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Phytochemical Screening of Different Extracts of Eclipta prostrata (Bringaraja)

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Abstract

Eclipta prostrata is a perineal weedy plant grown in wet lands in Sri Lanka. (Sanskrit name– Bringaraja, Sinhala name– Keekirindiya). This is a well-known medicinal plant used in Ayurveda as well as Sri Lankan Indigenous Medicine. Fresh juice of this whole plant is used as a home remedy for liver disorders. The objective of this study is to provide an overview of the chemical constituents present in the crude dried whole plant extract of Eclipta prostrata. Phytochemical screening of Eclipta prostrata was done by extracting the powder of whole plant with four different solvents; water,ethanol, hexane and ethyl acetate. Preliminary phytochemical screening revealed the presence of Carbohydrates, Aminoacids, Alkaloids, Tannis, Phenolic compounds, Terpenoids, Steroids, Flavanoids Cardiac glycosides, Saponins and Anthraquinone glycosides. Phytochemical analysis of Eclipta prostrata reveals absence of Resins. Presence of above phytochemicals may be responsible for the therapeutic effects of Eclipta prostrata on liver diseases. Finally, it is concluded that Eclipta prostrata plant contains medicinally important bioactive compounds and this finding justifies the ethnomedical use of this plant for various diseases of human.

Keywords: Eclipta prostrata; Phytochemical analysis; Sri Lankan Indigenous Medicine; Ethnomedicine; Bringaraja.

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Introduction

Eclipta prostrata is an erect or prostrate annual herb commonly found in marshy lands, abundant paddy fields and road sides. It is very common weedy plant used as a home remedy for different types of diseases by Sri Lankan indigenous medical practitioners. Plant is well known for its hair growth and hair colouring effect as mentioned in Ayurveda due to which the plant is termed "Bringharaja" in Ayurveda texts.[1] In Unani medicine, this plant is named as "Bhangra".[2] The plant is commonly seen in Sri Lanka, India, Malaya, Philippines and other tropical countries. In India, Eclipta prostratais used externally in chronic skin diseases, ulcers, elephantiasis, conjunctivitis.The plant stimulates hair growth. Internally, the plant is an essential ingredient of compound drugs used for arthritis, dropsy and indecoctions used for hepatic and splenic enlargement. In Philippine, the decoction of the leaves and flower tops is given for hepatitis.[3] This plant is keen stimulant to digestive system which augments appetite and digestion. It is an effective cholegouge, hence it is beneficialin hepato-spleenomegaly as well as hepatitis.[1] Presence of different phytochemicals may be responsible for different therapeutic effects of the plant.

Objective

The objective of this study was to screen phytochemical constituents present in Eclipta prostata whole plant by using aqueous and different solvent extracts.

Materials and Methods

Collecting plant materials: Fresh plants of Eclipta prostrata were collected randomly from Rajagiriya area in Colombo district. The taxonomic identities of the plantwere identified with the voucher specimen deposited at the Royal Botanical Garden Peradeniya, Sri Lanka. Plants were washed with tap water to remove soil and dust particles followed by shade dried under laboratory condition for three (3) weeks.

Preparation of aqueous extract: 200g of dried Eclipta prostrata were weighed and ground to a coarse powder using grinder (Diskmill Model FFC-234 China). 60 grams of coarse powder was weighed and put into a clay pot and added 1920ml distilled water. It is reduced into 120ml by giving moderate temperature. The decoction was filtered through Whatmann No 1 filter paper.[4]

Preparation of solvent extracts: A sample of shade dried Eclipta prostrata was finely powdered using a mechanical grinder (Diskmill, srlc23, China). 2000g of powdered material was subjected to sequential solvent extraction with increasing polarity using hexane, ethyl acetate and ethanol after keeping overnight in the shaker (Orbital shaker, Labline, UK) with each solvent separately. Each extract was filtered and the solvent was removed using a rotary evaporator (Buchi, R-114, Switzerland) at around 40C and obtained the dried extract. The yield of each dried extract was recorded (Citizen scale, CX, 220, USA) and all the extracts were kept in a refrigerator (below 0^{o}C) until they are used for bioassays and phytochemical analysis.

