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Evaluation Of Antimicrobial Activity Of *Calotropis Gigantea* Extracts On Two Main Skin Infection Causing Bacteria - *Escherichia Coli* And *Staphylococcus Aureus*

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ABSTRACT

Calotropis gigantea (*C. gigantea*) is a wild shrub that is a medicinal plant found in abundance india china and asia region. In this study, we investigated the phytochemical composition and antibacterial properties of the haxane and ethanolic extract of *C. gigantea*, in addition to the antimicrobial activity of the plant and its rhizospheric actinobacteria effects against pathogenic microorganisms. Each bacterial strain was subcultured overnight at 35 oC in Mueller–Hilton Agar slants. The bacterial growth was harvested using 5 ml of sterile saline water; its absorbance was adjusted at 580 nm and diluted to attain a viable cell count of 10^7 CFU/ml using a spectrophotometer, and antibacterial activity shown by the disc diffusion method was used to evaluate the antimicrobial activity of the plant extract. To obtain the final concentration, the plant extract residues are dissolved in 1 ml of ethanol and haxane, sterile through Millipore filters (0.22 m), and loaded onto a sterile filter paper disc (6 mm in diameter). 10 ml of agar medium was poured into a sterile petri plate, followed by 15 ml of seeded medium previously inoculated with bacterial suspension (100 ml of medium plus 1 ml

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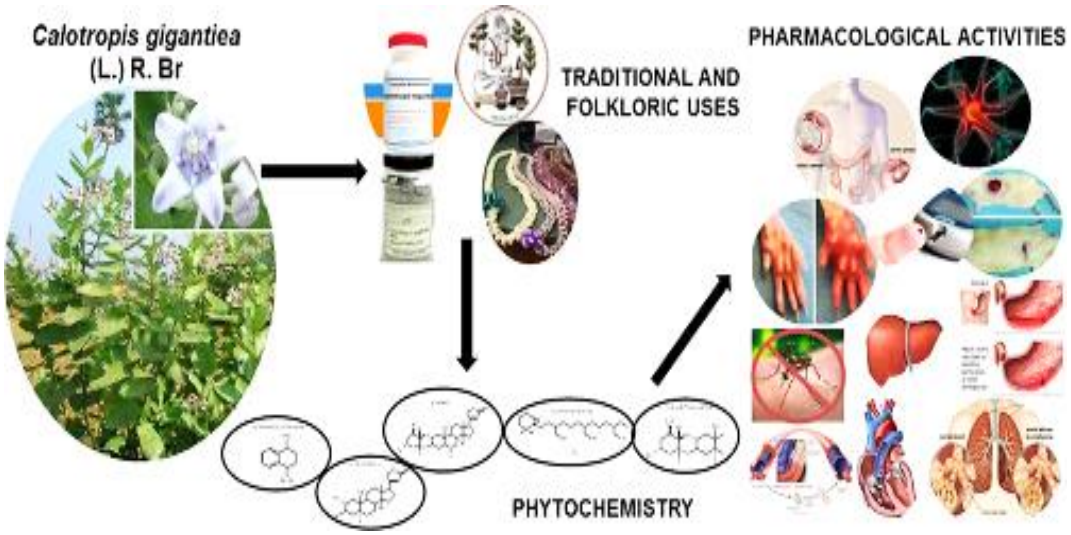
of 10^7 CFU) to achieve 10^5 CFU per ml of medium. *C. procera* plant extract's antimicrobial activity was investigated using an agar well diffusion assay and minimum inhibitory concentration (MIC) against six pathogenic microbial strains. The plant extract of *C. gigantea* was considered significantly active against *Staphylococcus aureus and Escherichia coli*, with inhibition zones of 18.66 mm, 21.26 mm, and 21.93 mm, respectively, in assays.

Keywords: Calotropis gigantea, Antibacterial activity, *E. coli*, Staphylococcus aureus.

