IJPSR (2020), Volume 11, Issue 7 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 August 2019; received in revised form, 16 December 2019; accepted, 18 April 2020; published 01 July 2020

COMPARATIVE PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *CALOTROPIS* SP. OF ETHNOMEDICINAL SIGNIFICANCE

Rasmita Sethy and Bandana Kullu *

Department of Botany, Utkal University, Bhubaneswar - 751004, Odisha, India.

Keywords:

Calotropis procera, Calotropis gigantea, Phytochemical screening, Anti-microbial activity, MIC, MBC

Correspondence to Author:
Dr. Bandana Kullu

Assistant Professor,
Department of Botany, Utkal University, Bhubaneswar - 751004, Odisha, India.

E-mail: bandkullu@gmail.com

ABSTRACT: The global prevalence of infectious diseases caused by microorganisms is a major public health concern worldwide. The emergence of antibiotic resistance and related toxicity issues are limiting the use of antibiotics and prompting research for alternative therapy. Plant-derived drugs are gaining popularity due to their efficacy and safety. *Calotropis procera* and *Calotropis gigantea* are two common species in the genus *Calotropis*, well known for their ethnomedicinal values. Present study comparatively evaluated the phytochemical screening and antimicrobial activity of *C. procera* and *C. gigantea*. The qualitative phytochemical screening of the ethanolic, methanolic, and aqueous extracts of *Calotropis* sp. indicated similar phyto-constituents of both the species. Secondary metabolites such as alkaloids, terpenoids, flavonoids, glycosides, cardiac glycosides, coumarin, steroid, phenol, tannin, saponin, and volatile oils were detected in both the plant extracts and ethanolic extracts contained the highest number of the plant metabolites. The plant extracts showed antimicrobial activity against Gram +ve and –ve human pathogenic bacteria, indicating their broad-spectrum activity. Comparatively, *C. procera* was more effective against the tested bacterial pathogens with lower MIC and MBC value than *C. gigantea.* The study suggested that aerial plant parts extracts of *Calotropis* sp. would provide therapeutic phytochemicals as anti-microbial agents and can serve as a viable alternative source of bioactive compounds with pharmaceutical relevance.

INTRODUCTION: Infectious diseases are the cause of mortality and morbidity, accounting for more than about 22% of the global disease burden [1]. Discovery of antibiotics, the wonder drug in the 20th century, is one among the significant achievements in medical science against infectious diseases.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(7).3243-51

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(7).3243-51

However, the indiscriminate use of antibiotics has led to the development of antibiotic-resistant bacteria population at alarming frequency [2, 3]. Additionally, antibiotics are also known to cause adverse side effects on the host [4]. In the current scenario, antibiotic resistance has increased substantially and is developing as an ever-increasing therapeutic challenge. Thus, alternative therapy is the need of the time. Plants are the basis of traditional medicine which have been in use since ancient time and are continuing to provide new remedies to mankind [5]. Hence, screening of safe and potent natural antimicrobial agents from plants is increasing throughout the world [6, 7].

Recently for the development of alternative therapy workers emphasized the control of antibiotic-resistant bacterial pathogens using phytochemicals [8, 9].

Calotropis a genus of flowering plants in the family Asclepiadaceae, is one among the native plants of India. Geographically it is widely distributed in southern Asia, northern Africa and northeastern South America. *Calotropis procera* (Ait.) R. Br. and *Calotropis gigantea* R. Br. are the two common species in the genus. Morphologically both *C. procera* (1-4 m) and *C. gigantea* (> 4.5 m) are erect shrubs with thick, sub-sessile, obovate leaves having a cordate base. The stems and leaves have a waxy appearance and contain milky latex. Flowers are 5 lobed, medium-sized, born on dense, multi-flowered, umbellate cyme arising from the nodes and appearing axillary or terminal. Corolla-lobes (1.2-1.5 cm) in *C. procera* is erect, white-colored with purple blotches on the upper half and are fragrant, whereas in *C. gigantea* corolla-lobes (3-3.5 cm) are spreading, uniformly greenish-white or bluish-purple without any fragrance.

The *Calotropis* sp. has been widely used in the Ayurvedic, Unani, Arabic, and Sudanese -Indian traditional system of medicine for the treatment of various ailments. In ancient ayurvedic medicine, the plant *Calotropis procera* was known as "Sweta Arka" and *Calotropis gigantea* as "Shyma Arka". Both the species are often similar in their botanical aspects and also have similar pharmacological effects. The latex of the *C. procera* has been shown wound healing activity [10, 11], protection against gastric ulcer [12], anti-inflammatory [13,] and antimicrobial activity [14]. The flower of *C. procera* has hepatoprotective activity [15], antipyretic, analgesic, and larvicidal activity [16, 17]. Many of the workers have reported antimicrobial activity of *C. procera;* however, similar reports on *C. gigantea* is scarce. Thus, the present investigation was aimed at comparative phytochemical screening and assessment of the antimicrobial activity of *C. procera* and *C. gigantea* against some human pathogenic bacterial strains.

