

# “ Extraction and Isolation ,Phytochemical analysis and Antimicrobial activity (against skin bacteria and some other bacteria ) of Calotropis gigantea in different organic solvent”

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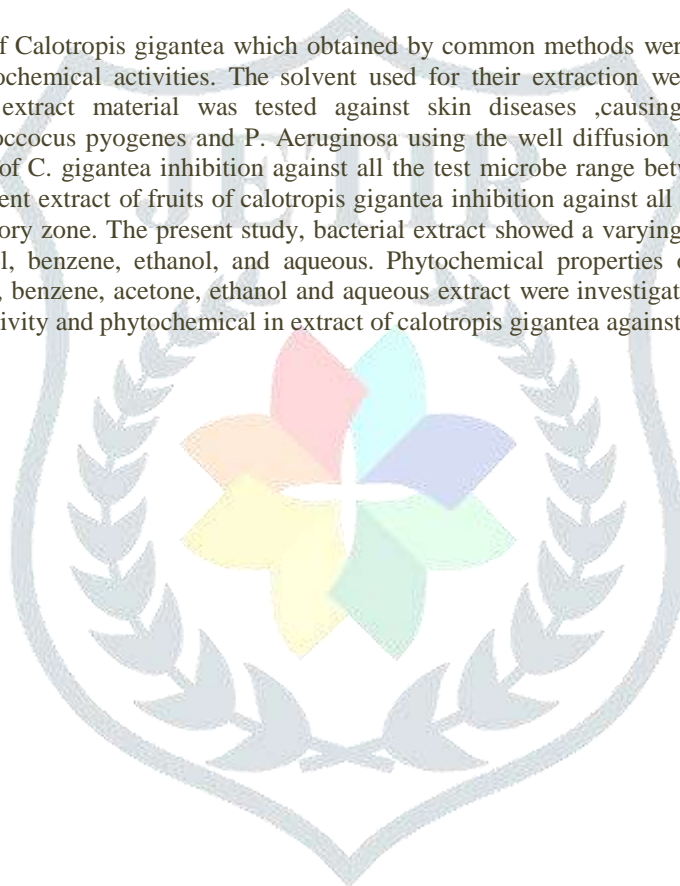
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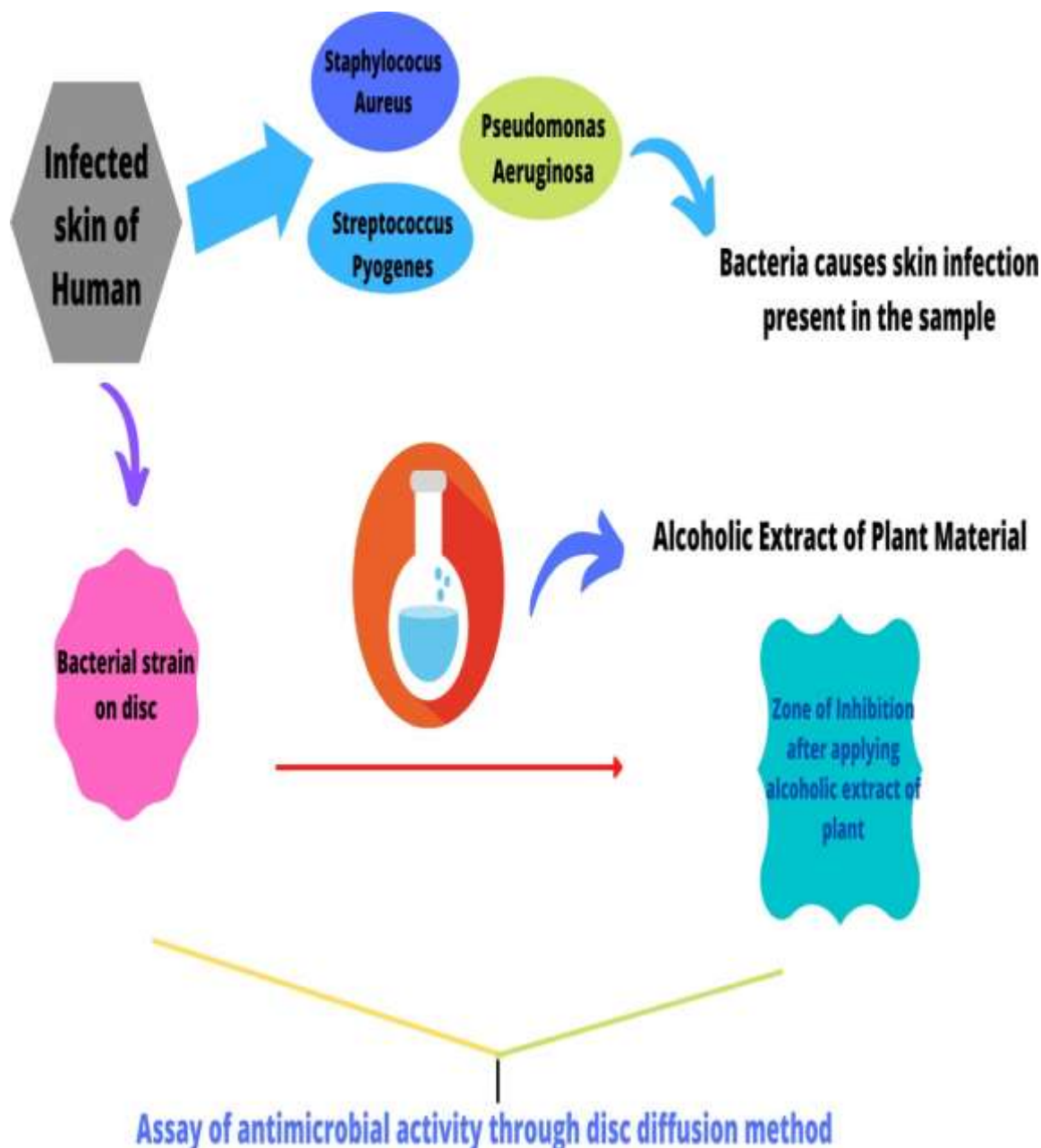
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## ABSTRACT:

