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Phytochemical analysis and antimicrobial screening studies of *Calotropis gigantea* leaves

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Abstract
Introduction: *Calotropis gigantea* (Apocynaceae) is a wild herb that can be used to cure a variety of ailments, including fever, indigestion, colds, coughs, asthma, and scabies.
Method: The powdered *C. gigantea* leaves was examined for ash value, extractive value, organoleptic properties, and microscopy. Also, the extract of the plant in several solvents (petroleum ether, diethyl ether, chloroform, ethyl acetate, ethanol, and water) was successively calculated. Each extract was screened for phytochemicals and evaluated for total phenolic content and total flavonoid content. The antioxidant activity of aqueous and alcohol extracts was assessed, along with the antibacterial properties of each extract.
Results: Alkaloids, steroids, saponins, terpenoids, glycosides, reducing sugars, non-reducing sugars, proteins, Alkaloids, steroids, saponins, terpenoids, glycosides, reducing sugars, non-reducing sugars, proteins, tannins, amino acids, phenols, coumarin, and quinones were found in preliminary phytochemical analyses of extracts. The aqueous extract had a greater total phenolic concentration and total flavonoids content. The DPPH scavenging experiment showed dosage dependent result in both the aqueous and alcohol extracts. The alcoholic and aqueous extracts of the plant were found to be effective against *E. coli* in a dose-dependent manner during antibacterial screening. On TLC analysis, ethyl acetate, alcohol, and aqueous extracts displayed distinct visible tailing in the TLC plates, spots of other extracts could only be seen under a UV light.
Conclusion: The recent study in the extracts of the *C. gigantea* leaves indicated the presence of antioxidants with phenolic and flavonoid compounds. Therefore, this could be potential lead molecule for antibiotic.

Keywords: Antimicrobial, *Calotropis gigantea*, TPC, TFC, DPPH

Introduction

Calotropis gigantea is a one of the six species of Calotropis weed that belongs to Apocynaceae family and has a lot of medicinal properties. This species usually grows in the wastelands of Asia and Africa and is native to South China, Pakistan, Malaysia, Indonesia, Cambodia, Vietnam, Nepal, Bangladesh, Sri Lanka, India, and Thailand. This plant is referred as "shallow wort", "giant milkweed" and "shallow wort". They have typical thick, wide leaves and odorless, purplish-colored flowers that makes their identification easier.

Calotropis is perennial plant that is about 4-6 m tall and has 4–8 inches long, decussate, obovate or shortly acute leaves that are cordate or often amplexicaul at the base. The white milky latex produce by this plant has cardiac glycosides calotopin, uscharin, calotoxin, calactin, and uscharidin and gigantin, are found to have great wound healing activity. While the leaves are that to possess antimicrobial and anti-inflammatory properties. The entire tree is thought to have therapeutic benefits and is used to treat a variety of ailments, including syphilis, boils, inflammation, epilepsy, hysteria, fever, muscle spasms, warts, leprosy, gout, snakebites, and cancer.

Generally, Ayurveda, Chinese, and homeopathic remedies employ *C. gigantea* to treat asthma, colds, coughs, diarrhea, fever, indigestion, leprosy, leukoderma, and rheumatism. In fact, it is used to cure toothaches, elephantiasis, purging, and vomiting, according to the homeopathic Materia Medica.

It has been discovered that the alcoholic extract of *C. gigantea* may improve skin permeability. This mixture has been investigated as a potential auto-debridement and tissue regeneration agent when combined with other bioactive components.

A variety of actions were produced by the ethanolic plant extract, including anti-inflammatory, antioxidant, antibacterial, enzyme inhibition, vasodilation, and wound healing. So, Researchers from all around the world are drawn to the *Calotropis gigantea*n because of its pharmacological properties, which include anti-diabetic, anti-toxin, anti-hepatotoxin, antioxidant, and wound-healing action.

Rationale of Study

The plant *Calotropis gigantea* is a traditional medicinal plant having immense therapeutic importance. As a hydrocarbon-rich plant, this plant needs more investigation on the aspect of energy conversion. A systemic research and development work should be undertaken for the conservation of *Calotropis gigantea* and development of products for their better economic and therapeutic utilization.

This research will provide data on herbal medicines to meet regulatory requirements and technical support and guidance in developing a framework for the promotion, development and regulation of herbal medicines. The framework will lay a strong foundation for the future development of herbal medicines in the health care system. Though there have been many studies in Calotropis species on international level but there are no satisfactory studies in Nepal till this date. The current study thus aims to establish data on the phytochemical and biological activities of the extracts of this plant to light up the numerous little known and unknown medicinal virtues of the plant.

