

Phytochemical, Physicochemical and Antifungal Activity of *Eclipta Prostrata*.Nasser M. Abd El-Salam¹, Iqbal Hussain², Riaz Ullah³, Shanzeb⁴, G A Marwat⁵, Shabir Ahmad²¹College of Science Research Centre, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia²Department of Chemistry, Islamia College University Peshawar, KPK Pakistan³Department of Chemistry Sarhad University of Science & Information Technology Peshawar, KPK, Pakistan⁴Department of Chemistry, Kohat University of Science & Technology, Kohat 26000, Kohat, Khyber Pakhtunkhwa, Pakistan.⁵PCSIR Labs complex Jamrud Road Peshawar, Khyber Pakhtunkhwa, Pakistanafriidriaz@yahoo.com

Abstract: *Eclipta prostrata*, is an important medicinal plant and their different parts are used for different health disorders. The triterpenoids isolated *E. prostrata* displayed antiproliferative and antimicrobial potentials. Phytochemically, *E. prostrata* is rich in wadeolactone, eclalbasaponin, b- amyryl, stigmaterol and luteolin-7-glucoside. Keeping in view the importance of *E prostrata*, it was analyzed quantitatively, qualitatively for the phytochemicals (alkaloids, flavonoids, saponins, tannins, glycosides, terpenoids, reducing sugars, anthraquinones, and cardiaglycoside) Physicochemicals, and anti-fungal activity. For anti-fungal activity four different strains including *Aspergillus niger*, *Aspergillus fumigatus*, *Fusarium solani* and *Aspergillus flavus* used has shown very promising results against the fungal strains.

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Key words: *Eclipta Prostrata*, Phytochemical, Physicochemical, Anti-fungal, *Aspergillus niger*

Introduction

Medicinal plants are used as herbs or traditional medicines for various types of diseases since ancient times. Recently the use of phytoterapics is considered to be safer and congenial to the human body. Medicinal plants are used for the preparation of various modern drugs or used as the principle sources of raw materials. Phytochemical progresses have been aided enormously by the development of rapid and accumulate methods of screening medicinal plants for particular chemicals. The medicinal values of these plants lie in bioactive phytochemical constituents that produce specific physiological action on the human body (Akinmoladun et al., 2007). Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world (Reddy et al., 2001, ErdoŪrul, 2002. Ateb and ErdoŪrul., 2003). Much work has been done on ethno-medicinal plants in India (Rai.1989. Maheshwari et al. 1986. Negi et al.1993). Interest in a large number of traditional natural products has increased (Taylor et al., 1996). It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents (Chung et al., 1995, Vlietinck et al., 1995). The selection of crude plant extracts for screening programs has the potential of being more successful in

initial steps than the screening of pure compounds isolated from natural products (Kusumoto et al., 1995).It has been used as a traditional medicine for treatment of human illnesses like kidney and liver weakness hyperlipidemia, atherosclerosis, hepatic disorders (Kim et al., 2008), inflammatory conditions, ophthalmic and digestive disorders (Arunachalam et al., 2009) including skin diseases. The plant species is also recognized as the best remedy for hair dying (Tewtrakul et al., 2007). Many studies showed that triterpenoids isolated from such plant displayed antiproliferative (Lee et al., 2008) and antimicrobial (Khanna and Kannabiran 2008) potentials. Phytochemically, *E. prostrata* is rich in wadeolactone, eclalbasaponin, b- amyryl, stigmaterol and luteolin-7-glucoside. (Kirtikar and Basu, 1998). In continuation to our previous study regarding the Phytochemicals (Iqbal et al., 2011, Farhat et al., 2011), the present study aimed at evaluating the phytochemical, Physicochemical and Anti-fungal activity of the *E. prostrate*.

Materials and Method

The plant was collected from the Peshawar district of Khyber Pakhtunkhwa, Pakistan. The collected samples were first washed with the tap water and then with the distilled water. After drying in the shade, was chopped, grind and stored till further use.