MATERIALS AND METHODS:

Plant Material: The aerial parts (leaves and young stem) of naturally grown (8-10 years old) *C. procera* and *C. gigantea* were used as plant material for phytochemical extraction. The *C. procera* (Bot-10575) and *C. gigantea* (Bot-10574) plant materials were collected from R. K. University Anantapuram, Andhra Pradesh (India), and Utkal University campus, Odisha (India) respectively, and herbarium was submitted to the Department of Botany, Utkal University. The collected aerial parts were cleaned under running tap water (15 min) and shade dried at room temperature for 5-7 days. Coarse powder of the dried plant material was prepared mechanically using mortar and pestle.

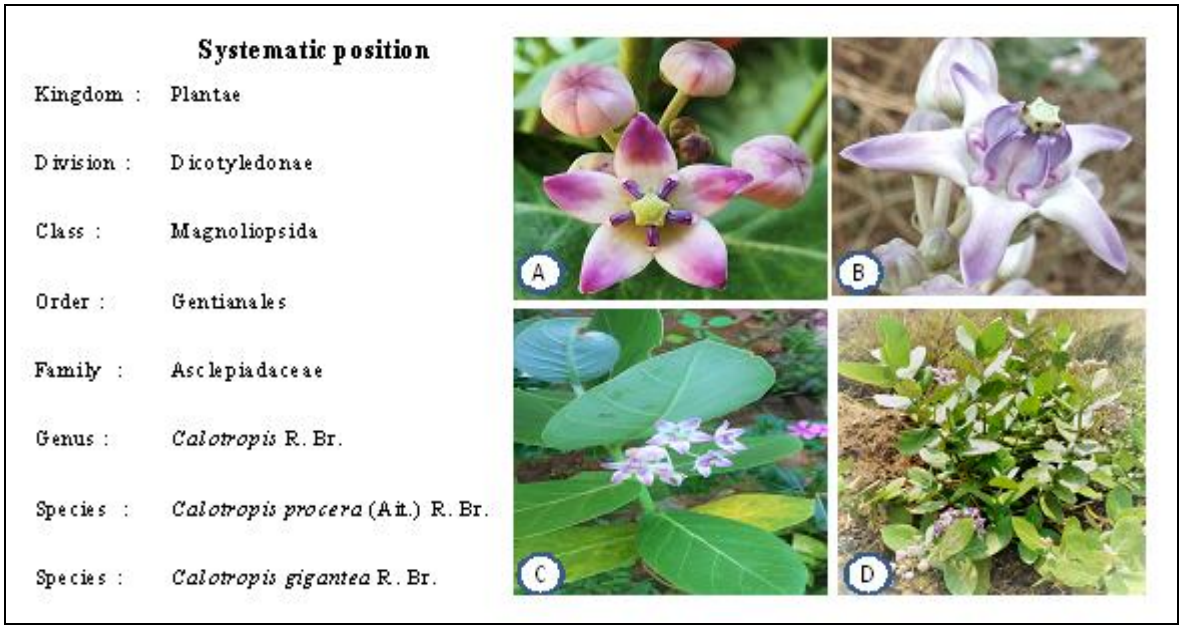

FIG. 1: FLORAL MORPHOLOGY OF *CALOTROPIS PROCERA* (A) & *CALOTROPIS GIGANTEA* (B). A FLOWERING TWIG OF *CALOTROPIS PROCERA* (C) & *CALOTROPIS GIGANTEA* (D)

Phytochemical Extraction: Phytochemical extraction was done in the Soxhlet apparatus by a hot continuous extraction method for 48 h [18]. Three different solvent systems in the order of increasing polarity such as ethanol (80%), methanol (80%) and distilled water (aqueous) were used sequentially for the extraction process. The extracts so obtained in each solvent were filtered separately using filter paper (Whatman no. 1) and the filtrates were concentrated at 40 °C using a water bath till sticky residue remained in the bottom of the flask. The residue was finally air-dried and stored at 4 °C until bioassay. The stock solutions of the extracts were prepared by dissolving 10mg of dried extract in 10ml of DMSO to get the concentration of 1 mg ml^{-1} for further experiments.

Phytochemical Screening: The ethanolic, methanolic and aqueous extracts of *C. procera* and *C. gigantea* were subjected to qualitative phytochemical screening for phytochemicals such as carbohydrates (Molisch's test), protein (Biuret test), an amino acid (Ninhydrin test), an alkaloid (Wagner and Dragandreff's test), terpenoids, flavonoids, glycosides, cardiac glycosides, steroid, phenol, tannin, saponin, and volatile oils [19].