Phytochemical analysis of plants extracts: Sequentially hexane, ethylacetate, ethanol extracts and the aqueous extract of Eclipta prostrata were subjected to phytochemical analysis as described below.

One (1) gram of extract of Eclipta prostrata was mixed with 100ml of each solvent (hexane, ethyl acetate, ethanol and water)separately to obtain stock solutions (1% w/v). These solutions were subjected to phytochemical analysis using the methods described by accepted methods.

Tests for Alkaloids

Three different tests were performed to isolate Alkaloids (Meyer's test, Hager's test and Wagner's test).

Mayer's Test: A few drops of Meyer's reagent was added to 2ml of each aqueous solution (test solution). The appearance of pale yellow/ white colour precipitate indicates the presence of alkaloids.[5] *Hager's test:* 2ml of each aqueous solution (test solution) is mixed with a few drops of the Hager's reagent. The appearance of pale-yellow precipitate indicates the presence of alkaloids.[5] *Wagner's test:* A few drops of the Wagner's reagent was added with 2–3 drops of concentrated HCl to 2ml of each aqueous solution (test solution). The appearance of brown precipitate indicates the presence of alkaloids.[5]

Tests for Tannins and Phenolic Compounds

Three (3) different tests were performed to identify tannins and phenolic compounds (Ferric chloride test, Gelatin test and Lead Acetate test).

Ferric chloride test: 2ml of each aqueous solution (test solution), was mixed with a few drops of 5% $FeCl_3$. The appearance of deep blue/dark green precipitate indicates the presence of tannins and phenolic compounds.[6] *Gelatin test:* 2ml of each aqueous solution (test solution) was added with a few drops of 10% Sodium Chloride solution and a few drops of 1% w/v gelatin solution. The appearance of white precipitate indicates the presence of tannins and phenolic compounds.[6] *Lead Acetate test:* 2ml of each aqueous solution (test solution) was mixed with a few drops of 10% w/v lead acetate solution. The appearance of bulky white precipitate indicates the presence of tannins and phenolic compounds.[5,7]

Tests for Terpenoids and Steroids

To identify the Terpenoids and Steroids Salkowski and Libermann Burchard's tests were performed.

Salkowski Test: 2ml of each aqueous solution (test solution) is addedto 1ml of concentrated H_2SO_4. The appearance of red colour in lower layer indicates the presence of steroids and yellow colour in the lower layer indicates the presence of triterpenoids.[8] *Libermann Burchard's test:* A portion of 10mg of dried extraction was dissolved in 8ml acetic anhydride and prepared test solution. 2ml of each test solutions were boiled separately and allowed to cool and then 1ml of concentrated H_2SO_4 was added along the wall of the test tubes. The appearance of a brown colour ring at the interface and upper layer turns to green colour indicates the presence of steroids and if upper layer turns to deep red colour indicates the presence triterpenoids.[8]

Tests for Flavonoids

To identify flavonoids four (4) different tests were performed such as NH_4OH test, alkaline test, Lead acetate test and Shinoda test.

Ammonia Test: 2ml of aqueous solution (test solution) was added to 2ml of dilute ammonia (10%) and a few drops of concentrated H_2SO_4. The appearance of yellow orange colour with the addition of dilute ammonia and the disappearance of that colour after some time indicates the presence of flavonoids.[7] *Alkaline Test:* A few drops of 5% NaOH solution and drops of dilute HCl were added to 2ml of each aqueous solution (test solution). The appearance of an intense yellow colour with the addition of few drops of 5% NaOH solution and the disappearance of that colour with the addition of few drops of dil. HCl indicates the presence of flavonoids.[5,7] *Lead acetate test:* 2ml of each aqueous solution (test solution) was added with 1ml of conc. HCl and a few pieces of magnesium. Disappearance of the colour of the solution with the addition of 1ml of HCl and the appearance of pink or red colour with the addition of few pieces of magnesium indicates the presence of flavonoids.[7]

Tests for Cardiac Glycosides

To identify Cardiac glycosides, two (2) different tests were performed (Legal test and Keller Killiani test).

