D-5914
[1-6]

RESEARCH ARTICLE Agricultural Science Digest, Volume Issue : ()

Exploration of Antibacterial and Antioxidant Potential of *Eclipta prostrata* Leaf Extract

H.M. Navya[1], Tathagath Waghmare[2], Ashajyothi Chavan[3], Jahanara Kudsi[4], M.M., Tejaswini[1], Prabhurajeshwar[1]

10.18805/ag.D-5914

ABSTRACT

Background: *Eclipta prostrate* also recognized as Bhringaraja is an annual herbaceous plant, commonly known as king of hairs. It is an erect or prostrate, much branched, roughly hairy, annual, rooting at the nodes; the leaves are opposite, sessile and lanceolate belonging to family Asteraceae. This plant is a significant medicinal plant in the tropical and subtropical areas. It is broadly used in treating different diseases of skin, liver and stomach in India, Nepal, Bangladesh and other countries.

Methods: The main aim of this work was to collect and analyze the available information on traditional uses, phytoconstituents and biological activities of *E. prostrata*. *Eclipta prostrata* L. methanol extract was evaluated for its phytochemical compounds, ferric reducing antioxidant power (FRAP) and antibacterial activity.

Result: Phytochemical analysis showed the presence of alkaloids, tannins, glycosides, steroids, glycoproteins and volatile oil the total saponin content was found to be 84.175 ± 0.575 in mg/g equivalent of diosgenin. The results of antioxidant activity measured by the FRAP method revealed stronger reducing power of the extract. Further antibacterial activity was observed against *K. pneumoniae* and *S. aureus* in potential of inhibiting the tested microorganisms. Crude methanolic extract of *E. prostrate* recorded a minimum inhibitory concentration of 2 mg/ml. The results of current study suggested that antioxidant activity and antibacterial activity could be due to polyphenols, however chiefly by various active compounds or substances present in the extract. Further isolation, characterization and identification of the potential phytochemical compound from crude extract needs to be addressed.

Key words: Antioxidant activity, *Eclipta prostrata, K. pneumoniae, S. aureus*.

INTRODUCTION

Eclipta prostrata (L.) commonly known as false daisy, yerba de tago, Guntakalagaraku, Karisalankanni and bhringraj, belongs to Asteraceae family, widespread across the world. It is rich in proteins, vitamins and antioxidants which help to protect the body against certain infections. The plant is known for its ability to support strong health. Bhringraj oil is effective remedy used to promote hair growth as well as various hair concerns for centuries. In fact, all the parts of this plant can be used for therapeutic purposes (Ambu *et al*., 2020; David *et al*., 2015).

This annual plant grows up to about a foot in height; it grows a solitary, white, winged flower reminiscent of a daisy. The flowers are quite delicate; the slightly curly leaves are strong and thick, with a layer of hairs that can cause the skin to itch when touched. A dark greenish-black liquid obtained up on crushing of flowers can be used to make dyes and inks for tattoos. In ayurvedic medicine, the leaf extract of bhringraj is also considered a powerful liver tonic and detoxifier. It contains several phytochemicals such as alkaloids, polyacetylenes, thiophene derivatives, flavonoids and triterpenes (Timalsina *et al*., 2021).

Eclipta prostrata (L.) is a source of antioxidants (Minh, 2019). Bioactive compounds of *Eclipta prostrata* are mainly contributed in medicinal value (Akhter *et al*., 2019). Traditionally *E. prostrata* was used in the treatment of inflammatory diseases. Methanolic extract of leaves of *E. prostrata was* proved to be anti-inflammatory activity in albino Wistar rats (Arunachalam *et al*., 2009). The leaf extract of *E. prostrate* known for antioxidant and antimicrobial activities (Nahid *et al*., 2017). Recent studies also revealed that *E. prostrata* has curative properties such nephroprotective activity, anti-diabetic, analgesic, antibacterial, anti-breast tumour activity, anticancer activity, anti-hepatotoxic, hair growth promoter activity and memory enhancing activity and it is considered as a potent medicinal plant with tremendous pharmacological significance (Pooja *et al*., 2020).