Plant Profile

Plant name: *Calotropis gigantea.*
Family name: Apocynaceae.
Synonyms: *Calotropis procera*, *Calotropis acia*.
Common name: Giant milkweed, Crown flower, Aakh
Parts used: barks, leaves, flowers.

Classification

Kingdom: Plantea
Subkingdom: Tracheobionata
Superdivision: Spermatophyta
Division: Magnoliopsida
Subclass: Asteridea
Order: Gentianales
Family: Apocynacea
Subfamily: Asclepiadoideae
Genus: *Calotropis*
Species: *gigantea, procera, acia*

Fig 1: Calotropis gigantea plant

Vegetative Characters

Calotropis gigantea is a shurb or a small tree up to 2.5m (max 6 meter) height. Its roots are simple, brancehed, woody at base Simple, branched, woody at base and covered with a fissured; corky bark; branches somewhat succulent and densely with tomentose; early glabrescent. All parts of the plant exude white latex when cut or broken. The leaves are Opposite, sessile, elliptic-oblong, acute, thick, bluish green, with a cottony, pubescent underside and are profusely milky.

The flower has no odor, purplish-white, in flat-topped clusters, arises from a stout stalk. Each flower has a central crown. A white flowered variant is found but rather rare. The plant is almost always in flower. And the fruit has paired boat-shaped capsules, about 8 – 10 cm in length. Dehisces when dry and exposes a large number of brown flattened seeds with silky hair attached at one end. The arrangement of the seeds in a young fruit is similar to the arrangement of fish scales.

Chemical Constituents

Phytochemical studies on Calotropis have afforded several types of compounds such as Cardenolide, triterpenoids, alkaloids, resins, anthocyanins and proteolytic enzymes in latex, flavonoids, tannins, sterol, saponins, cardiac glycosides.

But the leaves of *Calotropis gigantea* five major chemicals,

- methyl β-carboline-1-carboxylate,
- (+)-dehydrovomifoliol
- pleurone
- calotropagenin and
- calotoxin

Medicinal uses

Different extracts of the plant show different properties

1. Root and bark of *Calotropis gigantea* shows wound healing activity.
2. The cardenolide glycosides collected from the root *Calotropis gigantea* were reported to carry cytotoxic activity against several human and mouse cell lines.
3. The hydroalcoholic (50:50) extract of aerial part of *Calotropis gigantea* exhibits anti-diarrheal activity. Water: ethanol (50:50) extract of roots shows anti-pyretic activity.
4. Methanol extract of roots shows good insecticidal activity.
5. Ethanol extract of *Calotropis gigantea* shows anti-inflammatory activity.
6. Leaves of *Calotropis gigantea* carries profound amounts of antioxidants.
7. Ethanol extract of stems has hepatoprotective activity.
8. The leaves have anti-asthmatic property.
9. The crushed leaves are warmed and used as a poultice on sores, burns, headaches and rheumatic pains. The powdered flowers are valued for treating coughs, colds and asthma.
10. Aqueous extract of latex possesses antibacterial activity against *S. aureus*, *E. coli*, *B. cereus*, *P. aeruginosa*.

Traditional Uses

A fine fiber is obtained from the bark of the Calotropis plant, which is used for making textiles, fishing nets and bowstrings. The mature seed pods contain large quantity of floss, which is used to stuff pillows or mixed with other

fibers to make cloth. Similarly, the twigs are used as chewsticks for cleaning the teeth. The juice of the plant is used in making a yellow dye and in tanning.

Methods and Methodology

Plant Collection

The leaves of *Calotropis gigantea* was collected in the month of August, 2020 based on information given by local inhabitants. The plant was collected from Jhapa, Nepal.

Preparation of extract

The leaves were washed in running water several times. Then dried in a shaded place at the room temperature for about 2-3 weeks. After complete drying, the leaves were grinded into fine powder using grinding machine. The powder was then sieved through sieve no. 80. About 25 gm of powder was then subjected to successive extraction.

Method of Extraction

The extraction was done by successive extraction using Soxhlet apparatus. About 25 gm of powdered material was subjected to successive extraction with petroleum ether, diethyl ether, chloroform, ethyl acetate, ethanol and Distilled water. The extraction process continued until the solvent in the thimble was clear. After each extraction, the solvent was distilled off and the extract was concentrated by vacuum dryer at a temperature below 45 ºC. The percentage yield of petroleum ether, diethyl ether, chloroform, ethyl acetate, ethanol, and distilled water extract were recorded. The extracts were stored in refrigerator at 4 ºC until their biological activities were tested.

