



**STUDY COMPARATIVE ANTI-MICROBIAL ACTIVITY OF METHANOLIC EXTRACT
OF FLOWERS OF *CALOTROPIS GIGANTEA* AND *CALOTROPIS PROCERA***

P. B. Kadam*, D. S. Kachare and K. T. Sul

Department of Pharmacology, Yashoda Technical Campus, Wadhe, Satara, India.

***Corresponding Author: P. B. Kadam**

Department of Pharmacology, Yashoda Technical Campus, Wadhe, Satara, India.

Article Received on 25/03/2021

Article Revised on 15/04/2021

Article Accepted on 05/05/2021

ABSTRACT

Ayurveda is the oldest medicine system and includes numerous medicinal plants. Medicinal plants are massive source of biologically active compound and offering broad range of activity. The present study was aimed to evaluate and compare antimicrobial activity of methanolic extract of flowers of *Calotropis gigantea* and *Calotropis procera* which is also known as Rui. Its antimicrobial activity was performed by using gram negative bacteria *Escherichia coli*; gram positive bacteria *Bacillus subtilis* and fungi *Aspergillus niger* by cup plate method. Streptomycin and Itraconazole was used as a standard and to compare the antimicrobial activity of plants on pathogens. The antimicrobial activity was measured by different concentrations of methanolic extract of flowers of *Calotropis gigantea* and *Calotropis procera* with its minimum dose (20µg/ml) and maximum dose (100µg/ml) and Streptomycin (100µg/ml) and Itraconazole (100µg/ml). Antimicrobial activity was evaluated by measuring the zone of inhibition in millimeters. It can be concluded that methanolic extract of *Calotropis gigantea* and *Calotropis procera* was found to have antimicrobial activity against gram positive *Bacillus subtilis*; gram negative *E. coli* and fungi *Aspergillus niger*. It also indicated that methanolic extract of plants are potentially good for antibacterial resistant bacteria therapy.

KEYWORDS: Antimicrobial activity, Soxhlet extraction, Ultrasonication, cup plate method.

INTRODUCTION

In recent past, drug resistance to human pathogenic bacteria is being commonly reported from all over the world.^[1] There are number antibiotics available in market but some of them are having adverse effects like hypersensitivity, gene toxicity, and depletion of Normal gut flora.^[2] Ayurveda is the oldest system of medicines and includes numerous medicinal plants.^[3] As per WHO medicinal plant is a plant in which one or more of its parts, contains substances that can be used for therapeutic purposes or which are substrates for the synthesis of various useful drugs.^[4] Medicinal plants are used for the formation of various drugs and they have been used traditionally to cure various diseases.^[5,6] These plants contain many phytochemical constituents like flavonoids, alkaloids, tannins, and phenols etc.^[7], which show antimicrobial activity against the pathogens.^[5,6] As per the WHO, almost more than 80% of world's population depends upon the traditional medicine for primary care of health.^[8] *Calotropis* R. Br. species belongs to family Asclepiadaceae and is distributed in northern Africa, Arabia, and tropical Asia.^[9]

Calotropis procera also called as Sodom apple is a shrub about 6m high and is widely distributed in West Africa and other parts of the tropics. The plant is erect, large, tall, branched and perennial with milky latex throughout.

It is used to treat various diseases. The secretion from the root bark is usually used for the treatment of skin diseases, enlargements of abdominal viscera and intestinal worms. It is also used to treat elephantiasis, toothache, asthma, leprosy, rheumatism,^[5,10,11] It also possesses cardio tonic, hepatoprotective, antioxidant, analgesic and anticancer activities.^[4,12] It contain cardenolides, triterpenoids, resins, proteolytic enzymes, flavonoids, tannins, sterol, gallic acid, phenols and terpenes.^[12]

Calotropis gigantea also known as milkweed.^[13] has been used by tradition as an anthelmintic, carminative, astringent, digestive, stimulant, anti-inflammatory. It is also used to treat toothache, earache, sprain, anxiety, pain, epilepsy, diarrhea, mental disorders, cough, leprosy, and asthma.^[6,9,13] It contain cardenolides, flavonoids, terpenes, ester of α -and β -calotropeols and a nonprotein amino acid etc.^[9]

The aim of the present study was to prepare methanolic extract of flowers of *Calotropis gigantea* and *Calotropis procera* by using soxhlet and ultrasonication method of extraction and also to evaluate and compare their antimicrobial activity.

