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Investigation of antimicrobial activity of aqueous extracts of *Eclipta prostrata*

S. Prabagar[1], J.Nanthakumar[2], S. Thuraisingam[3], J. Prabagar[4]
[1]Industrial Technology Institute, Colombo 7, SRI LANKA.
[2]Department of Botany, University of Jaffna, SRI LANKA.
[3] Post Graduate Institute of Agriculture, University of Peradeniya, SRI LANKA.
[4]Department of Chemistry, University of Jaffna, SRI LANKA.
s.prabagar@yahoo.co.uk, jeyaraninandakumar@yahoo.com, tnsuganya@gmail.com, j_prabagar@yahoo.co.uk

ABSTRACT
Eclipta prostrata is a small herb distributed in tropical regions of the world. It is commonly known as false daisy. The plant is used in Ayurvedic medicine for skin infections and some non-communicable diseases. Aqueous extraction of plant material such as leaf and whole plant were investigated for anti-microbial activity without changing concentration. Results of study revealed that all extracts had inhibitory activity against *E.coli* with the clear zone of 1.3cm, which is similar to streptomycin control. Antifungal activity of leaf and whole plant extract of *Eclipta prostrata* against *Aspergillus* and *Rhizopus* showed the inhibitory growth ranged from 23.5 % to 33.30% whereas the leaf extract showed high inhibition than whole plant extract. The findings exhibit that leaf and whole plant aqueous extracts have narrow spectrum activity and there is a possibility in treatment of infectious diseases.

Key words: *Eclipta prostrata, leaf and whole plant extracts, antimicrobial activity*

INTRODUCTION
Eclipta prostrata (Family: Asteraceae) is a small herb distributed in tropical regions of Sri Lanka, India, Malaysia, China and Australia [1-3]. It is commonly known as false daisy (T - Karaisalankanni, S - Keekirindiya) and found in all parts of Sri Lanka. It has various synonyms to *Eclipta alba*. The branches are hairy and grow up to 40cm high. The leaves are opposite spike with a toothed edge and while cutting the leaves sap turns into black. Leaves are sessile, 2.5-7.25cm long and oblong. This is an erect much branched creeping and moisture-loving herb with small white colour flowers on a long stalk. The flowering stem rises from the axis of the leaf. Root is well developed, cylinder-shaped and grayish [4, 5,6,7].

E. alba is used in folk medicine as a hair nourishment, blackening and strengthening [8] hairs; self -medication for HIV infection; skin infection; hepatic diseases [9-12] in Thailand. The herb is used to cure jaundice, cancer, obesity and spleen enlargement [13] in India. People in China use this plant for cure of nose and gastric bleeding, knee pain, hypertension, cirrhosis of liver and cancer [14,15]. It is used for snake bites in Brazil, China, India [15, 16]. It functions against malaria, diabetic disease [17, 18, 19].

It has antimicrobial, antimytotoxic, antihaemorrahagic and immunomodulatory properties [20, 21,22]. It is used as an ailment for several diseases such as urinary infections, gastrointestinal disorders, cough and lung infections of this plant have been reported [23–25]. Most of the above literatures were related to *E.alba*. The present study was focused on *Eclipta prostrata's* anti-microbial activity of leaf and whole plant.

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Fig 1: whole plant of *Eclipta prostrata*

METHODOLOGY

1. Sample collection and extract preparation

Leaf and whole plant of *Eclipta prostrata* was collected from the premises of University of Jaffna, Sri Lanka. For aqueous extract preparation, 2.5g of plant material was weighed and washed well in tap water. Then they were sterilized by giving a quick dip in alcohol and washed with sterilized water again. The weighed plant material was crushed with 10mL of sterile water and it was filtrated using Whatman's filter paper No.1. The filtrate was collected in sterile beaker. Anti-microbial activity was carried out using disc diffusing method (26).

2. Anti-bacterial activity

The preliminary screening of antibacterial activity was done using well in agar method. *Bacillus sp, Staphyllococcus aureus, Klebsiella, E.coli* and *Pseudomonas* bacteria were selected for this study. These bacteria were streaked on pure nutrient agar plates separately and stored in refrigerator at 10^0C with labeling. Plant materials were sterilized using different methods such as UV sterilization (UV light at 336nm) and wet heat sterilization (autoclave at 121^0C for 15min). Then the petri dishes were used for the experiment. Peptone broth and agar standard solution were prepared and 100ppm streptomycin standard solution was used as positive control. The inoculum was spread in nutrient agar plates with bacterial strain and incubated at 37^0C for 24 hours. Wells were prepared with UV sterile and wet heat sterile extracts, streptomycin solution and sterile water in agar plates for each bacterial species. The diameter of the clear inhibitory zone around the well was measured.

