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Research Article

PRELIMINARY PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF *MILLINGTONIA HORTENENSIS BIGNONIA RADICANS* AND *BIGNONIA SUAVEOLENS*

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ABSTRACT

In the present study, an attempt was made to investigate the anti-bacterial activity of *Millingtonia hortenensis Bignonia radicans* and *Bignonia suaveolens* The crude drug powder extracts of the leaves of the above plants were taken for the study. The antibacterial activity was performed by using both gram positive and gram negative organism viz., *B.subtilis* and *E.coli* respectively. The Phytochemical Screening was done to the crude drug powder of the plants. Phenolic compounds, tannins, flavonoids, cardiac glycosides, saponins and alkaloids were present in *Millingtonia hortenensis* Steroids, alkaloids, flavanoids, carbohydrates, cardiac glycosides and tannins were present in *Bignonia radicans* Alkaloids, saponins, flavanoids, carbohydrates and anthraquinone glycosides were present in *Bignonia suaveolens*

Keywords: Antibacterial Activity, *Millintonia hortenensis Bignonia radicans Bignonia suaveolens.*

INTRODUCTION

Herbal medicine – It is also called botanical medicine or phytomedicine-refers to using plants seeds, flowers, roots for medicinal purpose. Herbalism has a long tradition of use of outside of conventional medicine. Bignoniaceae family plant (Bignonia) the trumpet creeper or catalpa family of the mint order of flowering plant. It contains about 112 genera and more than 725 species of trees ,shrub, and most commonly vines. Grown in India , America, tropical Africa ,and other places It Contains the species *Bignonia radicans Bignonia rosada Bignonia gracillis Bignonia grandiflora Millingtonia hortenensis*

EXPERIMENTAL

Plant Materials

The leaves of plants *Millingtonia hortenensis Bigonia radicans Bignonia suaveolens* were Authentified and were collected from different areas Guntur, , Prakasham districts of Andhra Pradesh.India .during the month August 2013

Solvent Extraction

The leaves of *Millintornia hortenensi Bignionia radicans* and *Bignonia suaveolens* were collected, washed, dried and powdered separately. 50g of dried powder of the leaves was weighed and transferred into a conical flask and it was macerated with sufficient amount of ethanol for about a week days. The whole mixture was filtered and filtrate was collected, concentrated in a china dish on a hot plate till the residue was obtained. The extract was collected, labelled and stored for further experimental use.

Microorganisms

The test organisms used were *E.coli* (ATCC 25922) a Gram –ve strain and *B.subtilis* (ATCC 21332) a Gram +ve strain. The strains were sub-cultured on nutrient agar slants and were incubated for 24 hrs.

Antibacterial activity

Agar well diffusion method

Required glass ware was washed and dried in a hot air oven. The sterilized agar medium was transferred into the Petri dishes, was allowed to solidify at room temperature. The selected test organism was spread over the solidified agar with the help of a swab stick. Sterile borer was used to make wells of 8mm diameter .The dilutions of ethanolic extracts of *Millintonia hortenensis, Bignonia radicans* and *Bignonia suaveolens* and solutions of combined ethanolic extracts of *Millintonia hortennensis* with *Bignonia radicans and Bignonia suaveolens respectively* were poured in the wells with the help of a sterile syringe needle. In each Petri plate a well was prepared for standard i.e., ciprofloxacin 10µg/ml solution. The Petri plates were placed in a refrigerator for 5min to allow diffusion. Later the Petri plates were incubated in inverted position at 37^0 C for 24 hours in the incubator. After 24hours the zone of inhibition was observed and diameter in mm was measured and recorded.

Qualitative analysis for Phytochemical Constituents

The extracts and crude dried powders of *Millintonia hortenensis Bignonia radicans Bignonia suaveolens* were subjected to following chemical tests.

TEST FOR ALKALOIDS

To 250 mg of each extracts, 10 ml of dilute HCl was added, mixed and filtered. To the filtrate the following reagents were added and tested.