Graphical abstract



1. Introduction

According to Ayurveda, the dried whole plant is a good tonic, expectorant, depurative, and antihelminthic. The dried root bark is a substitute for ipecacuanha. The root bark is febrifuge, antihelminthic, depurative, expectorant, and laxative. This root bark is also used in cutaneous infections, intestinal worms, helminthic infections, cough, and ascites. The powdered root is used in asthma, bronchitis, and dyspepsia, and it promotes gastric secretions. The leaves are useful for the treatment of paralysis, arthralgia, swellings, and intermittent fevers. The flowers are bitter, digestive, astringent, stomachic, antihelminthic, and tonic. Calotropis is also a reputed homoeopathic drug [1].

Herbal remedies for skin care with antibacterial and antifungal activities are prepared from a variety of plant parts, such as leaves, stems, roots, bark, or fruits. These medicines are administered topically, may be applied in the form of cream, lotion, gel, soap, sap, solvent extract, or ointment, and have been established to possess antimicrobial properties. Gels, creams, and soap formulations containing a variety of plant extracts have been used to treat various skin disorders caused by microbial infections [2].

Treatment of bacterial infections is achieved through the use of antibiotics, while fungal infections require antifungal pharmaceutical preparations such as clotrimazole solution (Buck

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et al., 1994). However, alternative treatment can be achieved by topical application of herbal extracts and herbal preparations in the form of soaps, gels, creams, and lotions. Skin infections and topical wounds require special attention as they make humans and animals prone to bacterial, fungal, and viral contaminations, thereby making them further susceptible to other types of secondary complications. The most common pathogens isolated from wounds are *Streptococcus* spp., *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus* spp., *Klebsiella*, *Enterobacter*, *Enterococci*, *Bacteroides*, *Clostridium*, *Candida*, *Peptostreptococcus*, *Fusobacterium*, and *Aeromonas*. These pathogens can seriously delay the wound healing process by disrupting the normal clotting mechanisms, promoting disordered leukocyte function and poor quality granulation tissue formation, reducing the tensile strength of connective tissue, and impairing epithelization. Medicinal plants are effective in the treatment of infectious diseases and infections of various types of external skin infections (chronic, deep suppurative, open, lacerated, incised, and ulcerated) and have been used for these purposes in humans and different species of animals. The use of medicinal plants has the added benefit of reducing many of the side effects often associated with synthetic antimicrobials [3].

The *Calotripis gigantea* plant is important in a global context today for its variety of medicinal uses and benefits. Almost every part of Madar is reported to be used for medicinal purposes, such as seeds, leaves, latex, and roots. Each of these parts of Madar has been used in traditional Indian medicine (Ayurveda and Unani systems) and is now used in the production of modern medicines, cosmetics, toiletries, and pharmaceuticals.Calotripis gigantea (Madar) is not only known for its herbal medicine use and environmental friendliness. Methanol, ethanol, aqueous, and petroleum ether extracts of the leaves of C. gigantea were reported to possess anti-Candida activity against clinical isolates of Candida albicans, Candida parapsilosis, Candida tropicalis, and C. krusei (Kumar et al., 2010a). C. gigantea latex also possesses potent fungicidal activity (Subramanian et al., 2010). Leaves and latex exhibited anti-bacterial activity (Kumar et al., 2010b; Kumar et al.,2010c). The ethanol Latex extract was active only against Staphylococcus aureus and Shigella dysenteries and did not show any significant antibacterial properties (Sarkar et al., 2013).

2.Materials and methods

2.1PLANT PROFILE:-

Kingdom: Plantae
Subkingdom: Tracheobionta,
Superdivision: Spermatophytes,
Division: Magnoliophyta,
Class: Dicotyledones ,
Sub class: Asteridae,
Series: Bicarpellatae,
Order: Gentianales,
Family: Apocynaceae,
Subfamily: Asclepidacea,
Genus: Calotropis,
Species: gigantea,

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Binomial name: Calotropis gigantea (Linn.)(Singh et al,1996

Figure.1 Calotropis gigantea

The Calotripis gigantea plant is important in a global context today for its variety of medicinal uses and benefits. Almost every part of Madar is reported to be used for medicinal purposes, such as seeds, leaves, latex, and roots. Each of these parts of Madar has been used in traditional Indian medicine (Ayurveda and Unani systems) and is now used in the production of modern medicines, cosmetics, toiletries, and pharmaceuticals. Calotripis gigantea (Madar) is not only known for its herbal medicine use and environmental friendliness [4].

