

PHYTOCHEMICAL, ANTIMICROBIAL AND ETHNOBOTANICAL STUDY OF *CALOTROPIS GIGANTEA*Hari Timilsina,¹ Bindu Modi,² Ram Chandra Basnyat²¹School of Health and Allied Science, Pokhara University²Department(s) and institution(s) Central Department of Chemistry, Tribhuvan University**ABSTRACT:**

Introduction: According to the WHO, more than 80% of world's population depends upon the traditional medicine for primary care of health. The increased interest in plant derived drugs is mainly because of 'herbal medicines' are safer than costly synthetic drug. In this study, *Calotropis gigantea* also known as 'Aank' in Nepali was selected as the plant for the research work. Plants were collected from different parts of the Chitwan district. The objective of the study was to prepare the methanol and hexane extracts of leaves and stem of *C. gigantea* and carry out phytochemical screening of those extracts. Similarly, antimicrobial activity of those extract were evaluated to find its potential as drug. The ethnobotanical survey was done to find out medicinal values of the plant.

Methods: Methanol and hexane extracts of the leaves and stem of *C. gigantea* was prepared by Soxhlet extraction method. Methanol was used for the extraction of various polar compounds and hexane for non-polar compounds. Phytochemical screening results showed the presence of secondary metabolites such as alkaloids, glycosides, flavonoids and terpenoids. The phytochemicals present in different plant extracts were analyzed by following the protocol given by Ciulei I. Inhibition of bacterial growth was tested by using agar well diffusion plate method (As per DPR/BS/SOP/Am/1) and measured in the form of zone of inhibition (ZOI).

Results: The ZOI shown by methanol extracts of leaves of *C. gigantea* for *Escherichia coli* and *Staphylococcus aureus* were measured 8 mm and 13 mm respectively. Similarly, the zones of inhibition shown by hexane extract of leaves of *C. gigantea* for *E.coli* and *S.aureus* were measured as 11 mm and 9 mm respectively in antibacterial assay. Antimicrobial activity was not seen against *Klebsiella pneumoniae* by any of the extracts. The ethnobotanical study conducted showed that the plant is being used for different medicinal purposes.

Conclusion : The findings of the study showed that the plant had high pharmaceutical importance. Traditionally, it is used alone or with other medicinal plants to treat common disease such as asthma, swelling rheumatism, diarrhoea, dysentery, syphilis, ulcer, leprosy etc. This study hopes to provide valuable information for different research.

Keywords: *Calotropis gigantea*, Methanol extract, Antimicrobial, Phytochemical screening, Ethnobotany

INTRODUCTION:

Nepal is rich in medicinal plants and is one of the richest countries in terms of genetic diversity of medicinal plants. It exhibits a wide range in topography and climate. Medicinal and aromatic plants have both domestic and international markets.¹ Among the various uses of the plants, the medicinal value is important since ancient medicinal procedure such as Ayurveda, Homeopathy, Unani, Chinese and Tibetan medicines.² According to the WHO, more than 80% of world's population depends upon the traditional medicine for primary care of health. The increased interest in plant derived drugs is mainly because of 'herbal medicines' are safer than costly synthetic drug.³

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of them based on their use in traditional medicine.⁴ People have been using plants and plant

products for healing of various diseases as medicine without knowing their chemical constituents and biological activities since prehistoric time.¹

The kingdom Plantae includes all land plants, mosses, flowering plants, ferns and so on. With an excess of 250,000 plant species, the kingdom Plantae is the second largest. The Asclepiadaceae is a large family comprising of 175-180 genera and 2200 species distributed mainly in the tropical and subtropical region of the world. *Calotropis* belong to it. It has two species *procera* and *gigantea*. Physically the main difference between the two species that are easily differentiated is their flowers' color while in bud, or bloomed condition. So, it is hard to recognize the species if the plant is not having flower.⁵