Test for Carbohydrates: To the 2 ml of extract, 2 drops of Molisch's reagent was added and mixed well. Subsequently 2 ml of conc. H_2SO_4 was added from the sides of the test tube. The appearance of a reddish violet color ring immediately at the junction of two liquid layers indicated the presence of carbohydrates.

Test for Proteins: Plant extract (2ml) was treated with 2ml of Biuret reagent. The formation of the violet color ring indicated the presence of peptide linkages of the protein molecule.

Test for Amino Acids: Ninhydrin reagent (2ml) was added to 2 ml of crude extract and the solution was kept in a hot water bath for 20 minutes. The appearance of purple color indicated the presence of amino acids.

Test for Alkaloids: The extract (2ml) was acidified by dilution with 1% HCl. The acid layer was used for testing the alkaloids. The acid layer was treated with a few drops of Mayer's reagent. The formation of creamy white precipitation indicates the presence of alkaloids.

Test for Terpenoids: To the 2 ml of extract, 2 ml of chloroform and 3 ml of conc. H_2SO_4 was added. The reddish-brown coloration at the interface indicated the presence of terpenoids.

Test for Flavonoids: To the crude extract 5ml of dilute ammonia solution and conc. H_2So_4 was added. A yellow coloration confirms the presence of flavonoids, which disappears on standing.

Test for Glycoside: To the 2 ml of extract, 1-2 ml of ammonium hydroxide was added and shaken well. The appearance of cherish red color indicates the presence of glycosides.

Test for Cardiac Glycosides: To the 5ml of extract, 2 ml of glacial acetic acid and one drop of ferric chloride solution was added. The layer formed was under-layed with 1ml of con. H_2SO_4. A brown ring of the interface indicated a deoxy sugar characteristic of cardenolides. A violet ring might appear below the brown ring, whereas in the acetic acid layer, a greenish ring might form just gradually throughout the thin layer.

Test for Steroids: Crude plant extract was mixed with 2 ml of chloroform and conc. H_2SO_4 was added sidewise. The appearance of red color in the lower chloroform layer indicated the presence of steroids.

Test for Phenols: To the 2 ml of extract, 3 ml of ethanol and a pinch of $FeCl_3$ was added. The formation of greenish-yellow color indicated the presence of phenols.

Test for Coumarins: Extract was dissolved in hot water. After cooling, the solution was divided in two test tubes. To one of the test tube, 10% (w/v) ammonium hydroxide was added, and the other test tube was used as control. The fluorescent color indicated the presence of coumarins.

Test for Tannins: To the 5ml of extract, a few drops of 1% of lead acetate were added. The appearance of a yellow precipitate indicated the presence of tannins.

Test for Saponins: The extract was diluted with 20 ml of distilled water and agitated in a graduated test-tube for 15 min. The formation of a 1cm thick foam layer indicated the presence of saponins.

Test for Volatile Oil: To the 2 ml of extract, 0.1 ml of dilute sodium hydroxide and few drops of diluted HCl were added. The formation of a white precipitate indicated the presence of volatile oils.

Antimicrobial Activity:

Bacterial Strains and Culture Media: For the assessment of antimicrobial activity of plant extracts, two Gram-positive human pathogenic bacteria such as *Streptococcus mutans* (MTCC-497^T) and *Bacillus circulans* (MTCC-490^T) and two Gram-negative bacteria such as *Salmonella enteric typhimurium* (MTCC-98), and *Vibrio cholera* (MTCC-3906) were procured from Institute of Microbial Technology (IMTECH) Chandigarh, India. All the bacterial strains were freshly cultured in nutrient broth according to the standard microbiological method [20].

Working Solution of Extracts and Standard Drug: The stock solution (1mg ml^{-1}) of ethanolic, methanolic and aqueous extracts was serially diluted with DMSO to the working solution concentration of 250 µgml^{-1}. The antibiotic Ciprofloxacin (250 µgml^{-1}) was used as a standard drug against the test of bacterial strains.

Agar Well Diffusion Assay: The agar well diffusion assay [21] was carried out to determine the growth inhibition of bacteria by plant extracts. The nutrient broth (Hi-Media, India) was used for broth culture and nutrient agar (Hi-Media, India) was used for the preparation of agar Petri plates (90 mm diameter). The bacterial suspensions were standardized by adjusting the optical density to 0.1 at 600 nm [22] in the UV-VIS spectrophotometer to get a cell suspension of about 1.5×10^8 CFU/ml. The bacterial inoculum of 100µl was seeded on the surface of the sterile agar plate aseptically and distributed evenly using sterilized glass spreader. Wells of 6.0mm diameter each were prepared on the inoculated plate by sterilized cork borer. Into the agar wells, 50µl of plant extracts and standard drug (250 µg ml^{-1}) were introduced and allowed to stand for 10 minutes for diffusion. Thereafter the plates were incubated at 37 °C for 24 h and examined for zones of inhibition (ZI). The diameter of the zone of inhibition formed by each agent was measured using an Antibiotic Zone Scale (Hi-Media) and the values recorded were expressed to the nearest millimeter. The average diameter of the zone of inhibition of more than 10 mm was considered as effective. Each extract was replicated four times.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): Minimum inhibitory concentration (MIC) was determined for those extracts that showed high efficacy (ZI ≥ 10mm) against microorganisms by the agar well diffusion assay. Ciprofloxacin was used as a reference drug. MIC is the lowest concentration of antimicrobial agent that inhibits visible growth of the microbes, was determined by microbroth dilution test [23]. Working solutions of extracts for MIC and MBC were achieved by two-fold serial dilution with DMSO to obtained different concentrations ranging from 1000µg ml^{-1} to 3.9 µg ml^{-1} and 1500 µg ml^{-1} to 1.46 µg ml^{-1}.