3. Anti - fungal activity

Candida sp, Aspergillus sp, Pencillium, Rhizopus fungus were selected for this study. Plant materials were sterilized using different methods such as UV sterilization (UV light at 336nm) and wet heat sterilization (autoclave at 121^0C for 15min). The sterilized extracts and Potato dextrose agar (PDA) media were mixed well and poured in petridishes. They were incubated at room temperature for 2 days. After it had grown enough, disc with the diameter of 7mm were cut using the sterile cork borer. The disc of each fungus was placed on the middle of the plate, which contain herbal product with PDA by using sterile loop. Control plates were also maintained without plant extract. These plates were incubated at room temperature for 2 days. Then the diameter of the clear inhibitory zone around the well was measured.

RESULTS AND DISCUSSION

1. Anti-bacterial activity

The extract of *Eclipta prostrata* found to be effective against the pathogenic bacteria. The inhibition zone is shown in Table 1 has exposed the power against pathogenic bacteria.

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Table 1: The diameter of the zone obtained from samples plant material under UV sterilization

Bacteria	Diameter of inhibitory zone (cm)		
	Leaf extract	Whole plant extract	Streptomycin
E.coli	1.3	1.3	1.3
Bacillus sp 1	Reduced growth	Reduced growth	2.5
Bacillus sp 2	0	0	1.55
Pseudomonas	0	0	1.95
Staphylococcus aureus	0	0	2.7
Klebsiella	0	0	2.4

Table 2: The diameter of the zone obtained from samples plant material under wet heat sterilization

Bacteria	Diameter of inhibitory zone (cm)		
	Leaf extract	Whole plant extract	Streptomycin
E.coli	1.3	1.3	1.3
Bacillus sp 1	Reduced growth	Reduced growth	2.7
Bacillus sp 2	0	0	1.45
Pseudomanas	0	0	1.95
Staphylococcus aureus	0	0	2.6
Klebsiella	0	0	2.2

Eclipta alba contains wedelolactone components which have significant antimicrobial activity against pathogens (27). This study further indicated that the methanol and ethyl acetate extract of plant found that the compound recorded the highest zone of inhibition against *Staphylococcus epidermidis*, followed by *Salmonella typhimurium*, *Staphylococcus aureus*, *E. coli, Pseudomonas aeruginosa* and *Shigella flexneri [27].*

Similar studies (Uddin et al, 2010 cited in Pandey et al, 2011, Chitravadivu et al, 2009; Karthikumar et al, 2007 cited in Udayashankar et al, 2019) stated that ethanol extract of *E. alba* leaves revealed significant antibacterial activity against *E.coli, Salmonella typhi,* and *Staphylococcus aureus* [28,29,30].

The current study revealed that the antimicrobial activity of aqueous extract of *E. prostrata* is very narrow. *E.coli* had a clear zone area for the leaf and whole plant extracts of *Eclypta prostrata*. Both of the extracts of leaf and whole plant reduced the growth of *Bacillus sp 1* without producing clear zone. All other bacteria showed resistance to both leaf and whole plant extracts. The inhibitory activity of extracts compared with the standard reference of antibiotic streptomycin activity.

Investigators have reported the antimicrobial properties of *E. alba* extracts against bacterial and fungal pathogens [31]. Akhtar Nahid et al stated that 100mg/mL of *E. prostrata* plant methanolic extract showed the ability to inhibit the growth of bacteria species. It has formed a zone of inhibition with diameter 1.4, 1.3, 0.9, 1.2 and 1.5 cm for *Escherichia coli, Staphylococcus aureus, Pseudomonas putida, Salmonella enterica and Streptococcus pyogenes* respectively. In this study 2.5mg/mL of aqueous plant extract formed 1.3cm inhibition zone for *E.Coli.* [32]

The antibacterial activity of leaves of E. prostrate was studied using acetone, ethanol, methanol, aqueous and hexane extracts. The hexane extract showed high antibacterial activity against *S.aureus, B.cereus, E.coli, S.thyphi* and *Pseudomonas sp* and other extracts showed intermediate antibacterial activity [33].