[1]Department of Studies in Biotechnology, Davangere University, Shivagangothri, Davangere-577 007, Karnataka, India.
[2]Department of Studies and Research in Biotechnology, Gulbarga University, Kalaburagi-585 106, Karnataka, India.
[3]Department of Studies in Biotechnology, Vijayanagara Srikrishna Devaraya University, Bellary-583 105, Karnataka, India.
[4]Department of Studies in Biotechnology, Khaja Bande Nawaz University, Kalaburagi-585 104, Karnataka, India.

Corresponding Author: Prabhurajeshwar, Department of Studies in Biotechnology, Davangere University, Shivagangothri, Davangere-577 007, Karnataka, India.
Email: p.v.chidre@gmail.com

How to cite this article: Navya, H.M, Waghmare, T., Chavan, A., Kudsi, J., Tejaswini, M.M and Prabhurajeshwar. (2024). Exploration of Antibacterial and Antioxidant Potential of *Eclipta prostrata Leaf Extract* Agricultural Science Digest. doi: 10.18805/ag.D-5914.

Submitted: 08-11-2023 **Accepted:** 26-04-2024 **Online:** 12-08-2024

This present investigation aims at phytochemical analysis of methanol extract of *E. prostrate* leaves, antioxidant activity and its antibacterial against *Klebsiella pneumoniae* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Microorganisms

Two bacterial isolates used in this study *i.e., Klebsiella pneumonia* and *Staphylococcus aureus* culture were provided by Skanda life sciences Pvt. Ltd., Bangalore.

Preparation of methanolic extract of *Eclipta prostrata*

Eclipta prostrata leaves were collected from local market of Bangalore. The leaves were shade dried, powdered and subjected to soxhlet extraction using methanol. After the extraction, crude extract was concentrated under reduced pressure using rotary evaporator and evaporated to dryness. The dried material was weighed and stored at 4°C until further use.

Phytochemical analysis

Phytochemical analysis of methanolic extract of *E.prostrata* were carried out by reconstituting the semisolid extract in methanol and analyzed for the presence of different constituents such as alkaloids, carbohydrates, tannins, terpenoids, glycosides, steroids, saponins, flavonoids, glycoprotein, volatile oil, phenols by following standard procedures (Riazunnisa *et al*., 2013).

Estimation of total saponin content

Standard saponin solution was prepared by dissolving 10 mg of diosgenin with16 ml of methanol and 4 ml of distilled water. Further, 0.25 ml of 8% vanillin reagent was added to the aliquots for each tube, followed by addition of 2.5 ml of 72% sulphuric acid (v/v,). The solutions were mixed well and the tubes were incubated at 60°C under water bath for 10 mins. After incubation period the tubes were cooled in ice cold water bath. The absorbance was measured at 544 nm against the reagent blank. 0.1 g of freeze dried *E. prostrata* methanol was dissolved in aqueous methanol (80%, 0.1 ml). Total saponins were determined spectrophotometrically at 544 nm.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was carried out calorimetrically using DetectX ® FRAP™ (Ferric Reducing Antioxidant Power) Colorimetric Detection Kit (2 Plate Kit Catalog Number K043-H1). Experiment was carried out as per the manufacturer's instructions. Briefly, working assay buffer was prepared by diluting concentrated assay buffer (1:10) with distilled water. FRAP colour solution was prepared by mixing 10 ml of assay buffer and 1 ml each of FRAP reagent A and FRAP reagent B reagent respectively and mixed well. Vitamin C (M.W=176.12 g/mol) was served as control.

Different concentrations tested for reference standard *i.e.,* 100, 50, 25, 12.5, 6.25 and 3.125 µg. To this sample 400 µl of assay buffer was added. Then 20 µl of samples/ standards was pipette into 96 well plates and for control 20 µl of diluted assay buffer was added. Freshly prepared FRAP colour solution (75 µl) was added to each well and incubated at room temperature for 30 minutes. The absorbance was measured at 560 nm. A stronger absorbance will indicate increased reducing power.

