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In vitro evaluation of antiulcer activity, nutritional analysis and antioxidant activity of *Eclipta prostrata*

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Abstract

Ecliptaprostrata (L.) (Asteraceae) is a extensively disbursed annual herb. This plant has been applied as a conventional remedy in numerous conventional remedy structures in India. Powdered leaves of *E.prostrata* had been extracted with extraordinary solvents i.e. chloroform, methanol and aqueous. The extracts are further analysed for antioxidant and *in vitro* antiulcer appraisal regards to their phytochemical constituents. Methanolic extract confirmed greater promising antiulcer property in addition to antioxidant activity as compared to different extracts.Maximum quantity of phytochemicals as well as total phenolic, alkaloid and flavonoid contents was also higher in case of methanolic extract followed by the chloroform and aqueous extract. As methanolic extract confirmed greater antioxidant and antiulcer efficacy, this extract may be applied as an effective and secure antioxidant supply and antiulcer drug on a industrial foundation for the improvement of latest phytodrugs.

Keywords: *Ecliptaprostrata (*L.*)* L., antiulcer activity, antioxidantactivity

Introduction

Human beings have always been depended upon nature and natural products especially plants for all their requirements. plants are significant member of ethnopharmacology and directly used for treating numerous health ailments by majority of cultures around the world. Plant based products are of great interest due to being fee effective, with lower side effects and multifaceted applications. Even today, plants are not only indispensable in health care butare also the best source for safe future drugs. With 24000 to 30000 accepted species and 1600-1700 genera, the Asteraceae family is the largest flowering plant in the world, extending to Antarctica as well. (Funk *et al*., 2005). This herb is also known as bhringaraj, a member of the same family, commonly known as false daisy. This herb is also known for its antihepatotoxic qualities, antihyperglycaemic properties, immuno modulatory properties, antipyretic activities, and antioxidant properties. (Mithun *et al*., 2011). Despite the absence of literature describing the bioactive profile, antiulcer and antioxidant potential of *E.prostrata*, this study was conducted to determine the potential antiulcer and antioxidant effects of the different extracts of *E.prostrata* collected in Tamilnadu for its antiulcer and antioxidant potentials in invitro condition with regards to its phytochemicals and total phenolic and flavonoid contents along with its phytochemical analysis.

Materials and Methods

Collection of plant materials and Extract preparation

Eclipta Prostrata (L.) have been gathered from villupuram, Tamilnadu, India.

Preparation of plant extracts

The experiment was done in three solvent Aqueous, chloroform and Methonol. The plant source was washed and dried in sunlight for 2 days. Then grinded in electronic blender and sieved it.1 g of plant was dissolved in 25 ml. Solvent 70% concentration then all the three prepared solution was kept for 14 hours at room temperature in a closed tube. After 24 hours centrifuge and filtered the solution using Watman No.1 filter paper then kept in airtight bottles at 4 degree Celsius for further experiment. A phytochemical screening was conducted on the extract and *in vitro* antiulcer activity and antioxidant power was assessed.

Qualitative phytochemical analysis of extracts

The plant extracts of different solvents were qualitatively analysed for carbohydrates, proteins, Amino acids, Alkaloids, Tannins, Saponin, Flavanoids, Glycosides, Steroids, and Phenols using standard procedures for phytochemical screening.

Detection of carbohydrate

Molish test: 2 ml of Molish's reagent and 2 ml of extracts were boiled and then few drops of sulfuric acid was added. A radish ring indicates the presence of carbohydrates

Detection of reducing sugar

5 ml of extracts was added with 5ml of boiling Fehling solution for 2-5min. A brick red precipitate indicates the presence of reducing sugar

Detection of tannin
2 ml of extracts was taken into a test tube and added few drops of 0.1% or 1m ferric chloride. A blue black or greenish black coloration indicates the presence of tannin.

Detection of saponins
Foam test: 2 ml of extract was shaken with 5 ml of distilled water in a test tube. If foam persist for ten minutes which indicates the presence of saponins.