The extract of root and fruits of *Calotropis gigantea* which obtained by common methods were screened to check the activity against microbes and its phytochemical activities. The solvent used for their extraction were benzene, acetonic methanol, ethanolic and aqueous. The extract material was tested against skin diseases ,causing bacterial pathogens such as *Staphylococcus aureus*, *Streptococcus pyogenes* and *P. Aeruginosa* using the well diffusion method. The aqueous, ethanolic and acetone extraction of root of *C. gigantea* inhibition against all the test microbe range between 7 mm to 13 mm diameter inhibitory zone. The same solvent extract of fruits of *calotropis gigantea* inhibition against all the test microbe ranging from 8 mm to 15 mm diameter inhibitory zone. The present study, bacterial extract showed a varying zone of inhibition of growth of tested organism than methenol, benzene, ethanol, and aqueous. Phytochemical properties of root and fruits of *calotropis gigantea* obtain from methanol, benzene, acetone, ethanol and aqueous extract were investigated.The obtained results indicate the presence of antibacterial activity and phytochemical in extract of *calotropis gigantea* against the human pathogenic bacteria.



## Graphical Abstract:



**KEYWORD:** Screened, Phytochemical activity, *Calotropis gigantea*, *S. aureus*, *P. areogenosa*, *S. pyogenes*.

## 1. INTRODUCTION

Plants which are present in huge amount can play an important role as a source for new drugs is still largely unexplored. Among the estimated millions of plants species, only a small percentage has been investigated phytochemically and the fraction which are submitted to biological or pharmacological screening is also on small scale [1]. Thus, any type of phytochemical investigation of a given plant will reveal only a very contract spectrum of constituents which it have. In the area of antibiotics there is very important tool in discovering new biologically active molecules is screening. Medicinal plants play an important role as as antimicrobial agents [2]. Only few species of plant have been tested for antimicrobial properties, the vast majority of have not been evaluated in abundance. Considering the plants as sources for antimicrobial drugs with reference to antibacterial agent, an investigation in systematic way is needed to screen the flora for antibacterial activity [3]. In latest work the antimicrobial activity of various extracts parts of a medicinal plant *Calotropis gigantea* is studied systematically on some common pathogenic microorganisms, which may result into the development of natural remedy against different types of microbial infections after advance studies in future.

*Calotropis gigantea* (L.) Br. is a traditional medicinal plant which belongs to the family Apocyanaceae is a common plant of Asian countries that includes India, Malaysia, Indonesia, Thailand, china and shrilanka. It is a xerophytic shrub growing widely in tropical and subtropical regions. It is commonly known as milkweed. The plant grows up 2- 4.2 meters long [4-6].

The various part of the *C. gigantea* are have been considered to cure various disease such as skin diseases, use as anticancer, antifertility and antidote for snakebites. On the basis of research *C. gigantea* is informed to have wound healing, cytotoxic activities. This plant is useful in cough, fever, cold, asthma, diarrhea, nausea vomiting indigestion, leprosy, rheumatism etc. Various parts like leaves, roots, fruit flowers, sap etc of this plant are containing various medicinal properties which are responsible for the curative nature if use with proper knowledge [7-9].

Plants are used medicinally in different countries in which India is one of them and are a source of potent and powerful drugs. The parts of plants in wide range is used for extract as raw drugs and they possess various properties and one of them is medicinal properties. The different parts of plant used such as root, stem, flower, fruit, and modified plant organs. Some of these raw drugs are collected on smaller scale by the local communities for local used, many other raw drugs are collected on larger scale and traded in the reachable market as the raw material for lots of herbal industries. Thus this and the present study of “Phytochemical and antimicrobial activity of *calotropis gigantea*” was under taken to find out the antimicrobial activity of herbal drug against some pathogenic bacteria in humans [10].

## 2. Materials and Methods

### 2.2- Collection of Plant Material:

Collection of plant material the root and fruit of *Calotropis gigantea* also known as crown flower; was done from the areas around the district Shahjahanpur, Uttar Pradesh. The identification of the whole plant and its parts were done by department of Chemistry Gandhi Faiz-E- Aam College, Shahjahanpur.

### 2.3 -Extraction of Plant

The root and fruits was collected in the field and cleaned by a sterile muslin cloth, cuts into very small pieces by sterile razor blade, and stored polythene bags which should be sterile.

Thus the fruits and roots of *C. gigantea* were washed and allowed to dry in shade for some days or a week and then only the follicular covering of the fruits and complete roots were grounded into fine powder with the help of grinder and used for the purpose of extraction. 15 grams of dried powder of follicular covering of the fruits and roots was subjected to Soxhlet extraction with 250 ml of solvents starting from methanol, followed by extraction with other suitable solvents, here used Benzene and Ethanol Acetone and pure distilled water used in separate ways [11-13].

Soxhlet process by soxhlet extractor was carry out till the complete exhaustion of used sample material use for extraction process and temperature maintain at (which is important) below the boiling points of the solvents used [14]. The obtained extract is subjected for evaporation of solvent to get the extract in slurry or in crystalline form which were diluted suitably and used for preliminary phytochemical analysis and studies of their antimicrobial activity [15].