Legal Test: 2ml of each aqueous solution (test solution) was added with 2ml of pyridine and 1ml of alkaline sodium nitroprusside solution. The appearance of pink to red colour in the solution indicates the presence of glycosides.[8] *Keller Killiani test:* A few drops of glacial acetic acid and a few drops of concentrated H_2SO_4 anda trace amount (1 drop) of $FeCl_3$ was added to 2ml of each aqueous solution (test solution), The appearance of reddish brown colourring at the interface and upper layer turns to greenish colourindicates the presence of glycosides.[8,11]

Tests for Saponins

Two (2) different tests were performed (Foam test and olive oil test) to identify saponins.

Foam Test: 5ml of each aqueous solution (test solution) was shaken vigorously until a stable persistent foam.[9] *Olive oil Test:* The above frothing was mixed with 3 drops of olive oil and shaken vigorously. Formation of emulsion should be observed.[9]

Test for carbohydrates

To identify carbohydrates, three (3) tests were performed (Fehling's Test, Benedict 's test and Seliwanoff's test).

Fehling's Test: Fehling's A–1ml and Fehling's B–1ml was heated for 1 minute. Heated Fehling's solution was added to 2ml of each aqueous solution (test solution). Then this setup was heated for 2 minutes in a water bath. The appearance of brick red precipitate indicates the presence of carbohydrate.[8,6] *Benedict's Test:* 2ml of each aqueous solution (test solution) was added to 1ml of Benedict's reagent and heated for 2 minutes in a water bath. The appearance of red colour precipitate indicates the presence of carbohydrates.[8,6] *Seliwanoff's test:* 2ml of each aqueous solution (test solution) was mixed with 1ml of Seliwan off's reagent. Then it was heated for 2 minutes in a water bath. The appearance of rose colour precipitate indicates the presence of carbohydrates.[6]

Tests for Amino acids

Presence of Amino acids was tested by Ninhydrine test and Millon's test.

Ninhydrine test: 2ml of each aqueous solution (test solution) was added to Ninhydride solution and then heated for 2 minutes in a water bath. The appearance of a blue or violet colour in the solution indicates the presence of amino acid.[10] *Millon's test:* 2ml of each aqueous solution (test solution) and 1ml of Millon's reagent was mixed. Then it was heated for 2 minutes. The appearance of white colour precipitate with the addition of 1ml of Millon's reagent and the colour turn to red with heating indicates the presence of amino acid.

Test for Anthraquinone Glycosides

Presence of Anthraquinone Glycosides were tested by Hydroxyanthraquinone and Borntrager's test.

Hydroxyanthraquinone test: 2ml of each aqueous solution (test solution) was added to 1ml of 10% Potassium hydroxide and mixed. The appearance of red colour in the solution indicates the presence of hydroxyantraquinone.[5] *Borntrager's test:* 2ml of each aqueous solution (test solution) was added to 2ml of dil. Sulphuric acid. Then it was boiled and

filtered and allowed to cool. The filtrate was added equal volume of chloroform and shaked well. The organic solvent was separated and ammonia solution was added. The appearance of a pink or red colour in the ammonia layer indicates the presence of hydroxyantraquinone glycosides.[8,11]

Results and Discussions

The phytochemical tests were done on aqueous extracts and solvent extracts of dried whole plant of Eclipta prostrate by colour test and the results of the extractions were presented in the following tables (Table 1).