Results

Organoleptic Characteristics Of Powdered Leaves

The plant powder was sieve through 80 mesh size. Then, the macroscopic characteristics of powdered was evaluated (Table 1).

Table 1: Organoleptic Character of *C.gigantea* leaves

S. No.	Characteristics	Inference
1	Color	Greyish green
2	Sand and silica	Absent
3	Odor	Pungent
4	Taste	Bitter
5	Insect infection	Absent
6	Texture	Coarse

Fig 2: *C. gigantea* leaves

Fig 3: *C. gigantea* flower

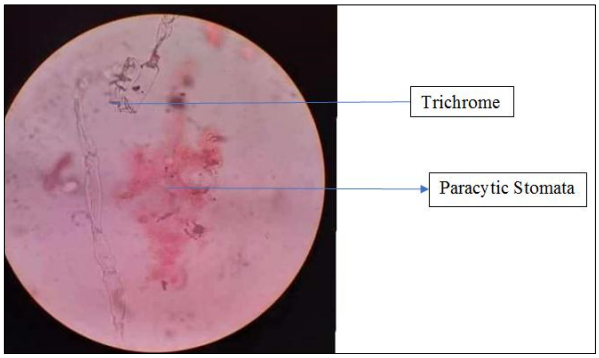

Fig 4: Powdered microscopy of *C. gigantea*

Microscopic of Powdered *C. gigantea* Leaves

The powdered leaves of *Calotropis gigantea* was analyzed under the microscope which showed single, capsulated, glandular and multicellular trichome. Many rosette crystals of starch grains and calcium oxalate was observed. The reddish spots that were found, indicated the presence of tannin. The stomata found in the powdered leaves of *C. gigantea* was paracytic.

Phytochemical Parameters Powdered Leaves Of *C. gigantea*

The 1gm powdered leaves of *Calotropis gigantea* was weighed and kept in the muffle furnace at a temperature of 500 ºC for about six hours. The total ash obtained was calculated. The ash was dissolved in water, acid, and alcohol and different phytochemical parameters were evaluated (Table 2).

Table 2: Phytochemical Parameters

S. No.	Physical Constants	Result (Mean±SD)
A	**Ash value (%w/w)**	
	• Total ash	12.7±0.86
	• Acid-insoluble ash	3.22±0.09
	• Water-soluble ash	2.76±0.15
B	**Extractive values (%w/w)**	
	• Alcohol soluble	5.22±0.72
	• Water soluble	18.45±0.13
C	Loss on drying (%w/w)	14.09±0.46

Fluorescence Test Of *C. gigantea* Powder In Different Solvents

Fluorescence test was carried out in the powdered leaves of *Calotropis gigantea* to detect the presence of fluorescence analytes. The powdered leaves were dissolved in different extracts and viewed from naked eyes and through UV-lamp of wavelength 365nm and 254nm. The result obtained through analysis was:

Table 3: Fluorescence test of *C. gigantea* leaf powder in different solvents

S. No.	Reagent	Visible	UV (365nm)	UV (254 nm)
1	Powder	Green	No change	No change
2	Powder + H_2O	Green	No change	No change
3	Powder + CH_3Cl	Yellowish green	Brownish green	Dark brown
4	Powder + CH_2OH	Dark green	Light green	Dark brown
5	Powder + Alcohol	Light green	Light green	Dark brown
6	Powder + 1N NaOH in H_2O	Green	Light green	Dark brown
7	Powder + 1N HCl	Brownish green	Brownish green	Dark brown
8	Powder + 1N NaOH in CH_2OH	Green	Light green	Dark brown
9	Powder + H_2SO_4	Brownish black	Black	Greenish black
10	Powder + HNO_3	Yellow	Light green	Dark brown

Percentage Yield Different Extracts

25 gm of powder was weight and successively extracted by petroleum ether, diethyl ether, chloroform, ethyl acetate, alcohol and distilled water respectively and the percentage yield were calculated (Table 4).

Table 4: Percentage Yields of Different Extracts

S.N.	Solvent	%Yield
1.	Petroleum ether	3.5
2.	Diethyl ether	2.9
3	Chloroform	6.24
4.	Ethyl acetate	5.5
5.	Alcohol	11.84
6.	Distilled water	9.97

Qualitative Phytochemical Screening

Qualitative phytochemical screening was carried out by chemical method based on the color formation or precipitation with the sample. The results obtained were tabulated below (Table 5).