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Calotropis gigantea is a glabrous or hoary, lactiferous shrubs or small trees, about 3-4 m tall commonly known as the swallow-wort or milkweed. Its stems are erect, up to 20 cm in diameter. The leaves are broadly elliptical to oblongobovate in shape, with the size of 9-20 cm x 6-12.5 cm but sub sessile. It is native to continental Asia and South East Asia and has been introduced in the Pacific Islands, Australia, Central and Northern South America and Africa, Asian ornamental near villages and temples and as a weed. However, its distribution is incompletely known, and it probably occurs in other countries as well. It is widely distributed in Nepal too.⁶ It has different local names as Sweta Arka in Sanskrit, Bang bein in Vietnam, Akada, Mandar in India and commonly as Aank in Nepal.

Phytochemical studies on genus *Calotropis* started in 1915 when Ernest and Co-worker reported the presence of akundrol isovalerate in the root bark of *C.gigantea*. In 1934 Kali and Madhab isolated a sterol, calosterol from the milky juice of *C.gigantea*. The same authors in 1936 reported the presence of proteinase in the latex of *C.gigantea*.¹¹

The plant was screened for its antimicrobial and phytochemical activities in different solvents. The extract was tested against infectious disease causing bacteria such as *E. coli*, *Pseudomonas aeruginosa* and *S. aureus* using the well diffusion method. The results confirmed that presence of antibacterial activity and phytochemicals in the shade dried extract of *C. gigantea* against the human pathogenic organisms.⁷

Ethnobotanical survey of this plant was carried out in different places and found that the plant was used as a cure for asthma patient. Likewise, another ethno botanical investigation of this plant carried out and found that the plant can be used for the treatment of snake bite.⁸

The objective of the study was to prepare the methanol and hexane extracts of leaves and stem of *C. gigantea* and carry out phytochemical screening of those extracts. Similarly, antimicrobial activity of those extract were evaluated to find its potential as drug. The ethnobotanical survey was done to find out medicinal values of the plant.

Materials and Methods

Material

The fresh leaves and stems of *C. gigantea* were collected from the fields of Terai region (Chitwan district, Province no. 3), Nepal. The taxonomic identification of the plants was done at the Central Department of Botany, Tribhuvan University, Kirtipur comparing with the preserved herbariums carefully.

METHODS

Extraction

The collected fresh leaves and stems were washed with tap water to remove the contaminants. The leaves were shade dried and grinded into the powder form and stored in a clean zipped plastic bag until further use. The phytochemicals present in the powdered leaves and stems were extracted by percolation method using widely used Soxhlet extraction method. The powdered sample was taken in two separate thimbles of Soxhlet extractor. The round bottom flask was filled two-third with hexane and was adjusted to the extractor. Finally, the solvent was heated at around 40° C and the extraction process was allowed to run for about an hour. After the completion of the extraction process, the solvent with extract was subjected to concentration process using the rotary evaporator at 40° C. Thus obtained hexane extract was dried over heating source and then stored properly for further use. Similarly, the methanol extract was also obtained by the similar process by heating the solvent at around 70° C.

Phytochemical Analysis

Freshly prepared extracts were subjected to standard phytochemical analysis to determine the presence of the following phytoconstituents, i.e., alkaloids, phenols, flavonoids, glycosides, tannins, saponins, steroids, terpenoids, sugar, and proteins by following the protocol given by Ciulei I.⁹

