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Research Article

PROXIMATE ANALYSIS AND ANTIMICROBIAL ACTIVITY OF ECLIPTA ALBA (L.) HASSK. - A TRADITIONALLY USED HERB

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ABSTRACT

Eclipta alba is an herb that is widely used in traditional cuisine and in folk medicine for the treatment of various ailments. Proximate analysis of the plant was carried out using the fresh material by the standard methods. Preliminary qualitative phytochemical analysis of the extracts was carried out by standard methods. Antimicrobial activity of petroleum ether, ethyl acetate, ethanol and aqueous extracts of *E. alba* was evaluated against selected strains of bacteria and fungi using agar well diffusion method against *Bacillus subtilis MTCC 441, Staphyllococcus aureus MTCC 96, Proteus mirabilis MTCC 1429, Bacillus cereus MTCC 430, Escherichia coli MTCC 739, Salmonella enterica serv.typhi MTCC 3917, Pseudomonas aeruginosa MTCC 1688, Staphyllococcus epidermidis MTCC 435* and *Candida albicans MTCC 3017*. The proximate analysis yielded moisture (79.02 ± 0.03 %), ash (15.78 ± 1.12 %), organic matter (84.22 ± 1.12 %), fibre (2.22 ± 0.04 %), carbohydrates (67.50 ± 1.50 %), proteins (2.6 ± 0.05 %) and lipids (4.87 ± 0.42%) and major phytochemicals *viz.* alkaloids, saponins, flavonoids, phenols, tannins, sterols, cardiac glycosides and anthraquinone glycosides were detected in the extracts. The extracts exhibited antimicrobial activity differently against the tested microorganisms. Maximum number of test strains were inhibited by the ethyl acetate extract (PI= 1) with zones of inhibition in the range of 11 ± 1 mm to 22 ± 1 mm with maximum activity against *Bacillus cereus.* It was followed by ethanol extract (PI= 0.89) with zone of inhibition in the range of 9 ± 1 mm to 27 ± 1 mm and maximum activity against *Escherichia coli*. Petroleum ether extract exhibited inhibition of only *Bacillus cereus* (10 ± 2 mm). Aqueous extract inhibited four strains (*Bacillus subtilis, Staphyllococcus aureus, Bacillus cereus* and *Candida albicans*) with zone of inhibition in the range of 10 ± 0 to 11 ± 1 mm. This study justifies the use of the plant in traditional system and also shows that the plant could be a potential source of new antimicrobial agent/s.

Keywords: *Eclipta alba,* Nutritive value, Phytochemical analysis, Antimicrobial activity

INTRODUCTION

Plants have been of great importance due to their nutritive value and as a major source of medicines. An impressive number of herbal products and modern drugs have been isolated from plants. In developing nations, numerous types of edible wild plants are exploited as sources of food and hence provide an adequate level of nutrition to the inhabitants. Recent studies on agro pastoral societies in Africa indicate that these plant resources play a significant role in nutrition; food security and income generation [1]. According to FAO report, at least one billion people are thought to use wild foods in their diet [2]. In Ghana alone, the leaves of over 300 species of wild plants and fruits are consumed. In Swaziland, wild plants provide a greater share of the diet than domesticated cultivars. In India, Malaysia and Thailand, about 150 wild plant species have been identified as sources of emergency food [3]. Similarly, in South Africa about 1400 edible plant species are used. In Sahel region of Africa, over 200 wild foods have been identified to be used by the rural communities [4]. In most of these reports, it was emphasized that nutritionally, these non-conventional plant foods could be comparable to or even sometimes superior to the introduced cultivars [5]. It is, therefore, worthwhile to note that the incorporation of edible wild and semi-cultivated plant resources could be beneficial to nutritionally marginal populations or to certain vulnerable groups within populations, especially in developing countries where poverty and climatic changes are causing havoc to the rural populace. Many of the local vegetable materials are under-exploited because of inadequate scientific knowledge of their nutritional potentials. Though several works reporting compositional evaluation and functional properties of various types of edible wild plants in use in the developing countries abound in literature [6, 7, 8, 9]much still need to be done.

Plants also continue to be a major source of medicines. Thirty to forty percent of today's conventional drugs used in the medicinal and curative properties of various herbal supplements, botanicals, nutraceuticals and drug are derived from plants [10]. Herbal medicine, based on their traditional uses in the form of powders, liquids or mixtures, has been the basis of treatment for various ailments in India since ancient times. According to World Health Organization (WHO) traditional medicines are relied upon by 65–80% of the World's population for their primary health care needs. The use of herbs as complementary and alternative medicine has increased dramatically in the last 20–25 years [7]. Moreover, emergence of multiple drug resistant strains of microorganisms due to indiscriminate use of antibiotics to treat infectious diseases has generated a renewed interest in herbal medicine [10]. Antimicrobial potential of different medicinal plants is being extensively studied all over the world [11, 12, 13, 14, 15] but the subject needs in depth studies in a systematic manner since, the absence of scientific proof of their effectiveness, the validity of these remedies remains questionable and their use locally restricted [11].