Detection of Phenols
Ferric chloride test: 2 ml of extract were treated with 3-4 drops of 0.1%or 1m ferric chloride solution. Formation of bluish black color indicates the presence of Phenols

Detection of flavonoids
About 2ml of extract was taken and add few drops of 10% of ferric chloride solution. The occurence of green or blue colouration indicates the presence of phenolic hydroxyl group.

Quantitative analysis of secondary metabolites
The plant extract of different solvents were estimate dquantitatively for total Alkaloid content, Total Flavanoid content amd total tannin content using the standard phytochemical screening procedures.

Estimation of Alkaloid (Harborn method)
5g of each plant sample was weighed separately into a 250 ml capacity beaker and added 100ml of 10% solvent of acetic acid in ethanol then covered the beaker to check evaporations of solvent and allowed to stand for 6 hour. This was filtered and extracts was concentratedon water bath to ¼ of original volume then cons. ammonium hydroxide was added drop wise into concentrated extracts until the precipitation was completed. The settled precipitate in solution was filtered and washed with dil. NH_4OH and filtered again. This precipitate residue is alkaloid which was dried andweighed.

Estimation of Flavanoid (Bohan and Kocipai method)
5g of leaf powder sample was weighed into a 250ml capacity beaker and added 100ml of 80% aqueous methanol for extraction at room temperature and then filtered through Whatman No. 42(125mm) filter paper and extracts was collected into another 250ml capacity beaker. Extraction procedure repeated in same used sample separately and extract was recollected. Collected extract then transferred into crucible and evaporated till dryness on water bath and weighed.

Estimation of Tannin (Peri and Pompei method)
2 ml of the sample extracts of concentration 2mg/ml was taken in a test tube. The volume was made up to 2ml with distilled water and 2 ml of water serves as the blank. To this 1 ml of Folin's phenol reagent (1:2) followed by 10ml of 35% sodium carbonate was added and kept at room temperature for 10 min. Blue colour was observed and the colour intensity was read at 640 nm. A standard graph (gallic acid - 1 mg/ml) was plotted, from which the tannin content of the extract was obtained. The total tannin content was expressed in mg/g dryweight of extract.

Estimation of ferric ion reducing antioxidant power (FRAP) (yildrin *et al* (2001))
About 100mg of samples was dissolved in 10ml of distilled water. To 2.5 ml of the extract (100-1000µg/ml, added 2.5ml of phosphate buffer (0.2M) and 2.5ml of 1% potassium ferricyanide. Boil the mixture in a water bath at 500 ^{0}C for 20 minutes, then rapidly cooled, mixed with 2.5ml of 10% trichloro acetic acid and centrifuged at 300rpm for 10 minutes. The supernatant was used for the analysis. Pipette out 0.5ml of supernatant, was taken in a test tube and made upto 2.5ml of distilled water. 0.5ml of 0.1% trichloro acetic acid was added, mixed well and allowed to stand for 10 minutes.The increase in the absorbance at λ 700nm was used to measure the reducing power. Ascorbic acid was used as standard solution.

***In vitro* Evaluation of Antiulcer activity by Acid neutralization method**
The acid neutralizing capacity value for chloroform extract fermented rice water mixture (50mg, 100mg, 150mg, 200mg) was compared with the standard antacid Aluminium hydroxide + Magnesium hydroxide (100mg). To the 2ml quantity of this mixture, water was added to make up the total volume 50ml and then mixed for two minute. There after 30ml of 1.0N HCl was added into the standard solution and test was prepared and stirred for 15minutes, drops of phenophthalein solution was droped and mixed. The excess HCl was titrated with 1N Sodium hydroxide solution drop wise until a pink color is observed. The moles of acid neutralized is calculated by, Moles of acid neutralized = (vol. of HCl ×Normality of HCl) - (vol. Of NaOH × Normality of NaOH) Acid neutralizing capacity (ANC) per gram of antacid = moles of HCl neutralized / Grams of Antacid Extract.