Each test tube was filled with 5 ml of nutrient broth, 10µl of bacterial suspension and 50µl of antimicrobial agents of different concentrations. Positive control contains inoculums with no antimicrobial agents whereas, DMSO used as a negative control with no microbes. The test tubes were incubated at 37 °C for 16-20 h. After incubation MIC was determined to be where growth was no longer visible by assessment of turbidity with optical density readings at 595 nm with a Beckman DU-70 UV-Vis Spectrophotometer.

The minimum bactericidal concentration (MBC) was defined as the lowest concentration of antimicrobial agent at which 99.9% of the bacteria were killed [24]. MBC was determined by transferring 20µl of each culture medium from the test tube (with no visible growth) from the MIC assay and thereafter inoculating into nutrient agar plates. The inoculated plates were then incubated aerobically at 37 °C for 16–20 h for any colony-forming unit (CFU). The concentration of extract where no CFU appeared on petriplate is considered as MBC.

RESULTS AND DISCUSSION:

Phytochemical Screening: The qualitative phytochemical screening of ethanolic, methanolic and aqueous extracts of *C. procera* and *C. gigantea* revealed the presence of 14 pharmaceutically active plant metabolites namely carbohydrates, protein,

amino acid, alkaloids, terpenoids, flavonoids, glycosides, cardiac glycosides, coumarin, steroid, phenol, tannin, saponin and volatile oils **Table 1**. The primary plant metabolites such as carbohydrates, protein and amino acid were detected in all the three extracts such as ethanolic, methanolic and aqueous extracts of both *C. procera* and *C. gigantea.* Among the secondary plant metabolites, glycosides and cardiac glycosides were detected in all the three extracts of both the *Calotropis* sp. The other secondary metabolites such as alkaloid, flavonoids, coumarin, steroids, phenols, and tannins were detected in ethanolic and methanolic extracts but were not detected in aqueous extracts of both the species. The terpenoids, saponin and volatile oils were detected only in ethanolic extracts.

Extraction is a critical step for recovering and isolating phytochemicals from plant materials. Extraction efficiency is affected by the chemical nature of phytochemicals, the extraction method used, sample particle size, the solvent used, as well as the presence of interfering substances [25]. For the extraction of biomolecules from plants, the solvents used are chosen on the basis of the polarity of the solute of interest. A solvent of similar polarity to the solute may properly dissolve the solute. Multiple solvents can be used sequentially to limit the amount of analogous compounds in the desired yield [26].

According to previous reports, methanol and ethanol can dissolve polar compounds, such as sugar, amino acid, glycoside [27], phenolic compounds with low and medium molecular weights having medium polarity [28], flavonoid [29], terpenoid, saponin and tannin [30]. Ethanol has been known as a good solvent for phenol extraction, whereas methanol has been generally found to be more efficient in the extraction of lower molecular weight phenols [31].

TABLE 1: COMPARATIVE PHYTOCHEMICAL SCREENING OF *C. PROCERA* AND *C. GIGANTEA*

S. no.	Phytochemicals	Ethanolic extract		Methanolic extract		Aqueous extract	
		C. procera	*C. gigantea*	*C. procera*	*C. gigantea*	*C. procera*	*C. gigantea*
1	Carbohydrates	+	+	+	+	+	+
2	Protein	+	+	+	+	+	+
3	Amino acid	+	+	+	+	+	+
4	Flavinoids	+	+	+	+	-	-
5	Alkaloids	+	+	+	+	-	-
6	Terpenoids	+	+	-	-	-	-
7	Glycosides	+	+	+	+	+	+
8	Cardiac Glycosides	+	+	+	+	+	+
9	Coumarin	+	+	+	+	-	-
10	Steroids	+	+	+	+	-	-
11	Phenols	+	+	+	+	-	-
12	Tannin	+	+	+	-	-	-
13	Saponin	+	+	-	-	-	-
14	Volatile oils	+	+	-	-	-	-

[(+) indicates presence, (-) indicate absence]

