



Extraction of the Metabolites from Medicinal plant *Euphorbia leaf*

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ABSTRACT

The antimicrobial, phytochemical contents of extracts obtained from the leaf extract of mature *Euphorbia pilulifera* (*E. pilulifera*) were examined in this research work. Using ethyl acetate extracts of the leaves of *Euphorbia pilulifera*, phytochemical metabolites were studied. Further using chromatography, different components were separated and its antimicrobial activity was studied. Different types of bacterial organisms were used for evaluating the antimicrobial activity. The results indicated that leaf extracts are more sensitive towards organisms. Thus the presence of phytochemicals in the leaf extract can be used for the treatment of different diseases.

Keywords: Leaf extract, *Euphorbia pilulifera*, Antimicrobial activity, Phytochemicals.

INTRODUCTION

Since ancient times, people have used plants as sources of chemicals, for therapeutic and recreational purposes and for poisoning^{1,2}. Based on the knowledge of medicinal plants human being has been investigating throughout the globe a

traditional medicine since long time. From many generations due to observations and research on animal character this knowledge got enhanced. In most instances these facts remains verbal and that's why it is most of the time, this information is only orally inherited and is therefore in risk of being disoriented in favour of allopathic medicine.



However, it represents for the local population a possibility of simple and cheap treatment. It is a origin of imaginably dominant contemporary pharmaceutical compound for the local residents and it may be a uncomplicated^{1,2,3} and low cost treatment for them. Numerous plant derived substances have demonstrated physiological and behavioral activity against insect pests and they can provide new sources^{4,5,6} for the development of natural pesticides. Ancient people utilised chemicals extracted from plant origin as animal poisons, vermifuges and insect repellents. The presence of toxic substances as secondary metabolites in plants emerges from an extensive and remarkable process known as co-evolution: changes that take place in 2 or many generations as a end result of the populations particular action of the community on one another. *Euphorbia* in India is used in Ayurveda medicine for the diseases like tumors, jaundice, asthma, bronchitis and leprosy, its juice is particularly used as purgative^{5,6}. So many chemical components from *Euphorbia pilulifera* has been isolated and exhibited anitmicrobial activity^{3,7-11}. Our present investigation explains the separation of active phytochemicals from leaf extracts and its antimicrobial activity.

MATERIALS AND METHODS

Chemicals and reagents

All the reagents and chemicals used were of analytical grades. Different biochemical reagents were obtained from Sigma-Aldrich.

Collection of plant material and extraction of secondary metabolites *E. pilulifera* plant is common in India. Fresh leaves were procured from Karnataka in the month of August (Fig. 1.) The leaves were washed in fresh water separately and air dried in dark condition for 7 days at room temperature ($35 \pm 2^\circ\text{C}$). The dried leaves were powdered using an electrical stainless steel grinder and subsequently sieved.



Fig. 1. Complete plant

Extraction and isolation of leaf metabolites

The bioactive compounds from powdered leaves (50 g) were extracted with HPLC grade Ethyl acetate (300 mL) by soxhlet extractor (60–80°C) for 3 hours. The extract was filtered and further concentrated under reduced pressure using rotary evaporator (Billy scientific with Stuart thermostat water bath) at 60°C and stored at 4°C for further analysis^{9,12}. The extract was then stored in the centrifuge tubes.

The leaf extract metabolites checked in silica thin layer chromatography and the solvent used is (ethyl acetate 8:2 methanol) and we observed four metabolites in chromatography and they were subjected to silica gel column chromatography, and fractions were separated using ethyl acetate, benzene, methanol and only two metabolites subjected to antimicrobial activity.

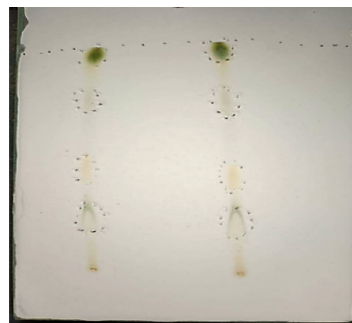


Fig. 2. TLC of crude leaf extract

Microorganisms used

In this investigation four standard microbial cultures used.