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2. *Anti-fungal activity*

Table 3: The diameter of the Mycelial disc in cm and % of growth reduction of samples obtained from *Eclipta prostrata* under UV sterilization

Fungus	Diameter of inhibitory zone (cm)			% of growth reduction in leaf extract	% of growth reduction in whole plant extract
	Leaf extract	Bark extract	Control (without plant extract)		
Yeast	2.8	2.8	2.8	0	0
Rhizopus	6.0	6.5	8.5	29.40	23.5
Aspergillus	2.0	2.2	3.0	33.30	26.50
Pencilliu m	1.5	1.5	1.5	0	0

Table 4: the diameter of the Mycelial disc in cm and % of growth reduction of samples obtained from *Eclipta prostrata* under wet heat sterilization

Fungus	Diameter of inhibitory zone (cm)			% of growth reduction in leaf extract	% of growth reduction in whole plant extract
	Leaf extract	Bark extract	Control (without plant extract)		
Yeast	2.8	2.8	2.8	0	0
Rhizopus	6.5	6.5	8.5	23.5	23.5
Aspergillus	2.0	2.2	3.0	33.30	26.60
Pencilliu m	1.4	1.5	1.5	0	0

Among the four plants tested *Eclypta prostrata* showed narrow range of anti-fungal activity. The inhibition growth against *Rhizopus* was identified by 29.4% and *Aspergillus* by 33.3%. Yeast and *Penicillium* showed resistance to this plant extract.

Muthukumaram mentioned that the antibacterial activity of both the extracts were found to be moderate (0.8-1.3cm) however the antifungal activity was good (1.4–1.9cm)[34]. There are little studies have done for anti-fungal activity of *E.prostrata.*

CONCLUSION

The present study revealed that the extracts obtained from leaf and whole plant of *Eclipta prostrata* figure out antimicrobial activities against the gram negative bacteria *E.coli* from the tested bacterial species. The aqueous plant extract has a narrow range of anti-bacterial and anti-fungal activities. This study leads to the possibility for the treatment of infectious diseases; however, alcoholic extracts enhance the anti-microbial activity due to high solubility of active components in alcohols.

References

[1]. Jahan R, Al-Nahain A, Majumder S, Rahmatullah M,(2014). "Ethnopharmacological Significance of *Eclipta alba* (L.) Hassk. (Asteraceae)". International Scholarly Research Notices, vol 22p.
[2]. Lansdown, R.V. Patzelt, A. Knees S, Juffe Bignoli D. *Eclipta prostrata*. (2014). The IUCN Red List of Threatened Species.
[3]. Wiart, C. Mogana, S. Khalifah, S. Mahan, M. Ismail, S. Buckle, M. Narayana, A.K. Sulaiman, M. (2004). Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia. Fitoterapia. 75(1):68-73.
[4]. Chopra, R.N. Nayar, S.L., Chopra I.C. (1996). In: Glossary of medicinal plants. SIR Publication, New Delhi, 104.
[5]. Trease, G. E. and Evans, W.C. Text book of Pharmacognosy. (1996).14th Edition, London. 545p.
[6]. Shri, S. P. Ambasta, Useful Plants of India, CSIR Publications, 1986, 189p.
[7]. Kirtikar, K.R. Basu, B. D. (1935). Indian Medicinal Plants, Allahabad, 3,1361p.
[8]. Sivarajan, V.V and Balachandran, I. (1994). Ayurvedic Drugs and their Plant Sources. 1st Edn, Oxford IBH Publishings Co.Pvt. Ltd, New Delhi, 119p.
[9]. Sawangjaroen N, Subhadhirasakul S, Phongpaichit S, Siripanth C, Jamjaroen K, Sawangjaroen K. (2005). The in vitro anti-giardial activity of extracts from plants that are used for self-medication by AIDS patients in southern Thailand. Parasitol Res. 95(1):17-21.
[10]. Tewtrakul, S. Subhadhirasakul, S. Cheenpracha, S. Karalai, C. (2007). HIV-1 protease and HIV-1

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DOI: http://doi.org/10.5281/zenodo.4024936