Based on preliminary phytochemical assay it can be concluded that high molecular weight compounds were extracted through high polarity solvent like water, whereas small and medium molecular weight compounds extract through less polar solvents like methanol and ethanol. Generally, most of the primary and secondary metabolites in plant samples are polar in nature with characteristic small to medium molecular weight; thus most of the phytochemicals of *Calotropis* sp. were extracted in ethanol and methanol. Further, ethanol (80%) was used as a least polar solvent in the first step of the extraction process; thus, ethanolic extract of *C. procera* and *C. gigantea* contained a majority of the secondary metabolites present in their aerial plant parts. Qualitative phytochemical analysis indicated that the phytochemical constituents of *C. procera* are similar to that of *C. gigantea.*

Antimicrobial Activity: For the qualitative evaluation of the antimicrobial activity of *C. procera* and *C. gigantea*, the ethanolic, methanolic and aqueous extracts (250 µg ml^{-1}) were tested against two Gram +ve and two Gram -ve human pathogenic bacterial strains and antimicrobial

activity was compared with that of standard drug Ciprofloxacin (250 µg ml^{-1}). The antimicrobial activity was assessed in terms of zone of inhibition (ZI) formed by agar well diffusion assay and the findings were presented in **Table 2**. The study revealed variable antimicrobial activities of the plant extracts against the tested bacterial strains. It was recorded that all the three extracts of *C. procera* and *C. gigantea* showed highest anti-bacterial activity against *S. enterica typhimurium* and *V. cholera*, followed by *B. circulans.* The least antimicrobial activity of the plant extracts was against the bacterial strains *S. mutans*. Comparatively, *C. procera* extracts were showing higher antimicrobial activity than *C. gigantea.* The antimicrobial efficiency of the plant extracts was in the order of ethanolic > aqueous > methanolic extracts.

TABLE 2: COMPARATIVE ANTI-BACTERIAL ACTIVITY* ASSESSMENT OF ETHANOLIC, METHANOLIC AND AQUEOUS EXTRACTS OF *C. PROCERA* AND *C. GIGANTEA* AGAINST HUMAN PATHOGENIC BACTERIAL STRAINS BY AGAR WELL DIFFUSION ASSAY

Bacterial strains	Reference antibiotic	Ethanolic extract		Methanolic extract		Aqueous extract	
	Ciprofloxacin ZI (mm)	*C. procera* ZI (mm)	*C. gigantea* ZI (mm)	*C. procera* ZI (mm)	*C. gigantea* ZI (mm)	*C. procera* ZI (mm)	*C. gigantea* ZI (mm)
S. mutans	26.5±0.64^{b}	12.3±0.62^{c}	9.8±0.25^{c}	10.3±0.47^{c}	10.3±0.25^{c}	12.3±0.47^{d}	11.5±0.64^{d}
B. circulans	24.5±0.86^{c}	13.3±0.62^{b}	12.8±0.47^{b}	14.0±0.40^{b}	12.0±0.47^{b}	15.0±0.64^{c}	12.8±0.47^{c}
S. enteric typhimurium	27.0±0.91^{a}	18.3±0.62^{a}	16.0±0.91^{a}	15.0±0.40^{a}	13.3±0.40^{a}	17.3±0.94^{a}	17.0±0.40^{a}
V. cholera	27.8±0.47^{a}	18.0±0.40^{a}	16.3±0.75^{a}	15.3±0.62^{a}	13.5±0.64^{a}	16.3±0.62^{b}	15.5±0.40^{b}

(Mean ± SDE) pooled from 4 independent experiments (n=4) * determined as Zone of Inhibition: ZI (mm). Mean values within the column with the same superscript alphabets are not significantly different ($p \leq 0.05$) using Duncan's new multiple range test.

The antimicrobial activity of *C. procera* and *C. gigantea* was quantitatively evaluated in terms of MIC and MBC **Table 3**. The concentration range of MIC and MBC for different plant extracts varied from 46.87 - 375 µgml^{-1}. For an ethanolic and aqueous extract of *C. procera* the lowest value of MIC (46.87 µgml^{-1}) was against *S. enteric typhimurium* and *V. cholera*. However, lowest value of MBC for ethanolic extract was against *S. enteric typhimurium* (46.87 µgml^{-1}) and that of aqueous extract *was* against *V. cholera* (93.75 µgml^{-1}).

For a methanolic extract of *C. procera* lowest value of MIC and MBC was against *V. cholera* (62.5 µgml^{-1}). For *C. gigantea* lowest value of MIC and MBC of ethanolic extract (62.5 µgml^{-1}) and methanolic extract (93.75 µgml^{-1}) was against both *S. enteric typhimurium* and *V. cholera*. For aqueous extract of *C. gigantea* lowest value of MIC (46.87 µgml^{-1}) and MBC (93.75 µgml^{-1}) was against *V. cholera.*

The study suggested that ethanolic and aqueous extracts were having lower MIC value than methanolic extracts of *C. procera* and *C. gigantea* against the tested bacterial pathogens. Comparatively, the extracts of *C. procera* were more efficient in bacterial growth inhibition than *C. gigantea* in terms of MIC and MBC. The bacterial pathogens *S. enterica typhimurium* and *V. cholera* were highly sensitive to the plant extracts of *C. procera* and *C. gigantea*. The MBC ≤ 125µgml^{-1} was considered to be very active, and all the extracts were very active against *S. enterica typhimurium*, *V. cholera* and *B. circulans.* However, compared to the reference antibiotic, the extracts of *C. procera* and *C. gigantea* have much higher MIC and MBC values.

