



# EVALUATE EFFICIENCY OF EUPHORBIA TIRUCALLI L. AGAINST EAR INFECTION CAUSING PATHOGENS

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**Abstract:** *Euphorbia tirucalli* L. is a shrub or small tree with pencil-thick, green, smooth, succulent branches. It has abundant medicinal values. Stem and latex portion of the plant have been used as antimicrobial, antimalarial and antitumorous. In accordance with this information antimicrobial, MIC and Phytochemical analysis were tested. Plant extract were tested for antibacterial activity against ear infection causing pathogens. The present study reveals that methanol extract were highly effective against test organism. Whereas aqueous extract was not showed pronounced effect on test organism.

**Keywords:** *Euphorbia tirucalli* L. stem extract, Phytochemical analysis, Agar well diffusion, Bacterial strains.

## Introduction

In spite of great development in modern scientific medicine, traditional medicine is still the primary form of treating disease of majority of people in developing countries (Ankita wal *et al.*, 2013). The world health organization estimates that about 80% of population were rely on traditional medicine for their primary health care needs (Harbone *et al.*, 1984). Plant synthesis secondary metabolites like phenols, quinines, flavonoids, tannins, terpenoids, alkaloids it act as antimicrobial, insecticidal, herbicidal, and other biological activity (Tonk *et al.*, 2006; Leeja *et al.*, 2007). *Euphorbia tirucalli* is used to treat gonorrhea, whooping cough, asthma, and leprosy, enlargement of spleen, jaundice, tumors and bladder stones. Stem latex is used to treat earache, toothache, skin disease and intestinal worms (Prasad *et al.*, 2011). The species has been patented for modern drugs such as prostate cancer (Aylward *et al.*, 2008). The root is used for colic pains in traditional medicine (Rao *et al.*, 2000).

*Euphorbia tirucalli* belongs to euphorbiaceae family and is a succulent plant from which white viscous latex exudes, it has pencil like smooth branches without any thorns, and the arbour has a distinctive shape that is easy to remark with a tall of up to 10-15 m (Gupta *et al.*, 2013). Research found that flavonoids present in *Euphorbia tirucalli* effectively inhibited the bacterial growth due to its ability to form complex with extracellular proteins of the cell wall and disrupt microbial membrane, phenols and polyphenol was too toxic to microorganism (Yi Qs *et al.*, 2017). They also found that tannins in *Euphorbia tirucalli* able to inhibit bacteria by inactive microbial adhesion enzymes and cell envelope transport proteins (Upadhyay *et al.*, 2010).

The composition of *Euphorbia tirucalli* stem latex includes terpenes and sterols, some of which have already been isolated, such as taraxasterols and tirucallol, and euphol and alphaeuphorbol (Diego Pinha Alves da Paz *et al.*, 2020).

*Euphorbia tirucalli* has been reported to present numerous pharmacological activities such as the latex of this plant exhibited strong oxytocic activity against isolated strips of the gravid rat uterus (Mwine *et al.*, 2013). The crude extract of *Euphorbia tirucalli* modulates the cytokine response of leukocytes, especially CD4+ T lymphocytes (Avelar *et al.*, 2011) and it also has a promising activity in modulation of myelopoiesis there by enhancing the resistance of tumor-bearing mice (Valadares *et al.*, 2006).

## MATERIALS AND METHOD

### Collection of sample

The plant same was collected from tirupur and commonly known as pencil tree. The collected plant was washed thoroughly with running tap water and once in distilled water.

### Identification of sample

The plant sample was identified and authenticated (BSR/SRC/320) at Botanical Survey of India, South Zone, Tamil Nadu Agriculture University campus (Ministry of Environment, Forest and Climate Change), Coimbatore.

### Processing and Extraction of sample

The collected stem of *Euphorbia tirucalli* L. plant were allowed to shadow dry in room temperature for 3-4 days to reduce the moisture content and later dried by hot air oven at 60°C for 48 hours. The dried plant stem were powdered by using Mechanical blender. Store the powder in a air tight container for further analysis. Different solvents (Methanol, Chloroform, Hexane and Aqueous) were used for the extraction process. About 30g of *Euphorbia tirucalli* stem powder was weighed and mixed with different solvents, serially extracted by using Soxhlet apparatus at 60°C until the plant extract become colourless and then the extract were evaporated under the rotary vaccum evaporator at 50°C for about 15 minutes.

### Microbial strains

Bacterial strains used for the assay were as follows: *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Staphylococcus aureus*. Microbial strains were obtained from KMCH, Coimbatore. The bacterial stock cultures were maintained on nutrient agar medium at 4°C.

### Phytochemical analysis of stem extract

Qualitative phytochemical analysis of the plant extract was determined as follows:

**Alkaloids:** 2 ml of plants extract was added with few ml of dil.Hcl and filtered and few drops indicates the presence of alkaloids.

**Flavonoids:** 2ml of extracts were added to few drops of NaOH and then few ml of dil.Hcl was added. Yellow colour becomes colourless while adding Hcl.

**Tannins:** In 2ml of extract 5ml of distilled water were added. In diluted extract few drops of 5%  $\text{FeCl}_3$  were added. Dark green colour indicates the presence of tannins.

**Phenols:** In diluted extract 3ml of 10% leads acetate were added white precipitate indicated the presence of phenolic compounds.

**Saponins:** In few ml of extract distilled water were added. The suspensions were shaken for 15 minutes. A two layer of foam indicated the presence of Saponins (Raman *et al.*, 2005).

