

“Evaluation of Anti Microbial Activity and Phytochemical analysis of different organic solvent of *Calotropis gigantea*”

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ABSTRACT: The leaf and fruits extract of *Calotropis gigantea* were screened for its anti microbial and phytochemical activities. The solvent used for the leaves and fruits extraction were n-hexane, benzene, acetone, ethanolic and aqueous. The extract was tested against infectious diseases causing bacterial pathogens such as *S. aureus*, *E. Coli* and *P. Aeruginosa* using the well diffusion method. The aqueous, ethanolic and acetone extract of leaf of *calotropis gigantea* inhibition against all the test microbe ranging from 8 mm to 14 mm diameter inhibitory zone. The aqueous, ethanolic and acetone extract of fruits of *calotropis gigantea* inhibition against all the test microbe ranging from 7 mm to 16 mm diameter inhibitory zone. In present study, bacterial extract showed a varying zone of inhibition of growth of tested organism than n-hexane, benzene, ethanol, and aqueous. Phytochemical properties of leaf and fruits of *calotropis gigantea* obtain from n-hexane, benzene, acetone, ethanol and aqueous extract were investigated. The results confirmed that presence of antibacterial activity and phytochemical in shade dried extract of *calotropis gigantea* against the human pathogenic bacteria.

KEYWORD: Antimicrobial and Phytochemical activity, *Calotropis gigantea*, *S. aureus*, *P. areogenosa*, *E. Coli*.

I. INTRODUCTION

The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Thus, any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics.⁽¹⁾ Medicinal plants represent a rich source of antimicrobial agents.⁽²⁾ Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated. Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial agent, a systematic investigation is needed to screen the local flora for antibacterial activity. In current work the antimicrobial activity of various extracts of a valuable medicinal plant *Calotropis gigantea* is systematically studied on some common pathogenic microorganisms, which may result into the development of potent natural remedy for many infections after advance studies in future.⁽³⁾

Calotropis gigantea (L.) Br. is a traditional medicinal plant it belongs to the family Apocyanaceae of *Asclepiadaceae* habitat of Asian countries that includes India, Indonesia, Malaysia, Thailand, Srilanka and china. It is a xerophytic shrub growing widely throughout the tropical and subtropical regions. It is commonly known as milkweed or swallows worth. The plant grows up 2- 4.3 meters long.⁽⁴⁾ From classical system of treatments the various part of the *C. gigantea* are have been considered to cure cardiovascular diseases, various skin diseases, use as anticancer, antifertility and antidote for snakebites⁽⁵⁾ According to researchers this plant *C. gigantea* is informed to have wound healing, antipyretic cytotoxic activities This plant is used for fever, cough, cold, asthma, nausea vomiting, diarrhea, indigestion, leprosy, rheumatism etc. Various parts of this plant like leaves, roots, flowers, fruits, sap etc are containing various chemical metabolites which are responsible for the curative properties of this plant if use with proper knowledge. The plant possesses potential pharmacological properties.⁽⁶⁾

Plants are used medicinally in different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries.⁽⁷⁾ Thus the present study “Phytochemical and antimicrobial activity of *calotropis gigantea*” was under taken to find out the antimicrobial activity of herbal drug against some human pathogenic bacteria.

Materials and Methods Collection of Plant Material:

Collection of plant material the leaves and fruits of *Calotropis gigantea* also known as crown flower; was done from the areas around Bhabra of District Alirajpur, Madhya Pradesh. The identification of the whole plant and its parts were done by department of Botany Holkar Science College, Indore, Madhya Pradesh.

Extraction of Plant

The leaf and fruits of *Calotropis gigantea* are follicular i.e., it is dry leaf, fruit formed of single carpel only that contains several seeds, dehisces by a suture for the dispersal of seeds by wind. Thus the fruits of *C. gigantea* were washed and allowed to dry in shade for a week and then only the follicular covering of the fruits and leaf were grounded into fine powder in mixer grinder and used for the purpose of extraction. Similar as the extraction for leaves & fruits. 10 grams of dried powder of follicular covering of the fruits was subjected to Soxhlet extraction with 200 ml of solvents starting from n-Hexane, followed by extraction with other solvents Benzene and Acetone Ethanol and pure distilled water in separate ways.

Soxhlet process was allowed to carry out till the complete exhaustion of sample material use for extraction with the maintenance of temperature below the boiling points of the solvents used. The extract so obtained is subjected to evaporation of solvent to get the extract in crystalline/slurry form which were suitably diluted and used for preliminary phytochemical analysis and studies of their antimicrobial activity.