Phytochemical Screening
Quantitative determination
Saponin determination

The samples were ground. 50 g of each plant samples were dispersed in 800 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 800 ml of 20% ethanol. The combined extracts were reduced to 40 ml by rotary evaporator at about 90 °C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. Two layers, aqueous and ether layer are formed. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of *n*-butanol was added. The combined *n*-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated in percentage (Kirtikar and Basu., 1998).

Flavonoid determination

50 g of the plant samples were extracted repeatedly with 800 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through what man filter paper no. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed. (Boham, and Kocipai, 1994)

Alkaloid determination

50 gm of the sample were weighed into a 1000 ml beaker and 800 ml of 20% acetic acid in ethanol was added and covered to stand for 4 h. Again the same process was repeated until three times. This was filtered and the extract was concentrated using Rotary to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. (Horborne. 1973).

Preliminary phytochemical screening

Phytochemical screenings were performed using standard procedures.

Test for reducing sugars (Fehling's test)

The aqueous ethanol extract (0.5 ml in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a color reaction.

Test for anthraquinones

0.5 ml of the extract was boiled with 10 ml of sulphuric acid (H₂SO₄) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for color changes.

Test for terpenoids (Salkowski test)

To 0.5 ml each of the extract was added 2 ml of chloroform. Concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for flavonoids

Three methods were used to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappears on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminum solution were added to a portion of the filtrate. A yellow colouration indicates the presence of flavonoids. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration indicates the presence of flavonoids.

Test for cardiac glycosides (Keller-Killianitest)

To 0.5 ml of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Test for saponins

To 0.5 ml of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for tannins

About 0.5 ml of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for alkaloids

0.5 ml of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions.

Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Physicochemical analysis of *Eclipta Prostrata*

The percentage of loss of weight on drying, total ash, water-soluble ash, acid-insoluble ash, and residue

on ignition were determined by employing standard methods of analysis as described in pharmacopoeia of India (1966). The percentage of extractive values of the leaf powder in various solvent systems was also determined.

Anti-fungal Activity

Acetic Acid-Ethanol Extract

Required amount of the plant sample was mixed with 20% acetic acid in ethanol for Alkaloids then treated with ammonium hydroxide solution for precipitation.

Methanol-Aqueous Extract

Required amount of the plant sample was mixed with 80% aqueous methanol. The filtrate so obtained was condensed by Rota vapor.

Aqueous-n-Butanol Extract

Required amount of the plant sample was mixed with 20% aqueous ethanol then washed with di-ethyl ether, n-Butanol and brine solution. The n-Butanol layer contain saponins, each extract was evaporated to dryness under reduce pressure using rotary evaporator.

Alkaloids = 4.5 g

Saponins = 2.5 g

Flavonoids = 5 gm

Microorganism

The microorganisms employed in the current study were procured from the department of I.P.S.

Media

Nutrient agar method was used for antifungal activity

Antifungal Assay

By using agar tube dilution method, the antifungal activity of the extracts was evaluated. *A. niger*, *A. fumigatus*, *F. solani* and *A. flavus* are four strains, were used for this assay. In this method, four dilutions (4.5 mg/ml, 3.5 mg/ml, 2.5 mg/ml and 1.5 mg/ml) of the crude extracts and fractions were prepared in DMSO.

Four dilutions, Incorporated into sterile media at 45 °C, poured in previously sterile test tubes. In each tube, a small piece of previously grown fungus were placed and incubated for 5 days at 25°C. After 6 days, the fungal growth was observed in each tube. In a whole procedure negative and positive control with and without any fungi were included.

Results and Discussion

Crude phytochemicals (alkaloids, flavonoids and saponins) were determined quantitatively in the *E. prostrata*. The phytochemicals were determined quantitatively using the literature methods.