2.2 Geographıcal dıstrıbutıon

It is native to India, China, and Malaysia, but it is found almost everywhere on the planet. In India, they are found chiefly in lower Bengal, the Himalaya, Punjab, Assam, Madras, and South India. These plants are common in waste land, on road sides, and on railroad embankments, ascending to about 1000 m in the Himalayas from Punjab to Assam. *Calotropis gigantea* Linn is a well-known medicinal herb commonly known as milkweed and has been used in the Unani, Ayurvedic, and Siddha systems of medicine for years. It is a native of India, China, and Malaysia, and it is distributed almost everywhere in the world. All parts of the plant have been used as medicine and as an important ingredient in a number of unani formulations used for the treatment of various ailments [5, 6].

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2.3 Descrıptıon

Macroscopic features *Calotropis gigantea* is an erect, much-branched shrub about 1–5 m tall. The roots are cylindrical, tortuous, and often branched, externally yellowish grey while internally ceramic white, and about 90 cm in length and 2.5–10 cm in diameter. The root bark is short, curved, and rarely quilled, measuring 2-5 mm thick and 3-5 cm wide, and has a distinct mucilaginous, bitter taste. The leaves are simple, opposite-decussate, subsessile, and extipulate, with blades ranging from oblong-obovate to broadly obovate and measuring 5-30 2.5-15.5 cm. Flowers are bisexual, bracteate, actinomorphic, pentamerous, hypogynous, and pedunculate; the calyx has five sepals and a lobe that is briefly united at the base; and the corolla is gamopetalous. Fruit is simple, fleshy, inflated, and subglobose to obliquely ovoid. The seed is approximately 6 5 mm in diameter, flat, and compressed with silky white pappus [7].

2.4 Chemıcal constıtuents

The chemical study of this plant has manifested the presence of cardiac glycosides, saponins, flavonoids, steroids, and terpenoids. Cardenolides, calotropin, -amyrin, -amyrin, taraxasterol, -sitosterol, -amyrin methylbutazone, -amyrin methylbutazone, -amyrin acetate, taraxasterol acetate, lupeol acetate B, gigantursenyl acetate A, gigantursenyl acetates, Flavonol glycosides such as akundarol, uscharidin, calotropin, frugoside, and calotroposides A to G are responsible for many of its activities. Chemical constituents are calactin, calotoxin, calotropagenin, proceroside, syriogenine, uscharidin, uscharin, uzarigenin, and voruscharin. Flavonoids, triterpenoids, alkaloids, steroids, glycosides, saponins, terpenes, enzymes, alcohol, resin, fatty acids, esters of calotropeol, volatile long-chain fatty acids, glycosides, and proteases have been isolated from the various parts of the plant Calotropis gigantea. [8,9].

3 Methods

3.1 Collection and authentication of plant material

The leaves of *Calotropis gigantea were* collected from waste land and roadside wild areas in Kanpur, U.P., India, in the month of October 2022. The plant identification and authentication were carried out at the Department of Botany, Christ Church College, Kanpur. After its collection and washing thoroughly with water to ensure the absence of foreign

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organic matter, fungi, and other organisms, 1 kg of total leaves were collected for the present study.

3.2 Processing of plant material

The fresh leaves of Calotropis gigantea collected were thoroughly washed with tap water and finally rinsed with distilled water. The leaves were dried on trays at 50-70 degrees Fahrenheit and powdered with an electric grinder. After collection, the plant materials were air dried for 7–15 days. The leaves were ground into a very smooth powder.

Figure2. Tray Dryer for drying plant material.

