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Full Length Research Paper

Phytochemical, physiochemical and anti-fungal activity of *Eclipta alba*

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Eclipta alba, **commonly known as False Daisy is an important medicinal plant and its different parts are famous for the treatment of different health problems including digestion, headache, asthma, cough and normalizing skin colour. Keeping in view the importance of *E. alba*, it was analyzed quantitatively, qualitatively for its phytochemicals (alkaloids, flavonoids, saponins, tannins, glycosides, terpenoids, reducing sugars, anthraquinones, and cardiacglycoside) physiochemicals, 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 strain.**

Key words: *Eclipta alba, Staphylococcus aureus, Aspergillus niger*, phytochemicals, physiochemicals.

INTRODUCTION

Plant parts, which have in one or more of its organs containing substances that can be used for therapeutic purposes, are called medicinal plants (Sofowora, 1982). Plants derived medicines are widely used because they are relatively safer than the synthetic alternatives, and are easily available and cheaper (Iwu et al., 1999). These plants are used as a whole or their parts like leaves, barks, flowers, and seeds are either swallowed or the extracts are inhaled or applied to the skin or drank and are supposed to work as medicine (Westh et al., 2004). The search for natural products to cure diseases represents an area of great interest in which plants have been the most important source. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these plants bioactive chemical constituents are alkaloids; tannins, flavonoids, and phenolic compounds (Hill, 1952).

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; ErdoUrul, 2002). It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumor and antimicrobial agents (Ateb and ErdoÛrul, 2003; Chung 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 (Vlietinck et al., 1995).

Eclipta alba commonly known as False Daisy, belonging to the family Asteraceae (Kusumoto et al., 1995). *Eclipta* is a small and erect annual herb. Its stem is usually erect, flat or round, blackish green, profusely branched and pubescent. Leaves are opposite, serrate, 3 to 5 cm long and blackish green in colour (Foster, 1990). The whole plant and seeds have great medicinal value. *E. alba* is equally useful both, internally as well as externally. In glandular swellings and filariasis, it is used to mitigate swelling and pain, by applying the paste. The chronic and infected wounds get cleansed and heal better with application of its paste. The nasal drops of its

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Table 1. Quantitative determinations of crude alkaloids, saponins and flavonoids (g kg^{-1}) in *E. alba.*

S/N	Crude extract	Wt in g	Wt of crude extract	Percentage (%)
1	Flavonoid	50	3.0	6
2	Alkaloid	50	2.4	4.8
3	Saponin	50	2.1	4.2

Table 2. Preliminary phytochemical screening of powder of *E. alba.*

S/N	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 glycodides	+	-	-	-	-

juice, mixed with milk, are beneficial in migraine. The medicated oils of *E. alba* is widely used as hair tonic and to prevent hair fall and premature graying of the hair. Bhrngaraja is the most common ingredient incorporated in numerous market preparations of various hair oils. Internaly, *E. alba* is useful in many diseases. It is keen stimulant to digestive system. It is an effective cholegouge, hence benevolent in hepatosplenomegaly as well as hepatitis.

The medicinal importance of the afore-mentioned herb was evaluated for the phytochemicals, physiochemicals and anti-fungal activity.

EXPERIMENTAL

The plant was collected from Peshawar district of Khyber Pakhtunkhwa, Pakistan. The collected sample was first washed with tap water and then with distilled water and allowed to dry in shade. The dried sample was crushed powdered and stored in bottles for further uses.

Phytochemical screening

Quantitative determination

Alkaloid determination: To a 50 g sample was added 200 ml of 20% acetic acid in ethanol and covered to stand for 4 h. Again the same process was repeated three times. The combined 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, dried and weighed. The results obtained are presented in Table 1.

Saponin determination

After dispersing 50 g in 200 ml of 20% ethanol, the suspension was heated over a hot water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with another 200 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 separating funnel and 20 ml of diethyl ether was added and shaken vigorously to form two layers, aqueous and ether layer.

The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and then 60 ml of n-butanol was added. The combined *n*-butanol extracts was washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was weighed. The saponin content was calculated in percentage.

