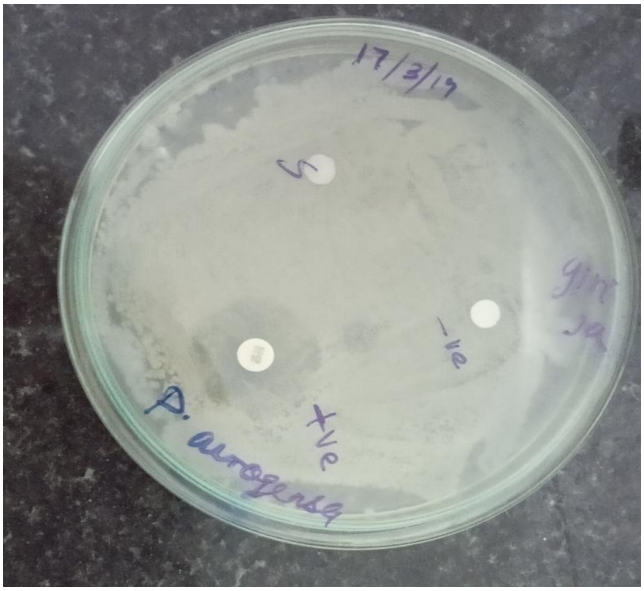
The anti-fungal screening was done by poisoned food technique while disc diffusion method was followed for anti-yeast screening. 80 mg of each extract was mixed uniformly with 100 ml of sterile PDA to get a concentration of 0.8 mg/ml and immediately poured into the Petri dishes aseptically. After solidification, 10 mm agar blocks of the test mould were placed in the centre of the treated plate. For each of test fungi, one such plate was prepared. Similarly, another set of plates were prepared using a standard antibiotic clotrimazole at a

concentration of 80 µg/ml. A set of control plates were also prepared using PDA plates alone. All of the plates were incubated at 24°C for 4 days after which the radial growth of fungal colony was measured with a transparent scale in mm and the percentage of inhibition of mycelia growth was calculated. Finally the antibiotic clotrimazole was employed as a standard for comparison (Rashmi and Mathew, 2012).

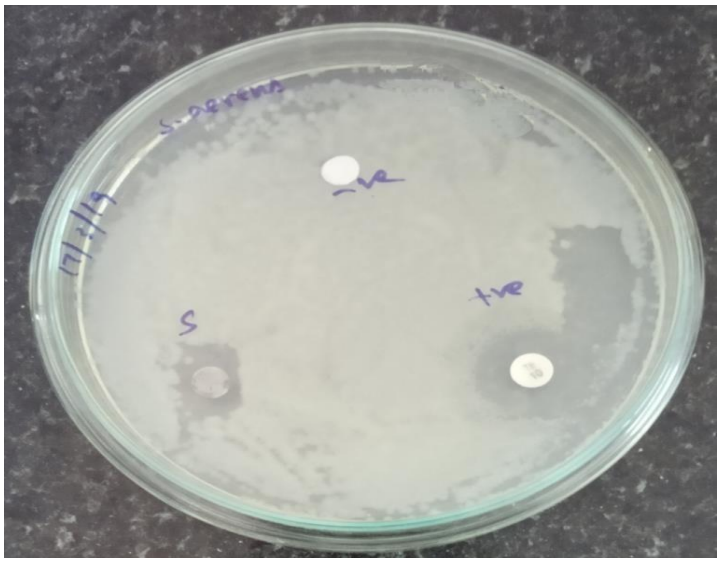
3. RESULTS AND DISCUSSION

Table: 1.Antibacterial activity of Methanolic extract of *Eclipta prostrate*

Name of the bacteria	**Methanolic extract of *E. prostrate* (Zone of inhibition)**	**Standard drug Tetracycline (Zone of inhibition)**
Staphylococcus aureus	**7.1**	**18.3**
Pseudomonas aeruginosa	**-**	**16.2**



Pseudomonas aeruginosa

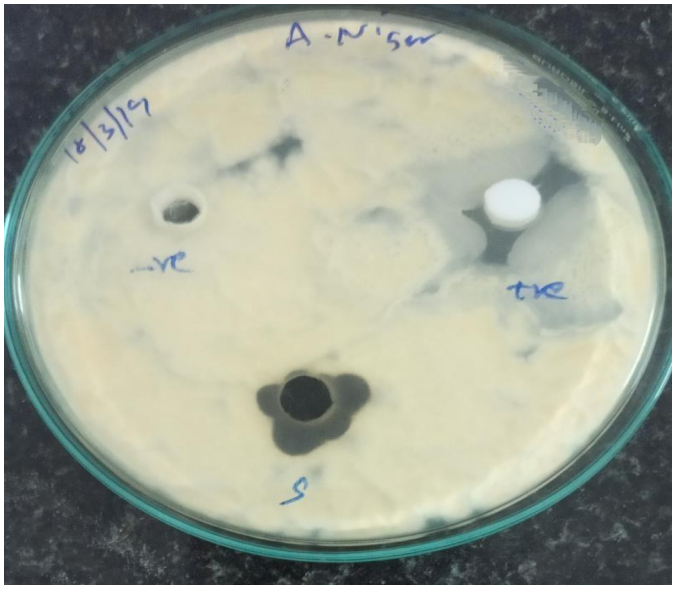


Staphylococcus aureus

The *E. prostrate* extract were tested for antibacterial activities against two pathogenic bacteria including gram-positive and gram-negative using disc diffusion method and the results are tabulated in Table 1. The zone less than 7.1mm was considered as resistant. All the studied pathogens were found to be moderately susceptible to *E. prostrate* extract with a zone of inhibition ranging 18.3 mm. The highest activity was found against *Staphylococcus aureus* (with a zone of inhibition of 7 mm). However, the standard antibiotic tetracycline showed very strong inhibition against almost all of the test bacteria.