### 2.4- Phytochemical Analysis Of The Extract:

Small amount of the dry extracts were subjected to the phytochemical test using Harbourne’s methods to test for glycosides alkaloids, tannins, saponins terpenoids, and flavonoids

**Test for alkaloids:** About 0.5 g extract warmed with 3% H<sub>2</sub>SO<sub>4</sub> for three minutes, filtered and few drops of Dragendorff’s reagent added orange red precipitate indicates the presence of alkaloids. Another method in which filtrates were treated with Wagner’s reagent (solution of Iodine in KI). Brown precipitate formation indicates the presence of alkaloids [16].

**Test for glycosides:** Obtained extracts hydrolyzed with hydrochloride (HCl) and neutralized with Sodium hydroxide (NaOH) solutions. Some drops were added of Fehling solution A and B. The formation of Red precipitate indicates the presence of glycoside. Benedict’s test also can be used, in which the filtrates were treated with Benedict’s reagent and heated gently. If orange red precipitate obtained then it indicates the presence of reducing sugars.

**Test for tannins:** Small quantity of extracts mixed with water and heated after that filtered and ferric chloride added. If A dark green solution obtained then it indicates the presence of tannins.

**Test for saponins:** About 0.5g of the extracts shaken with 6ml of distilled water and then heated to boil appearance of creamy mix of small bubbles (frothing) shows the presence of saponins [17].

**Test for flavonoids:** 0.5 g of the extracts shaken with 6ml of distilled water and then some drops of 6% lead acetate solution is added. A dirty white or yellow precipitate indicates the presence of flavonoids.

### 2.5- Antimicrobial Activity:

The well diffusion method which is very common method was used for the assay antibacterial activity of the extracts prepared from the *Calotropis gigantea* roots and latex using standard procedure [18-20]. This is the method, in which broth of bacteria which is called bacteria broth are used on the nutrient agar plates for inoculation with the help of sterile cotton swabs to develop the lawn culture. These plates of 5 mm diameter well are punched in agar plates pre- inoculated with test microorganisms, over night broth cultures of undiluted should never be used as an inoculum routine direct application of suitably diluted extracts are stream into the well. The plates were incubated at 35°C for 25 hr. and then examined zones of inhibition. Sterile water was used as control [21].

### 3. RESULTS AND DISCUSSION

#### 3.1- Phytochemical Analysis Of Bioactive Compound In Different Solvent Extracts Of *C. Gigantean*

Different solvent of plant root extract were screened for the presence of various bioactive phytochemical compounds. The analysis evident the presence of glycosides, tannins alkaloids, saponins, terpenoids and flavonoids were flavonoids is present in enough quantity according to first phytochemical analysis [22-23]. Flavonoids are the common phytochemicals that are present in all the extracts of this plant except the hexane extract.

The plant fruits extract in different solvent were screened to evaluate of various bioactive phytochemical compounds. This analysis evident the ethanolic and aqueous extract of the fruits were rich in tannins, , flavonoids ,saponins and terpenoids. The benzene extract were poor in their phytochemical content.This were documented in given Table 1&2.

**Table 1: Analysis of Phytochemicals in *Calotropis gigantea* extracts from root**

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

[(+) present, (+2 to +4) Prominent, (+5) high prominent and (-) means absent.]