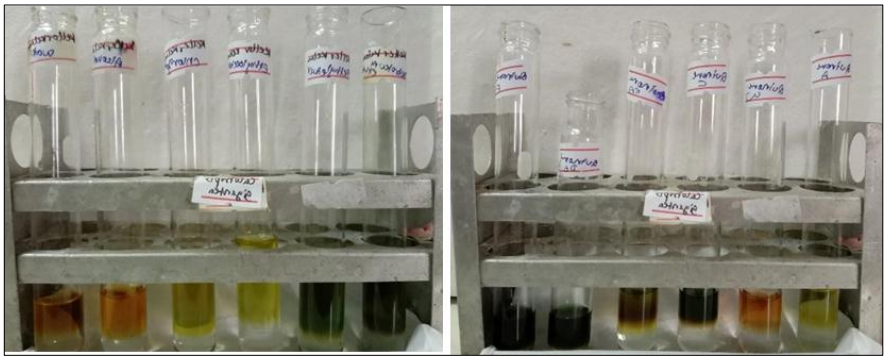

Fig 5: Phytochemical Analysis

Table 5: Phytochemical Screening of *Calotropis gigantea* Extracts

S. N.	Phytoconstituent	Pt. ether	Di.ether	$CHCl_3$	E. acetate	Alc.	D.H_2O
1.	Alkaloids (Mayer's)	-	-	+	+	-	-
	Wagner's	-	+	-	+	-	-
	Dragendroff's	-	-	-	+	-	-
2.	Carbohydrates (Fehling's)	-	-	+	+	+	-
	Molisch	+	+	+	+	+	+
3.	Glycosides (Anthraquinone)	-	-	-	+	-	+
	Killer-killani test	+	+	+	+	+	+
4.	Flavonoids (Lead acetate)	-	-	-	-	+	+
	Shinoda	-	-	-	-	+	+
5.	Proteins (Milon's)	-	-	+	-	-	-
	Ninhydrin	-	-	-	-	-	-
6.	Terpenoids	+	+	+	+	+	+
7.	Tannins	+	+	+	+	+	-
8.	Phobatanins	-	-	+	-	-	+
9.	Starch	-	-	-	-	-	-
10.	Fats and oils	-	-	-	-	-	-
11.	Saponin	-	-	-	-	+	-
12.	Amino acids	+	+	-	-	-	-
13.	Phenols	+	+	+	+	+	-

14.	Coumarins	-	-	-	+	+	+
15.	Quinones	-	-	+	+	-	-

Note: (+) sign indicate the presence and (-) sign indicate the absence of phytochemical

Thin Layer Chromatography Analysis

The qualitative analysis different extracts of *C. gigantea* were done by TLC using the mixture of ethyl acetate, methanol and water as solvent. Then, the plates were kept in UV lamp and iodine chamber for visualization.

The TLC report is illustrated in the Table 6.

Table 6: TLC Analysis of *C. gigantea* Extracts

S. N.	Extracts Of *C. gigantea*	No. of spots	Visualizing agents		Rf values
			UV Lamp (365 nm)	Iodine chamber	
1	Petroleum ether	3	Green	Green	0.72
			Light Brown	Brown	0.84
			Yellow	Dark Brown	0.90
2	Diethyl ether	1	Green	Green	0.96
3	Chloroform	3	Green	Green	0.95
			Greenish	Yellowish	0.86
			Yellow	Brown	0.68
4	Ethyl acetate	4 spots with tailing	Green	Brown	0.96
			Greenish	Brownish	0.88
			Yellow	Brown	0.60
			Red	No color	0.32
5	Alcohol	3 clear spots with tailing	Green	Green	0.80
			Yellow	Brown	0.60
			Orange	Dark Brown	0.40
6	Water	3 spots with tailing	Light Yellow	Brown	0.90
			Brown	Dark Brown	0.36
			Yellowish	Brown	0.81

After TLC test, tailing of the spots were observed in the TLC plates of ethyl acetate, ethanol, and water extracts of *Calotropis gigantea*.

Quantitative Phytochemical Screening

Determination of Total Phenolic Content

Total phenolic content of different extracts of *Calotropis gigantea* are expressed in terms of gallic acid equivalent (mg GAE/gm dry weight of extract) with calibration curve of gallic acid. The aqueous extract showed the highest TPC value (38.45±0.03 mg GAE/gm) and petroleum ether showed the lowest TPC value (7.11±0.01mg GAE/gm).