Antibacterial activity assay

The antibacterial activity of methanol extract was assessed by well diffusion assay (Valgas *et al.,* 2007). The extract was dissolved in methanol to get 1 and 2 mg/ml concentration. The pre-sterilized, Soya Bean Casein Digested (SBCD) agar plates were poured into 90 mm Petri plates. After solidification, (SBCD) agar plates were inoculated with *K. pneumoniae*/ *S. aureus* with pre-adjusted cell density of 2×10^6 cells/ml. Further, plates werepunched with a sterile cork borer (6 mm diameter) to make open wells at the four edges of the Petri plate. Open wells were filled with *E. prostrata* extract (1 mg/ml or 2 mg/ml), Standard Ciprofloxacin (0.1 mg/ml). Wells added with 50% methanol serves as control. The treated plates were incubated in at 37°C for 24-36 hrs. After incubation period plates were observed for antibacterial activity and evaluated by measuring the zones of inhibition of microorganism growth surrounding the extract using a millimeter ruler. The antibacterial analysis was carried out under strict aseptic conditions in triplicates and repeated thrice (Eagle and Musselman, 1948; Barry and Sabh, 1974; Bergeron *et al*., 1973).

Determination of minimum inhibitory concentration (MIC)

Cell suspension of *K. pneumoniae* and *S. aureus* culture were prepared by growing on tryptone broth at 37°C for 24 hrs and cell density was adjusted to 1-2 $\times10^8$ cells/ml using 0.5 Mc Farland standards, serves as inoculum. Test concentration was prepared with different concentrations of Ciprofloxacin (8, 4, 2, 1, 0.5, 0.25 and 0.125 µg) in 90 µL tryptone broth. Similarly different concentrations of methanolic extract 1, 0.5, 0.25, 0.125, 0.625, 0.3125 and 0.156 mg in 90 µL tryptone broth. Tryptone broth inoculated only with respective bacterial culture and without any test compounds or standard served as control.

Minimum inhibitory concentration was determined by mixing 90 µl test samples/standard of different test concentration with 10 µL inoculum in 96 well plates (100 µl/ well). Mix 90 µl tryptone broth with 10 µl inoculum served as control. Treated plates were incubated at 37°C for 24 hours. After incubation optical density (O.D.) at 590 nm was measured using plate reader. Resazurin (0.015 %) was added to all wells (30 µl per well) and further incubated for 2-4 hours for the observation of colour change. On completion of the incubation, columns with no colour change (blue resazurin colour remained unchanged) were scored as above the MIC value. Metabolism of resazurin by active bacterial cells leads to reduction of resazurin (Purpleblue) to resorufin (pink-colourless) pink colour. Determine MIC

as minimum concentration of drug giving 50% inhibition of optical density as compared with control (John *et al*., 2000; Wikler 2006).

RESULTS AND DISCUSSION

Phytochemical analysis

The qualitative phytochemical analysis of methanolic extract of *E. prostrata* exhibited the presence of alkaloids, tannins, glycosides, steroids, saponins, glycoproteins and volatile oil (Table 1).

Total saponin

The total saponin content was determined using vanillin reagent. Diosgenin was used as a standard compound and the total saponin content were expressed as mg/g diosgenin equivalent using the standard curve equation: y = 0.001× + 0.117, R 2 = 0.929, where y is absorbance at 544 nm and × is total saponin content in the methanolic extract expressed in mg/g. The total saponin content in the methanol extract of *E. prostrata* leaves was found to be 84.175 ± 0.575 in mg/g equivalent of diosgenin.

In vitro antioxidant activity; Ferric reducing antioxidant power (FRAP) assay

The *in vitro* antioxidant activity of *E. prostrata* clearly demonstrated leaves have prominent antioxidant properties. The absorbance increases at 560 nm and can be measured to test the amount of ferric reduced and can be expressed as µmolar Fe^{2+} equivalents or relative to an antioxidant reference standard Ferrous (Fe^{2+}) in the methanolic extract of *E. prostrata*. Higher absorbance value indicates a stronger reducing power of the sample (Table 2).

Antibacterial activity by well diffusion method

Among the varied concentrations of methanolic extract of *E. prostrata* at 2 mg concentration exhibited potential inhibitory activity against *K. pneumonia* and *S. aureus* (Table 3; Fig 1 a and b) however, standard antibiotic, Ciprofloxacin exhibited its inhibitory activity at 2 µg/ml.