***In vitro* evaluation of Antiulcer activity by Anti-Helicobacter pylori activity Disk diffusion test (Helicobacter pylori sensitivity test)**
Freshly prepared pre-incubated agar plates were spread over by bacterial suspensions of each isolate. Disks of filter paper (6 mm) soaked with 25µl of stock solutions of different extract was placed over agar plates and incubated at 37 degree Celsius in microaerophilic condition for 3-6 days. The anti helicobacterpylori activity was estimated by measuring zone of inhibition (mm) around the disks (disk + zone) using digital patchy meter. To verify neomycin (positive control) sensitivity of isolates concentration of 1µg/ml was prepared where phosphate buffer saline (PBS) is used as solvent.

Evaluation of minimum inhibitory concentration (MIC)
Minimum Inhibitory Concentration was determined by using agar dilution method for the extracts that showed minimum 6 mm zone of inhibiton. DMSO based stock solution of different extract was diluted with distilled sterile water to make a volume of 1000µl to get final concentrations of different extract in the mixture as 125, 250, 500 and 1000 µg/ml respectively and was added with 100µl of bacterial suspension with turbidity corresponds to 0.5 McFarland's standard ($1.5x10^{8}$CFU/ml). After incubation of 1h 100µl of this mixture was spreaded onto freshly prepared preincubated blood agar plates and incubated at 37oC under microaerophilic conditions for 3 days and the colonies formed were subsequently identified.

To determine MIC which is defined as minimum concentration of plant extract that can completely inhibit the growth of bacteria, plants that showed complete inhibition at 125µg/ml were further subjected to the same procedure with two folds dilution i.e. 62 µg/ml and more. DMSO (100µl) was used as negative control. Amoxicillin (100µl) with a concentration of 1µg/ml contributed as standard drug or positive control for comparison. All the experiments were performed in duplicate.

Results and Dicussion

Phytochemical analysis of different solvents like methanol, acetone and aqueous extracts of *lemno minor* was carried by qualitative tests and the data were presented in Tables 1. The phytochemical constituents the solvent extract of of *E.prostrata* showed presented in all the tested solvent extractand showed results in carbohydrates, proteins, tannins, flavonoids, saponins, terpenoids, Quinone, Steroids and Phenolic compounds were and showed the results in Table 1. Naturally occurring substance of plant origin have been reported to inhibit the growth of microorganisms. Plants extracts have been used in folk and even modern medical practices for the treatment of different ailments, most of which are due to microbial activities (Irobi 1992). Bacterial infection seems especially controllable due to good hygiene and the availability of effective antibacterial drugs. The development of resistance to antibiotics is an almost inevitable consequence of their application (Ekhaise and Okoruwa 2001). The speed of resistance depends on the respective class of antibiotics and their product use. For many years, medicine depended exclusively on leaves, flowers and barks of plants, only recently have synthetic drugs come into use and in many instance, these are carbon copies of chemical identified in plants Phytochemical analysis of different solvent like Aqueous, Chloroform, methanol extract of *E.prostrata* was carried by qualitative test. This result in Carbohydrate, protein, tannin, phenolic compounds.

The antibacterial activity of different solvents extracts *E.prostrata was* carried out and the results. The methanolic leaf extract of *E.prostrata* showed highest antibacterial activity against *Proteus vulgaris* (21±1.22mm), *Salmonella typhi* (12±0.81mm)and Helicobacter pylori at the concentration of 5mg/100µl. The ethanol extarct of *E.prostrata* showed the highest zone of inhibition against *Salmonella typhi*(9±1.63mm) and *Proteus vulgaris* (9±1,22mm) and Helicobacter pylori at the concentration of 5mg/100µl. The aqueous leaf extract of *E.prostrata* showed highest antibacterial activity against *Proteus vulgaris* (20±1.63mm) and Helicobacter pylori From the above study, it can be concluded that this plant has immense potential to be used in the area of pharmacology and as a source of valuable drug. The extract showed antibacterial and antifungal activities against the tested microorganisms. The data clearly depicts the use of plant in treating various bacterial and fungal diseases and also which is indicating its use in the traditional system of medicine since ancient times.

