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Phytochemical analysis and Antibacterial activity of *Eclipta Prostrata* found in Chhattisgarh region

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Abstract: ***Eclipta prostrata (bhringraj) widely known for its hair growth effects also have medicinal properties. Being one of the tribal state, herbal plants had been used by locals as well as tribals of Chhattisgarh. Present study focus on the study of phytochemicals and antibacterial property of Eclipta prostrata so as to amplify the knowledge of its medicinal properties which might be useful in further research. Results revealed the presence of active phytochemicals like alkaloids, flavonoids, terpenoids, anthraquinones, proteins, phenols, tannins, saponins and glycosides in the plant. TLC analysis showed the presence of important phytosterol: stigmasterol . Methanolic extract of plant showed antibacterial property against E coli, Bacillus s and streptococcus which indicates that the plant might show assuring results in the field of antibiotics. Above data might also help to spread awareness among the tribal people of Chhattisgarh about the medicinal values of Eclipta prostrata.***
Keywords: Antimicrobial activity, Hot Percolation Method, phytochemical analysis, Sterols, Local tribes

I. INTRODUCTION

Plants had been used for Traditional and Modern medicinal purpose since ages. It has been recorded that about 450-500 or more plants growing or available in Indian Forest possess therapeutic values (Yagik.D and Verma.R, 2018). Government had declared Chhattisgarh as a Herbal State in July 2001. Rich in biodiversity, about 44% of state's land area is covered with forest. Being one of the Tribal states in India, plants have been widely utilized by locals for medicinal purposes. Moreover advancement in the field of pharmaceuticals through research achievements clearly states that, bioactive phyto-chemical constituents of the medicinal plant provide them medicinal value (Prabhas.L *et al*, 2016). *Eclipta prostrata* also known as false daisy/Bhringraj belongs to the family *Asteraceae*. This plant has cylindrical grayish root with solitary flower heads and white florets. Species grow commonly in moist places in warm temperature to tropical areas worldwide.

Bhringraj has traditional uses in Ayurveda. (Zubair *et al*, 2017) reported the presence of phytol and citronellyl butyrate. The antibacterial potential of aerial part extract of bhringraj was previously studies in solvent like acetone, ethanol, methanol, aqueous and hexane against selected gram positive and gram negative bacteria (Yusuf *et al*, 2013). Bhringraj also said to have anti-venom property, its leaves have been used in treatment of scorpion stings. The methanol extract of bhringraj promotes hair growth by inducing anagen in telogen (resting) phase hair follicles (Yusuf *et al*, 2013). Since literature mentioning bioactive profile of bhringraj from Chhattisgarh origin were few. The present study intends to study about the phytochemical and antibacterial activity of the plant extract of *Eclipta prostrata*.

II. MATERIALS AND METHODS

A. Collection and Sample Preparation

Leaves of *Eclipta prostrata* were takes from rural localities of Durg district of Chhattisgarh state. Leaves were washed thoroughly under running water and then with distilled water to remove all the dirt and debris from the surface and shade dried, since some plant constituents are photosensitive (Khandelwal.S and Koche.V, 2018). The dried leaves were further incubated at 36 ˚C for 48hours followed by fine powdering by a mixer.

B. Extraction Method

The powdered sample was then subjected to successive cycles of Soxhlet's apparatus using 3 solvents i.e. ethanol (99%), petroleum ether, aqueous(distilled water); 200ml each with 20gms of dried sample. The resulting extract is then filtered and concentrated in hot water bath.

C. Determination of Extractive Value

The extract was filtered by using whatmann filter paper no 1 and filtrate was then dried in hot water bath and weighted. Extractive values in percentage were calculated by using following formula:

$$\text{Extractive value (\%)} = \frac{weight\ of\ extract}{weight\ of\ plant\ material} \times 100$$

III. PRELIMINARY SCREENINGTEST

A. Millons's Test for protein

5ml of millon's reagent were added to 3ml of sample extract, white precipitate was obtained. After heating precipitate turns brick red.

B. Molisch's test for Carbohydrates

The extract was treated with 2-3 drops of 1% alcoholic alpha-napthol and 2ml of sulphuric acid (H_2SO_4) was added along the side of the test tube, violet ring formed at the junction.

C. Ferric Chloride Test for Phenols

A small amount of extract was treated with aqueous 5% ferric chloride. Formation of deep blue or black color indicated the presence of phenols.

IV. SECONDARY METABOLITES SCREENING TEST

A. Salkowski test for Sterols

3ml of chloroform ($CHCl_3$) and concentrated sulphuric acid (H_2SO_4) was mixed and when added with crude sample, red color appears in lower layer indicated the presence of sterols.