One of the high value herbaceous plant with a long history of traditional medicinal uses in many tropical and subtropical countries is *Eclipta alba* (L) Hassk. The decoction of the leaves is used as laxative and source of a black dye [16]. The herb has been known for its curative properties against various ailments [8, 17-32]. Considering the spectrum of medicinal value of *E. alba*, the present study was initiated to investigate its nutritive potential and to evaluate the effect of different solvents on the extraction of antimicrobial substances from the plant.

MATERIALS AND METHODS

Preparation of plant extracts

The plant samples were collected locally and processed. The cleaned and shade dried plant material was ground into fine powder using electric blender. Plant extracts in the different organic solvents were prepared by successive cold maceration method. Fifty grams of dried powder was extracted successively by soaking in 500 ml petroleum ether, ethyl acetate and ethanol for 48 hours with intermittent shaking. The extracts were filtered through Whatman No. 1 filter paper into pre-weighed beakers. The filtrates were dried in a rotatory vacuum evaporator until a constant dry weight of each extract was obtained. The residues were stored aseptically at 5°C for further use. The aqueous extract was prepared by soaking 50 g of dried plant material in 500 ml of sterile distilled water containing 1% chloroform for 48 hours with intermittent shaking. The extract was filtered through a double layer muslin cloth and then

centrifuged at 3500 rpm for 20 minutes. The supernatant was then filtered through Whatman No. 1 filter paper and then by 0.2 µ membrane filter. The extract was dried and preserved aseptically at 5^{0}C for further use.

Proximate analysis

The moisture and organic matter of plant sample was determined by AOAC methods [33]. Total ash was determined by the methods of WHO [34], total carbohydrate content by Anthrone method [35], total protein content by the Lowry method [36], total lipids and crude fibre by the methods suggested by Aberoumand [37]. All the proximate values were reported in percentage.

The nutritive value was determined from the percentage of carbohydrates, proteins and fats by the following equation [38] and expressed as calories per 100 g.

Nutritive value= 4x % Carbohydrates + 4x % Proteins + 9x % Fats

Qualitative phytochemical analysis

Preliminary qualitative phytochemical analysis was performed with the petroleum ether, ethyl acetate, ethanol and aqueous extracts of the plant for alkaloids, saponins, flavonoids, phenols and tannins, sterols, cardiac glycosides and anthraquinone glycosides by following the standard methods [39, 40].

Alkaloids: (Extract + 1% HCl, filtered) 1ml of filtrate+ few drops of Dragendorff's reagents/Mayer's reagents/ Hager's reagents/ Wagner's reagent, Orange brown precipitate/ Cream colored precipitate/ Yellow precipitate/ Red brown precipitate respectively indicated the presence of alkaloids.

Tannins and Phenolics: a) Small quantity of the extract dissolved in distilled water + 10% Lead acetate solution, white precipitate indicated the presence of tannins, b) Small quantity of the extract dissolved in distilled water + few ml of 1% gelatin + 10% sodium chloride, white precipitate indicated the presence of tannins.

Flavonoids: a) Plant residue + 10% NaOH, yellow coloration indicated the presence of flavonoids. (b) Extract + con. H_2SO_4, formation of yellow or orange color indicated the presence of flavonoids.

Saponin: a) Foam Test: small quantity of the residue was diluted with distilled water to 20 ml and shaken vigorously, formation of one cm layer of foam which was stable for 10 minutes indicated the presence of saponin. b) Alcoholic extract + Sodium bicarbonate + shaken well, honey comb like frothing confirmed the presence of saponin.

Cardiac glycosides: Kellar-Killani test: 2 ml of extract + 1 ml glacial acetic acid + one drop 5% $FeCl_3$ + Conc. H_2SO_4, reddish brown color appears at junction of the two liquid layers and upper appears bluish green, indicates the presence of cardiac glycosides.

Anthraquinine glycosides: 5 ml of extract + 5 ml 5% $FeCl_3$ + 5 ml dil HCl + Heat, cool + chloroform, shaken well, separated the organic layer and added dilute ammonia solution. Pinkish color indicated the presence of anthraquinone glycosides.

Antimicrobial screening

The petroleum ether, ethyl acetate, ethanol and aqueous extracts of the plant were screened against 8 bacterial strains, four of which were Gram positive and four Gram negative and one fungal strain. The test organisms were *Bacillus subtilis MTCC 441, Staphyllococcus aureus MTCC 96, Proteus mirabilis MTCC 1429, Bacillus cereus MTCC 430, Escherichia coli MTCC 739, Salmonella enterica serv.typhi MTCC 3917, Pseudomonas aeruginosa MTCC 1688, Staphyllococcus epidermidis MTCC 435* and *Candida albicans MTCC 3017.* The test strains were obtained from the MTCC, Chandigarh, India.