MATERIALS AND METHODS

• Selection and authentication of plant

The fresh flowers of plants *Calotropis gigantea* and *Calotropis procera* were collected from the Satara city, from Satara district of Maharashtra. The plants collected were authenticated botanically in department of Botany at Yashwantrao Chavan Institute of Science, Satara.

• Preparation of *C. gigantea* and *C. procera* plant extract

Fresh flowers were collected and washed in water. Flowers were air dried for 15-20 days and then powdered.

Soxhlet extraction

10 gm of powdered plant material (flowers) of *Calotropis gigantea* and *Calotropis procera* were weighed and uniformly packed into two separate thimbles of Soxhlet extractor. The round bottom flasks were filled two-third with methanol and were adjusted to the extractor. The extraction process was allowed to run. After the extraction process completion, extract was filtered and collected in different petri plates. The petriplates were kept aside for evaporation at room temperature and collected extract was stored in refrigerator for further use.

Ultrasonic extraction

10gm powdered plant material (flowers) of *Calotropis gigantea* and *Calotropis procera* were weighed and taken in four different beakers respectively. After 50 ml methanol was added in each beaker, the beakers were kept in sonicator for sonication extraction at different time intervals 15, 30, 45, 60 minutes respectively. After completion of extraction process the extract was filtered and collected in different petri plates. The petriplates were kept aside for evaporation at room temperature and collected extract was stored in refrigerator for further use.

OBSERVATION

Antimicrobial activity by using soxhlet method *E. coli* (gram negative)



Fig.1 *Calotropis gigantea* flower extract.

• Phytochemical constituents present in the extract

Phytochemical constituents present in the extract were determined by performing chemical test for alkaloids, steroids, carbohydrate, saponins, flavanoids, cardiac glycosides, anthraquinone glycosides etc.

• Collection of bacterial sample

The stock cultures of gram positive *Bacillus subtilis*; gram negative *E. coli* and fungi *Aspergillus niger* were collected from department of Microbiology of Yashwantrao Chavan institute of science Satara.

• Antimicrobial activity

The antibacterial activity was carried out by using cup plate method. The nutrient agar media was sterilized for 30 min. at 121 °C under 15 psi pressure. Then cooled to about 65 °C. Then 25 ml of the medium was poured in Petri-dish. The plates were kept at room temperature for solidification and stored at 4 °C, for further use. Bacterial culture was spread over the nutrient agar plates by using separate sterile spreader. Holes were made in the medium with the help of 7 mm corn borer. The dried plant extract was dissolved in methanol to final extract of 100 µg/ml. Each hole in plate was filled with 1 ml of plant extract. The plates were incubated at 37 °C for about 24-48 hours. Also Streptomycin and Itraconazole was used as standard. The antibacterial activity was recorded at the end of incubation period based on the inhibition of bacterial growth by the extract. After incubation period is completed the zones of incubations were measured to the nearest millimeter. The inhibition zone is the area surrounded the hole and there is no growth of inoculated micro-organisms. Each test was performed in duplicate to confirm the results.



Fig.2 *Calotropis procera* flower extract.

bacillus subtilis (gram positive)



Fig.3 *Calotropis gigantea* flower extract.

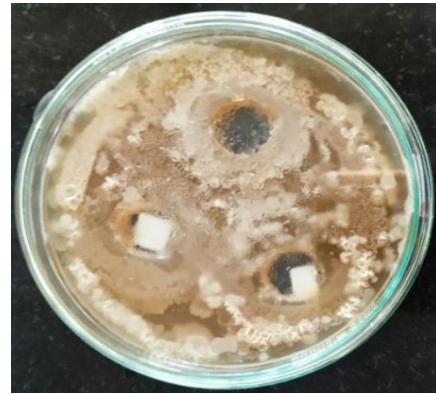


Fig.4 *Calotropis procera* flower extract.

aspergillus niger



Fig. 5 *Calotropis gigantea* flower extract.

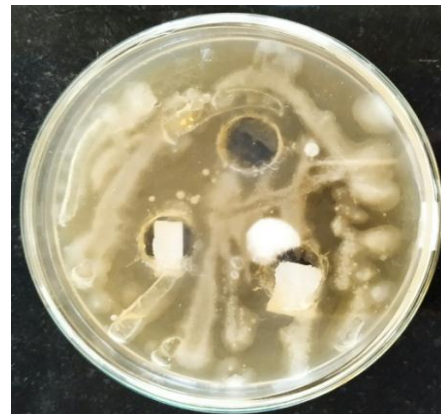


Fig. 6 *Calotropis procera* flower extract.