Flavonoid determination

50 g of the plant samples was extracted repeatedly with 200 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was dried and weighed.

Preliminary phytochemical screening

Phytochemical screening was performed using standard procedures (Thomas et al., 2008).

Test for reducing sugars (Fehling's test)

The aqueous ethanol extract (0.5 ml in 5 ml water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction. The phytochemical tests performed and the result obtained is presented in Table 2.

Test for anthraquinones

0.5 ml of the extract was boiled with 10 ml of sulphuric acid (H_2SO_4) 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 colour changes.

Test for terpenoids (Salkowski test)

To 0.5 ml each of the extract was added 2 ml of chloroform. Concentrated H_2SO_4 (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 flavonoid. Second, a few drops of 1% aluminum solution was added to a portion of the filtrate. A yellow colouration indicates the presence of flavonoid. 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 flavonoid.

Test for cardiac glycosides (Keller-Killianitest)

To 0.5 ml of extract diluted with 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed 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 colouration indicating the presence of tannins.

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 alkaloid 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 (Table 2).

Physicochemical analysis

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 (Thomas et al., 2008). The percentage of extractive values of the leaf powder in various solvent systems was also determined.

Anti-fungal activity

The plant material was air dried and ground to moderately fine powder after washing with tap water and distilled water. The sample was stored in refrigerator for further uses.

Fungal strains: The fungal (*Aspergillus niger, Aspergillus fumigatus, Fusarium solani and Aspergillus flavus)* strain was procured from Microbiology department at our University campus.
The 7 mm wells were punched in the medium by using sterile metallic borer. Stock solutions of crude extract and fractions were prepared in DMSO at concentration of 20 mg/ml and 200 µl from each stock solution was added into respective wells. The Petri dishes were incubated at 37ºC for 24 h and control wells containing a standard antibiotic (doxycycline) (positive control) was also run 24 h and the anti-fungal activity were measured by measuring the diameter of the zones of inhibition. To measure the zone of inhibition of crude extract and fractions, these zones of inhibition was compared with the zones of inhibition of standard drug (Doxycycline).

RESULTS AND DISCUSSION

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

As can be seen from Table 1, the concentration of flavonoids was found *E. alba* which is 6%, followed by alkaloids, 4.8% and saponins 4.2%. These are the main constituents responsible for the therapeutic value of the medicinal plants. They are also contributing biological activities, analgesic, diuretic and other activities and are mostly active against human pathogens.

For ethanolic extract, all tests were positive except anthraquinones, which was absent in *E. alba*. 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, terpenoids and saponins were positive and all other tests were negative. In petroleum ether extract, tests for anthraquinones, alkaloids, flavonoids and cardiac glycosids were negative and the rest were found positive. In water extract, test for anthraquinones, tannins and cardiac glycosides were found negative and the rest were positive.

Table 3. Fluorescence characters of *E. alba* powder and their extracts in different solvents.

Particulars of treatment	Under ordinary light	Under UV light
Powder as such	Dark green	Green
Powder + 1 N NaOH (aqueous)	Dark brown	Dark green
Powder + 1 N NaOH (ethanol)	Yellow green	Dark green
Powder + 1 N HCl	White	Light green
Powder + H2 SO4 (1:1)	Black	Black
Powder + HNO3 (1:1)	Light brown	Light green
Extract		
Petroleum ether	Light green	Green
Benzene	Light green	Green
Chloroform	Light green	Dark green
Ethanol	Dark green	Green
Water	Light yellow	Light green

Table 4. Physiochemical characters of the *E. alba*.

S/N	Particular	E. alba (%)
1	Loss of weight on drying	2.4
2	Total ash	16.65
3	Acid – insoluble ash	1
4	Water-soluble ash	35
5	Residue on ignition	42.63
	Extractive values	
a	Petroleum ether	2.8
b	Benzene	13
c	Chloroform	10.8
d	Ethanol	9.4
e	Water	26

Fluorescence analysis

The plant powder of *E. alba* and the extracts of the powder in various solvents were examined under ordinary light and UV light (365 nm). The powder was also treated with various chemical reagents and the changes in colour were recorded (Thomas et al., 2008). These results are presented in Table 3.