Preparation of inoculum

Stock cultures were maintained at 4°C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of culture from the stock to test tubes of Nutrient Broth for bacteria and Malt Yeast Broth for fungi and incubating for 24 hours at 37°C and 25°C respectively. The cultures were diluted with fresh Nutrient Broth and Malt Yeast Broth to achieve optical densities corresponding to 0.5 McFarland standard.

Antimicrobial susceptibility test

The agar well diffusion method [41, 42] was used to screen the antimicrobial activity of the extracts. In vitro antibacterial activity was screened by using Nutrient Agar obtained from Himedia (Mumbai) and in vitro antifungal activity assay was performed by using Malt Yeast Agar obtained from Himedia (Mumbai). The plates were prepared by pouring 25 ml of molten media into sterile petri-plates (diameter 100 mm). The plates were allowed to solidify at room temperature and 100 µL inoculum suspension was spread uniformly with the help of a sterile glass spreader and allowed it to dry. The extracts were dissolved in DMSO to obtain a final concentration of 500 mg/ml. Five 6 mm diameter wells were bored into the medium with the help of a sterile glass well borer and 100 µL of each of the four extracts was loaded into each well. These were allowed to diffuse for 45 minutes at room temperature after which the plates were transferred for incubation at 35^{0}C for bacteria and 25^{0}C for fungi. After the 24 hours of incubation, inhibition zones formed around the well were measured with transparent ruler in millimeter. The experiment was performed in triplicate and mean along with standard deviation was calculated. The activities of the extracts were compared with the standard drugs- Ciprofloxacin (10 µg/ml) for bacteria and Clotrimazole (30 µg/ml) for fungi. DMSO was used as negative control.

Determination of activity index and proportion index [43]

The activity index of the crude plant extract was calculated as

$$\text{Activity Index (A. I)} = \frac{\text{Mean of zone of inhibition of the extract}}{\text{Mean of zone of inhibition of standard antibiotic drug}}$$

The proportion index was calculated as

$$\text{Proportion Index (P. I)} = \frac{\text{Number of positive results obtained for extract}}{\text{Total number of tests carried out for each extract}}$$

Determination of minimum inhibitory concentration (MIC) [44]

MIC is defined as the lowest concentration of a compound/extract/drug that completely inhibits the growth of the microorganism in 24h. MIC of the extract with highest P.I. was determined by the agar well diffusion method [40]. The Nutrient Agar and Malt Yeast Agar plates were prepared as mentioned earlier for antimicrobial susceptibility testing. 0.1ml of the adjusted inoculums of the microbes were then inoculated and spread over the medium. Two-fold serial dilutions of the extract in DMSO were prepared from the stock concentration (400 mg/ml) of the extract to obtain 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 mg/ml. The different concentrations of the extract were then dispensed into the wells made in the plates and the plates were incubated at 35^{0}C for bacteria and 25^{0}C for fungi for 24 hours. The lowest concentration which showed a zone of inhibition was recorded as the MIC.

Statistical Analysis

Statistical analysis was performed using SigmaStat 3.5 and the results were expressed as the mean of the three replicates ± standard deviation of the replicates.

RESULTS

The findings of the present study are laid down below. The proximate analysis yielded moisture (79.02 ± 0.03 %), ash (15.78 ± 1.12 %), organic matter (84.22 ± 1.12 %), fibre (2.22 ± 0.04 %), carbohydrates (67.50 ± 1.50), proteins (2.6 ± 0.05) and lipids (4.87 ± 0.42%)(Table 1). The nutritive value was calculated to be 204.24 ± 15.64 calories per 100 grams. The ethanol extract showed the presence of most of the secondary metabolites viz. alkaloids, flavonoids, saponins, tannins, sterols and cardiac glycosides (Table 2).

Table 1: Proximate analysis and nutritive value of *E.alba*

	% content
Total ash	15.78 ± 1.12
Organic matter	84.22 ± 1.12
Moisture content	79.02 ± 0.03
Fibre	2.22 ± 0.04
Carbohydrates	37.1 ± 1.60
Proteins	0.46 ± 0.06
Lipids	6 ± 1
Nutritive value (calorie/100g)	204.24 ± 15.64

Table 2: Qualitative phytochemical analysis of extracts of *E. alba*

Extract	Alkaloids	Saponins	Flavonoids	Phenols and Tannins	Sterols	Cardiac glycosides	Anthraquinone glycosides
Petroleum ether	-	-	+	-	+	+	-
Ethyl acetate	-	-	+	+	+	+	-
Ethanol	+	+	+	+	+	+	-
Water	+	-	+	+	-	-	+

All the extracts exhibited antimicrobial activity against one or more of the tested microorganisms (Figure 1). Maximum number of test strains were inhibited by the ethyl acetate extract (P.I = 1) with zones of inhibition in the range of 11 ± 1 mm to 22 ± 1 mm with maximum activity against *Bacillus cereus* (A.I = 1.16). It was followed by ethanol extract (P.I = 0.89) with zone of inhibition in the range of 9 ± 1 mm to 27 ± 1 mm and maximum activity against *Escherichia coli* and *Pseudomonas aeruginosa* with activity index of 1 (Figure 2).