Phytochemical Analysis Of The Extract:

A small portion of the dry extracts were subjected to the phytochemical test using Harbourne's (1983) methods to test for alkaloids, tannins, terpenoids, saponins, flavonoids and glycosides.

Test for alkaloids: About 0.2 g extract warmed with 2% H₂SO₄ for two minutes, filtered and few drops of Dragendorff's reagent added orange red precipitate indicates the presence of alkaloids. And or filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Test for glycosides: The extracts hydrolyzed with HCl solutions and neutralized with NaOH solutions. A few drops of Fehling solution A and B were added. Red precipitate indicates the presence of glycoside. Another test use was Benedict's test, in which the filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Test for tannins: Small quantity of extracts mixed with water, heated, filtered and ferric chloride added. A dark green solution indicates the presence of tannins.

Test for saponins: About 0.2g of the extracts shaken with 5ml of distilled water and then heated to boil frothing (appearance of creamy mix of small bubbles) shows the presence of saponins.

Test for flavonoids: Extract of about 0.2 g of the extracts shaken with 5ml of distilled water and then a few drops of 10% lead acetate solution is added. A yellow or dirty white precipitate shows the presence of flavonoids.⁽⁸⁾

Culture Media And Inoculum Preparation:

Nutrient agar broth cultures of the pure culture isolates of *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa* were prepared by transferring a loop of culture into sterile nutrient broth and incubated at 37°C for 48hours. A loop full was taken from these broths and seeded onto sterile nutrient agar plates through sterile cotton swab to develop diffused heavy lawn culture.

Antimicrobial Activity:

The well diffusion method was used to determine the antibacterial activity of the extracts prepared from the *Calotropis gigantea* roots and latex using standard procedure. In this method, first the test bacteria broth of bacteria are used to inoculate on the nutrient agar plates with the help of sterile cotton swabs to develop the lawn culture. Then to these plates 6 mm diameter well are punched in agar plates pre-inoculated with test microorganisms Undiluted over night broth cultures should never be used as an inoculum .routine direct application of suitably diluted extracts are poured into the well. The plates were incubated at 37°C for 24 hr. and then examined for clear zones of inhibition. Sterile water was used as control.⁽⁹⁾

II. RESULTS AND DISCUSSION:

Phytochemical Analysis Of Bioactive Compound In Different Solvent Extracts Of *Calotropis Gigantea*

The plant leaf extracts in different solvent were screened for the presence of various bioactive phytochemical compounds. The analysis revealed the presence of alkaloids, glycosides, tannins, saponins, terpenoids and flavonoids. Were flavonoids is present in sufficiently enough quantity according to preliminary phytochemical analysis. Flavonoids are the phytochemicals that are present in all the extracts of leaves except the hexane extract.

The plant fruits extract in different solvent were screened for the presence of various bioactive phytochemical compounds. The analysis revealed the aqueous and ethanolic extract of the fruits were rich in tannins, saponins, flavonoids and terpenoids. The n-hexane and benzene extract were poor in their phytochemical contents. This were documented in Table 1&2.

Table 1: Phytochemical Analysis of *Calotropis gigantea* extracts from Leaf.

S.N	Phytochemicals	Soxhlet Extractions of Leaf Samples to <i>C. gigantea</i>				
		n-Hexane Extract	Benzene Extract	Acetone Extract	Ethanolic Extract	Aqueous Extract
1	Alkaloids	–	–	+2	+2	+2
2	Glycosides	–	–	–	+	+
3	Tannins	–	±	+	+5	+
4	Saponins,	–	–	+	+	+3
5	Flavonoids	–	+4	+2	+5	+5
6	Terpenoids	+	–	–	+2	–

[(+) means present, (+2 or +3) means Prominent, (+5) means highly prominent and (-) means absent

Table 2: Phytochemical Analysis of *Calotropis gigantea* extracts from Fruits.