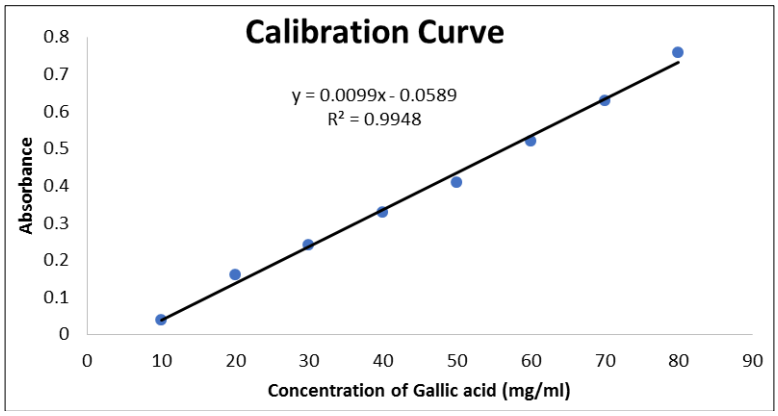

Fig 6: Calibration curve for gallic acid. From the standard curve of quercetin, line of regression was found to be: Y= 0.0099x-0.059 and R2= 0.994

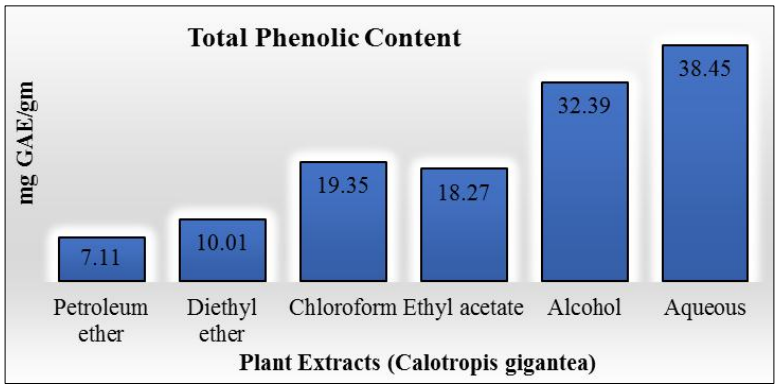

Fig 7: Total Phenolic Content of *C. gigantea* Extracts.

Determination of Total Flavonoid Content

TFC of the extract of different plants is expressed in terms of quercetin equivalent (mg QE/g) which is given in Table 8. Aqueous extract was found to be highest with the TFC value of 26.28±1.87 mg QE/gm. Diethyl ether extract of *C. gigantea* leaves showed the lowest TFC value of 9.15±2.11 mg QE/gm.

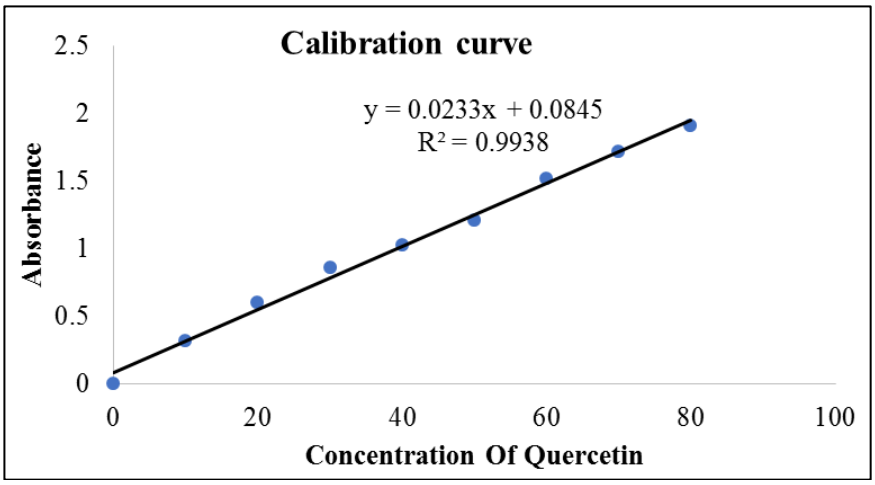

Fig 8: Calibration curve for Quercetin. From the standard curve of quercetin, line of regression was found to be: Y= 0.0233x + 0.0845 and R2= 0.9938