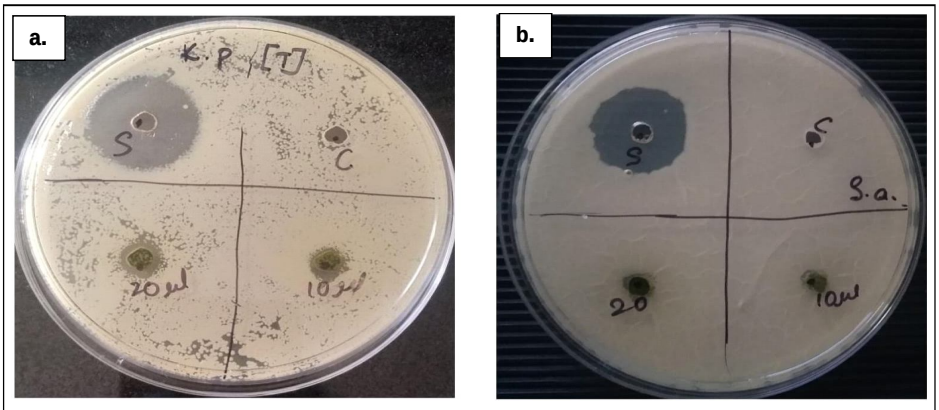
Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the *E. prostrate* against *K. pneumoniae* and *S. aureus* was 0.0625 mg/ml and 0.0312 mg/ml respectively. However, with ciprofloxacin MIC was 1.0 µg/ml and 0.5 µg/ml against K. pneumoniae and *S. aureus* respectively (Table 4).

Eclipta prostrata (Bhringraj) is considered to be having medicinal importance because of the presence of various secondary metabolites. This study was made to evaluate the methanol extract of *E. prostrata* leaves for its phytochemical analysis as well as antibacterial and antioxidant activity. The qualitative phytochemical test of methanolic extract of leaves of *E. prostrata* (L.) revealed the presence of presence of alkaloids, tannins, glycosides, steroids, saponins, glycoproteins and volatile oil. Recent report by Priya *et al*. (2018) also in support of current investigation that they reported presence of steroids, tannins, saponins, flavonoids, diterpenes and triterpenes further the GC-MS analysis exposed the compounds propanedinitrile dimethyl, Pentadecane, citronellyl butyrate, Citronellyl propionate, Heptadecanoic acid, methyl ester. *Eclipta alba* ethanol extract exhibited large zone of inhibition in disc diffusion for antibacterial screening against Gram-negative

Table 1: Phytochemical analysis of eclipta prostrata.

Types of tests	Eclipta prostrata
Alkaloid	Positive
Carbohydrates	Negative
Tannin	Positive
Terpenoids	Negative
Glycoside	Positive
Steroid	Positive
Saponin	Positive
Flavonoids	Negative
Proteins	present
Glycoprotein test	Positive
Volatile oil	Positive



S- Standard (Ciprofloxacin); C- Control (Methanol)

Fig 1: Inhibitory activity of *E. prostrata* methanol extract against a. *K. pneumonia,* b. *S. aureus.*

Table 2: FRAP assay with eclipta prostrata methanol extract.

Sample	Conc. (µg/ml)	Absorbance at 560 nm	Sample Abs-control Abs	FRAP value Vitamin C C Equivalent (µg/ml)
Control	0	0.010	0.000	-
	125	0.173	0.163	1.646
E. prostrata	250	0.191	0.181	4.415
	500	0.203	0.193	6.262
	1000	0.266	0.255	15.877

Table 3: Inhibitory activity of test compounds against test organisms.

Test organisms	Test compounds	Conc./well	Zone of inhibition (mm)
K. pneumoniae	Control (Methanol)	50%	-
	Ciprofloxacin (Standard)	2 µg	22
	E. prostrata methanol extract	2 mg	12
	E. prostrata methanol extract	1 mg	10
S. aureus	Control (Methanol)	50%	-
	Ciprofloxacin (Standard)	2 µg	22
	E. prostrata methanol extract	2 mg	8
	E. prostrata methanol extract	1 mg	6

Table 4: Determination of minimum inhibitory concentration (MIC).