Antimicrobial Activity

Inhibition of bacterial growth was tested by using an agar well plate method and measured in the form of zone of inhibition (ZOI) as given by Dingle et al.¹⁰ The antibacterial assay was performed at Central Department of Microbiology, Tribhuvan University, Kirtipur, Nepal. The bacterial strain on which antibacterial assay was carried out on three bacteria were *E. coli*, *K. pneumoniae*, and *S. aureus*. Methanol extract of *C. gigantea* (100 mg) were dissolved in 50% dimethyl sulfoxide (DMSO) in water. Tubes were capped and stored in refrigerator at 4°C until further use. Preparation of Muller Hilton Agar (MHA) Plates, nutrient broth (NB) solution, preparation of standard culture inoculums, transfer of bacteria in petri plates, screening and evaluation of antibacterial activity was carried out according to the standard protocol.¹⁰ After 24 hours, petri plates were then observed for zone of inhibition (ZOI) produced by antibacterial activity of plant extracts. The inhibition zones were measured by the use of a scale. Ampicillin was taken as the positive control and methanol as that of negative control. The crude extract of different plant parts, which showed antibacterial activity were subjected to serial dilution method to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Ethno-botanical Survey

For the ethnobotanical survey questionnaire were prepared and answer were collected through direct interviews and emails .12 Field visit to different places were conducted at Chitwan district. More than 150 direct interviews were conducted within the different wards of Ratnanagar Municipality and more than 100 emails were sent to different people. The emails were specially sent to the local herbal healers and ayurvedic doctors.

Table 1: Phytochemical screening of *C. gigantea*

Phytochemicals	Colour	M (L)	M (S)	H(L)	H(S)
Polyphenols	Greenish Blue	+	+	+	+
Alkaloids	Reddish gray	+	+	-	-
Carbohydrates	Violet	+	+	-	-
Terpenoids	Reddish gray	+	-	+	+
Steroids	Yellowish	-	+	+	+
Saponins	Light Maroon	+	+	+	+
Tannins	Dark Maroon	+	-	+	+
Flavonoids	Orange	+	+	+	+

(+) indicates present and (-) indicates absent

Where,

M(L) = Methanol extract of leaves of *C. gigantea*

M(S) = Methanol extract of stem of *C. gigantea*

H(L) = Hexane extract of leaves of *C. gigantea*

H(S) = Hexane extract of stem of *C. gigantea*

Antimicrobial activity

Pathogenic bacteria are the ones that cause diseases to plants, animals and mostly human beings. Diarrhoea, dysentery, tuberculosis, pneumonia, respiratory infections etc. are common disease caused by pathogenic microorganisms. These have long terrorized the world proving fatal in several time periods and are

RESULTS**Phytochemical Analysis**

The analysis of the presence of main groups of natural constituents present in the different plant extracts was done by the color reaction using different specific reagents. This result shows the presence of most of the phytochemicals in the polar extracts in both samples.

still potent causing high mortality rate in third world countries. Anti-microbial agents are anything that inhibits or kills the growth of these micro-organisms and prevent from any kind of disease. Anti-microbial activity of a plant extract is evaluated by calculating several parameters such as ZOI, MIC and MBC. The area around the antimicrobial disk where there is no growth of micro-organisms is called zone of inhibition. The minimum concentration of the plant extract that hinders the growth of microorganisms is called minimum inhibitory concentration while the minimum concentration that kills the microorganisms completely is called minimum bactericidal concentration.

Table 2: Antimicrobial activity of methanol extract of *C. gigantea* leaves

S. N.	Bacteria	Reference culture	ZOI Value (mm)			MIC mg/mL	MBC mg/mL
			Positive control Ampicillin	Negative Control DMSO	CG stems extract		
1	<i>Escherichia coli</i>	ATCC 25922	27	0	8	50	50
2	<i>Klebsiella pneumoniae</i>	ATCC 600703	18	0	0	-	-
3	<i>Staphylococcus aureus</i>	ATCC 8534P	23	0	13	12.5	12.5

ZOI= Zone of Inhibition, MIC= Minimum Inhibitory Concentration, MBC= Minimum bactericidal Concentration

Table 3: Antimicrobial activity of methanol extract of *C. gigantea* stem

S.N.	Bacteria	Reference culture	ZOI Value (mm)			MIC mg/mL	MBC mg/mL
			Positive control Ampicillin	Negative Control DMSO	CG stems extract		
1	<i>Escherichia coli</i>	ATCC 25922	27	0	10	50	50
2	<i>Klebsiella pneumoniae</i>	ATCC 600703	18	0	0	-	-
3	<i>Staphylococcus aureus</i>	ATCC 8534P	23	0	9	12.5	12.5

