

**PRELIMINARY PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY  
OF *ACALYPHA INDICA* *ACALYPHA WILKESIANA* MILK WHITE  
*ACALYPHA WILKESIANA TRICOLOR***

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**ABSTRACT**

In the present study, an attempt was made to investigate the anti-bacterial activity of ethanolic extracts of *Acalypha indica*, *Acalypha wilkesiana milk white*, *Acalypha wilkesiana tricolor*. 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 *Acalypha indica*. Steroids, alkaloids, flavanoids, carbohydrates, cardiac glycosides and tannins were present in *Acalypha wilkesiana tricolor*. Alkaloids, saponins, flavanoids, carbohydrates and anthraquinone glycosides were present in *Acalypha wilkesiana milk white*.

**Keywords:** Phytochemical screening, Antibacterial Activity, *Acalypha wilkesiana tricolor*.

**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. Herbal medicine, sometimes referred to as herbalism which is the use of herbs for their therapeutic or medicinal values. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body. Herbal medicine is the oldest form of health care known to mankind. Herb had been used by all cultures throughout history. Many drugs commonly used today are of herbal origin. Used in treatment and prevention of diseases. Popular among rural and urban community of india due to its safe use. Non toxic, less side effects and easily available at a affordable prices. Indicated in both prophylactic and curative treatment throughout the world.



***Acalypha indica***



***Acalypha wilkesiana Milk white***



*Acalypha wilkesiana tricolor*

## EXPERIMENTAL SECTION

### Plant Materials

The leaves of plants *Acalypha indica* *Acalypha wilkesiana* *milk white* *Acalypha wilkesiana tricolor* were Authenticated and were collected from different areas Guntur, , Prakasam districts of Andhra Pradesh.India .during the month August 2014.

### Solvent Extraction

The leaves of *Acalypha indica* *Acalypha wilkesiana* *milk white* *Acalypha wilkesiana tricolor* 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 *Acalypha indica* *Acalypha wilkesiana* *milk white* *Acalypha wilkesiana tricolor* and solutions of combined ethanolic extracts of *Acalypha indica* *Acalypha wilkesiana* *milk white* *Acalypha wilkesiana tricolor* 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<sup>o</sup> 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 *Acalypha indica* *Acalypha wilkesiana* *milk white* *Acalypha wilkesiana tricolor* were subjected to following chemical tests.

## 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 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 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 <sub>3</sub> solution were added, heated and then cooled. This was transferred to a test tube containing 2ml conc. H <sub>2</sub> SO <sub>4</sub> and observed.

#### TEST FOR ANTHRAQUINONE GLYCOSIDES

TEST	PROCEDURE
BORNRAGER'S TEST	To 200 mg of each extracts, dil. H <sub>2</sub> SO <sub>4</sub> 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 ammoniacal 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.

- 5 ml of 5% w/v FeCl<sub>3</sub> solution.
- 5 ml acetic acid solution.
- 5 ml dil. KMnO<sub>4</sub> solution.

#### TEST FOR STEROIDS

TEST	PROCEDURE
SALKOWSKI TEST	To 100 mg of each extracts, 2 ml of CHCl <sub>3</sub> , 2 ml of conc. H <sub>2</sub> SO <sub>4</sub> were added, mixed thoroughly and both the layers were observed for colour.
LIBERMAN AND BURCHARD TEST	To 200 mg of each extracts, 5ml CHCl <sub>3</sub> , 5 ml acetic anhydride were added. Two drops of H <sub>2</sub> SO <sub>4</sub> was added from the sides of test tube and observed.

Table A: Phytochemical Screening of *Acalypha indica*

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

Table B: Phytochemical Screening of *Acalypha wilkesiana milk white*

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

Table C: Phytochemical Screening of *Acalypha wilkesiana Tricolor*

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

Table D: Antibacterial Activity of *Acalypha indica*  
*Acalypha wilkesiana* milk white *Acalypha wilkesiana tricolor*

S.No	COMPONENT	DOSE	Zone of Inhibition (mm)	
			E.COLI	B.SUBTILIS
1.	STANDARD CIPROFLOXACIN	10 µg/ml	20mm	22mm
2.	Ethanolic extract of <i>Acalypha indica</i>	500µg/ml	-	-
		750µg/ml	-	-
		1000µg/ml	3mm	4mm
3.	Ethanolic extract of <i>Acalypha wilkesiana</i> Milk white	500 µg/ml	-	-
		750µg/ml	-	-
		1000µg/ml	5mm	6mm
4.	Ethanolic extract of <i>Acalypha wilkesiana Tricolor</i>	500 µg/ml	-	-
		750µg/ml	-	-
		1000µg/ml	-	-
5.	Combined ethanolic extracts of <i>Acalypha wilkesiana</i> Milk white <i>Acalypha wilkesiana Tricolor</i>	1000µg/ml	-	-
		1500µg/ml	9mm	11mm
		2000µg/ml	13mm	16mm
6.	Combined ethanolic extracts of <i>Acalypha indica</i> <i>Acalypha wilkesiana</i> Milk white, <i>Acalypha wilkesiana Tricolor</i>	1000µg/ml	-	-
		1500µg/ml	4mm	4mm
		2000µg/ml	3mm	3mm

## 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 *Acalypha indica* in combination with the leaf extracts of *Acalypha wilkesiana white* separately were performed. Earlier studies on *Acalypha wilkesiana tricolor* 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 not that significant the combined leaf extract of *Acalypha indica Acalypha wilkesiana milk white Acalypha wilkesiana tricolor* were used which showed a synergistic effect i.e., *Acalypha indica* increased the antibacterial activity of the *Acalypha wilkesiana*. While this combination showed more synergistic activity than the other combination *Acalypha indica Acalypha wilkesiana milk white Acalypha wilkesiana tricolor*

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