S.N	Phytochemicals	Soxhlet Extractions of Fruits Samples to <i>C. gigantea</i>				
		n-Hexane Extract	Benzene Extract	Acetone Extract	Ethanolic Extract	Aqueous Extract
1	Alkaloids	–	–	+2	+	+
2	Glycosides	–	–	+3	–	+
3	Tannins	+	+	+4	+3	+4
4	Saponins,	–	+	+4	+5	+4
5	Flavonoids	–	–	+5	+5	+5
6	Terpenoids	–	–	+5	+5	+5

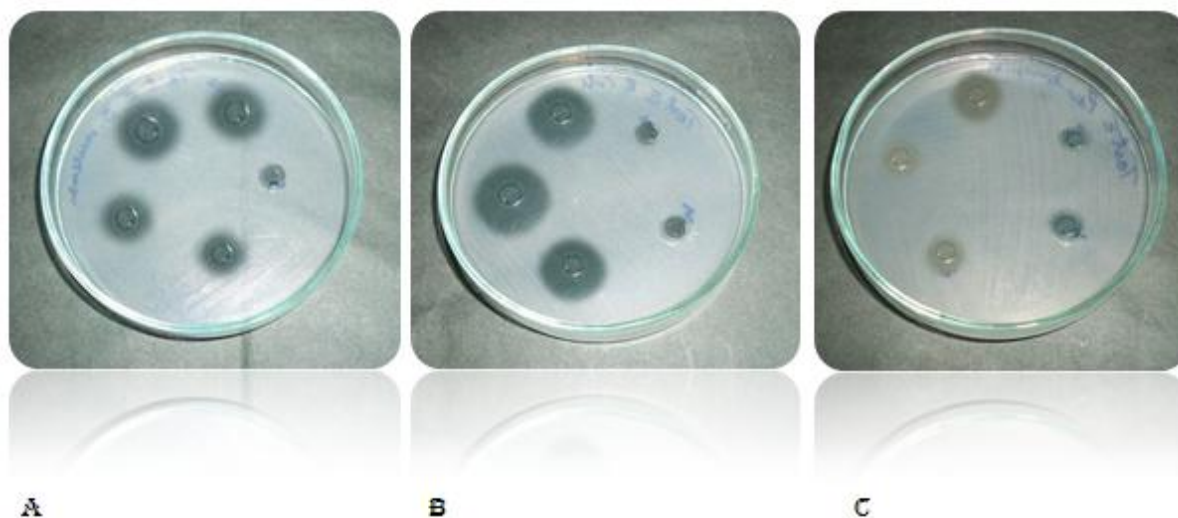
[(+) means present, (+2 or +3) means Prominent, (+5) means highly prominent and (-) means absent]

Antimicrobial efficacy analysis of different organic solvent extracts of *Calotropis gigantea*.

Antimicrobial efficacy of different solvent extract of *Calotropis gigantea* is shown. The results from leaf extracts of *C. gigantea*, ethanolic extract shows maximum antimicrobial activity against *E. coli* out of the all test microbes with zone of inhibition lying in the range of 14 mm approx. on the basis of results depicted in the table 1. The least inhibitory range was 8 mm for ethanolic extract against *P. aerogenosa* and n-haxane extract agains *S. aureus*. But there was no inhibition observed against *E.coli* and *P. aeruginosa* in hexane leaf extract and in benzene extracts in all test microorganisms.

The fruits extract of *C. gigantea*, ethanolic and aqueous extract shows maximum antimicrobial activity against *S. aureus* out of the all test microbes with zone of inhibition lying in the range of 14mm & 16mm approx. on the basis of results depicted in the table 2. The least inhibitory range was 7mm for n-hexane extract against *E. coli* and benzene extract agains *P. aeruginosa*. But there was no inhibition observed against *E.coli* and *P.aeruginosa* in hexane fruit extract and in benzene extract in all microorganisms.

LEAF



- Images A, B and C are revealing the Antimicrobial activity of leaf extracts on *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa* respectively.
- ❖ Reading from top well as sample 1 and moving towards left of anti-clock.

Table 1: Result of the antimicrobial activity of leaf extracts of *Calotropis gigantea*.

S.N	Test microbes	Zone of Inhibition due to <i>C. gigantea</i> (L)leaf extracts 1mg/ml (in mm)				
		n-Hexane extract	Benzene Extract	Acetonic Extract	Ethanolic Extract	Aqueous extract
1.	<i>Staphylococcus aureus</i>	8	Nil	11	12	13
2.	<i>Escherichia coli</i>	Nil	Nil	13	14	12
3.	<i>Pseudomonas aeruginosa</i> .	Nil	Nil	9	8	10