Dried whole plant of Eclipta prostrata with four different solvents; aqueous (water), ethanol. Hexane and ethyl acetate revealed that the number of primary and secondary phytochemicals such as carbohydrates, amino acids, alkaloids, tannin, phenolic compounds, terpenoids, steroids, flavonoids, cardiac glycosides, saponins and anthraquinone glycosides present in different concentrations. Eclipta prostrata aqueous extract and other solvent extracts were not positive for the resins. According to the above table, aqueous extract and different solvent extracts of dried whole plant of Eclipta prostrata contains alkaloids, tannins, phenolic compounds, terpenoids, steroids, flavonoids, cardiac glycosides, saponins, carbohydrates and Anthraquinone glycosides. Terpenoids, steroids, flavonoids and cardiac glycosides in aqueous extract were present in high concentrations in comparison to that of ethanol, hexane and ethyl acetate extracts. Tannin and phenolic compounds were present in high concentration in ethanolic and hexane extract in comparison to aqueous extract.

Conclusion

These phytochemicals having specific therapeutic effects to the human body. Therefore, due to presence of these phytochemicals Eclipta prostrata whole plant can possess Anti-malarial, anti-cancer, anti-microbial, anti-fungal, anti-hyperglycemic, anti-inflammatory, anti-oxidant as well as cardiac protective action. Presence of this phytochemical compounds suggests that the whole plant of Ecliptaprostatais rich in primary and secondary metabolites which are directly responsible for its effecton different disorders (Table 2).

Table 1: Phytochemical screening of aqueous extract and different solvent extracts of dried whole plant of Eclipta prostrata.[5,6,7,8,9,10,11]

Phytochemical Tests		Aqueous extract	Ethanol extract	Hexane extract	Ethyl acetate extract
Alkaloids	Mayer's Test	+	++	++	+
	Hager's Test	+	++	++	+
	Wagner's Test	+	++	++	+
Tannins and Phenolic compounds	$FeCl_3$ Test	+	+++	+++	+
	Gelatin Test	±	++	++	±
	Lead Acetate Test	–	++	++	–
Terpenoids and Steroids	Salkowski Test	+++	+	+++	+
	Libermann Burchard's Test	+++	++	+++	++
Flavonoids	Ammonia Test	+	+	+	+
	Alkaline Test	+	+	+	+
	Lead Acetate Test	++	–	++	–
	Shinoda Test	+++	++	+++	++
Cardiac Glycosides	Legal Test	+	++	+	++
	Keller Killiani Test	+++	–	+++	–
Saponins	Foam Test	++	++	++	++
	Olive Oil Test	+	+	+	+
Carbohydrates	Fehling's Test	++	++	++	++
	Benedict's Test	++	+	++	+
	Seliwanoff's Test	+	+	+	+
Amino Acids	Ninhydrine Test	++	–	++	–
	Millon's Test	++	–	++	–
Antraquionone glycosides	Hydroxyantraquinone Test	+	±	+	±
	Borntrager's Test	++	±	++	±

Table 2: Importance of different phytochemicals containing in Eclipta prostrata whole plant.

Phytochemicals	Importance
Carbohydrate and proteins[12]	Play a vital role in satisfying human needs for energy and life processes
Alkaloids[13]	Anti-malarial, Anti-cancer
Tannins[14]	Anti-viral, Anti-bacterial, Anti-tumour activity
Phenolic compound[15]	Delayed aging, Decreased risk of chronic disease development E.g. Cardio vascular diseases, Arteriosclerosis, Cancer, Diabetes, Cataract, Disorders of cognitive function and Neurological diseases.
Terpenoids[16]	Anti-microbial, Antifungal, Anti-viral, Anti hyperglycaemic, Anti-inflammatory, Antioxidants, Antiparasitic, Immunomodulatory,
Steroids[17]	Anti-inflammatory
Flavanoids[18]	Anti-oxidant effect, Inhibit the initiation, promotionand progression of tumours
Cardiac glycosides[19]	Treatment of congestive cardiac failure due to its direct action which increases the force of myocardial contraction. Also, it acts directly on the smooth muscles.
Saponins[20]	Useful in treating yeast and fungal infections.
Anthraquinone glycosides[21]	Antimalarial, Laxative, Antineoplastic.

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