Well number	K. pneumoniae		S. aureus	
	1	2	3	4
	Sample(mg/ml)	Std (µg/well)	Sample(mg/well)	Std(µg/well)
A	Control	Control	Control	Control
B	0.0156	0	0.0156	0
C	0.0313	0.125	0.0313	0.125
D	0.06265	0.25	0.0625	0.25
E	0.125	0.5	0.125	0.5
F	0.25	1	0.25	1
G	0.5	2	0.5	2
H	1	4	1	4
MIC	0.0625 mg	1 µg	0.0313 mg	0.5 µg

S.typhi, *S. sonnei, E.coli, S.paratyphi, Pseudomonas species (I)* and *Pseudomonas species (II) and* Gram-positive *B.subtilis, B.cereus, B. megaterim* and *S.aureus* and antifungal activity was observed against *Aspergillus ochraceus* (Uddin *et al*., 2010). In a similar kind of study phyto-chemical screening of the extract revealed the presence of tannins, flavonoids, saponins, alkaloids and the fractionated ethanol extract from *Eclipta prostrata* could be used against *S. typhi* pathogen (Karthikumar *et al*., 2007). The chemical composition of the essential oils from the leaves and stem bark of *E. prostrata* (L.) led to the identification of 33 and 30 compounds respectively (Ogunbinu *et al*., 2008).

The present study revealed the antimicrobial efficiency of methanol extract of *E. prostrata* against target pathogens *i.e., K. Pneumonia and S.aureus*. Shekokar and Nayak (2017) reported the solvent extracts of *E.alba* exhibited intermediate activity against *S. aureus, B. cereus, E. coli, S. typhi, K. pneumoniae, P. aeruginosa, P. mirabilis and S. pyogenes* in comparison with standard antibiotics (Ciprofloxacin). Ethanolic extract and active principle compound of *E. prostrata* was tested for *in vitro* antimicrobial studies (Sunita and Sudhir, 2010). Zone of inhibition studies and minimum inhibitory concentration exhibited activity against studied strains. The antimicrobial spectrum of *E. prostrata* was proved against *Aspergillus niger, Candida albicans* and *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* (Raghuwanshi *et al*., 2018).

Eclipta prostrata is an important natural source of antioxidants (Sinha and Raghuwanshi 2016). Antioxidant activity of *E. prostrata* was determined by FRAP, radical scavenging activity, reducing activity and DPPH assay. The antioxidant capacity was increased by increasing the concentration of the extracts similar results were recorded by Jaglan *et al*. (2013).

In the present study from the results of antimicrobial assay it was evident that *K. pneumonia* 2 mg of *E. prostrata* methanol extract showed highest inhibitory activity against

K. pneumoniae and *S. aureus.* The minimum inhibitory concentration of the *E. prostrata* against *K. pneumoniae* was 0.0625 mg for *K. pneumoniae* and 0.03125 mg for *S. aureus* whereas with ciprofloxacin standard it was 15 mg/ml and 0.5 mg/ml respectively. Similar kind of results were observed by Sonia *et al*. (2013) where ethanolic leaf extract of *Catharanthus roseus* had recorded the antimicrobial activity against *Candida albicans, Pseudomonas aeruginosa and Aspergillus niger* with zone of inhibition as 14, 13 and 8 mm respectively.

CONCLUSION

Eclipta prostrata is an important traditional herb used in the treatment of many diseases, which are usually free from side effects, are economical and also easily accessible to humans. On the basis of our results of the present study it is concluded that the methanolic extract of *E. prostrate* has significant antimicrobial and antioxidant activity. *E. prostrate* could be considered for the preparation of nutraceuticals with potent antimicrobial activity suitable for the prevention of plant as well as human diseases too. The extract can be subjected to identification and isolation of the potential antimicrobials and further screening can be used as sources for new drugs or formulations against human or phytopathogens respectively. The results obtained in the current study proved the antimicrobial and antioxidant potential of *E. prostrata* which is in support of earlier published reports.