Antimicrobial activity by using ultra sonication method
E. coli



Fig. 7 *Calotropis gigantea* flower extract.

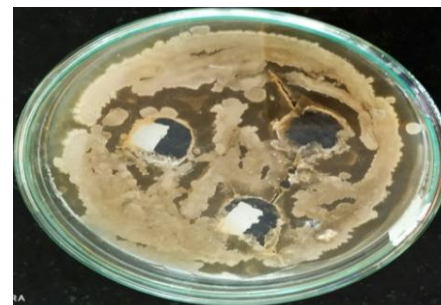


Fig. 8 *Calotropis procera* flower extract.

bacillus subtilis



Fig. 9 *Calotropis gigantea* flower extract.

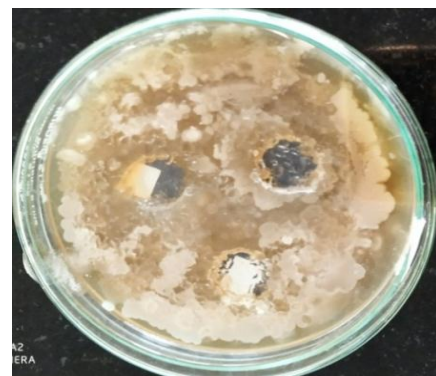


Fig. 10 *Calotropis procera* flower extract.

aspergillus niger

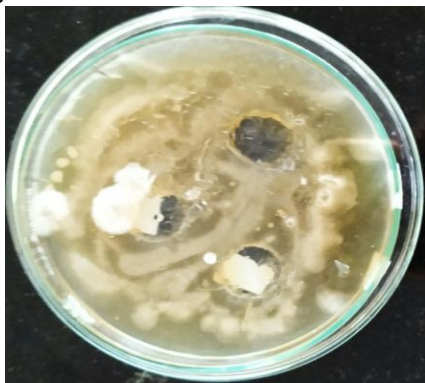


Fig. 11 *Calotropis gigantea* flower extract.

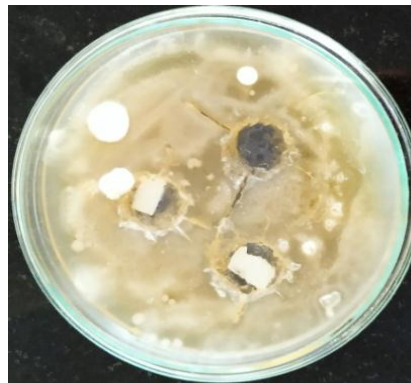


Fig. 12 *Calotropis procera* flower extract.

RESULTS

Gram positive: *Bacillus subtilis*.

Gram negative: *Escherichia coli*.

Fungi: *Aspergillus niger*.

A. Antimicrobial Activity by Soxhlet Extraction Technique.

Sr. No.	Pathogens	Zone of Inhibition in mm					
		Flowers of <i>C. gigantea</i>			Flowers of <i>C. procera</i>		
		Std. (100 µg/ml)	Min. (20 µg/ml)	Max. (100 µg/ml)	Std. (100 µg/ml)	Min. (20 µg/ml)	Max. (100 µg/ml)
1.	<i>E. coli</i>	26mm	30mm	25mm	16mm	22mm	30mm
2.	<i>Bacillus subtilis</i>	31mm	22mm	24mm	26mm	24mm	28mm
3.	<i>Aspergillus niger</i>	26mm	30mm	32mm	30mm	34mm	20mm

B. Antimicrobial Activity by Ultrasonic Extraction Technique.

Sr. No.	Pathogens	Zone of Inhibition in mm					
		Flowers of <i>C. gigantea</i>			Flowers of <i>C. procera</i>		
		Std. (100 µg/ml)	Min. (20 µg/ml)	Max. (100 µg/ml)	Std. (100 µg/ml)	Min. (20 µg/ml)	Max. (100 µg/ml)
1.	<i>E. coli</i>	15mm	18mm	25mm	35mm	25mm	38mm
2.	<i>Bacillus subtilis</i>	30mm	28mm	26mm	32mm	28mm	30mm
3.	<i>Aspergillus niger</i>	30mm	32mm	26mm	34mm	30mm	32mm

DISCUSSION

The extracts obtained from Flowers of *C. gigantea* and *C. procera* exhibited activity against *E. coli* which is gram negative, *Bacillus subtilis* which is gram positive fungi *Aspergillus niger*. The finding of current study demonstrates that the methanolic extract of *C. gigantea* and *C. procera* showed better activity against gram positive and gram negative organisms and fungi *Aspergillus niger*.