TEST	PROCEDURE
WAGNER'S TEST :	2 ml of Wagner's reagent was added to the above filtrate solution and observed.
HAGER'S TEST :	To the 2 ml of above filtrate solution, 2 ml of picric acid was added and observed.

TEST FOR CARBOHYDRATES

TEST	PROCEDURE
MOLISCH'S TEST :	200 mg of extracts were dissolved separately in 5ml of water and filtered. 2 ml of the above sample solution is placed in a test tube. Two drops of the Molisch reagent is added. The solution is then poured slowly into a tube containing 2 ml of concentrated sulphuric acid and observed.
FEHLING'S TEST :	1ml of Fehling's solution A and 1ml of Fehling's solution B were added to 100mg of extracts separately. They were heated on a boiling water bath for 5 min and observed.
BENEDICT'S TEST :	To the 150 mg of each extracts, 2ml of Barfoed's reagent was added. Then the mixture was heated on a boiling water bath for 5 min, cooled and observed.

TEST FOR GLYCOSIDES

The extract was tested for the presence of Saponin glycosides Cardiac glycosides Anthraquinone glycosides

TEST FOR SAPONIN GLYCOSIDES

TEST	PROCEDURE
FOAM TEST	To 200 mg of each extracts, 15 ml of distilled water was added, shake it well and observed.

TEST FOR CARDIAC GLYCOSIDES

TEST	PROCEDURE
LEGAL'S TEST:	To 50 mg of each extracts, 1 ml of pyridine, 1 ml of Sodium nitro prusside solution were added and observed.
KELLER-KILIANI TEST:	To 50 mg of each extracts, 2 ml of glacial acetic acid, 1 ml $FeCl_3$ solution were added, heated and then cooled. This was transferred to a test tube containing 2ml conc. $H_2SO_4$and observed.

TEST FOR ANTHRAQUINONE GLYCOSIDES

TEST	PROCEDURE
BORNTRAGER'S TEST:	To 200 mg of each extracts, dil. $H_2SO_4$was added and boiled. Then it was filtered and cooled. To the cold filtrate, 3 ml of benzene was added and mixed. The benzene layer was separated and to it, ammonia (2 ml) was added and ammonical layer was observed.

TEST FOR FLAVANOIDS

TEST	PROCEDURE
LEAD ACETATE TEST:	To the 100 mg of each extracts, lead acetate (5 ml) was added and observed.

TEST FOR TANNINS

To 100 mg of each extracts, the following reagents were added and observed.

a) 5 ml of 5% w/v $FeCl_3$ solution.
b) 5 ml acetic acid solution.
c) 5 ml dil. $KMnO_4$ solution.

TEST FOR STEROIDS

TEST	PROCEDURE
SALKOWSKI TEST:	To 100 mg of each extracts, 2 ml of $CHCl_3$, 2 ml of conc. H_2SO_4were added, mixed thoroughly and both the layers were observed for colour.
LIBERMAN AND BURCHARD TEST:	To 200 mg of each extracts, 5ml $CHCl_3$, 5 ml acetic anhydride were added. Two drops of H_2SO_4 was added from the sides of test tube and observed.

Table 1: Phytochemical Screening of *Millintonia hortenensis*

S.NO.	CHEMICAL TESTS	RESULT
1	**TEST FOR CARBOHYDRATES** A. Molisch's test B. Fehling's test C. Benedict's test D. Barfoed's test	 Positive Positive Positive Positive
2	**TEST FOR ALKALOIDS** A. Hager's test B. Wagner's test	 Positive Positive
3	**TEST FOR FLAVANOIDS** Lead acetate test	 Positive
4	**TEST FOR SAPONINS** A. Foam test	 Negative
5	**TEST FOR STEROIDS** A. Lieberman burchard test B. Salkowski test	 Negative Negative
6	**TEST FOR CARDIAC GLYCOSIDES** A. Legal test B. Keller-killiani test	 Positive Positive
7	**TEST FOR ANTHRAQUINONE GLYCOSIDES:** A. Borntrager's test	 Positive