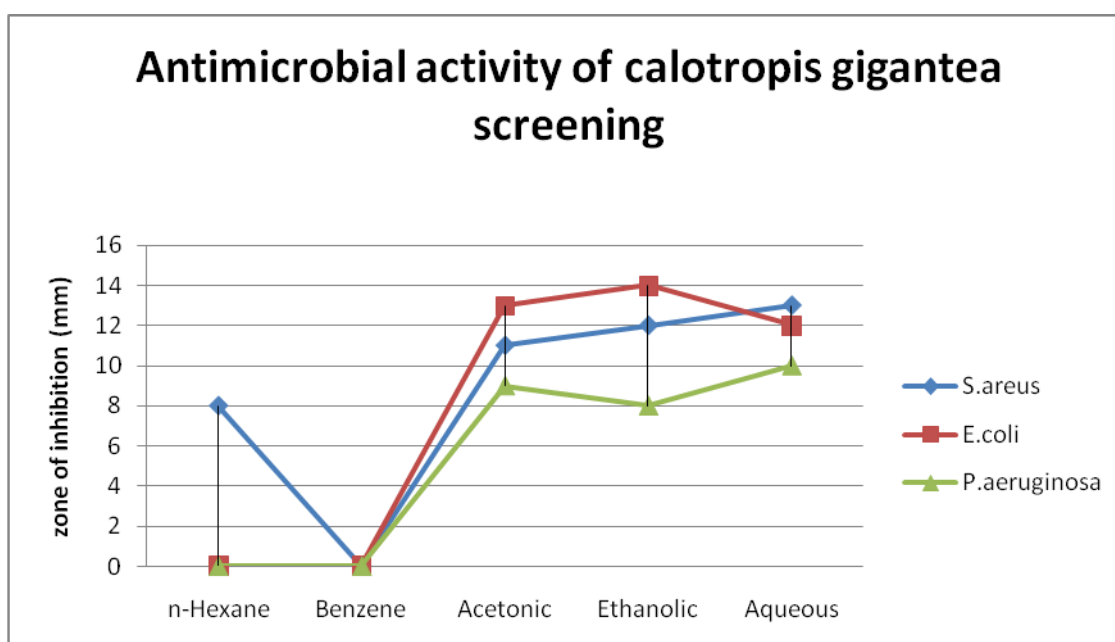


Figure 1: Graphical representation Antimicrobial Activity of *Calotropis gigantea* leaf Extracts on three Test species.

FRUIT

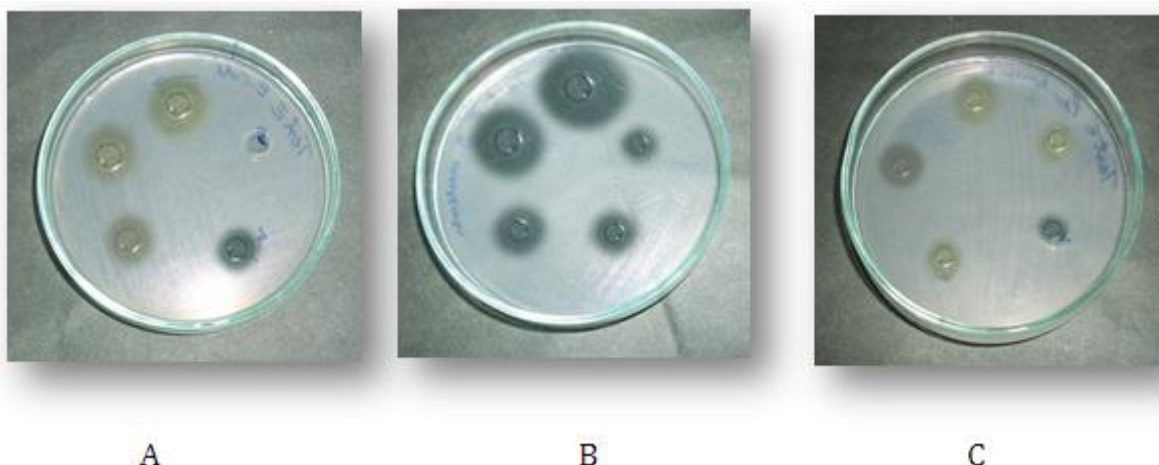


Plate – V (b) details: Results of antimicrobial activity of fruit and latex extracts of *C. gigantea*.
 ➤ Images A, B and C are revealing the Antimicrobial activity of fruit extracts on *E. coli*, *Staphylococcus aureus* and *Pseudomonas aerogenosa* respectively.
 ❖ Reading from top well as sample 1 and moving towards left of anti-clock.

Table 2: Results of the antimicrobial activity of fruits extracts of *Calotropis gigantea*.