TABLE 3: MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF ETHANOLIC, METHANOLIC AND AQUEOUS EXTRACTS OF *C. PROCERA* AND *C. GIGANTEA* AGAINST HUMAN PATHOGENIC BACTERIAL STRAINS

Bacterial strain	Reference antibiotic (µg ml^{-1})		Ethanolic extracts (µg ml^{-1})				Methanolic extracts (µg ml^{-1})				Aqueous extracts (µg ml^{-1})			
			C. procera		*C. gigantea*		*C. procera*		*C. gigantea*		*C. procera*		*C. gigantean*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S.m	11.71	11.71	125.0	125.0	187.5	375.0	187.5	375.0	187.5	375.0	187.5	187.5	187.5	187.5
B.c	7.81	15.62	125.0	125.0	125.0	125.0	93.75	125.0	125.0	187.5	62.5	125.0	93.75	125.0
S.et	3.9	7.81	46.87	46.87	62.5	62.5	62.5	93.75	93.75	125.0	46.87	93.75	62.5	125.0
V.c	7.81	7.81	46.87	93.75	62.5	62.5	62.5	62.5	93.75	93.75	46.87	93.75	46.87	93.75

S. m: Streptococcus mutans, B. c: Bacillus circulans, S. et: Salmonella enterica typhimurium, V. c: Vibrio cholerae
Data pooled out from 4 independent experiments (n=4); extracts with values in bold font considered as very active (MBC ≤125 µg/ml)

The development of antimicrobial drugs from higher plants plays a central role in the healthcare system over the world [32, 33]. There has been an increased interest in the study of medicinal plant extracts as a potential source of new antimicrobial agents. The discovery of such new antimicrobial drugs makes an important field of research, as there is an increase in resistance to existing antibiotics by several pathogenic bacteria [34]. The present study revealed that two Gram-negative bacterial pathogens, such as *Salmonella enterica typhimurium* and *Vibrio cholera* were highly sensitive to extracts of *C. procera* and *C. gigantea*. The gram-negative bacterial pathogen *S. enterica typhimurium* causes infection in the intestinal tract, typhoid fever, food poisoning and gastroenteritis. Whereas, the *V. cholera* causes serious diseases like cholera.

The Gram-positive bacteria *Bacillus circulans*, an opportunistic pathogen known to cause sepsis, wound infection, bacteremia, abscesses and meningitis in immune-compromised persons [35, 36] was also sensitive to all the extracts of *C. procera* and *C. gigantea*. However, the *Streptococcus mutans*, a Gram-positive pathogen reported to cause dental caries [37] is not much sensitive to the plant extracts compared to the other tested pathogens. These findings indicated broad-spectrum antimicrobial activity of the plant extracts against both Gram-positive and Gram-negative bacteria, particularly very effective against Gram-negative enteric pathogens.

Drugs derived from plants are generally secondary metabolites and their derivatives [38]. From the phytochemical screening, it was clear that the *Calotropis* sp. contained numerous bioactive compounds, including alkaloids, steroids, coumarin, flavonoids, glycosides, tannins and saponins. These secondary metabolites act as a defense mechanism against several micro-organisms, insects and herbivorous [39]. Phenolic and flavonoids contents in various fruits and vegetables are reported to help immune-modulator organs for killing microorganisms [40]. Tannins are polymer of phenolic compounds, found in *Calotropis* sp. have natural antifungal and antibacterial properties [41, 42]. Thus, the antimicrobial activity of the crude ethanolic, methanolic and aqueous extracts of *Calotropis* sp. was due the presence of secondary metabolites.

As the plant extracts were crude, which contains active ingredients responsible for antimicrobial activity along with other phytochemicals, the MIC and MBC values of plant extracts were much higher than the highly purified reference antibiotic. Thus, the isolation, purification and identification of active principles of crude extracts can lead to novel drug development from *Calotropis* sp. Further, both *in-vitro* and *in-vivo* research is required to evaluate the biological effects of such formulated drugs before application in clinical use.