Petroleum ether extract exhibited lowest proportion index of 0.11 and inhibition of only *Bacillus cereus* (A.I = 0.53). Aqueous extract inhibited four strains (*Bacillus subtilis, Staphyllococcus aureus, Bacillus cereus* and *Candida albicans*) with zone of inhibition in the range of 10 ± 0 to 11 ± 1 mm and activity index in the range of 0 to 0.65 (Figure 1 & 3). The three extracts- ethyl acetate, ethanol and water exhibited similar antifungal activity against *C. albicans* with an activity index of 0.65 (Figure 2).

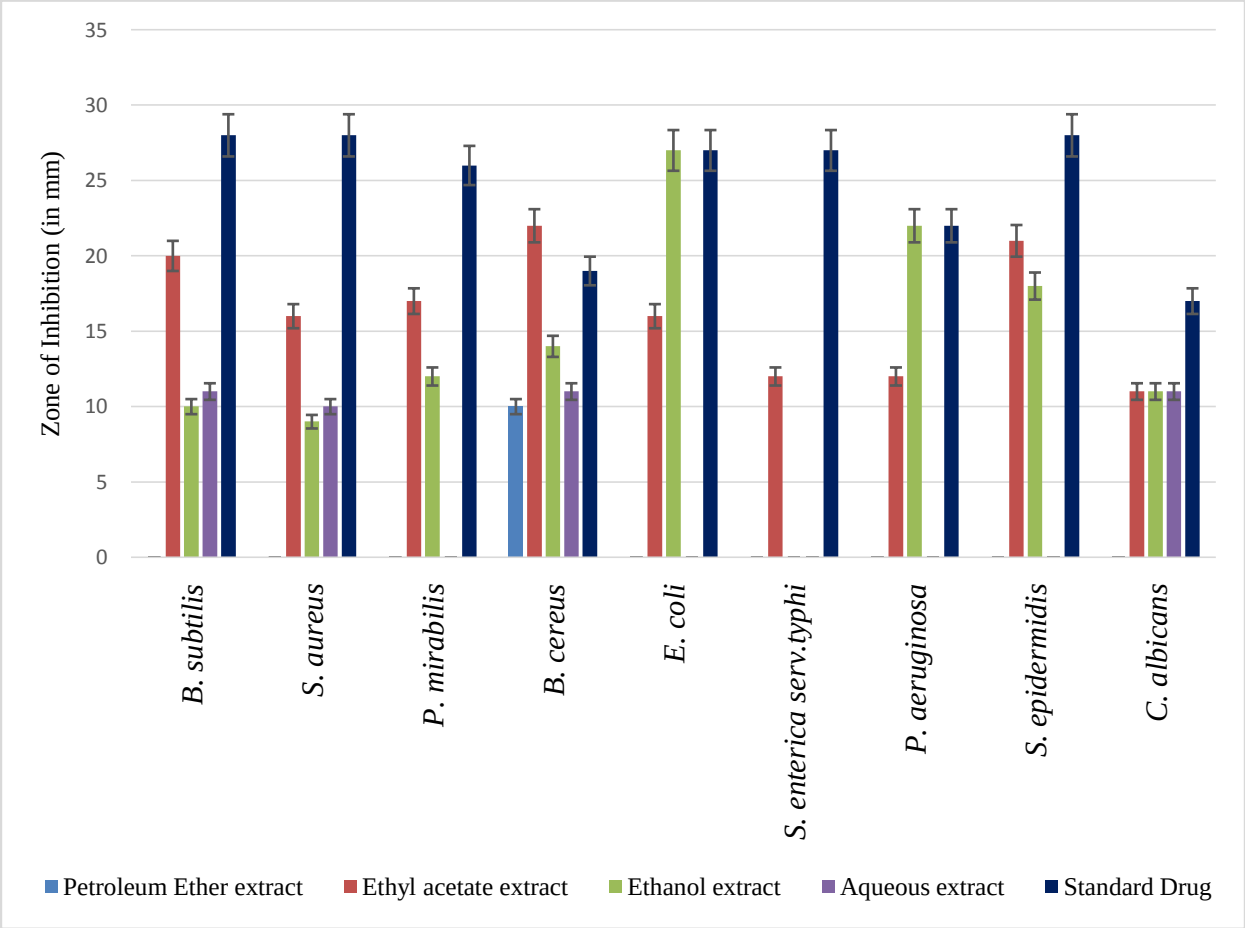

Fig. 1: Antimicrobial activity of the four extracts of *E. alba*

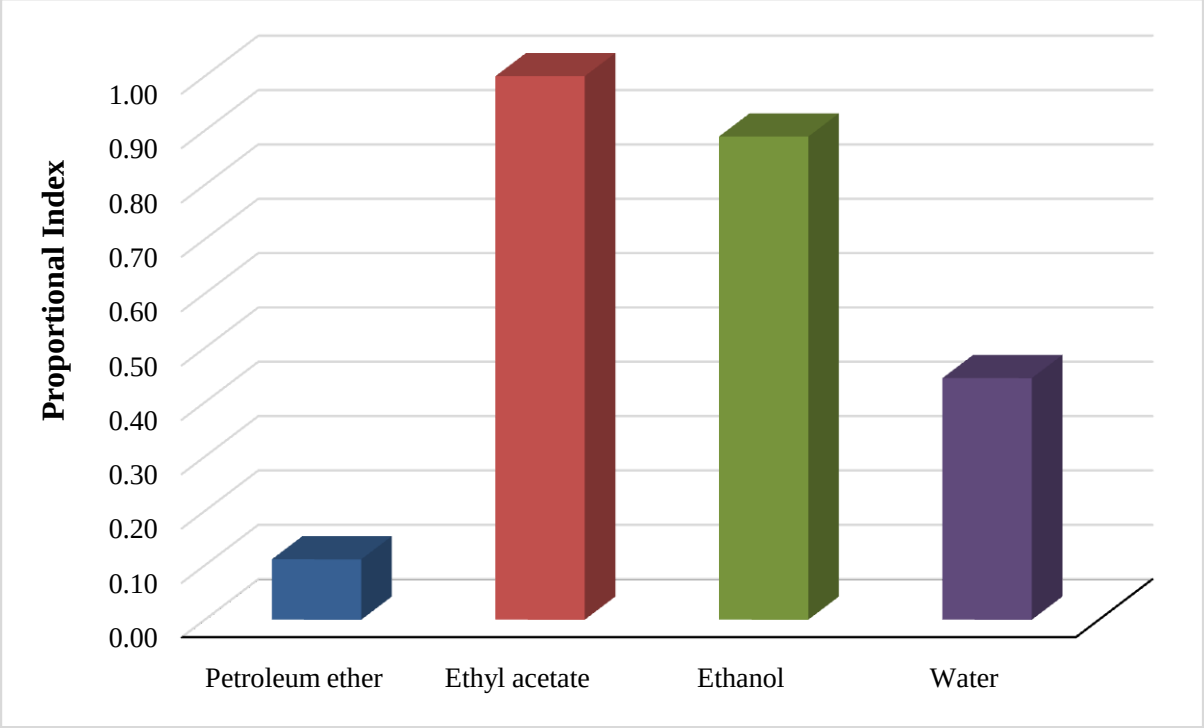

Fig. 2: Activity Index of the four extracts of *E. alba*

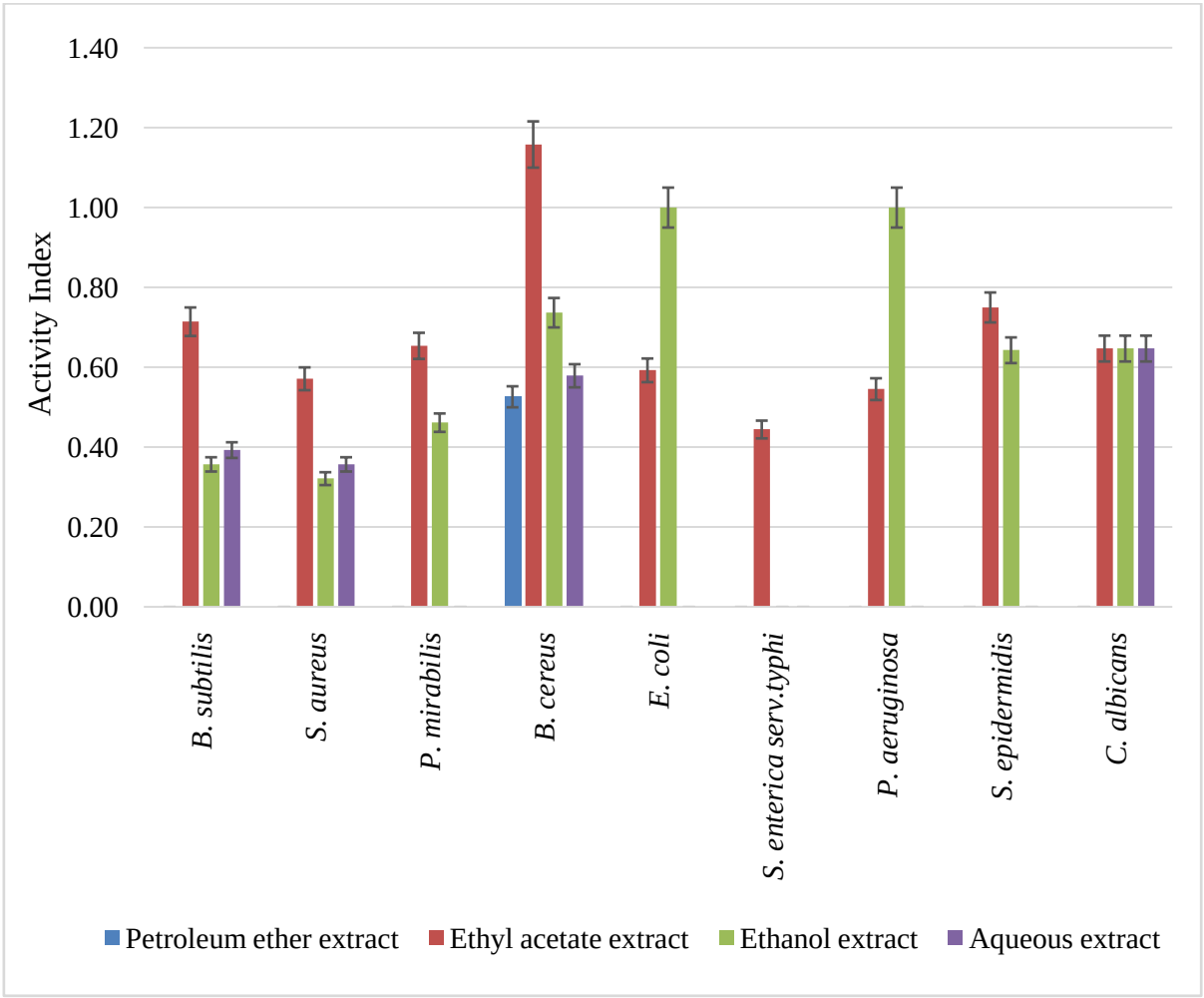

Fig. 3: Proportional Index of the four extracts of *E. alba*

The minimum inhibitory concentration of ethyl acetate extract recorded a lowest value of 1.56 mg/ml for *B. cereus* and *S. epidermidis* **(Table 3)**, hence suggesting a greater effectiveness of the ethyl acetate extract in inhibiting these strains. The negative control did not inhibit the test strains.

Table 3: MIC of ethyl acetate extract of *E. alba*

Strain	MIC (in mg/ml)
B. subtilis	6.250
S. aureus	3.125
P. mirabilis	25.000
B. cereus	1.560
E. coli	12.500
S. enterica serv.*typhi*	25.000
P. aeruginosa	12.500
S. epidermidis	1.560
C. albicans	12.500

DISCUSSION