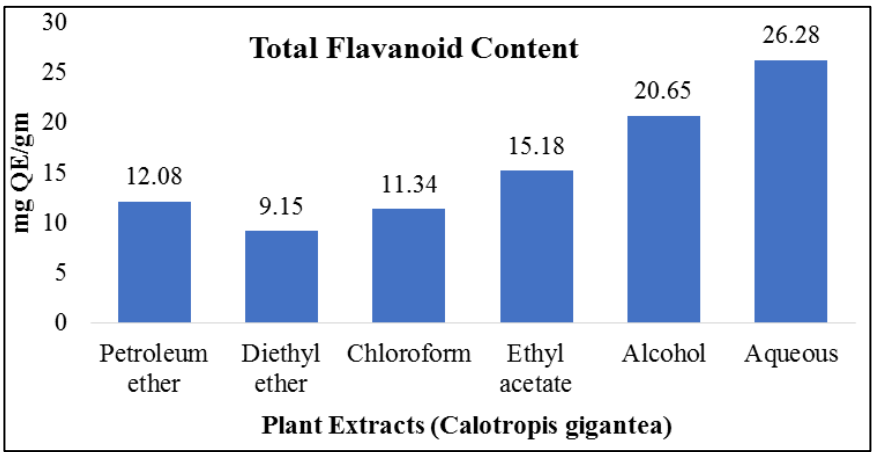

Fig 9: Total Flavonoid Content of *C. gigantea*

Biological Activity

DPPH Free Radical Scavenging Assay

The antioxidant potential is in an inverse relation with the IC_{50} value, which can be calculated from linear aggression of the % inhibition versus antioxidant activity. Lower the IC_{50} value, higher the antioxidant activity. All the calculations are based on the standard method. Absorbance was measured at 517 nm.

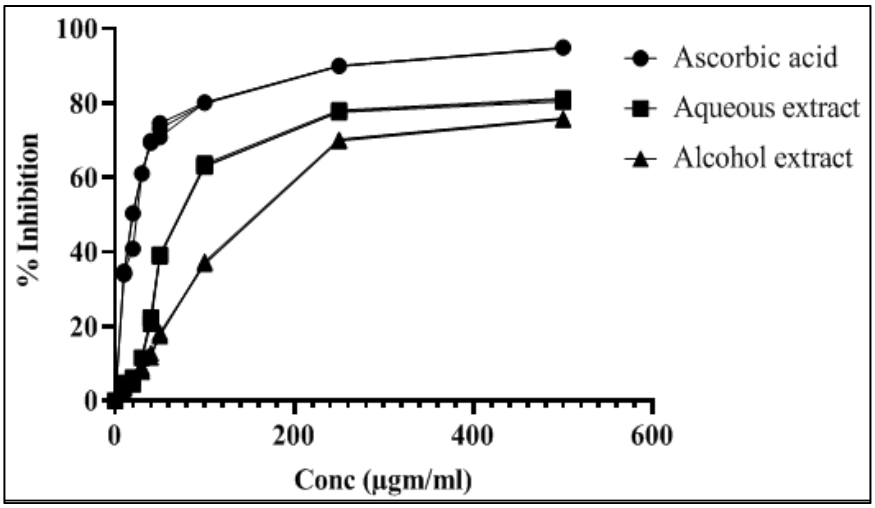

Fig 10: Graphical representation of DPPH assay of the extract with ascorbic acid

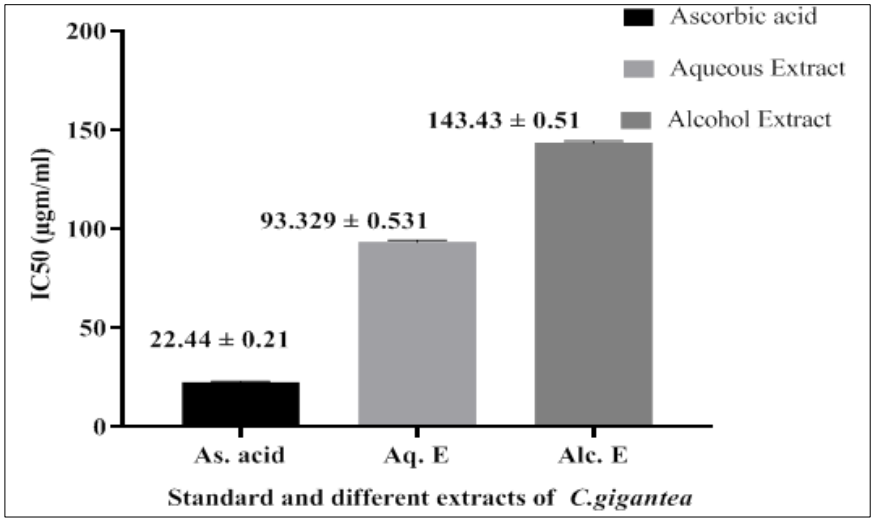

Fig 11: Comparative bar diagram of standard and *C. gigantea* extracts. Data are mean±S.D. of three similar experiments. ***p<0.001

Graphical representation of DPPH assay of the extract is shown above. The curve in figure 12 and the bar diagram for IC_{50} values of ascorbic acid and standards were prepares by using GraphPad Prism 8.0.1.