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. The percentage of extractive values in petroleum ether, benzene, chloroform, ethanol and water were also determined and the results are depicted in Table 4.

Thin layer chromatography

Thin layer chromatographic studies have been performed for the petroleum ether, benzene, chloroform, ethanol and water extracts of the plant powder of *E. alba*. The plates were first viewed through UV-fluorescence viewing cabinet (365 nm) before keeping in an Iodine chamber and the Rf values of the fluorescing spots were noted.

Then the plates were developed in the Iodine chamber and Rf values were noted of the various solvent systems used for thin layer chromatographic studies. There was no common solvent system for all the four different extracts. Different solvent systems have been found to be effective to get the maximum number of spots for the various extracts. Petroleum ether extracts show one spot in ordinary light and no spot under UV and in iodine chamber. Benzene extract showed two spots in ordinary light, one spot in iodine chamber and no spot under UV light. Chloroform extract show two spots in ordinary light, one spot in iodine and no spot under UV light. Ethanol and water extract show no spot. The results are presented in Table 5.

Table 5. Thin – layer chromatographic behavior of the extracts of *E. alba*.

Name of the extract	Solvent system used	Rf values of the spots			
		Ordinary light		Under UV light	In iodine chamber
Petroleum ether	Benzene:Chloroform (1:1)	0.02			
Benzene	Chloroform:Ethanol (9.5:0.5)	0.777, 0.533			0.422
Chloroform	Chloroform:Ethanol (9.5:0.5)	0.822, 0.533			0.4
Ethanol	Chloroform:Ethanol (8:2)				
Water	1 – Butanol:Acetic acid:Water (4.0:1.1:4.9)				

Table 6. Antifungal activity of crude alkaloids, flavonoids and saponins from *E. alba*.

S/N	Name of fungus	Activity of saponins	Activity of alkaloid	Activity of flavonoid
1	*Aspergillus niger*	+	-	-
2	*Aspergillus fumigates*	-	-	-
3	*Fusarium solani*	+	+	+
4	*Aspergillus flavus*	+	+	+