**Table 2: Analysis of Phytochemicals from fruit extract of *C. gigantea***

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

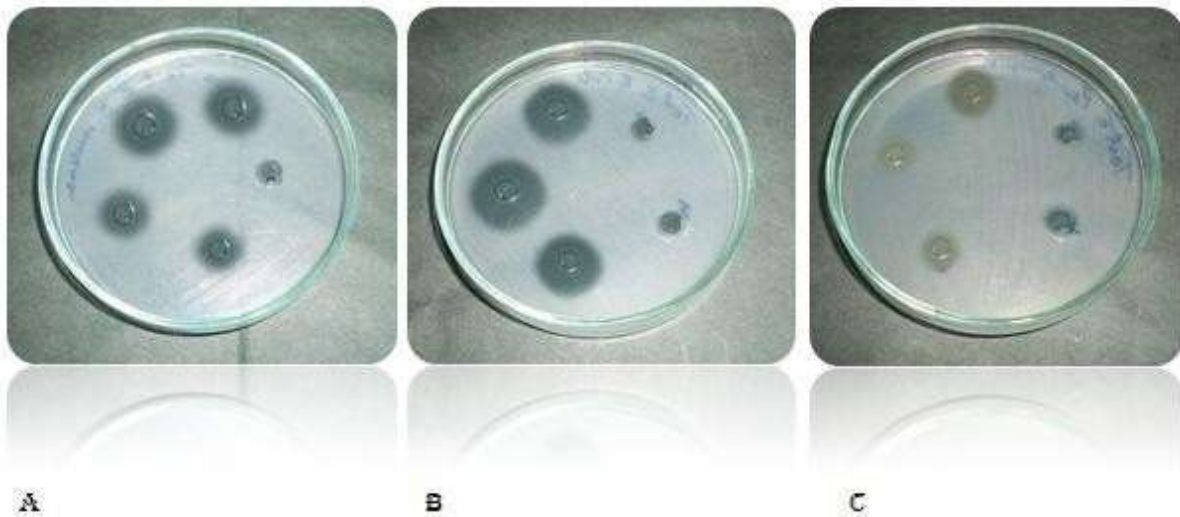
[(+) present, (+2 to +4) Prominent, (+5) high prominent and (-) absent Antimicrobial assay

#### 3.2- Analysis of extracts of Different organic solvent of *Calotropis gigantea*.

Antimicrobial assay of extract of *Calotropis gigantea* in different solvent is shown. The results from extraction of roots of *Calotropis gigantea*, aqueous extract shows maximum antimicrobial activity against *S.aureus* out of the all test microbes with zone of inhibition lying in the range of 16 mm approx. on the basis of results given in the table 1. The least inhibitory range was 8 mm for Acetonic extract against *S.pyogenes* and *P. aeruginosa* [24-26]. But there was no inhibition observed for all in Benzene.

The fruits extract of *C. gigantea*, aqueous extract shows maximum antimicrobial activity against *S. aureus* and *S.pyogenes* out of the all test microbes with zone of inhibition of 15mm approx. on the basis of results given in the table 2. The least inhibitory range was 7mm for benzene extract against *P.aeruginosa* [27]. But there was no inhibition observed against *S.pyogenes* in Benzene extraction of fruit.