B. Test for Alkaloids (Dry Extract Precipitation Test)

4ml Methanol and 400 ml of Glacial acetic acid, along with a few drops of Ammonia was added to the small quantity of dry plant extract. The precipitation indicated the presence of alkaloids. (Husain.N and Kumar.A, 2016)

C. Test for Flavonoids

To the aqueous extract, 2.5ml of dilute ammonia was added, followed by few drops of concentrated sulphuric acid (H_2SO_4). Yellow color appears indicated the presence of flavonoids.

D. Test for Cardiac Glycosides

0.5 ml of extract was dissolved in 2ml glacial acetic acid along with 1-2 drops of 1% ferric chloride ($FeCl_3$). Furthermore 1ml of sulphuric acid (H_2SO_4) was added to this solution. Formation of brown ring at the interface indicates the presence of cardiac glycosides.

E. Test for Anthraquinones

2ml of concentrated sulphuric acid was added to 0.5ml of extract, the solution was then heated. 2.5ml of chloroform was added to the solution and shaken well followed by the addition of the 1ml of dilute ammonia. The change in color indicates the presence of anthraquinones (Shabeer.M *et al*, 2011).

F. Salkowski test for Terpenoids

0.5 gms of extract was taken and mixed with 2ml of chloroform. 3ml of concentrated sulphuric acid was added. Appearance of reddish brown color at interface indicates the presence of terpenoids.

G. Test for Tannins

Few drops of 1% ferric chloride ($FeCl_3$) were added to the 5ml of aqueous filtrate of the extract. The appearance of brownish green or blue black coloration confirmed the presence of tannins.

H. Test for Saponins (Frothing Test)

0.5 ml of extract was mixed with 5ml of distilled water and shaken vigorously. Appearing of froth indicated the presence of saponins.

I. TLC (Thin Layer Chromatography)

TLC analysis of extract of medicinal plants from Chhattisgarh region, showed characteristic spots and Rf value of 0.88 which were comparable to the standard stigmasterol (Khandelwal.S and Koche.V, 2018). In present analysis of TLC, silica gel coated plates were used on which the plant extract was applied using a capillary. The plates were then dipped carefully in an organic solvent methanol and left undisturbed for a while. The movement of samples was characterized by a retardation factor (Rf):

$$\mathbf{Rf} = \frac{\text{distance moved by the analyte}}{\text{distance moved by the mobile phase front}}$$

V. ANTIBACTERIAL ASSAY

A. Media Preparation

Nutrient Agar Media (NAM) was prepared by mixing peptone (5.0g), beef extract (3.0g), NaCl (5.0g) and agar (15.0g) in distill water (1L) and sterilized in autoclave at 15lb pressure for 15min. The sterilized media were then poured into petri dishes and wells were created in the media using a borer.

B. Antibacterial Activity

About 70µg of methanolic extract was pour into each wells and were tested against three different pathogenic bacterial strains (24 hours old) i.e. *E coli, Bacillus subtilis and Streptococcus.*

VI. RESULT

Solvents	Color and consistency	Extractive value (in gms)
Aqueous	Dark greenish brown, viscous	3.31
Ethanol	Dark green, sticky	3.46
Petroleum ether	Dark green, sticky	1.65

Table1:- Extractive values of air dried plant extract from various solvents.

Test	Aqueous	Ethanol	Petroleum ether
Protein	+	+	+
Carbohydrate	+	+	+
Phenol	+	+	+

Table2:- Preliminary Screening of Extract of Eclipta prostrata in different solvents

Test	Aqueous	Ethanol	Petroleum ether
Sterols	+	+	+
Alkaloids	+	+	+
Flavonoids	+	+	-
Glycosides	+	+	-
Anthraquinones	+	+	-
Terpenoids	+	+	+
Tannins	+	+	+
Saponins	+	+	-

Table3:- Phytochemical Screening of Extract of Eclipta prostrata in different solvents.

Tested strain	Zone of Inhibition (in cms)
	70µL
E coli	5.12
Bacillus subtilis	4.87
Streptococcus	4.10

Table4:- Antibacterial activity of methanolic extract against different bacterial strains

The data obtained above allude that the extractive value of ethanolic extract was higher than the aqueous extract followed by petroleum ether extract (table1). Preliminary screening tests showed that the plant extract contains proteins, carbohydrate and phenolic constituents (table2), furthermore on phytochemical analysis of extract it was observed that photo active components like alkaloids,sterols, flavonoids, terpenoids, anthraquinones tannins, glycosides andsaponins were present in ethanolic and aqueous extract where as anthraquinones, glycosides, flavonoids and saponins were absent in petroleum ether extract (table3). TLC analysis of extract revealed the presence of stigmasterol as a characteristic spot was observed with a Rf value of 0.862 which is approximately near and comparable standard value of stigmasterol (0.88). Antibacterial activity of methanolic extract was a bit higher against *E coli* followed by *B subtilis* and *streptococcus* (table4).