S.N	Test microbes	Zone of Inhibition due to <i>C. gigantea</i> (L.) Br. fruits extracts 1mg/ml (in mm)				
		n-Hexane extract	Benzene Extract	Acetonic Extract	Ethanolic Extract	Aqueous extract
1.	<i>Staphylococcus aureus</i>	8	8	10	14	16
2.	<i>Escherichia coli</i>	7	Nil	8	11	13
3.	<i>Pseudomonas aeruginosa</i> .	Nil	7	8	12	10

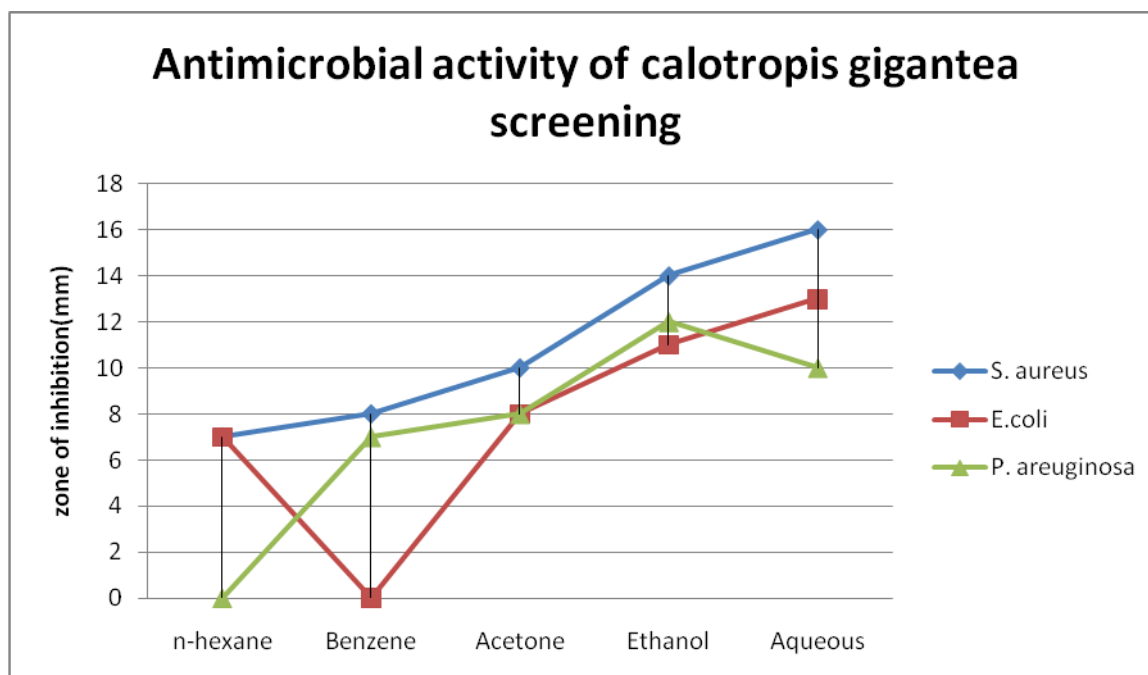


Figure 2: Graphical representation Antimicrobial Activity of *Calotropis gigantea* fruits Extracts on three Test species.

Antibacterial activity of different solvent extracts of *C. gigantea* showed varying degrees of antibacterial activity against all microorganisms tested.⁽¹⁰⁾ There are many reports of plants in the family *Asclepiadaceae* possessing anti-microbial activity.^(11, 12) From this study it can be said that, acetone ethanol and aqueous shade dried leaf extract and ethanol and aqueous shade dried fruits extract of *C. gigantea* showed wide range of antibacterial activity can be used and administered in the ethano medical practice.^(13, 14) The results obtained from our study showed an effective inhibition against the test organism which justify the traditional use of the plant for infectious diseases.

III. CONCLUSION

The phytochemical analysis revealed the bioactive compounds which are responsible for the invitro antimicrobial of *calotropis gigantea* our all bacteria strains in all extracts could be alkaloids glycosides tannins, saponins, flavonoids and terpenoids. The encouraging results indicate that the aqueous, ethanolic, acetic, n-hexane and benzene extracts of various parts of *Calotropis gigantea* might be exploited as a natural drug for the treatment of several infectious diseases caused by these organisms and could be useful in understanding the relations between traditional cures and current medications.

Our results showed that in present work that extracts obtained different parts of the plant *Calotropis gigantea* using various solvents are rich sources of potent phytochemicals especially the leaves and fruits extract and has inhibitory effects on the experimental microbes. From previous studies and the current work it is clear that the plant is rich source of alkaloids, glycoside, tannins, saponins, flavonoids, terpenoids etc. These bioactive complex phytochemicals can be used for the development of potent drugs, medicines or antimicrobial agents that can be used for various purposes for human welfare upon further extensive & systematic studies.

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