Conflict of interest

The authors declare that they have no potential conflicts of interest to disclose.

REFERENCES

Akhter, S. and Sharika, S. (2019). Spectroscopic profiling of *Eclipta prostrata* ethanolic leaf extract by UV and FT-IR.GSC Biological and pharmaceutical sciences. 8(1): 029-034.

Ambu, G., Chaudhary, R.P., Mariotti, M and Cornara L. (2020). Traditional uses of medicinal plants by ethnic people in the Kavrepalanchok District, Central Nepal. Plants. 9: 759.

Arunachalam, S. and Pazhaniand, R. (2009). Anti-inflammatory activity of methanolic extract of *Eclipta prostrata L*. (Astearaceae). African Journal of Pharmacy and Pharmacology. 3(3): 097-100.

Barry and Sabh. (1974). Special Tests: Bactericidal Bctivity and Activity of Antimicrobials in Combination. InIE. H. Lennette, E. H.Spaulding and J. P. Truant (Ed.), Manual of Clinical Microbiology, 2nd Edition. American Society for Microbiology, Washington, D.C. 431-435.

Bergeron, Brusch, Barza and Weinstein. (1973). Bactericidal activity and pharmacology of cefazolin antimicrobial agentschemother. Antimicrob Agents Chemotherapy. 4: 396-401.

David, B., Wolfender, J.L. and Dias D.A. (2015). The pharmaceutical industry and natural products: Historical status and new trends. *Phytochem. Rev.*14: 299-315.

Eagle and Musselman. (1948). The rate of bactericidal action of penicillin *in vitro* as a function of its concentration and its paradoxically reduced activity at high concentrations against certain organisms. Journal of experimental medicine. 88(1): 99-131.

Jaglan, A. and Gill. (2013). Pharmacological activity and chemical constituents of *Ecliptaalba*. Global Journal of Medical Research Pharma, Drug discovery, Toxicology and Medicine. 13(7): 2249-4618.

John, B., David, B., Steven, V., Mahmoud. C., Luis, M., Daniel, T. (2000). Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved Standard-Third edition M27-A3.Clinical and Laboratory Standards Institute (CLSI). 28(14): 1-13.

Karthikumar, Vigneswari and Jegatheesan. (2007). Screening of antibacterial and antioxidant activities of leaves of *Eclipta prostrata* (L). Scientific Research and Essay. 2(4): 101-104.

Minh. (2019). Dried herbal tea production from Eclipta *prostrata*. Journal of Pharmaceutical Sciences and Research. 11(3): 684-687.

Nahid, Neelabh and Kumar. (2017). Evaluation of antioxidant and antimicrobial potentials of *Eclipta prostrata* collected from the Nepal region. The Pharma Innovation Journal. 6(11): 04-07.

Ogunbinu, F., Cioni, O.O. (2008). Essential oil constituents of *Eclipta prostrata* (L.) and *Vernonia amygdalina Delile*. Natural Product Communications. 4(3): 421-424.

Pooja, R. and Rajani. (2020). Pharmacological and therapeutic importance of *Eclipta alba:* A review. Journal of Pharmacognosy and Phytochemistry. 9(4): 577-579.

Priya, J., Usha, K., Uma and Hogale. (2018). Phytochemical analysis of *Eclipta prostrata (L.)* leaves. International Journal of Current Microbiology and Applied Sciences. 7 (8): 1069-1075.

Raghuwanshi, P. and Gupta. (2018). Anticandidal activity of phytochemicals extracted from medicinal plant *Eclipta prostrata*. International Journal of Pharma and Bio Sciences. 9(4): 50-56.

Riazunnisa, Chandra, Sudha and Habeeb. (2013). *In-vitro* Antibacterial activity and Phytochemical studies of some medicinal plants. International Journal of Pharmaceutical sciences Review and Research. 23(2): 77-80.

Shekokar and Nayak. (2017). A Phytopharmacological review of prospective of Bhringraj (*Eclipta alba* Hassak).International Journal of Ayurvedic Medicine. 8(1): 01-07.