Common medicinal plants have been compiled from translations of ancient Indian texts on health and healings [6]. The present study also evaluated the nutritional profile of *E. alba* and explored its potential for use in daily diet as an alternative food. The moisture content is comparable to that of *Amaranthus virdis* Linn, *A. caudatus* Linn., *Achyranthus aspera* Linn., *Chenopodium album* Linn., *Centella asiatica* Linn. and *Cassia tora* Linn. [45]. The nutritive value has been found to be higher than that of *Clerodendrum colebrokianum, Oenanthe linearis, Sanchus arvensis* and *Zanthoxylum acanthopodium* [46]. The presence of carbohydrates, proteins, fats and fiber makes the plant a wholesome food and potent enough to be a part of our daily diet. Its nutritive value is comparable to the other foods usually included in the daily diet.

Validation and selection of solvents used for extraction guarantees a sound selection of extracts and active principles with relevant pharmacological action and worthy following up. The present study therefore offers a scientific basis for selection of solvents for extraction of antimicrobial molecules from *E. alba* [47]. Although the traditional medicines are usually prepared in water as a solvent [48], the present study shows that the plant extracts prepared in organic solvents - ethyl acetate and ethanol, exhibited higher antimicrobial activity than the aqueous extract. These findings support the works of Vaghasiya and Chanda [49] but contradict the findings of Samy *et al.* [50] who reported a significant activity of the aqueous extracts of medicinal plants towards some Gram negative bacteria.

The proportion index suggests that the antimicrobial activity of the plant extracts varies with the solvent used for the purpose of extraction and hence antimicrobial activity may be regarded solvent-specific. This variation in the activity of the extracts may be due to the different phytochemicals contained in them [51, 52, 53, 54]. The higher activity of the ethyl acetate and ethanol extracts may be due to the different phytochemicals present at different percentages. The flavonoids in the aqueous extracts are reported to have no antimicrobial significance and the water soluble phenolics are only important as antioxidant compounds [47]. This justifies the lower antimicrobial activity of the aqueous extract as compared to the other solvent extracts.

CONCLUSION

The results clearly indicate that *E. alba* has a high nutritive value and the ethyl acetate extract of the plant possesses broad-spectrum antimicrobial activity. Therefore, further investigation in this regard is needed to identify the active principle present in the organic solvent extract. Hence the present study justifies the use of the plant in traditional system of medicine and encourages its use in diet as an alternative food for protecting and maintaining human health.