CONCLUSION: The present investigation supports and validates the antimicrobial aspect of two important ethnomedicinal species *C. procera* and *C. gigantea* of the genus *Calotropis*. The comparative phytochemical screening showed similar phytoconstituents of *C. procera* and *C. gigantea*. Several secondary plant metabolites have been qualitatively detected in crude extracts of the plants. The antimicrobial assay indicated higher antimicrobial activity of *C. procera* than *C. gigantea* terms of larger zone of inhibition and lower MIC/MBC values. The secondary metabolites detected in the crude extracts of *Calotropis* sp. responsible for the antimicrobial activity could be a viable alternative source of bioactive compounds with pharmaceutical relevance for the control of antibiotic-resistant human pathogenic bacteria.

ACKNOWLEDGEMENT: Authors acknowledge the University Grant Commission (UGC), New Delhi, for financial support to RS in the form of Rajiv Gandhi National Fellowship (RGNF). Authors thank Dr. T. Ravi Kumar, Department of Botany, R. K. University, Anantapuram, Andhra Pradesh, India, for providing *C. procera* plant samples.

CONFLICTS OF INTEREST: The authors declare that there are no potential conflicts of interest.

REFERENCES:

1. Murray CJ and Lopez AD: Alternative projections of mortality and disability by cause 1990-2020. Global Burden of Disease Study Lancet 1997; 349: 1498-04.
2. Susanne AK, Arthi R and Gabriel GP: Antibiotic pollution in the environment: from microbial ecology to public policy. Microorganisms 2019; 7(6): 180.