Figure 3. Powdered form *Calotropis Gigantea* leaves

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3.3 Extractıon process

3.4 Sequentıal extractıon method

After collection, the plant materials were air dried for 7–15 days. The leaves were ground into a very smooth powder. Plant powder (25 g) was extracted with 200 ml of four different solvents according to their increasing polarity, i.e., hexane, chloroform, ethyl acetate, and ethanol, in a soxhlet apparatus at 60 °C for 8 to 9 hours. The mixture was stirred at 2-hour intervals using a sterile glass rod [10]. Finally, the extract was concentrated using a rota evaporator at 40-55 oC. The concentrated extract was stored in a cool place prior to use. **(Kothari S., et al., 2011).**

4.In-vıtro antıbacterıal actıvıty

4.1 Inoculums preparatıon

Each bacterial strain was sub cultured overnight at 35ºC in Mueller –Hilton Agar slants. The bacterial growth was harvested using 5 ml of sterile saline water, its absorbance was adjusted at 580nm and diluted to attain viable cell count of 10^7 CFU/ml using spectrophotometer[11].

4.2 Antıbaceterıal actıvıty of plant extract by dısk dıffusıon method

The disc diffusion method is used to evaluate the antimicrobial activity of each plant extract. The plant extract residues dissolve in 1 ml of ethanol, which is sterilised through a Millipore filter (0.22 m) and then loaded onto a sterile filter paper disc (6 mm in diameter) to obtain the final concentration. 10 ml of agar medium was poured into a sterile petri plate, followed by 15 ml of seeded medium previously inoculated with bacterial suspension (100 ml of medium plus 1 ml of 10^7 CFU) to achieve 10^5 CFU per ml of medium. A sterile filter paper disc (6 mm) loaded with plant extract concentration is placed on top of agar plates. Gentamycin was used as a positive control. The plates were kept in the fridge at 5 oC for 2 hours to permit plant extract diffusion, then incubated at 35 oC for 24 h. inhibition in the zone as measured with vernier callipers [12].

5 Results and discussion

5.1 Extractıve value of *calotropıs gıgantea* by soxhlet method

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Sequential extraction of *Calotropis gigantea* with hexane, chloroform, ethyl acetate, and ethanol (according to polarity of solvent). The percentage yield was found to be as follows-

Table .1 Percentage yield of different extracts of Argemone mexicana *and calotropis gigantea*

S.No		Extracts	Extractive value(%) of *Calotropis Gigantea*
1.		Hexane	12%
2.		Chloroform	14%
3.		Ethyl acetate	10%
4.		Ethanol	**16%**

The highest percentage yield obtained with due to first sequence extract by different solvent was shown in table ,Extractive value of ethanol (14.%) was maximum comparison to other solvent. *Calotropis gigantea* extract was follow by sequential solvents use and the extractive value of ethanol (16%) was maximum in comparison to other solvents[13].

5.2 Phytochemıcal screenıng of majour phyto-constıtuents of *argemonemexıcana* and *calotropıs gıgantea* plant leaf extracts

Table .2 Test of Alkaloids

S.No	ALKALOIDS TEST NAME	HEXANE		CHLOROFORM		ETHYL ACETATE		ETHANOL	
		A. Mexicana	**C. Gigantea.**	**A. Mexicana**	**C. Gigantea.**	**A. Mexicana**	**C. Gigantea.**	**A. Mexicana**	**C. Gigantea.**
1.	**Dragendroff's test**	+	++	+	+	++	++	++	++
2.	**Mayer test**	+	+	+	+	-	+	+	+
3.	**Hager test**	+	++	+	++	++	++	++	++

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4.	**Wagner test**	+	++	+	++	++	++	++	++

(-) Absent (+) Present (++)