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REFERENCES

1. Asibey-Berko E, Tayie F. The antibacterial properties of some plants found in Hawaii.Ghana J Sci 1999; 39: 91-92.
2. Burlingame B. Comparison of total lipids, fatty acids, sugars and nonvolatile organic acids in nuts from Castanea species.J Food Comp Anal 2000;13:99-100.
3. Faruq U, Sani A, Hassan L. Composition and distribution of deadly nightshade.Niger J Basic Appl Sci 2002;11:157-164.
4. Sena L, VanderJagt D, RiveraC, Tsin A, Muhammad I,Mahamadou Oet al.Nutritional profile of some edible plants from Mexico.Plant Food HumNutr 1998;52: 17-30.
5. Edmonds J, Chweya J.Black Nightshades, *Solanum nigrum* L. and related species. Promoting the conservation and use of underutilized and neglected crops (Taylor andFrancis, London), 1995.
6. Aberoumand A.A comparative study of nutrients and mineral molar ratios of some plant foods with recommended dietary allowances.Advance Journal of Food Science and Technology 2010;2(2): 104-108.
7. Jimoh FO, Adedapo AA, Afolayan AJ.Comparison of the nutritive value, antioxidant and antibacterial activities of *Sonchus asper* and *Sonchus oleraceus.*Rec Nat Prod2011;5(1): 29-42.
8. Bakht J, Islam A, Ali H, Tayyab M, Shafi M. Antimicrobial potentials of *Eclipta alba* by disc diffusion method.African Journal of Biotechnology2011;10(39):7658-7667.
9. Rios JL, Recio MC. Medicinal plants and antimicrobial activity.J Ethnopharmacol2005;100: 80-84.
10. Chopra I, Hodgson J, Metcalf B, Poste G. The search for antibacterial agents effective against bacteria resistant to multiple antibiotics.Antimicrob Agents Chemother1997;4:497-503.
11. Ahmad I, Mehmood J, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. JEthnopharmacol 1998;62: 183-193.
12. Arora DS, Kaur J. Antimicrobial activity of spices.IntJ Antimicrob Agents 1999;12: 257-262.
13. Arora DS, Kaur G J, Kaur H. Antibacterial activity of tea and coffee: their extracts and preparations.Int J Food Properties2009;12:286-294.
14. Rojas JJ, Ochoa VJ, Ocampo SA, Munoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections.BMC Complement Altern Med2006; 6(2).
15. Kaur G J, Arora DS. Antibacterial and phytochemical screening of *Anethum graveolens, Foeniculum vulgare* and *Trachyspermum ammi*.BMC Complement Altern Med2009;9(30).
16. Dutta AC.Dictionary of economic and medicinal plants. 2nd ed.(Jorhat): Assam Printing Works (P) Ltd, Assam; 2006.
17. Banji O, Banji D, Annanmalai AR ,Manavalan R. Investigation on the effect of *Ecliptaalba* on animal models of learning and memory.Indian J Physiol Pharmacol2007;51: 274-280.
18. Tewtrakul S, Subhadhirasakul S, Cheenpracha S, Karalai C.HIV-1 protease and HIV-1 integrase inhibitory substances from *Eclipta prostrata*.Phytother Res2007;21(11):1092-95.
19. Tabassum N, Agrawal SS. Hepatoprotective activity of *Eclipta alba* Hassk. Against paracetamol induced hepatocellular damage in mice.JK-Practic2004;11:278-280.
20. Malhotra S, Singh AP. A review of pharmacology of phytochemicals from Indian medicinal plants.Internet Journal of Alternative Medicine 2007; 5(1). DOI: 10.5580/75c
21. Arunachalam G, Subramanian N, PazhaniGP, Ravichandran V. Anti-inflammatory activity of methanolic extract of *Eclipta prostrata* L. (Asteraceae).Afr J PharmPharmacol2009; 3:97-100.
22. Bapna S, Adsule S, Shirshat MS, Jadhav S, Patil LS,Deshmukh RA. Anti-malarial activity of *Eclipta alba* against *Plasmodium berghei* infection in mice. J Commun Dis2007; 39: 91-94.
23. Chenniapan K & Kadarkarai M. In vitro antimalarial activity of traditionally used Western Ghats plants from India and their interactions with chloroquine against chloroquine-resistant Plasmodium falciparum.Parasit Res2010; 107(6): 1351-1364.