Identification of phytochemical components

The phytochemical compounds were quantified using a UV–Vis spectrophotometer (Shimadzu UV 1900) Fig. 3. Using standard biochemical^{12,13,14} procedures, phytochemical components like phenol, soluble starch, steroids, tannins, flavonoids, alkaloids, were identified from the *Euphorbia leaf* extract. Quinone test there was no appearance of violet, pink or red colour. Tannin test gave bluish black ppt, flavonoid test gave yellow to colourless inference, frothing test for saponins was negative as there was no froth formation, Fehling's test gave reddish brown ppt. to indicate presence of reducing sugar, Mayer's test gave brown ppt which indicate alkaloid presence, ferric chloride test gave violet colour for phenol, starch test gave yellow colouration and steroids test gave blue colour.

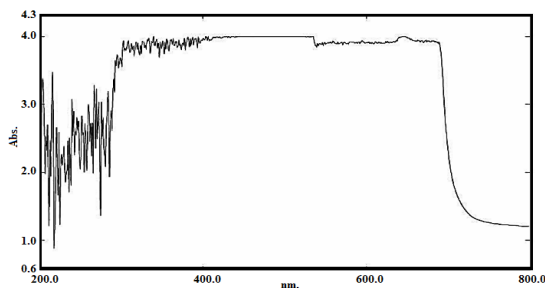


Fig. 3. UV-Vis Spectra of *E. pilulifera* leaf ethyl acetate extract

RESULTS AND DISCUSSION

The ethyl acetate crude leaf extracts of *Euphorbia pilulifera* exhibits antimicrobial activity. The crude fractions shows a very good antibacterial activity as shown in Table 1 and Figure 4.

Table 1: Antimicrobial activity of crude leaf extract

Microorganism Strain	Leaf extract (mm)
<i>Bacillus</i>	18
<i>E. coli</i>	14



Fig. 4. Antimicrobial activity of crude leaf extract

Table 3: Antimicrobial activity of leaf extract.

Leaf extract No.	<i>Bacillus cereus</i> (mm)	<i>E. coli</i> (mm)	<i>Staphylococcus aureus</i> (mm)	<i>Bacillus Subtilis</i> (mm)
1	7	7	14	17
2	7	16	15	5

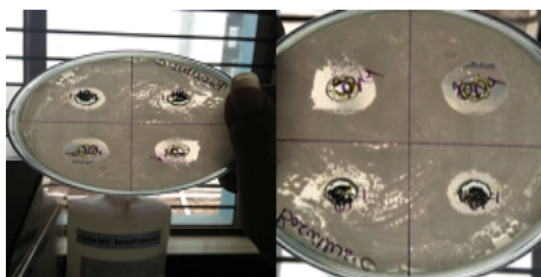


Fig. 5. Antimicrobial activity of leaf extract

The Leaf extract fraction 1, 2 of *Euphorbia pilulifera* portrayed a wide antimicrobial activity towards all the strains of the experimental *Bacillus cereus*, *Bacillus subtilis*, *E. coli* and *Staphylococcus aureus* as shown in the above Table 3 and Fig. 5. The

Further phytochemical components like phenol, soluble starch, steroids, tannins, flavonoids, alkaloids, etc., were identified from the leaf extract as shown in the below Table 2.

Qualitative UV-Vis spectral data (Fig. 3) of ethyl acetate leaf extract showed the peaks between 200 to 400 nm indicates presence of flavonoids and phenolic components.