3. Nair R and Chand S: Antimicrobial activity of *Punica granatum* exhibited in different solvents. Pharmaceutical Biology 2005; 43: 21-25.
4. Richard JF and Yitzhak T: Antibiotics and bacterial resistance in the 21st Century. Perspective in Medicinal Chemistry 2014; 6: 25-64.
5. Teixeira F M, Ramos MV, Soares AA, Oliveira RSB, Almeida-Filho LCP, Oliveira JS, Marinho-Filho JDB and Carvalho CPS: *In-vitro* tissue culture of the medicinal shrub *Caloptropis procera* to produce pharmacologically active proteins from plant latex. Process Biochemistry 2011; 46: 118-24.
6. Cohen ML: Changing patterns of infectious disease. Nature 2002; 406: 762-67.
7. Hancock EW: Mechanism of action of newer antibiotics for Gram positive pathogens. Lancet Infectious Diseases 2005; 5: 209-18.
8. Mallik T, Yadav AS and Shabir AL: Antibacterial activity of ethylacetate extract of leaves of *Clerodendrum serratum* linn, against pathogenic bacterial strains. International Journal of Recent Scientific Research 2018; 3(A): 24653-56.
9. Lillehoj H, Liu Y, Calsamiglia S, Miyakawa MEF, Chi F, Cravens RL, Oh S and Gay CG: Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Vetenary Research 2018; 49: 76.
10. Aderounmu AO, Omonisi AE, Akingbasote JA, Makanjuola M, Bejide RA, Orafidiya LO and Adelusola KA: Wound-healing and potential anti-keloidal properties of the latex of *Calotropis procera* (Aiton) Asclepiadaceae in rabbits. African Journal of Traditional, Complementary and Alternative Medicine 2013; 10(3): 574-79.
11. Mali RP, Rao PS and Jadhav RS: A review on pharmacological activities of *Calotropis procera*. Journal of Drug Delivery and Therapeutics 2019; 9(3): 947-51.
12. Amandeep P and Antul K: Review on pharmocological properties of Aaka (*Calotropis procera*). International Journal of Economic Plants 2018; 5(3): 157-62.
13. Zohara Y and Hinanit K: *Calotropis procera*, apple of sodom ethnobotanical review and medicinal activities. Israel Journal of Plant Sciences 2018; 65: 55-61.
14. Morsy N, Sherif Al, Emad A, Abdel R and Tayseer MA: Phytochemical analysis of *Calotropis procera* with antimicrobial activity investigation. Main Group Chemistry 2016; 15(3): 267-73.
15. Setty RS, Quereshi AA, Viswanath Swamy AH, Patil T, Prakash T, Prabhu K and Gouda AV: Hepatoprotective activity of *Calotropis procera* flowers against paracetamol-induced hepatic injury in rats. Fitoterapia 2007; 78(7-8): 451-54.
16. Syed IA, Saber H, Mohammad A, Hikmat UK, Baharullah K and Hatem F: Evaluation of the larvicidal potential of *Calotropis procera* plant extract against *Culex pipiens*. International Journal of Mosquito Res 2016; 3(6): 1-5.
17. Ramasubramania RR, Kishore N, Sreenivasulu M, Rasoolbee SK, Nandini S, Ooha L and Chaitanya N: *Calotropis gigantea*- botanical, pharmacological view. Journal of Medicinal Plants Studies 2016; 4(2): 87-89
18. Azwanida NN: Review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicinal & Aromatic Plants 2015, 4:3.
19. Farina M, Preeti B and Neelam P: Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos*. Bio Med Research International 2014; 497606.
20. Patrice N, Aurélie J and Laurent P: A universal culture medium for screening polymyxin-resistant gram-negative isolates. Journal of Clinical Microbiology 2016; 54(5): 1395-99.
21. Mounyr B, Moulay S and Saad KI: Methods for *in-vitro* evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis 2016; 6(2): 71-79.
22. Tereschuk ML, Riera MVQ, Castro GR and Abdala LR: Antimicrobial activity of flavonoid from leaves of *Tagetes minuta*. Journal of Ethnopharmacol 1997; 56: 227-32.
23. Felde O, Kreizinger Z, Sulyok KM, Hrivna´k V, Kiss K, Jerzsele A, Imre B and Miklo SG: Antibiotic susceptibility testing of *Mycoplasma hyopneumoniae* field isolates from Central Europe for fifteen antibiotics by microbroth dilution method. PLoS One 2018; 13(12): e0209030.
24. Kuete V, Ngameni B, Simo CC, Tankeu RK, Ngadjui BT, Meyer JJ, Lall N and Kuiate JR: Antimicrobial activity of the crude extracts and compounds from *Ficus chlamydocarpa* and *Ficus cordata* (Moraceae). Journal of Ethnopharmacology 2008; 120: 17-24.
25. Stalikas CD: Extraction, separation, and detection methods for phenolic acids and flavonoids. Journal of Seperation Science 2007; 30(18): 3268-95.
26. Quy DD, Artik EA, Phuong LT, Lien HH, Felycia ES and Suryadi I: Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic*. Journal of food and drug analysis 2014; 22: 296-02.
27. Houghton PJ and Raman A: Laboratory Hand book for the Fraction of Natural extracts New York : Chapman & Hall, Edition 1998.
28. Yu Lin H, Kuo YH, Lin YL and Chiang W: Antioxidative effect and active component from leaves of lotus (*Nelumbo nucifera*). Journal of Agriculture and Food Chemistry 2009; 57: 6623-29.
29. Dehkharghanian M, Adenier H and Vijayalakshmi MA: Analytical methods study of flavonoids in aqueous spinach extract using positive electrospray ionisation tandem quadrupole mass spectrometry. Food Chemistry 2010; 121: 863-70.
30. Cowan MM: Plant products as antimicrobial agents. Clinical Microbiology Review 1999; 12(4): 564-82.
31. Dai J and Mumper RJ: Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 2010; 15(10): 7313-52.
32. Evans CE, Banso A and Samuel OA: Efficacy of some nupe medicinal plants against *Salmonella typhi*: *in-vitro* study. Journal of Ethnopharmacology 2002; 80: 21-24.
33. Koduru S, Jimoh FO, Grierson DS and Afolayan AJ: Ethnobotanical information of medicinal plants used for treatment of cancer in the Eastern Cape Province. South Africa Current Science 2007; 92: 906-08.
34. Riffel A, Medinal LF, Stefani V, Santos RC, Bizani D and BrandelliIn A: *In-vitro* antimicrobial activity of a new series of 1,4-naphthoquinones. Brazilian Journal of Medical and Biological Research 2002; 35(7): 811-8.
35. Logan N, Old D and Dick H: Isolation of Bacillus circulans from a wound infection. Journal of Clinical Pathogens 1985; 38(7): 838-39.
36. Alebouyeh M, Orimi P, Azimi-rad M, Tajbakhsh M, Tajeddin E, Sherafat S and Zali M: Fatal sepsis by Bacillus circulans in an immunocompromised patient. Iranian Journal of Microbiology 2015; 3(3): 156-8.
37. Gurmeet K, Shrinidhi R and Adline SP: Plausible drug targets in the *Streptococcus mutans* Quorum Sensing pathways to combat dental biofilms and associated risks. Indian Journal of Microbiology 2015; 55(4): 349-56.
38. Ramawat KG and Merillon JM: Secondary plant products in nature, In: Biotechnology: Secondary Metabolites;

Plants and Microbes Ramawat, K.G. and. J.M. Merillon (Ed.). Science Publishers, Enfield, NH, 2007; 21-57.
39. Vaghasiya Y, Dave R and Chanda S: Phytochemical analysis of some medicinal plants from western region of India. Research Journal of Medicinal Plant 2011; 5(5): 567-76.
40. Lin JY and Tang CY: Determination of total phenolic and flavonoid conents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chemistry 2007; 101: 140-47.
41. Hammer KA, Carson CF and Rileg TV: Antimicrobial activity of essential oils and other plant extracts. Journal Applied Microbiology 1999; 86: 985-90.
42. Savoia D: Plant-derived antimicrobial compounds: Alternatives to antibiotics. Future Microbiology 2012; 7: 979-90.

How to cite this article:
Sethy R and Kullu B: Comparative phytochemical screening and antimicrobial activity of *Calotropis* sp. of ethnomedicinal significance. Int J Pharm Sci & Res 2020; 11(7): 3243-51. doi: 10.13040/IJPSR.0975-8232.11(7).3243-51.