



PHYTOCHEMICAL SCREENINGS, ANTIBACTERIAL ACTIVITY AND PHYSICO CHEMICAL CONSTANTS OF ETHANOLIC EXTRACT OF *EUPHORBIA THYMIFOLIA LINN*

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ABSTRACT

The present study designed for phytochemical screening, antibacterial activity and physic chemical constants of ethanolic extracts of whole plant of *Euphorbia thymifolia linn* belongs to the family Euphorbiaceae, which is widely used in Ayurveda to cure many remedies. The antibacterial activity was studied against *Bacillus Subtilis*, *Staphylococcus Aureus*, *Proteus Species* and *E. coli* were tested by cup-plate agar diffusion method. Erythromycin and tetracycline was used standard antibacterial agent. Physic chemical evaluation was carried out by ash values, extractive values and phytochemical screenings. The results of the study revealed that, the ethanolic extracts exhibited antibacterial activity against both gram positive and gram negative bacteria's.

Key words: *Euphorbia thymifolia*, Phytochemical, Antibacterial, Ash values, Extractive values.

INTRODUCTION

Euphorbia thymifolia linn belongs to the family euphorbiaceae. The plant is used as laxative, diuretic, antihelmintics, constipation, skin diseases, bitter and antiviral. Review of literature did not reveal any information on studies. Hence, in the basic present work in phytochemical screenings, antibacterial activity and physic chemical constants of the ethanolic extract of whole plant of *Euphorbia thymifolia Linn* was studied.

MATERIALS AND METHODS

The whole plant of *euphorbia thymifolia linn* were collected from the local area of Anantapur district of Andhra Pradesh during june 2009 and authenticated by Dr. Prasad Rao, department of botany, sri krishnadevaraya university, Anantapur.

Preparation of extracts

The whole plant was shade dried, coarsely powdered by using cutter mill and extracted with ethanol in soxhlet extractor. The extract were concentrated and dried by using Rotavapour (Heidolph) under vacuum. The yield of the concentrated ethanolic was 9.2%.

Preliminary phytochemical screening

The crude drug was dissolved in distilled water and subjected to preliminary phytochemical screening. The study was carried out by using standard procedure described by Kokate.C.K (1986 b)¹ and Harborne (1998)².

Physicochemical analysis

Physic-chemical values such as the percentage of ash values and extractive values were performed according to official methods prescribed in IP 1996 and the WHO guidelines on quality control methods for medicinal plant materials (WHO/QCMMPM guidelines, 1992)³.

Test micro organisms

The gram positive and gram negative bacteria strains were obtained from the microbiology department, OTRI-JNTU, Anantapur. The selected strains of bacteria and reference antibiotics were given below.

S. No	Micro organisms	Test organisms	Antibiotics
1	Gram positive bacteria	<i>Bacillus subtilis</i> <i>Staphylococcus aureus</i>	Tetracycline Tetracycline
2	Gram negative bacteria	<i>Proteus species</i> <i>E.coli</i>	Erythromycin Erythromycin

Table 1: preliminary phytochemical screening of ethanolic extract of whole plant of *E. thymifolia linn*

Chemical tests	Inference	Chemical tests	Inference
<u>Test for carbohydrates</u>		<u>Test for alkaloids</u>	
Molishch test	Positive	Hager's test	Positive
Fehling's test	Positive	Wagner's test	Positive
Benedict's test	Positive	Mayer's test	Negative
<u>Test for steroids</u>		<u>Test for indole alkaloids</u>	
Libermann-buchard's test	Negative	Van urk's test	Negative
<u>Test for amino acids</u>		<u>Test for tannins</u>	
Ninhydrine test	Negative	Ferric chloride test	Negative
<u>Test for glycosides</u>		<u>Test for flavonoids</u>	
Baljet test	Positive	Shinoda test	Positive
Borntrager's test	Positive	Lead acetate test	Positive
Modifies borntrager's test	Positive	Sodium hydroxide test	Positive
<u>Test for fixed oils</u>		<u>Test for resins</u>	
Spot test	Negative	<u>Test for gums</u>	
<u>Test for saponins</u>		Hydrolytic test	Negative
Froth test	Positive		

Antibacterial activity

The anti bacterial activity was evaluated by employing 24 hrs cultures of *b. Subtilis*, *S. Aureus*, *Proteus Sp* and *E.coli* using nutrient agar medium. The bacterial strains were transferred to sterile plates aseptically. The plates were left at room temperature and allowed for solidification. In each plate one well of 6mm diameter were made using a sterile borer. Accurately 1001 different dilutions of ethanolic extracts of *euphorbia thymifolia linn* (25mg, 50 mg, 75 mg and 100mg) and single concentration of erythromycin (5mg/ml) and tetracycline (5mg/ml) solutions were transferred to wells aseptically and labeled accordingly. The plates were incubated at 37°C for 24 hrs. The diameter of zones inhibition surrounding each of wells was recorded ^{4,5,6}

RESULTS AND DISCUSSIONS

Preliminary phytochemical screenings

The preliminary phytochemical screenings of ethanolic extracts of whole plant of *E. thymifolia linn* mainly revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids (Table: 1).

Anti bacterial activity

The results of anti microbial activity revealed that significant antibacterial activity was showed against both gram positive and gram negative bacterias in comparision with standards erythromycin and tetracycline (table no: 2).

Table 2: antibacterial activity of ethanolic extract of whole plant of *E. thymifolia linn*

Micro organisms	Zone of inhibition (mm)					
	25 µg/ml	50 µg/ml	75 µg/ml	100µg/ml	5 µg/ml	5 µg/ml
Gram positive bacteria	09	11	12	09	--	19
<i>Basillus subtilis</i>	10	12	14	11	--	18
<i>Staphylococcus aureus</i>						
Gram negative bacteria	08		11	08	18	--
<i>Proteus species</i>	09	09	16	10	17	--
<i>E.coli</i>		12				

Table 3: Ash Values and Extractive Values of ethanolic extract of whole plant of *E. thymifolia linn*

Ash values			Extractive values	
S. No	Parameters	Values % (w/w)	Parameters	Values % (w/w)
1	Total ash	4.37	Alcohol soluble extractives	2.91
2	Acid insoluble ash	0.95		
3	Water soluble ash	1.04	Water soluble extractives	4.16
4	Sulphated ash	7.35		

Physico-chemical content

Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The ash values (table no: 3) of the ethanolic extract showed higher content of sulphated ash followed by the total ash the extractive values are primarily useful for the determination of exhausted or adulterated drug. The water soluble extractive was high (table no: 3).

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