Soxhlet extract of *Calotropis gigantea* flowers at minimum concentration shows better activity against *E. coli*; but it shows less activity as compared to standard against *Bacillus subtilis*; its maximum concentration shows better activity against fungi *Aspergillus niger*.

Ultrasonication extract of *Calotropis gigantea* flowers shows better activity against *E. coli* at maximum concentration; but it does not shows activity superior

than standard against *Bacillus subtilis*. Its minimum concentration shows better activity against *Aspergillus niger*.

Soxhlet extract of *Calotropis procera* flowers shows activity superior to the standard at maximum concentration against *E. coli* and *Bacillus subtilis* and at minimum concentration against *Aspergillus niger*.

Ultrasonication extract of *Calotropis procera* flowers shows activity superior than standard at maximum concentration against *E. coli* but it is less effective than standard against *Bacillus subtilis* and *Aspergillus niger*.

CONCLUSION

Calotropis procera flowers extract by ultrasonication method showed better activity against *E. coli* as compared to standard and *Calotropis gigantea*. Soxhlet extract of *Calotropis gigantea* flowers shows better

activity against *Bacillus subtilis* as compared to standard and *Calotropis procera*. Soxhlet extract of *Calotropis gigantea* and *Calotropis procera* shows better activity against *Aspergillus niger* than standard.

Therefore, *C.gigantea* and *C. procera* may be considered as plant having various health benefits. These plants showed good Antimicrobial activity. The methods of the extraction were easy. Their extracts showed Antimicrobial activity against *E. coli*, *Bacillus subtilis* and *Aspergillus niger*. The extracts possessing high Antimicrobial activity should be used further for therapeutic purpose. These plants are easily available and economically affordable and have many medicinal values. Hence these plants can be used to minimize health problems and for achieving healthy life.

REFERENCES

- Jahan F, Lawrence R, Kumar V. Journal of Chemical and Pharmaceutical Research susceptible and resistant Staphylococcus aureus strains, 2011; 3(4): 777-789.
- Bhokare P, Khadke A, Kulkarni S, Kuchekar G. Study of antibacterial activity of leaf , stem , root methanolic extract from delonix regia, 2018; 7(3): 904-909.
- Dhane N, Campus YT, Havaladar V, et al. Antibacterial activity of methanolic extracts of Nyctanthes Abortristis ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACTS OF, 2016.
- Bilal H, Ali I, Uddin S, et al. Biological evaluation of antimicrobial activity of Calotropis procera against a range of bacteria, 2020; 9(1): 31-35.
- Joshi M, Kaur S. IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF CALOTROPIS PROCERA , EICHHORNIA CRASSIPES AND DATURA INNOXIA LEAVES, 2013; 6.
- Ahmad W. Preliminary phytochemical, antimicrobial and photochemical study of Calotropis gigantea leaf extract, 2020; 9: 105-112. doi:10.5267/j.ccl.2019.10.001
- Dhivya R, Manimegalai K. Preliminary Phytochemical Screening and GC- MS Profiling of Ethanolic Flower Extract of Calotropis, 2013; 2(3): 28-32.
- Science A. Phytochemical, antimicrobial and ethnobotanical study of, 2020; 10(2): 23-27.
- Wang M, Yang Q, Yan X, Wang Q, Wang J, Wang Z. CHEMICAL CONSTITUENTS OF Calotropis gigantean, 2017; 53(5): 821-822. doi:10.1007/s10600-017-2170-5
- Length F. Antimicrobial Activities of Calotropis procera on Selected Pathogenic Microorganisms, 2008; 11: 105-110.
- Parihar G, Balekar N. Calotropis procera: A phytochemical and pharmacological review, 2016; 40(3): 115-131.
- Ranjit PM. AN OVERVIEW OF PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITIES AN OVERVIEW OF PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITIES OF, 2014.
- Kumar G, Karthik L, Venkata K, Rao B. Antibacterial activity of aqueous extract of Calotropis gigantea leaves - An in vitro study, 2010.