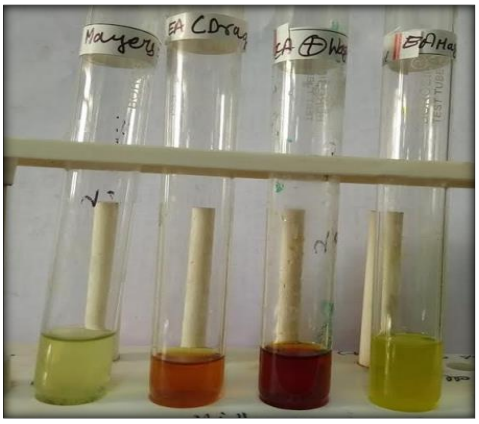
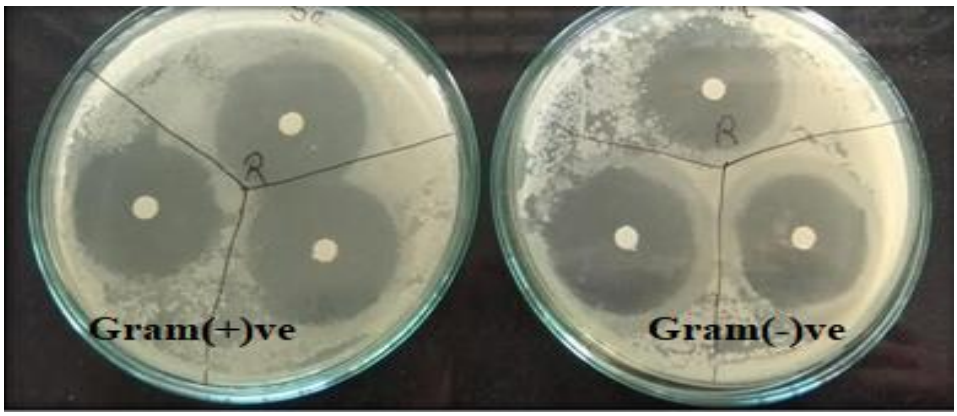
(a)  (b) 

Figure .4(a) and (b) Test of Alkaloids: *Calotropis gigantea* plant

All the tests viz. Dragendroff's, Mayer, Hager and Wagner tests, perform for the detection of alkaloids , plant extract in solvents Hexane ,chloroform Ethyl-acetate and ethanol gives positive and negative result; Hager test was found more intensive for Ethanolic extract , then ethyl acetate extract . And *Calotropis gigantea* plants leaves in more intensive alkaloids found DuttaM. et al 2014, phytochemical analysis confirm the presence of alkaloids, in both plant extract of ethanol. The satisfactory result of present investigation was fetched out of number of physical and chemical parameters.

5.3 In -vitro antibacterial activity



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Figure–5 Antibacterial activity of reference (Gentamycin) zone of inhibition in GRAM +ve (*Staphlococcus aureus)* and GRAM_ve (*Escherichia coli*) bacteria

5.4 Antıbacterıal actıvıty of plants extract

The disc diffusion method is used to evaluate the antibacterial activity of each plant extract. The plant extract residues (100 mg and 50 mg) were re-dissolved in 1 ml of DMSO and loaded over sterile filter paper discs (8 mm in diameter) to obtain a final concentration of 300 g/disc and 150 g/disk. Ten millilitres of Mueller-Hilton agar medium were poured into sterile Petri dishes (as a basal layer), followed by 15 millilitres of seeded medium previously inoculated with bacterial suspension. Sterile filter paper discs loaded with plant extract concentrations of 100 mg/ml and 50 mg/ml were placed on top of Mueller-Hilton agar plates. Filter paper discs loaded with 5 mg of Gentamycin were used as references. The plates were kept in the fridge at 5 °C for 2 hours to permit plant extract diffusion, then incubated at 35 °C for 24 hours. The presence of inhibition zones was measured by Vernier callipers, recorded, and considered an indication of antibacterial activity.