### Antibacterial activity

The antimicrobial activity of *Euphorbia tirucalli* L. was determined by using different solvents against ear infection causing pathogens (*Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Staphylococcus aureus*). It was determined by using agar well diffusion method. Bacterial inoculums were grown in nutrient broth for overnight. Muller Hinton Agar plates were prepared. The test organism was swabbed on the Muller Hinton Agar plates and well made by gel puncher. The plant extract were added into the well and incubated at 37°C for 24 hours. The zone of inhibition was measured with ruler after 24 hours of incubation.

### Minimum inhibitory concentration of stem extract

The minimum inhibitory concentration was used to determine the lowest inhibition concentration of visible microbial growth. Different solvent extract of *Euphorbia tirucalli* L. were subjected to determine the Minimum inhibitory concentration. Methanol and chloroform extract of *Euphorbia tirucalli* L. were taken in different concentrations 50µl, 45µl, 40µl, 35µl, 30µl and 25µl in test tubes to determine MIC. 2ml of Muller Hinton Agar broth were added to sterile test tubes. Inoculum was added to each test tube. Then the inoculated tubes were incubated at 37°C for 24 hours. The lowest concentration of the extract which inhibits microbial growth (no turbidity) was recorded as Minimum inhibitory concentration.

## RESULTS AND DISCUSSION

### Phytochemical analysis of plant extract

Plant synthesis secondary metabolites to protect themselves from pests, disease, drought etc, it is not important for metabolic process but help to face the stressful condition. Some secondary metabolite has the ability to fight microorganism and can be used for medicinal purpose (Muthu *et al.*, 2006). In this present study, Preliminary phytochemical screening of methanol extract revealed the presence of alkaloids, flavonoids, phenol, tannins. Most of the secondary metabolites were identified in methanol extract. The concentration of polar metabolites is higher than non polar metabolites. The chloroform extract revealed the presence of tannins and flavonoids. The hexane extract revealed the presence of tannins. The aqueous extract revealed the presence of Saponins. The result was mentioned in table 1.

Table 1 Phytochemical analysis of *Euphorbia tirucalli* L. extract

Tests	Methanol extract	Chloroform extract	Hexane extract	Aqueous extract
Alkaloids	+	-	-	-
Flavonoids	+	+	-	-
Tannins	+	+	+	-
Phenols	+	-	-	-
Saponins	-	-	-	+

### Antibacterial activity

In this study, Different solvent extract were assessed for their antibacterial activity using agar well diffusion method. The extent of zone of inhibition represents the antibacterial activity of each solvent. Among four solvents methanol was very effective in inhibiting the bacterial growth. The result of antimicrobial activity was consistent with previous reports (Saranya sugumar *et al.*, 2010). The zone of inhibition was mentioned in table2

Table 3 Antibacterial activity of stem extract

Pathogens	Methanol extract	Chloroform extract	Hexane extract	Aqueous Extract	Standard (+control)	DMSO (-control)
<i>Pseudomonas aeruginosa</i>	7±0.816	-	-	-	8.6±0.894	-
<i>Staphylococcus Aureus</i>	6.3±1.313	6±0.816	-	-	5±4.081	-
<i>Streptococcus Pyogenes</i>	5±3.558	-	3.6±0.475	-	8.6±0.774	-

Plate 1 Antibacterial activity of *Euphorbia tirucalli* L. stem extract by using agar well diffusion methodAntimicrobial activity against *Pseudomonas aeruginosa*Antimicrobial activity against *Streptococcus pyogenes*Antimicrobial activity against *Streptococcus aureus*

In this study it is not surprising that there are differences in antimicrobial properties in different extract of plant due to the phytochemical constituents. The outer lipo-polysaccharide layer of cell wall slows down the assessment of most phytochemical compounds to the peptidoglycan layer. This is the cause why the gram-negative strains have resistance to the toxic effect of plant extracts exhibiting antimicrobial activity (Upadhyay *et al.*, 2010). Methanol extract shows very effective against the growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes* at a concentration of 40µg/µl. Chloroform and Hexane shows less effective against pathogens compare to methanol. The aqueous extract did not show any inhibitory effect against pathogens.

#### **Minimum inhibitory concentration of *Euphorbia tirucalli* stem extract**

The plant extract were tested for its Minimum inhibitory concentration for all the pathogens. The Minimum inhibitory concentration of medicinal plants extract against various pathogens was identified. The MIC range was observed for the medicinal plant extract from 50µg/µl to 25µg/µl. The methanol extract of *Euphorbia tirucalli* L. inhibits *Streptococcus pyogenes* (40µg/µl), *Staphylococcus aureus* (35µg/µl) and *Pseudomonas aeruginosa* (40µg/µl). The chloroform extract of *Euphorbia tirucalli* L. inhibit *Staphylococcus aureus* (35µg/µl).

#### **Conclusion**

The present study was conducted to determine the ability of *Euphorbia tirucalli* L. on ear infection causing pathogen. The biologically active phytochemical were present in the methanol extract compare to other solvents of *Euphorbia tirucalli* L. stem extract. Methanol extract of *Euphorbia tirucalli* L. showed maximum concentration of all of these three organisms. In recent times traditional medicine value has increased and new pharmacological drugs were developed. According to WHO, nearly 25% of the modern medicines have been derived from plants being used in traditional medicine. This work is done to conclude that *Euphorbia tirucalli* L. extract effectively work against ear infection causing organisms.

#### **Acknowledgment**

The author thanks DST-FIST laboratory, DBT Star Scheme, chairman and managing Trustee of Dr. N.G.P Arts and Science College, Coimbatore, Tamil Nadu, for providing necessary facilities for carrying out this research work.

#### **Conflicts of Interest**

The authors have no conflicts of interest to publish this research article in this journal.



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