integrase inhibitory substances from *Eclipta prostrata*. Phytother Res. 21(11):1092-5p.
[11].Lirdprapamongkol, K. Kramb, J.P. Chokchaichamnankit, D. Srisomsap, C. Surarit, R. Sila-Asna, M. Bunyaratvej, A. Dannhardt, G. Svasti, J. (2008). Juice of *Eclipta prostrata* inhibits cell migration in vitro and exhibits anti-angiogenic activity in vivo. In Vivo. 22(3):363-8.
[12]. Bunyapraphatsara, N. Chokchaijaroenporn, A. (1996). Medicinal Plants Indigenous to Thailand. Vol. 1. Bangkok, Medicinal Plant Information Center, Faculty of Pharmaceutical Sciences, Mahidol University, 154-159p.
[13].Kumari, C.S. Govindasamy, S. Sukumar, E. (2006). Lipid lowering activity of *Eclipta prostrata* in experimental hyperlipidemia. Journal of Ethnopharmacology. 24; 105(3):332-5p
[14]. Li DL, Xing FW. (2016). Ethnobotanical study on medicinal plants used by local Hoklos people on Hainan Island, China. Journal of Ethnopharmacology. 24;194:358-368p.
[15]. Liu QM, Zhao HY, Zhong XK, Jiang JG. (2012). Eclipta prostrata L. phytochemicals: Isolation, structure elucidation, and their antitumor activity. Food Chem Toxicol. 50(11):4016-22
[16]. Pithayanukul P, Laovachirasuwan S, Bavovada R, Pakmanee N, Suttisri R. (2004). Anti-venom potential of butanolic extract of *Eclipta prostrata* against Malayan pit viper venom. J Ethnopharmacol. 90(2-3):347-52.
[17]. Samy RP, Thwin MM, Gopalakrishnakone P, Ignacimuthu S. (2008). Ethnobotanical survey of folk plants for the treatment of snakebites in Southern part of Tamilnadu, India. Journal of Ethnopharmacology. 2008(17); 115(2):302-12.
[18]. Pithayanukul P, Laovachirasuwan S, Bavovada R, Pakmanee N, Suttisri R. (2004). Anti-venom potential of butanolic extract of *Eclipta prostrata* against Malayan pit viper venom. J Ethnopharmacol. 90(2-3):347-52
[19]. Zhao Y, Peng L, Lu W, Wang Y, Huang X, Gong C, He L, Hong J, Wu S, Jin X. (2015). Effect of *Eclipta prostrata* on lipid metabolism in hyperlipidemic animals. Exp Gerontol. 62:37-44.
[20]. Mahesh, S. Jolly, C.I. and Shridhar, N. (2004). Analgesic studies on total alkaloids and alcohol extrcats of Eclipta alba (Linn) Hassak. Phytother.Res., 18, 111-113
[21]. Singh, B., A.K. Chandra, S.G. Agawal and K.K. Anand, 2001. Invitro hepatopratective fraction from ethanolic extract of Eclipta alba leaves. Indian J.Physiol. Pharmacol., 45: 435-441.
[22]. Anonymous. (1962). *The Wealth of India: Raw Materials, Vol 6*, Publications and Information Directorate, CSIR, New Delhi. 298–301p.
[23]. Warrier, P. K., Nambiar, V. P. K. and Ramakutty, C. (1995). *Indian Medicinal Plants:A Compendium of 500 Species, vol. 3*, Orient Longman Private Limited,Hyderabad. 393p.
[24]. Pareek, O. P., Sharma, S. and Arora, R. K. (1998). Underutilized edible fruits and nuts: an inventory of genetic resources in their regions of diversity. International Plant Genetic Resources Institute (IPGRI), New Delhi. 73p.
[25]. Uttara, B. Singh, A.V. Zamboni, P. Mahajan, R.T. (2009). Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol. 7(1):65-74.
[26]. Kirby, M.D.K. Sherris, J,C. Turck, M. (1966). Antibiotic susceptibility testing by standard single disc diffusion method. American Journal of Clinical Pathology. 45: 493-496
[27]. Dalal S, Kataria SK, Sastry KV and Rana SVS. (2010). Phytochemical screening of methanolic extract and antibacterial activity of active principles of hepatoprotective herb, *Eclipta alba*. Ethnobotanical Leaflets. 14: 248-58.
[28]. Pandey, M.K. Singh, G.N. Rajeev, K. R. Sharma and Sneh Lata (2011). Antibacterial activity of Eclipta alba (L.) HasskJournal of Applied Pharmaceutical Science. 01 (07): 104-107
[29]. Chitravadivu, C. Manian, S. Kalaichelvi, K. (2009). Antimicrobial Studies on Selected Medicinal Plants, Erode Region, Tamilnadu, India. Middle-East J. of Sci. Res. 4(3):147-152.
[30]. Udayashankar, A. C. Nandhini, M. Rajini, S. B. and Prakash, H. S. (2019). Pharmacological Significance Of Medicinal Herb Eclipta Alba L. - A Review. International Journal of Pharmaceutical Sciences and Research. 10 (8): 3592-3606.
[31]. Prabu, K. Shankarlal, S. Natarajan, E. and Mohamed-Sadiq, A. (2011). Antimicrobial and antioxidant activity of methanolic extract of *Eclipta alba*. Advances in Biological Research. 5: 237-40.
[32]. Akhtar Nahid, Choudhury Neelabh, Kumar Navneet. (2017). Evaluation of antioxidant and antimicrobial potentials of *Eclipta prostrata* collected from the Nepal region. The Pharma Innovation Journal. 6(11): 04-07
[33]. Pandy, M. K. Singh, G. N. Sharma, R. K. and Lata, S. (2008). Antibacterial activity of Eclipta alba (L.) Hassk Journal of Applied Phar,aceutical Science Sci. 21(2), 195-199.
[34]. Muthu Kumaran Peraman, Ramalingam, P. and Bapatla JNN Sai. (2011). Anti-inflammatory and antimicrobial activities of the extracts of *Eclipta alba* Leaves. European Journal of Experimental Biology. (2):172-177.