Sinha and Raghuwanshi. (2016). Phytochemical screening and antioxidant potential of *Eclipta prostrata*. International Journal of Pharmacy and Pharmaceutical Sciences. 8(3): 0975-1491.

Sonia, Garima and Anil. (2013). Study of antimicrobial properties of *Catharanthus roseus* by agar well diffusion method. International Research Journal of Pharmaceutical and Applied Sciences. 3(5): 65-68.

Sunita and Sudhir. (2010). Phytochemical screening of Ethanolic Extract and Antibacterial Activity of Eclipta *prostrata*. Asian Journal of Chemistry. 22(9): 7336-7342.

John, B., David, B., Steven, V., Mahmoud. C., Luis, M., Daniel, T. (2000). Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved Standard-Third edition M27-A3.Clinical and Laboratory Standards Institute (CLSI). 28(14): 1-13.

Karthikumar, Vigneswari and Jegatheesan. (2007). Screening of antibacterial and antioxidant activities of leaves of *Eclipta prostrata* (L). Scientific Research and Essay. 2(4): 101-104.

Minh. (2019). Dried herbal tea production from Eclipta *prostrata*. Journal of Pharmaceutical Sciences and Research. 11(3): 684-687.

Nahid, Neelabh and Kumar. (2017). Evaluation of antioxidant and antimicrobial potentials of *Eclipta prostrata* collected from the Nepal region. The Pharma Innovation Journal. 6(11): 04-07.

Ogunbinu, F., Cioni, O.O. (2008). Essential oil constituents of *Eclipta prostrata* (L.) and *Vernonia amygdalina Delile*. Natural Product Communications. 4(3): 421-424.

Pooja, R. and Rajani. (2020). Pharmacological and therapeutic importance of *Eclipta alba:* A review. Journal of Pharmacognosy and Phytochemistry. 9(4): 577-579.

Priya, J., Usha, K., Uma and Hogale. (2018). Phytochemical analysis of *Eclipta prostrata (L.)* leaves. International Journal of Current Microbiology and Applied Sciences. 7 (8): 1069-1075.

Raghuwanshi, P. and Gupta. (2018). Anticandidal activity of phytochemicals extracted from medicinal plant *Eclipta prostrata*. International Journal of Pharma and Bio Sciences. 9(4): 50-56.

Riazunnisa, Chandra, Sudha and Habeeb. (2013). *In-vitro* Antibacterial activity and Phytochemical studies of some medicinal plants. International Journal of Pharmaceutical sciences Review and Research. 23(2): 77-80.

Shekokar and Nayak. (2017). A Phytopharmacological review of prospective of Bhringraj (*Eclipta alba* Hassak).International Journal of Ayurvedic Medicine. 8(1): 01-07.

Sinha and Raghuwanshi. (2016). Phytochemical screening and antioxidant potential of *Eclipta prostrata*. International Journal of Pharmacy and Pharmaceutical Sciences. 8(3): 0975-1491.

Sonia, Garima and Anil. (2013). Study of antimicrobial properties of *Catharanthus roseus* by agar well diffusion method. International Research Journal of Pharmaceutical and Applied Sciences. 3(5): 65-68.

Sunita and Sudhir. (2010). Phytochemical screening of Ethanolic Extract and Antibacterial Activity of Eclipta *prostrata*. Asian Journal of Chemistry. 22(9): 7336-7342.

Timalsina, D., Devkota, H.P., *Eclipta prostrata* (L.) L. (2021) (Asteraceae): Ethnomedicinal Uses, Chemical Constituents and Biological Activities. Biomolecules. 11(11): 1738

Uddin, R., Ahmed, R., Akter., Masudul and Azad Chowdhury. (2010). Antioxidant, cytotoxic and antimicrobial properties of *Eclipta alba* ethanol extract. International Journal of Biological and Medical Research. 1(4): 341-346.

Valgas C., De Souza S.M., Smania E.F. (2007). Screening methods to determine antibacterial activity of natural products. Brazilian Journal Microbiolology. 38: 369-380

Wikler. (2006). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Approved standard-sixth edition M7-A6.Clinical and Laboratory Standards Institute (CLSI). 29(2): 1-10.