From the above study, the IC_{50} value of aqueous extract of *C. gigantea* was found to be 93.329±0.531 µgm/ml and IC_{50} value of alcohol fraction of plant extract was 143.43±0.513 µgm/ml. Therefore, it showed that both the extracts have good antioxidants.

Antibacterial Screening: Antibacterial activity was performed against gram positive organism: *Staphylococcus aureus* (ATCC 25923), MRSA and gram-negative organism: *Escherichia coli* (ATCC 25922), *Salmonella Typhimurium* (ATCC 14028), *Pseudomonas aeruginosa* (ATCC 27553).

Alcohol and aqueous extracts of *C. gigantea* was examined at two different concentrations viz 2.5% and 5%. Both the extracts showed some antibacterial activity for gram negative bacteria (*E. coli*) at two different concentrations. The Zone of Inhibitions (ZOI) of those extracts was compared with the ZOI of standard control line Ofloxacin (10 µg/ml).

Table 7: Antibacterial activity of C. gigantea

Plant extracts	Zone of Inhibition (in mm)			
	S. aureus	***P. aeruginosa***	***E. coli***	**S. typhi**
Petroleum Ether	-	-	-	-
Diethyl Ether	-	-	-	-
Chloroform	-	-	-	-
Ethyl Acetate	-	-	-	-
Alcohol (2.5 Mg/Disc)	-	-	6	-
Alcohol (5 Mg/Disc)	-	-	8	-
Aqueous (2.5 Mg/Disc)	-	-	8	-
Aqueous (5 Mg/Disc)	-	-	10	-
Ofloxacin (0.5 µg/Disc)	-	15	19	18

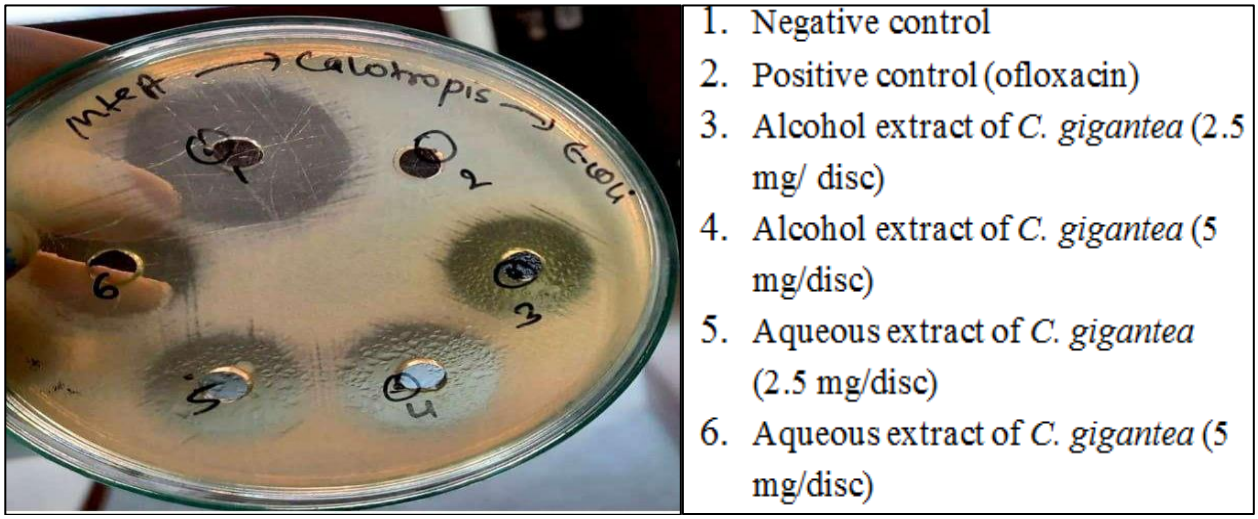

Fig 12: ZOI of alcohol and aqueous extracts of *C. gigantea*

Discussion

Nepal is known for its physiographic diversity and climatic variation that harbors various fauna and flora. Among the total floral wealth of Nepal, about 10% of species are reported with medicinal and aromatic properties.