Table 2: Phytochemicals Analysis

SNo	Phytochemical	Leaf extract	UV (wavelength(nm))
1	Quinones	-	-
2	Tannins	+	272
3	Flavonoids	+	285
4	Saponins	-	-
5	Reducing sugar	+	510
6	Alkaloids	+	254
7	Phenol	+	268
8	Soluble starch	+	590
9	Steroids	+	530

Further, leaf crude extracts were subjected to silica gel column chromatography, and fractions were separated using ethyl acetate, benzene, methanol. Only two metabolites separated pure extracted components were used for testing antimicrobial activity using different bacterial strains and the result is as shown in Table 3, Figure 5.

antimicrobial activity of the leaf extract towards the experimental strains of the *Bacillus cereus*, *Bacillus subtilis*, *E. coli* and *Staphylococcus aureus* were performed by the traditional way of antimicrobial assay in which the Muller-Hinton agar used for the assaying of the antimicrobial were divided quadrangle into four equal parts and inoculated each with the separate experimental strains. The first quarter were inoculated with the experimental strain of *Bacillus cereus*, the second with the *E. coli*, the third one with *S. aureus* and the fourth with *B. subtilis*. A well of 1 mm was digged in all the four quadrangles and the leaf fraction 1 was dispensed equally in all the four wells with 1 mL of the extract. The inoculated plates were incubated at 37°C for 24 hours. Similar set up of the antimicrobial

activity was performed for the fraction 2 of the leaf extract with the same experimental strains. The incubated plates were then observed for the antimicrobial activity of the leaf extract fraction 1, 2 of *Euphorbia pilulifera* which was measured by zone of inhibition and the results were exemplary. The experimental strain of the *Bacillus cereus* shown intermediate sensitivity of 7mm towards both the leaf extract fraction 1, 2 of *Euphorbia pilulifera* where as the experimental strain of the *E. coli* shown the same 7mm intermediate sensitivity the leaf extract fraction 1 of *Euphorbia pilulifera* but an excellent sensitivity of 16 mm towards the leaf extract fraction 2 of *Euphorbia pilulifera* which was a promising result. The experimental strain of the *S. aureus* shown excellent sensitivity of 14 mm and 15 mm towards the leaf extract fraction 1, 2 of *Euphorbia pilulifera* respectively which was a suggestive of the effectiveness of the extract. The experimental strain of the *B. subtilis* shown excellent sensitivity of 17 mm towards the leaf extract fraction 1 of *Euphorbia pilulifera* which was the highest by any strain recorded in this assay and intermediate sensitivity of 5 mm towards the leaf extract fraction 2 of *Euphorbia pilulifera*. The collected data were tabulated and the evidence of the sensitivity activity of the experimental strains towards the leaf extract by the traditional antimicrobial activity assay were photographed in Fig.5. The test assay results were satisfactory with 4 excellent sensitivities were observed with 4 intermediate sensitivities of the experimental strains towards both the leaf extract fraction 1, 2 of *Euphorbia pilulifera*. The experimental strain of *S. aureus* shown excellent promising sensitivity towards both the leaf extract fraction 1, 2 of *Euphorbia pilulifera* while the other experimental strains of *E.coli* and *B. subtilis* shown the mixed results of

sensitivity and intermediate sensitivity towards both the leaf extract fraction 1, 2 of *Euphorbia pilulifera*. The least susceptibility was observed with experimental strain of *Bacillus cereus* which shown intermediate sensitivity towards both the leaf extract fraction 1, 2 of *Euphorbia pilulifera* which was also a quite promising scenario as there were no resistant observed with any of the strain towards the extract.

CONCLUSION

The antimicrobial activity of the *Euphorbia pilulifera* towards the experimental strains of *Bacillus cereus*, *Bacillus subtilis*, *E. coli* and *Staphylococcus aureus* shown a promising display of the test results which boosts the researchers to experiment more about these types of phytochemical activities towards the existence of the diseases. As per the WHO due to the abuse of the antibiotics and self-medication many untreatable resistance organisms poses serious threat to the mankind and the experiment similar to this encourages one's belief on the traditional way of treating the disease with no side effects. This experiment was performed to attempt for a much awaited brighter future in eradicating many emerging diseases in align with the present day medicine. Extracts of plant may be utilized as food preservative in food industry and antimicrobial agent in medicines.

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Conflict of interest

None

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