24. Patel MB, Panchal SJ, Patel JA. Antianaphylactic activity of alcoholic extract of *Eclipta alba*. J Young Pharmacol2010;1: 244-250.
25. Sharma MC, Sharma S. Phytochemical screening of methanolic extract and antibacterial activity of *Eclipta alba* and *Morinda citrifolia* L.Middle-east journal of Scientific Research2010;6(5):445-449.
26. Sagoo MIS, KaurR, Gupta RC. Comparison of antibacterial activity of three morphotypes of medicinal herb *Eclipta alba* (L.) Hassk. Scholars Research Library2010;2(6):200-207.
27. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. (New Delhi): CSIR; 1956.
28. Wiart C, Mogana S, Khalifah S, Mahan M, Ismail S,Buckle M, Narayana AK, Sulaiman M. Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia, Fitoteropia2004; 75 (1):68-73.
29. Venkatesan S, Ravi R. Antifungal activity of *Eclipta alba.*Indian J Pharmaceutical Sci2004;66(1): 97-98.
30. Ananthi J, Prakasam A, Pugalendi KV. Antihyperglycemic activity of *Eclipta Alba* leaf on alloxan-induced diabetic rats.Yale J Biol Med (2003);76:97-102.
31. Karthikumar S, Vigneswari K, Jegatheesan K. Screening of medicinal plant extracts for antioxidant activity.J Med Plants Res2007;2(4): 101-104.
32. Veeru P, Kishor MP, Meenakshi M. Screening of medicinal plant extracts for antioxidant activity. J Med Plants Res2009;3: 608-612.
33. AOAC, Officialmethods of analysis.14th edition.(Washington DC):Association of Official Analytical Chemists;1990.
34. WHO, Quality control methods for medicinal plant materials,(Geneva): World Health Organization; 1998.
35. Sadasivam S, Manickam A.Biochemical methods for agricultural sciences.(New Delhi): New Age International (P) Ltd;1996.
36. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent.J Biol Chem 1951;193: 265-275.
37. Aberoumand A. A comparative study on composition analysis of plants consumed in India and Iran.American-Eurasian J Agric & Environ Sci 2010;8:3329-333.
38. Indrayan AK, Sharma S, Durgapal D, Kumar N,Kumar M. Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttraranchal.Current Science 2005;89(7):1252-1255.
39. Khandelwal KR. Practical Pharmacognosy–Techniques and Experiments.(Pune): Nirali Prakashan; 2005.
40. Kokate CK.Practical Pharmacognosy. (Delhi): Vallabh Prakashan; 1999.
41. Mbata TI, Debiao L, Saikia A. Antibacterial activity of the crude extracts of Chinese Green Tea (*Camellia sinensis*) on *Listeria minocytogenes*. Internet J Microbio2006 ;2(2)
42. Norrel SA, Messley KE.Microbiology Laboratory Manual Principles and Applications. (New Jersey): Prentice Hall Upper Saddle River; 1997.
43. Egharevba HO, Abdullahi MS, Okwute SK, Okogun JI. Phytochemical Analysis and Broad Spectrum Antimicrobial Activity of *Laggera pterodonta* (DC.) Sch. Bip. (Aerial Part).Researcher2010;2(10):35-40.
44. Rajasekaran C, Meignanam E, Vijayakumar V, Kalaivani T, Ramya S, Premkumar Net al, Investigations on antibacterial activity of leaf extracts of *Azadirachta indica* A. Juss (Meliaceae): a traditional medicinal plant of India.Ethnobotanical Leaflets 2008;12: 1213-1217.
45. Menghani E, Ojha CK, Negi RS, Agarwal Y,Pareek A. Screening of Indian Medicinal Plants and their potentials as Antimicrobial agents. Global Journal of Science Frontier Research2011;11 (2): 15-20.
46. Vishwakarma KL, Dubey V. Nutritional analysis of indigenous wild edible herbs used in eastern Chhattisgarh, India.Emir J Food Agric 2011;23 (6): 554-560.
47. Seal T.Determination of Nutritive Value, Mineral Contents and Antioxidant activity of some wild edible plants from Meghalaya State, India.Asian J Applied Sci2011;4(3): 238-246.
48. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H.Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia 2011;1 (1):98-106.
49. VaghasiyaYK, Chanda SV. Screening of Methanol and Acetone Extracts of Fourteen Indian Medicinal Plants for Antimicrobial Activity.Turk J Biol2007; 31: 243-248.
50. Samy RP, Ignacimuthu S, Sen A. Screening of 34 Indian medicinal plants for antibacterial properties.Journal of Ethnopharmacology1998;62:173-182.
51. Cowan MM.Plant Products as Antimicrobial Agents.Clinical Microbiology Reviews1999;12(4): 564–582.
52. Borkataky M, Sood K, Kardong D, Kakoti, BB, Saikia LR. Antibacterial activity and phytochemical analysis of two folklore medicinal plants of North-East India.Bulletin of Life Sciences2011;XVII: 73-80.
53. Abdullah S, Gobilik J, Chong KP. Preliminary phytochemical study and antimicrobial activity from various extract of *Cynodon dactylon* (L.) Pers. (Bermuda) against selected pathogens.Int J Pharm Pharm Sci 2012; 4(5): 227-230.
54. Natarajan D, Srinivasan R, Shivakumar MS. Phyllanthus wightianus Müll. Arg: a potential source for antibacterial and phytochemical analysis. Int J Pharm Pharm Sci 2012; 4(5): 241-245.