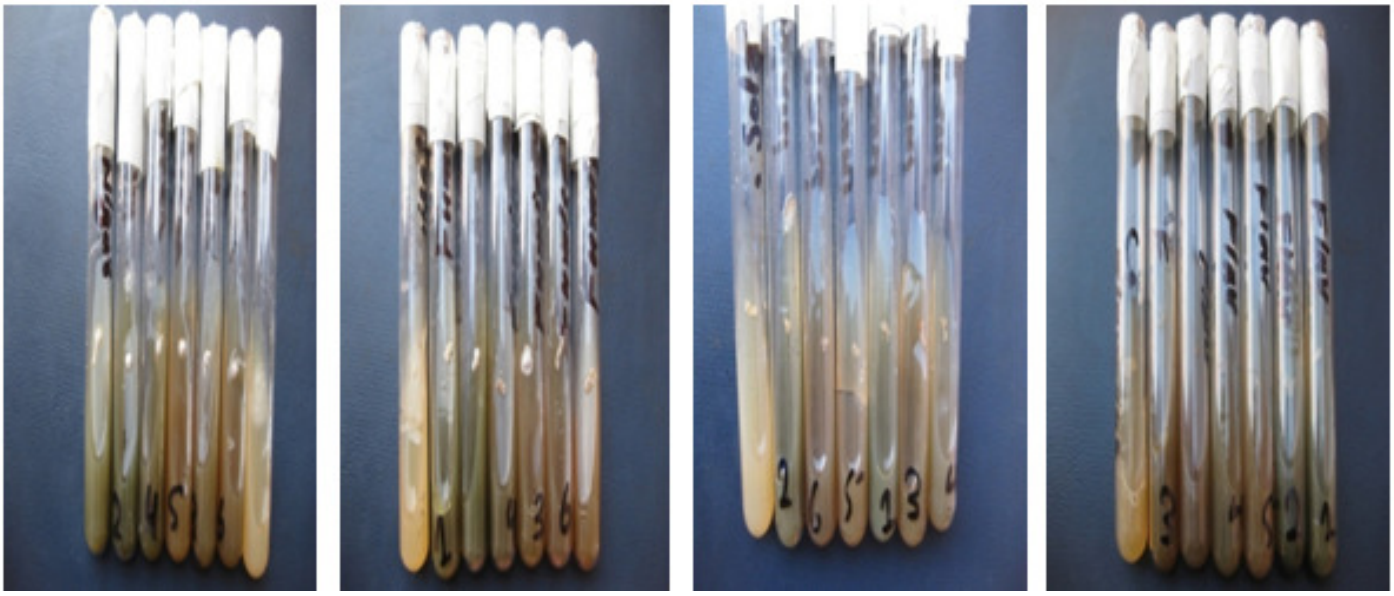

Figure 1. Antifungal activity of crude extract of alkaloids, saponins and flavonoids against *A. niger, A. fumigatus, F. solani and A. flav.*

Anti-fungal activities

Crude saponins extract of *E. alba* showed good antifungal activity. It is active against *F. solani* and *A. flavus* and *A. niger* and inactive against *A. fumigates*. Alkaloids crude extract of *E. alba* show good antifungal activity. The alkaloids crude extract was active against *F. solani* and *A. flavus* and inactive against *A. niger* and *A. fumigates*. Flavonoids crude extract was found active against *F. solani* and *A. flavus* and inactive against *A. niger* and *A. fumigates* (Table 6 and Figure 1).

Conclusion

The plant collected Peshawar area has demonstrated important information regarding the phytochemicals, physiochemical and anti-fungal parameters. To the best of our Knowledge, no study has reported this medicinal herb from Peshawar areas. This plant associated with a

variety of medicinal values can be used in traditional system of medicine. Furthermore the current study is also highly valuable by providing a scientific database besides making awareness among the local peoples and herbal practitioners.

REFERENCES

Ateb DA, ErdoUrul OT (2003). Antimicrobial activities of various medicinal and commercial plant extracts. Turk. J. Biol., 27: 157-162.

Chung TH, Kim JC, Kim MK (1995). Investigation of Korean plant extracts for potential phytotherapeutic agents against B-virus Hepatitis. Phytother. Res., 9: 429-434.

ErdoUrul OT (2002). Antibacterial activities of some plant extracts used in folk medicine. Pharmaceut. Biol., 40: 269-273.

Foster SD (1990). Filed Guide to medicinal Plants. Eastern Central N America.

Hill AF (1952). Economic Botany. A Textbook of useful Plant Products. 2nd edn. McGraw – Hill Book Company Inc, New York. p. 432

Iwu MM, Duncan AR, Okunji CO (1999). New antimicrobials of plant origin. In J. Janick (ed). pp. 457-462

Kusumoto IT, Nakabayashi T, Kida H (1995**)**. Screening of various plant extracts used in ayurvedic medicine for inhibitory effects on human immunodeficiency virus type 1 (HIV-1) protease. Phytother. Res., 9: 180-184.

Reddy PS, Jamil K, Madhusudhan Pl (2001). Antibacterial activity of isolates from *Piper longum* and *Taxus baccata*. Pharmaceut. Biol., 39: 236-238.

Sofowora A (1982). Medicinal Plants and Traditional Medicine in Africa. John Willy New York, p.289

Thomas S, Patil DA, Patil AG, Naresh Chanda (2008). Pharma-cognostic Evaluation and Physiochemical Analysis of *Averrhoa carabola* L. Fruit. J. Herb. Med. Toxicol., 2(2): 51-54.

Vlietinck AJ, Van Hoof L, Tott. J (1995). Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. J. Ethnopaharmacol., 46: 31-47.

Westh H, Zinn CS, Rosdahl VT (2004). An international multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. Microb. Drug Resist., 10: 169-176.