VII. DISCUSSION AND CONCLUSION

Eclipta prostrata had been used by locals as well as tribals of Chhattisgarh state for medicinal purpose. Present study revealed the presence of important secondary metabolites and phytosterols like stigmasterol in the plant extract. Stigmasterol is one of the major components responsible of the medicinal characteristics of the plant. It was also observed that the methanolic extract of *Eclipta prostrata* had antibacterial properties. With the presence of active phytochemicals and antibacterial property the plant might show assuring results in the field of antibiotics as well as in other medicinal effects if further researched more.

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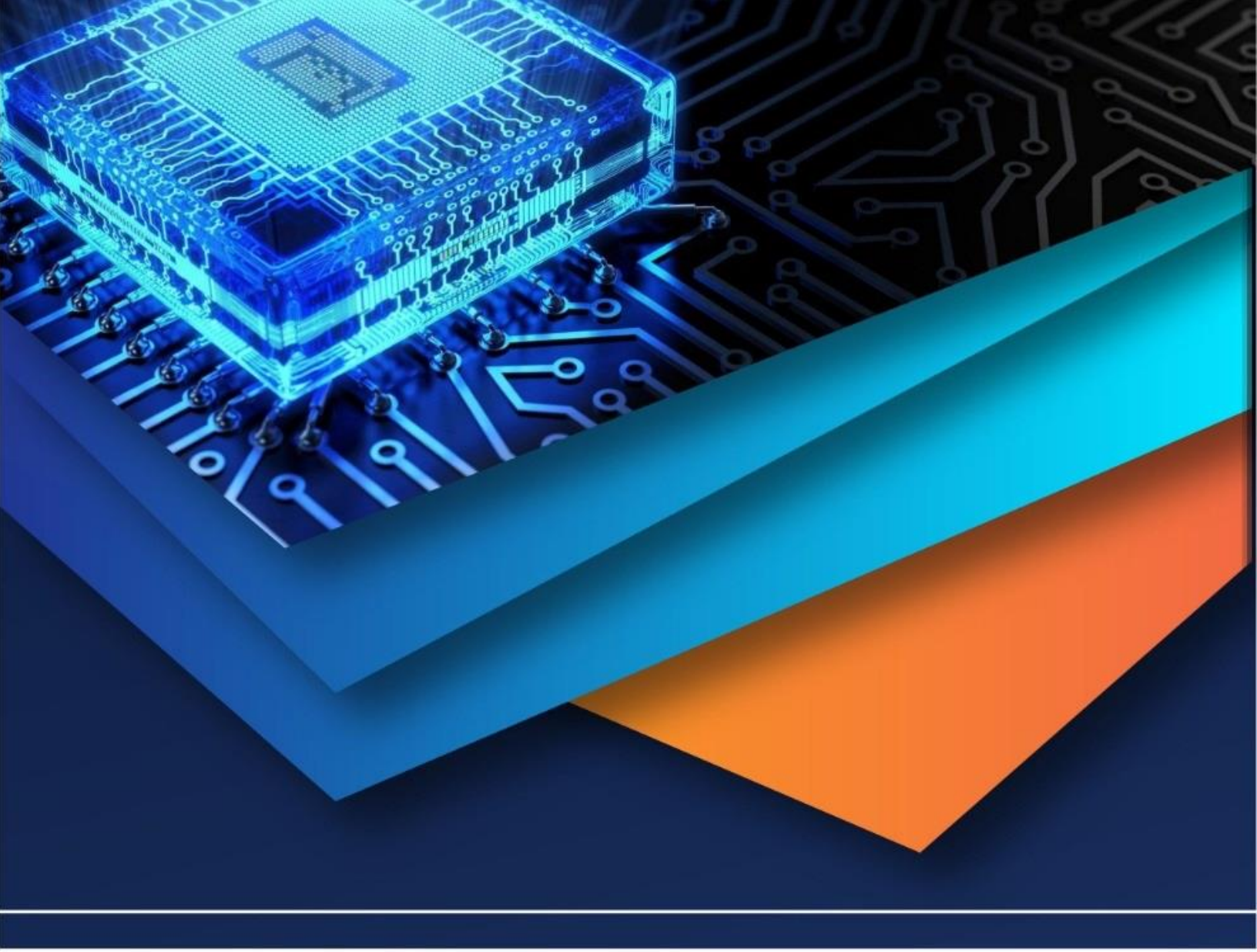

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