## ROOT

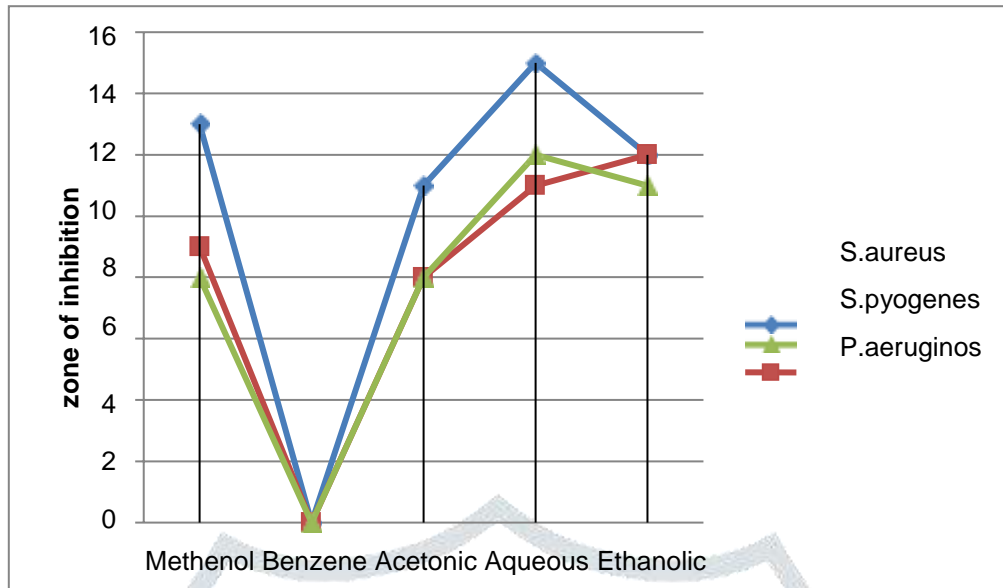


Img. A, B and C are Antimicrobial activity assay of root extracts on *S. aureus*, *S. pyogenes* and *P.s aeruginosa* respectively.

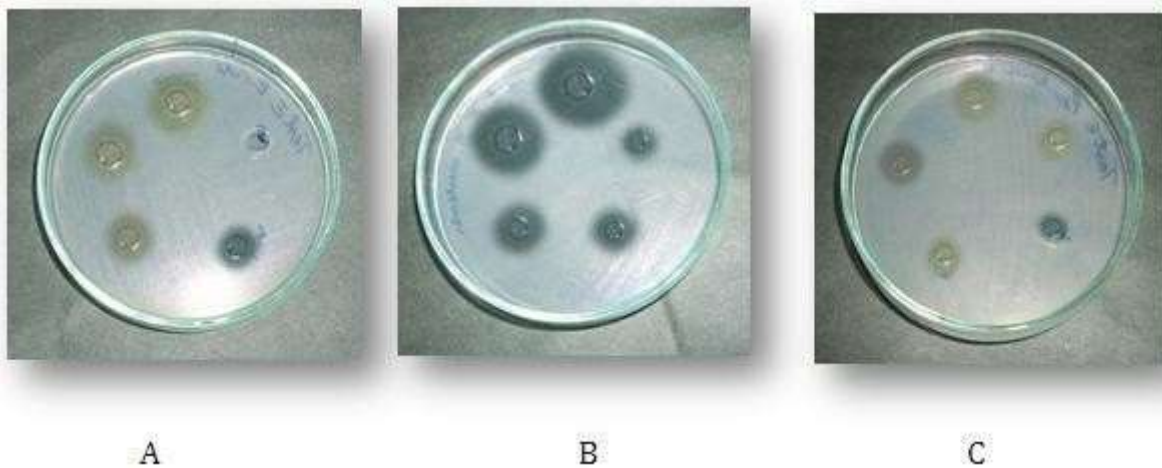
Table 1: Result of the antimicrobial activity of root extracts of *Calotropis gigantea*.

S. N.	Test microbes	Zone of Inhibition due to <i>C. gigantea</i> (L) root extracts 1mg/ml (in mm)				
		Methanol Extract	Benzene Extract	Acetonic Extract	Ethanollic Extract	Aqueous Extract
1.	<i>Staphylococcus aureus</i>	13	Nil	11	12	15
2.	<i>Streptococcus pyogenes</i>	9	Nil	8	12	11
3.	<i>Pseudomonas aeruginosa</i> .	13	Nil	8	11	12

Figure 1: A representation in graph of antimicrobial activity of *Calotropis gigantea* root Extracts on three Test species