ZOI= Zone of Inhibition, MIC= Minimum Inhibitory Concentration, MBC= Minimum Bactericidal Concentration

Table 4: Antimicrobial activity of hexane extract of *C. gigantea* leaves

S.N.	Bacteria	Reference culture	ZOI Value (mm)			MIC mg/ mL	MBC mg/ mL
			Positive control	Negative Control	CG leaves		
			Ampicillin	DMSO	extract		
1	<i>Escherichia coli</i>	ATCC 25922	27	0	11	50	50
2	<i>Klebsiella pneumoniae</i>	ATCC 600703	18	0	0	-	-
3	<i>Staphylococcus aureus</i>	ATCC 8534P	23	0	9	12.5	12.5

ZOI= Zone of Inhibition, MIC= Minimum Inhibitory Concentration, MBC= Minimum Bactericidal Concentration

Table 5. Antimicrobial activity of hexane extract of *Calotropis gigantea* stem

S. N.	Bacteria	Reference culture	ZOI Value (mm)			MIC mg/ mL	MBC mg/ mL
			Positive control	Negative Control	CG stem		
			Ampicillin	DMSO	extract		
1	<i>Escherichia Coli</i>	ATCC 25922	27	0	13	50	50
2	<i>Klebsiella pneumoniae</i>	ATCC 600703	18	0	0	-	-
3	<i>Staphylococcus aureus</i>	ATCC 8534P	23	0	9	12.5	12.5

ZOI= Zone of Inhibition, MIC= Minimum Inhibitory Concentration,

MBC= Minimum Bactericidal Concentration, (-) = No effective antibacterial activity

DISCUSSION

Phytochemical analysis

In the evaluation of antimicrobial activity for the phytochemical analysis of crude extract of *C. gigantea* leaves and stem in methanol and hexane extract depicted the presence of class of phytochemicals as shown in the table no.1. The presence of phytochemicals was confirmed by the appearance of specific colors as visualized by microscope. The phytochemical screening of the methanol extract of leaves and stems of *C. gigantea* exposed the presence of carbohydrates, polyphenols, terpenoids, saponins, tannins, alkaloids, quinones, glycosides, steroids, phenolic compounds and flavonoids. The presence of these secondary metabolites can be useful in the further investigation of its uses in pharmaceutical areas.

Antimicrobial analysis

In the evaluation of antimicrobial activity for methanol extract of leaves of *C. gigantea*, the zone of inhibition were found to be 8 mm, 0 mm and 13 mm for *E. coli*, *K. pneumoniae* and *S. aureus* respectively. The MIC and MBC values for the bacteria were found to be 12.5mg/ml for gram positive bacteria and 50mg/ml for gram negative bacteria for the methanolic leaf extract.

In the evaluation of antimicrobial activity for methanol extract of stems of *C. gigantea*, the zone of inhibition were found to 10 mm, 0 mm and 9 mm for *E. coli*, *K. pneumoniae* and *S. aureus* respectively. The MIC and MBC values for the bacteria were found to be 12.5mg/ml for gram positive bacteria and 50mg/ml for gram negative bacteria for the methnolic stem extract.

For the hexane extract of leaves of *C. gigantea*, the zone of inhibition were found to 11 mm, 0 mm and 9 mm for *Escherichia*

coli, *Klebsiella pneumoniae* and *Staphylococcus aureus* respectively. The MIC and MBC values for the bacteria were found to be 12.5mg/ml for gram positive bacteria and 50mg/ml for gram negative bacteria for the hexane leaf extract.