Table: 2.Antifungal activity of *E. prostrate* extract

Name of the fungus	Methanolic extract *E. prostrate* (Zone of inhibition)	Standard drug Clotrimazole (Zone of inhibition)
Aspergillus niger	**7.2**	**20.8**
candida albicans	**-**	**17.5**



Aspergillus niger



Candida albicans

The *E. prostrate* extract were tested for antifungal activities against two pathogenic fungal including using well diffusion method and the results are tabulated in Table 2. The zone less than 7.2mm was considered as resistant. All the studied pathogens were found to be moderately susceptible to *E. prostrate* extract with a zone of inhibition ranging from 7-20 mm. The highest activity was found against *Aspergillus niger* (with a zone of inhibition of 7mm). From above result, it was proved that *E. prostrate* extract had antifungal activity.).

Data revealed that maximum inhibition in the growth of antibacterial strains was noticed for Staphylococcus aureus, followed by Pseudomonas .However, the standard antifungal clotrimazole showed very strong inhibition against almost the entire test.

The use of herbal extracts as antioxidant and antimicrobial agents has two separate advantages: the natural origin and the related low risk. This means that they cause fewer side effects for people and the environment (Ahmad *et.al.* 2015). Based on the results, methanolic extracts of *E. prostrate* showed the maximum antibacterial activity against most of the tested bacteria pathogens, attributable to higher content of phenolic and flavonoid compounds. In addition, our findings were in agreement with those of Yang *et.al.* 2015.

4. CONCLUSION

Finally we concluded that the result of the study reveals the methanolic extract of *Eclipta prostrata* exhibits antibacterial and antifungal effect. These results can be the strong scientific evidence for the use of this plant as a useful source of antibacterial and antifungal references. Furthermore, all our studies were *in-vitro* for the better observation of its activities we need to study the *in vivo* activities of *Eclipta prostrata.*

REFERENCES

[1] *Ahmad N, Shinwari ZK, Husain J, Perveen R. Phytochemicals, antibacterial and antioxidative investigations of* Alhagi maurorum *medik. Pak J Bot. (2015); 47(1):121–124. .*

[2] *Ali NAA, Ju W. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J Ethnopharmacol. (2001); 8741:173–9.*

[3] *Ananthi J, Prakasam A and Pugalendi KV Antihyperglycemic activity of Eclipta alba leaf on alloxan induced diabetic rats. Yale J Biol Med, (2003), 76:97 – 102.*

[4] *B. Aslam, W. Wang, M. I. Arshad et al., "Antibiotic resistance: a rundown of a global crisis," Infection and Drug Resistance, vol. 11, pp. 1645–1658,(2018).View at: Publisher Site | Google Scholar*

[5] *CDC, People in the United States Dying from Antibiotic-Resistant, CDC, Atlanta, GA, USA, 2019, https://www.cdc.gov/media/releases (2019) p1113-antibiotic-resistant.html.*

[6] *Kuldeep Dhama, Ruchi Tiwari, Sandip Chakraborty, Mani Saminathan, Amit Kumar, K.Karthik, Mohd.Yaqoob wani, Amarpal, Shoor Vir Singh and Anu Rahal, Evidence Based Antibacterial Potentials of Medicinal plants and Herbs countering Bacterial pathogens Especially in the Era of Emerging Drug Resistance An integrated update, Volume 10, Issue:1, (2014), 1-43.*

[7] *M. Lethek, "The discoverer of the antibiotic effects of penicillin," Frontiers for Young Minds, vol. 7, 159 pages, (2020).View at: Publisher Site | Google Scholar*

[8] *Meignanalakshmi S, Vinoth Kumar S, Deepika J, Farida Begum I , Evaluation of antibacterial activity of methanol extract of leaves of Adhatoda vasica on mastitis pathogens, Hygeia.J.D.Med.(2013). 5:1-4.*

[9] *Pelgrifta, R.Y.; Friedmanbc, A.J. Nanotechnology as a therapeutic tool to combat microbial resistance. Adv. Drug Deliv. Rev. (2013), 65, 1803–1815. [CrossRef] [PubMed]*

[10] *Rashmi Pa, Linu Mathew, Antimicrobial activity of leaf extracts of Justicia adhatoda L. in comparison with vasicine, Asian Pacific J.Tropical Biomedicine, (2012), 1556-60.*

[11] *S. Valsalam, P. Agastian, G.A. Esmail, A.K.M. Ghilan, N.A. Al-Dhabi Arasu MV. Biosynthesis of silver and gold nanoparticles using Musa acuminata colla flower and its pharmaceutical activity against bacteria and anticancer efficacy J. Photochem. Photobiol. B: Biol. (2019).*

[12] *Samy RP, Ignacimuthu S , Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats in India, J. Ethnopharmacology (2000) , 69:63-71*

[13] *Savoia, D. Plant-derived antimicrobial compounds alternatives to antibiotics. Future Microbiol. (2012), 7, 979–990. [CrossRef] [PubMed]*

[14] *T.V. Surendra, S.M. Roopan, N.A. Al-Dhabi, M.V. Arasu, G. Sarkar, K. Suthindhiran Vegetable peel waste for the production of ZnO nanoparticles and its toxicological efficiency, antifungal, hemolytic, and antibacterial activities Nanoscale Res. Lett., 11 ,(2016), p. 546.*

[15] *Yang J-F, Yang C-H, Wu C-C, Chuang L-Y. Antioxidant and antimicrobial activities of the extracts from* Sophora flavescens*. J Pharmacog Phytochem. (2015); 3(6):26–31.*