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Appendix 1

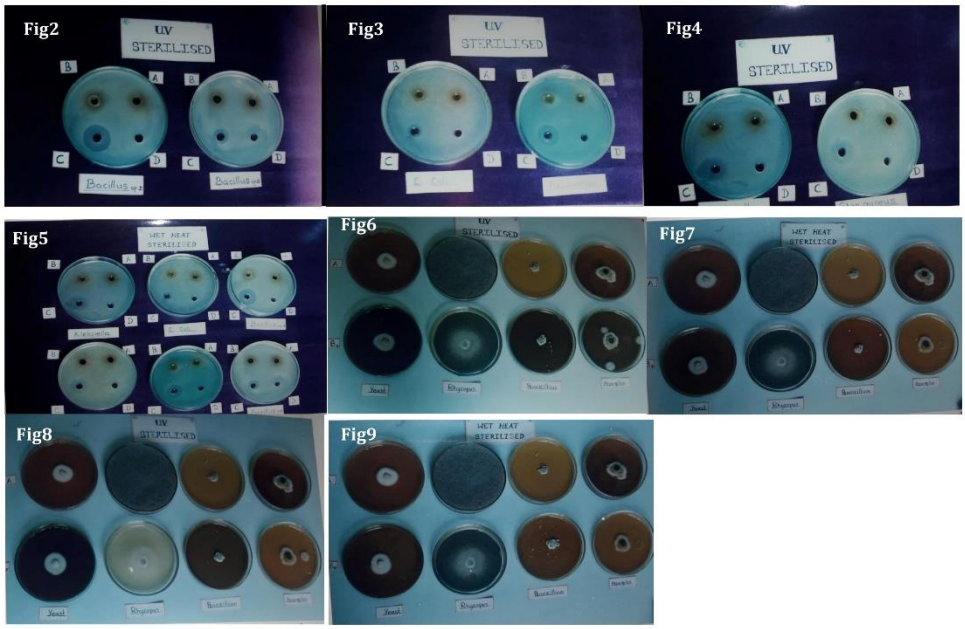

Fig 2: Effect of UV sterilised extract of leaf (A) and whole plant (B) of *Eclipta prostrata* on the growth of *Bacillus sp1* and *Bacillus sp 2*(C- Streptomycin, D- sterile water)
Fig 3: Effect of UV sterilised extract of leaf (A) and whole plant (B) of *Eclipta prostrata* on the growth of *E.coli* and *Pseudomonas*
Fig 4: Effect of UV sterilised extract of leaf (A) and whole plant (B) of *Eclipta prostrata* on the growth of *Klebsiella* and *Staphylococcus aureu*
Fig 5: Effect of wet heat sterilised extract of leaf (A) and whole plant (B) of *Eclipta prostrata* on the growth of *Klebsiella, E.coli, Pseudomonas, Staphylococcus aureus. Bacillus sp 1 & Bacillus sp 2*
Fig 6: Effect of UV sterilised leaf extract of *Eclipta prostrata* on the mycelial growth of Yeast, *Rhizopus, Aspergillus* and *Pencillium* (A- Control, B- extract added)
Fig 7: Effect of UV sterilised whole plant extract of *Eclipta prostrata* on the mycelial growth of Yeast, *Rhizopus, Aspergillus* and *Pencillium*
Fig 8: Effect of UV sterilised leafract of *Eclipta prostrata* on the mycelial growth of Yeast,*Rhizopus, Pencillium* and *Aspergillus*
Fig 9: Effect of wet heat sterilised whole plant extract of *Eclipta prostrata* on the mycelial growth of Yeast, *Rhizopus, Pencillium* and *Aspergillus*