In the evaluation of antimicrobial activity for hexane extract of stems of *C. gigantea*, the zone of inhibition were found to 13 mm, 0 mm and 9 mm for *E. coli*, *K. pneumoniae* and *S. aureus* respectively. The MIC and MBC values for the bacteria were found to be 12.5mg/ml for gram positive bacteria and 50mg/ml for gram negative bacteria for the hexane stem extract.

The value of ZOI of plant extracts for two gram negative bacteria (*E. coli* and *K. pneumoniae*) and one gram positive bacteria (*S. aureus*) and corresponding values for MIC and MBC shows it to be potent against all disease caused by these bacteria. As *K.pneumoniae* is a multidrug resistant bacteria it did not have any antimicrobial effect from the extract.

The values of ZOI, MIC and MBC differ for methanolic and hexane extract because the biologically active components are more potent in methanolic solvent than hexane. Similarly, the values of ZOI, MIC and MBC of leaves differ than stem suggest that components of leaves have high potential against microbes than stem for given three pathogens.

From these results, knowledge of the MIC and MBC will provide a drug researcher valuable information for further investigation as a potential medicinal plant. Accurate and precise usage of antimicrobials is also important in the context of multi drug resistant bacteria. Microbes such as bacteria have been gaining resistance to antimicrobials, they were previously susceptible to. Ethnobotanical study:

C. gigantea is used as a traditional medicinal plant with unique properties. Traditionally, it is used alone or with other medicinal plants to treat common disease such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, and diarrhoea. These findings are the responses collected from the surveys. These are the traditional and local ways people use the different parts of the *C. gigantea* plant for the treatment of different diseases.

Some of the traditional methods of use of the plant:

1. The seeds are used for the asthma treatment. Equal parts of ash and the powder of *Aschyranthus aspera* plant mixed in equal parts along with the lemon juice. 100mg is taken two times per day by mouth.
2. The leaves along with the pepper are used to treat snake bite. Dry leaf powder is mixed and boiled with oil and powdered turmeric and applied to the affected area.
3. Latex of this plants is used to cure dental problems, rat bite, swelling, gonococcal arthritis and other rheumatic complaints. Simply the latex is applied to the infected areas.
4. Latex is also used in fractures and sprains.
5. The leaves are crushed, warmed and applied on the burns, headaches, rheumatic pains and in the form of tincture for fever.
6. Decoction of flowers is used for cough and asthma. The plant powder mixed with cow milk is used for rheumatism, diarrhoea, dysentery, syphilis, ulcer, leprosy. Dosages were prescribed by the local healer.
7. The dried powder of flowering (about 2-4) are boiled in molasses and used in the treatment of asthma.
8. An oil prepared by boiling Sesame oil 8 parts, *C. gigantea* juice 6 parts and turmeric 1 part is used in eczema, eruptive skin, scorpion and insects bites, relieves from pain and burning sensation.
9. The folks use latex for various activities such as to stop bleeding for fresh cuts, anti-inflammation, and abortion easy delivery.
10. Fresh leaves were used for treating convulsions, fits in children and the extract of the leaf along with rock salt, oils used for ear-ache and fresh warmed leaves were useful in rheumatic pains.
11. Latex is used for several skin infections, root juice when applied on the abdomen and vaginal region during child birth reduces the labour pain.
12. The root juice warmed with coconut oil is applied for treating scabies.
13. The tribal communities use twigs as abortive agents by introducing them into vagina or uterus and seeds when taken on empty stomach along with country liquor (made from *Madhuca indica*) after mensuration prevents pregnancy up to one year of the time period.
14. Fermented mixture of *C. gigantea* and salt is used to remove the hair from the goat skins for production of “nari leather” and of sheep skins to make leather.
15. The plant yields a double fiber useful for ropes, carpets, fishing nets and sewing thread.

CONCLUSION

From this study, it is concluded that the phytochemical, antimicrobial activity showed that the plant have high pharmaceutical importance. The ethnobotanical survey shows that the plant has diverse use in treating different diseases. The traditional methods can be well documented along with the clinical trials.

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