Table 1: Phytochemical Analysis

Phytochemicals	Test performed	Methanolic extract	chloroform extract	Aqueous extract
Carbohydrate	Fehling test	+	-	+
Phenols	Ferric chloride test	+	+	+
Flavonoids	Ammonia test	+	+	+
Alkaloids	Wagner's test	+	+	-
Steroids	Salkowski test	+	+	+
Tannins	Lead acetate test	+	+	-
Saponins	Frothing test	-	+	+
Glycosides	Nitroprusside test	-	-	+
Amino acids	Ninhydrin test	+	+	+

Table 2: Estimation of secondary metabolites

S. No	*Eclipta prostrate Extract*	Total Alkaloid Content (mg/g dry weight)	Total Flavonoid Content (mg/g dry weight)	Total Tannin Conten (mg/g dryweight)
1	Methanolic Extract	0.60±0.002	0.780±0.022	0.04±0.0002
2	Chloroform Extract	0.50±0.006	0.645±0.006	0.13±0.004
3	Aqueous Extract	0.66±0.035	0.608±0.013	0.12±0.011

Table 3: Effect of aqueous extract of on acid neutralizing capacity

S. No	Concentration of Extract (mg)	Value of sodium hydroxide consumed (mg)	mEq of HCl consumed	ANC per gram of Extract
1	100	35.03	12.75	109.33
2	500	25.50	13.67	32.18
3	1000	36.3	7.60	10.70
4	1500	41	13.3	8.30
5	500 mg $Al(OH)_3$+$Mg(OH)_2$	46.3	6.89	15.6

Table 4: *In vitro* MIC (mg/ml) of Aqueous, Methanol and Chloroform extract of *Eclipta prostrate* against Helicobacter pylori isolates

Name of the bacterial species	Diameter of zone of inhibition (mm)					
	Negative control 100µl	Positive control 100µl (30µg)	Name of solvent extract (5mg/100µl)			
			Hexane extract	Ethanol extract	Chloroform extract	Aqueous extract
Salmonella typhi	-	17 ±1.22	12 ±0.81	9± 1.63	15± 1.25	11± 1.22

Staphylococcus epidermidis	-	18 ±1.63	13 ±1.63	7 ±1.63	11± 1.22	11± 1.22
Klebsiella pneumonia	-	19 ±1.22	15 ±1.25	7 ±0.81	-	10 ±1.22
Proteus	-	20± 1.22	19± 0.81	9 ±1.22	15± 1.25	20± 1.63
vulgaris	-	21± 0.81	21± 0.81	15± 1.25	18 ±1.63	18 ±1.63
Pseudomonas fluorescens	-					
Helicobacter pylori	-	24± 0.24	21± 0.81	22± 0.81	21± 1.22	21± 1.22

Values are expressed as mean ± standard deviation of triplicates
Negative control: Dimethyl sulfoxide (DMSO)
Positive control: Neomycin
Note - indicates: No activity

Summary and Conclusion

Findings of the present study clearly indicate that the lab experiments using Eclipta prostrata whole plant displayed appreciable gastro protective activity as demonstrated by *In vitro* Acid neutralization method and decreased percent inhibition in antihelicobacter pylori test. The present study therefore supports the claims of traditional medicinal practitioners as an antiulcer remedy. It could also be a prospective substitute for the existing synthetic antiulcer drugs which are to known to produce harmful adverse effects.Anyhow further studies are required to isolate, purify and characterize the active principles and an investigation of the detail biochemical pathway responsible for this antiulcer action is required to understand the precise mechanism of action of the fractions of different extracts of Eclipta prostrata and hence for the development of a effective antiulcer agent having less-toxicity.

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Conflict of Interests

Herewith all the authors declare that they do not have any conflict of interests.

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