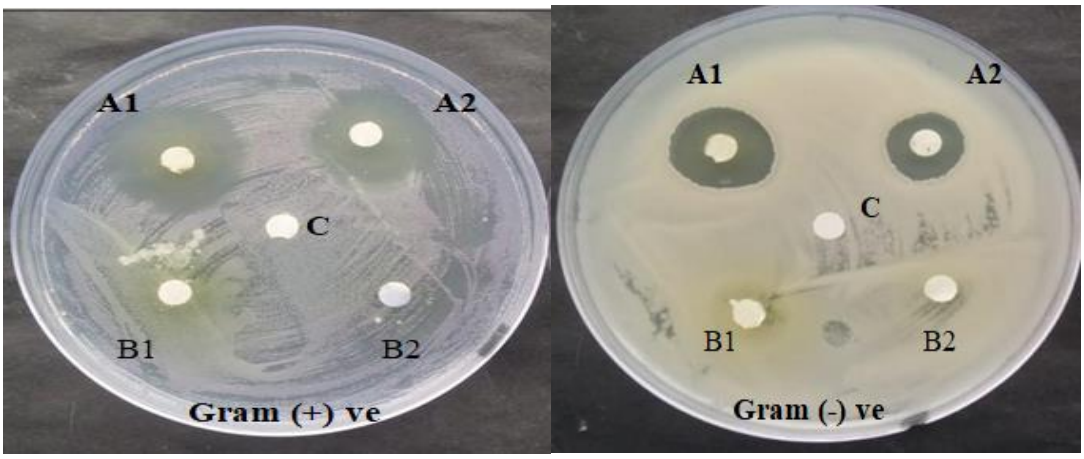

Figure 6,7. Zone of inhibition of *Calotropis gigantea* ethanol extract (A1 & A2) and *Calotropis gigantea* (B1& B2) haxane extract in S.aureous Gram +ve. And E.coli Gram-ve

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Table 3. Zone of inhibition of antibacterial study

S.No	Sample	Zone of inhibition							
		Concentrations							
		180µg/µl		25µg/µl		50µg/µl		100µg/ µl	
		5 µg/disc		75 µg/disc		150 µg/disc		300 µg/disc	
		Gram +ve	Gram-ve	Gram+ve	Gram-ve	Gram+ve	Gram-ve	Gram+ve	Gram-ve
		S. aureus	*E.coli*	*S. aureus*	*E.coli*	*S. aureus*	*E.coli*	*S. aureus*	*E.coli*
1.	Gentamycin	35 ± 0.7	34 ± 0.5	-	-	-	-	-	-
2.	*C.gigantean a (Ethanol)*	-	-	6±0.5	6±0.5	15 ± 1.07	12 ± 0.2	25 ± 1.04	20 ± 0.5
3.	*C. gigantea(ha xane)*	-	-	-	-	-	-	-	-
4.	Control(DM SO)	-	-	-	-	-	-	-	-

6 Conclusions

In the present study, an antibacterial assay of Calotropis gigentica against E.*oli and S. aureus* bacteria was done. The results were assessed by measuring the presence or absence of inhibition zones at various concentrations.

In our study, an ethanolic extract of *C. gigantea* was used, and it was found to be more efficient than other extracts. This might be due to the polar nature of the solvent, ethanol, which resulted in the leaching of more active ingredients during the extraction of antimicrobial active substances from Calotropis compared to other solvents.

Plants are an imperative source of potentially precious structures for the development of novel chemotherapeutic agents. Extracts from these plants have long been used to treat a wide range of infectious diseases, including those caused by bacteria, fungi, protozoa, and viruses.*Calotropis gigantea* is one such plant found widespread in most of the agricultural and nonagricultural fields, and the practise of this plant for medicinal purposes has been documented by various researchers.

Extracts of plants and phytochemicals are identified as having antimicrobial properties; these can be of immense significance in therapeutic treatments. Over the last decade, a number of studies have been conducted all around the world to demonstrate such efficiency. Various plants have been tried because of their antimicrobial traits, which are due to compounds synthesised in the secondary metabolism of the plant. Plant extracts have been found to have high antimicrobial potential against a wide range of microorganisms. [14,15].

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Declarations

Conflict of Interest -The authors declare no potential conflicts of interest.

Ethical Approval -This Article does not contain any studies with human participants or animals performed by the author.

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