Quantitative determinations of crude Alkaloids, Saponins and Flavonoids.

Table 1: Quantitative determinations of crude Alkaloids, Saponins and Flavonoids (g Kg⁻¹) in *E. prostrata*

S.No	Plant taken in grams	Name of crud extract	Wt. of crud extract	Percentage (%)
1	50	Flavonoids	3.00	6
2	50	Alkaloids	2.4	4.8
3	50	Saponins	2.1	4.2

Crude phytochemicals (alkaloids, flavonoids and saponins) were determined quantitatively in the *E. prostrata*. The phytochemicals were determined quantitatively using the literature methods. As can be seen from the Table 1 the concentration of flavonoids was found high in *Eclipta prostrata* which is 6%, followed by alkaloids, 4.8% and saponins 4.2%.

Quantitative determination

Table 2. Physico –chemical characters of the *E. prostrata*.

S.No	Particulars	% yield
1	Loss of weight on drying	2
2	Total ash	18.46
3	Acid – insoluble ash	.09
4	Water-soluble ash	40
5	Residue on ignition	42.65
6	Extractive values	
(a)	Petroleum ether	8.4
(b)	Benzene	2.6
(c)	Chloroform	2.4
(d)	Ethanol	10
(e)	Water	13.6

The percentage of loss of weight on drying, total ash, water-soluble ash, acid insoluble-ash and residue on ignition were obtained by employing standard methods of analysis as described in pharmacopoeia of India⁷. The percentage of extractive values in petroleum ether, benzene, chloroform, ethanol and water were also determined. The results are presented in Table 2.

Table 3: Preliminary phytochemical screening of powder of *E. prostrata*

S.No	TEST	Ethanol extract	Chloroform	Benzene	Petroleum ether	Water
1	Fehling test (for reducing sugars)	Fehling-A= + Fehling-B= +	Fehling (A) = - Fehling (B) = +	Fehling (A) = + Fehling(B) = +	Fehling (A) = - Fehling (B) = +	Fehling (A) = + Fehling (B) = +
2	Anthraquinones	-	-	-	-	-
3	Terpenoids (salkowski test)	+	+	+	+	+
4	Flavonoids	+	-	-	-	+
5	Saponins	+	-	+	+	+
6	Alkaloids	+	+	-	-	+
7	Tannins	+	-	-	-	+
8	Cardiac glycosides	+	+	-	-	-

5 g of the plant powder of *E. prostrata* was extracted with petroleum ether, benzene, chloroform, ethanol and water in soxhlet apparatus. The different extracts were tested for the presence of steroids, reducing sugars, cardiac glycosides, terpenoids, alkaloids, anthraquinones, tannins and flavonoids.

For ethanol extract all tests was positive except anthraquinones, which was absent in *E. prostrata*. In Chloroform extract the following tests were positive, Fehling (solution B), terpenoids, flavonoids and cardiac glycosides. All other tests were negative. In benzene extract test for reducing sugars, for terpenoids and for saponins were positive and all other tests were negative. In petroleum ether extract tests for reducing sugars, (Fehling sol-B), terpenoids and saponins were positive and all other tests were negative. In water extract, test for anthraquinones, and cardiac glycosides were negative and all other tests were positive. The phytochemical tests performed and the results obtained are presented in Table-3. The crude extracts of plant are shown in table 4. The Plants had shown excellent antifungal activity.

Antifungal activity

Table-4: Antifungal activity of crude alkaloids, flavonoids and saponins from *E. prostrata*.

S. No	Name of Fungus	Saponins	Alkaloids	flavonoids
1	<i>Aspergillus Niger</i>	+	-	-
2	<i>Aspergillus fumigates</i>	-	-	-
3	<i>Fusarium solani</i>	+	+	-
4	<i>Aspergillus flavus</i>	+	+	+

The *E. Prostrata*, crude extract was introduced for antifungal activity. The results shown by Table 4.

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