**FRUIT**

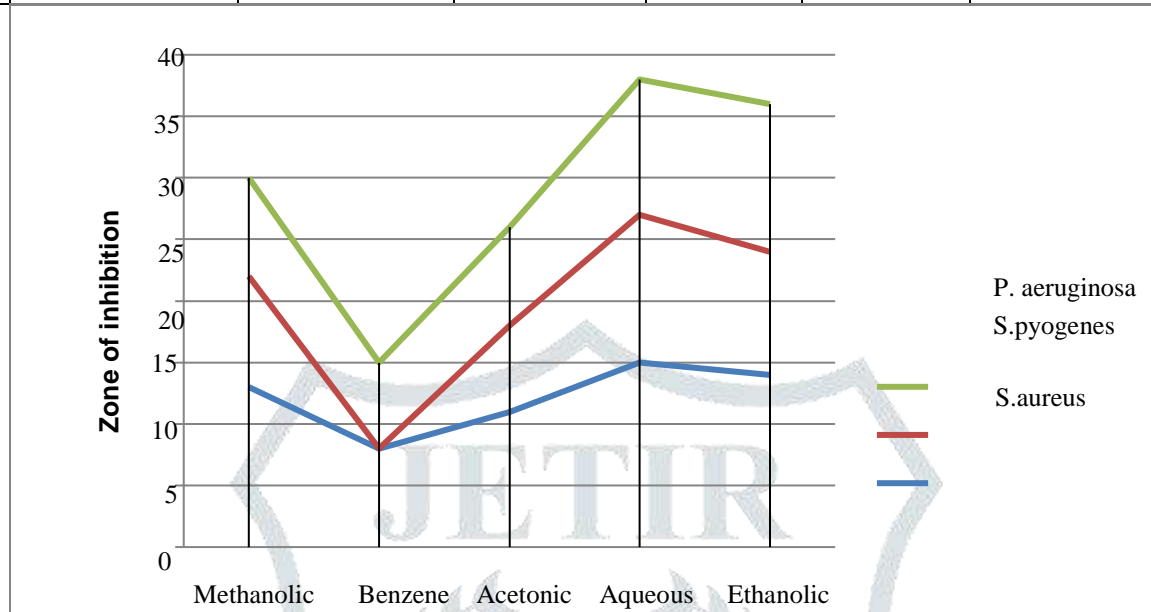


**Plate ; Results of antimicrobial activity of fruit extracts of *C. gigantea*.**  
**Images A, B and C are the assay of Antimicrobial activity of fruit extracts on *S.pyogenes* *Staphylococcus aureus* and *Pseudomonas aerogenosa* respectively.**

**Table 2: Antimicrobial activity of fruits extracts of *C. gigantean*.(Results).**

S.N.	Microbes	Zone of Inhibition due to <i>C. gigantean</i> (L.) Br. fruits extracts 1mg/ml (in mm)				
		Methanolic extract	Benzene Extract	Acetonic Extract	Ethanolic Extract	Aqueous Extract
1	<i>Staphylococcus aureus</i>	13	8	11	14	15

2.	<i>Streptococcus pyogens</i>	9	Nil	11	14	15
3.	<i>Pseudomonas aeruginosa.</i>	8	7	8	11	12



**Figure 2: A representation in graph of Antimicrobial Activity of *C.gigantea* fruits Extracts on three Test species**

Different extracts of *C. gigantea* in different solvent showed varying degrees of antibacterial activity against all microorganisms tested. Plants of the family *Asclepiadaceae* possessing anti-microbial activity according to many reports. By the proper study it can be said that, acetone, ethanol and aqueous shade dried root extract and ethanol and aqueous shade dried fruits extract of *C. gigantea* showed a broad range of antimicrobial activity [28-30]. The results obtained from our study showed an effective inhibition against the test organism which prove the traditional use of the plant for microbial diseases.

#### 4. CONCLUSION

The phytochemical analysis evident 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, acetonic, methenol and benzene extracts of various parts of *Calotropis gigantea* might be as a natural drug for the treatment of several microbial diseases caused by these organisms and could be useful in understanding the relations between traditional cures and current medications.

This Study showed that ,extracts obtained roots and fruits of the plant *C.gigantea* using various solvents are rich sources of phytochemicals create inhibitory zones on the tested microbes. Previous studies and the current work tells that the plant is rich source of tannins, saponins, flavonoids alkaloids, glycoside, terpenoids etc. These bioactive compounds phytochemicals can be used into the development of huming drugs, medicines or antimicrobial agents that can be used for various purposes for human welfare upon further extensive and systematic studies.

#### Declaration of conflict of interest

None

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