Table 2: Phytochemical Screening of *Bignonia radicans*

S.NO.	CHEMICAL TESTS	RESULT
1	**TEST FOR CARBOHYDRATES** A. Molisch's test B. Fehling's test C. Benedict's test D. Barfoed's test	 Positive Positive Positive
2	**TEST FOR ALKALOIDS** A. Hager's test B. Wagner's test	 Positive
3	**TEST FOR FLAVANOIDS** Lead acetate test	 Positive
4	**TEST FOR SAPONINS** Foam test	 Negative
5	**TEST FOR STEROIDS** A. Lieberman burchard test B. Salkowski test	 Positive Positive
6	**TEST FOR CARDIAC GLYCOSIDES** **A.** Legal test B. Keller-killiani test	 Positive Positive
7	**TEST FOR ANTHRAQUINONE GLYCOSIDES** Borntragers test	 Positive
8	**TEST FOR TANNINS** A. $FeCl_3$test B. Acetic acid test C. $KMnO_4$ test	 Positive Positive Positive

Table 3: Phytochemical Screening of *Bignonia suaveolens*

S.NO.	CHEMICAL TESTS	RESULT
1.	**TEST FOR CARBOHYDRATES** A. Molisch's test B. Fehling's test C. Benedict's test D. Barfoed's test	 Positive Positive Positive Positive
2.	**TEST FOR ALKALOIDS** A. Hager's test B. Wagner's test	 Positive

3.	**TEST FOR FLAVANOIDS** Lead acetate test	Positive
4.	**TEST FOR SAPONINS** A. Foam test	Positive
5.	**TEST FOR STEROIDS** A. Lieberman burchard test B. Salkowski test	Positive Positive
6.	**TEST FOR CARDIAC GLYCOSIDES** A. Legal test B. Keller-killiani test	Negative Negative
7.	**TEST FOR ANTHRAQUINONE GLYCOSIDES:** A. Borntrager's test	Positive

Table 4: Antibacterial Activity of *Millingntonia hortenensis Bignonia radicans Bignonia suaveolens*

S. No.	COMPONENT	DOSE	Zone of Inhibition (mm)	
			E.COLI	B.SUBTILIS
1.	STANDARD CIPROFLOXACIN	10 µg/ml	20mm	22mm
2.	Ethanolic extract of *Millingtonia hortenensis*	500µg/ml	-	-
		750µg/ml	-	-
		1000µg/ml	2mm	3mm
3.	Ethanolic extract of *Bignonia radicans*	500 µg/ml	-	-
		750µg/ml	-	-
		1000µg/ml	4mm	5mm
4.	Ethanolic extract of *Bignonia suaveolens*	500 µg/ml	-	-
		750µg/ml	-	-
		1000µg/ml	-	-
5.	Combined ethanolic extracts of *Bignonia radicans suaveolens suavNyctanthes* arbortristis	1000µg/ml	-	-
		1500µg/ml	8mm	10mm
		2000µg/ml	12mm	15mm
6.	Combined ethanolic extracts of *Millintonia hortenensis Bignonia sp*	1000µg/ml	-	-
		1500µg/ml	3mm	3mm
		2000µg/ml	2mm	2mm

RESULTS AND DISCUSSION

The study of the chemical constituents and the active principles of the medicinal plants have acquired a lot of importance all over the world. The present study includes the antibacterial activity of extracts of leaves of *Millintonia hortenenisis* in combination with the leaf extracts of *Bignonia radicans* and *Bignonia suaveolens* separately were performed. Earlier studies on *Nyctanthes arbortristis* indicated that the ethylacetate and chloroform extracts showed significant activity on both Gram +ve and Gram –ve strains. But the present study with the ethanolic leaf extract showed that the activity on bacterial strains was not that significant. But comparably the activity on *B.subtilis* was more than that of *E.coli.*

As the activity obtained for the leaf extract was mot that significant the combined leaf extract of *Millintonia hortenensis* and *Bignonia radicans* were used which showed a synergistic effect i.e., *Bignonia radicans* increased the antibacterial activity of the *Millingtonia hortenensis* While this combination showed synergistic activity the other combination *Millintonia hortenensis* and *Bignonia suaveolens* showed antibacterial acitivity

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