However, to get full-fledge advantages from these precious plants and to ensure the best of both the modern and traditional therapy, their scientific extraction and correlation with the modern therapy principle is necessary. Therefore, the present study that deals with the phytochemical, antibacterial, antifungal, and antioxidant activities of *Calotropis gigantea* leaves can help in the production of excellent medicine in the future.

Extraction

After drying and grinding the leaves of C. *gigantea*, the powder was extracted with petroleum ether, diethyl ether, chloroform, ethyl acetate, alcohol, and water successively with respect to their polarity. Soxhlation was chosen for the extraction process of the plant because continuously heating the powder with the solvents that can easily penetrate the powder for a longer time ensures higher extractive values among other process. The solvents are changed successively from the lowest polarity to highest polarity.

In the previous research paper of, the yield percentage was reported to be higher in aqueous extract, while my research showed higher yield in alcohol extract. The reason for this variation in both the studies might be due to the difference time of collection, geography, or climate.

Phytochemical screening

The values of the physiochemical parameters of the plant can be used as indicators in authentication and for assuring the quality of the powder form so that possibility of substitution and adulteration could be avoided.

The study of reported the presence of glycosides, flavonoids, tannins, terpenoids, alkaloids, and steroids in the methanolic extract *Calotropis gigantea* leaves that was similar to the result shown by the ethanolic extract of my plant. The research of, reported the presence of sterols, glycoside and carbohydrate in the chloroform extract, sterols and carbohydrates in ethanolic extract, and carbohydrate, sterols, flavonoids, and glycosides in the aqueous extract that resembled with my study.

Total Phenolic Content

Total phenolic content was analyzed by using Folin-coicalteu procedure which is widely used and acceptable procedure for determination of phenolic compounds in different extracts and drugs. In the present research, the aqueous extract of *Calotropis gigantea* leaves showed the maximum TPC value of 38.45±0.03 mg GAE/gm and petroleum ether extract showed minimum TPC value of 7.11±0.01 mg GAE/gm.

Total Flavonoid Content

The total flavonoid content of the extracts of the *Calotropis gigantea* leaves was also determined by colorimetric method using the mixture of aluminum trichloride and potassium acetate as reagent. Quercetin was used as standard for this test and the total flavonoid content of the Quercetin was compared with the different extracts. The total flavonoid content was found to be highest in the aqueous extract of the plant (26.28±1.87 mg QE/gm) and lowest in diethyl ether extract (9.15±2.11 mg QE/gm).

There was no study on the TPC and TFC of *Calotropis gigantea* leaves. But according to research report, the aqueous extract of *Calotropis procera* roots has lesser amount of TPC and TFC value than my study. Thus, we can conclude that leaves of *C. gigantea* might be more potent than roots of *C. procera.*

Thin Layer Chromatography

Chromatographic analysis of different extracts of *Calotropis gigantea* in thin liquid chromatography plates showed clear visible spots. When observed under UV lamp the spots were colorful and showed tailing. Thus, this indicates the presence of different volatile compounds that might have therapeutic benefit or might be impurities.

In the study of, the ethanol extract of the roots and stem showed the Rf values of 0.84 and 0.68 which was similar to my study in the ethanolic extract of *Calotropis gigantea leaves.*

DPPH Scavenging Free Radical Assay

DPPH is considered stable free radical due to the presence of odd electron and gives a strong absorption maximum at 517 nm imparting blue color. On accepting hydrogen atom from free radical scavenging antioxidant, DPPH radical changes its purple color to yellow for the formation of reduced DPPH.

In the present research, the antioxidant activity of the aqueous and alcohol fraction of the *Calotropis gigantea* extract was determined due to the presence of higher flavonoids and phenols in those extracts. The assay was done using antioxidant as a standard. Since the antioxidant activity is inversely proportional to IC_{50} value, the IC_{50} value of standard was lowest followed by aqueous extract and alcohol fraction of plant extract.

The IC_{50} values was found to be 22.49±0.218 µgm/ml for ascorbic acid, 93.325±0.531 µgm/ml for aqueous extract and 143.436±0.513 µgm/ml for alcohol extract of *C. gigantea*.

Antibacterial Activity

The antimicrobial screening was done by agar well diffusion method. The efficacy of plant was determined by measuring zone inhibition. For this test, ofloxacin of 1mg/ml was used as positive control and DMSO was taken as negative control. In the recent research, only the aqueous and alcoholic extracts of *Calotropis gigantea* showed activity only in gram negative bacteria *E. coli*. Both the extracts showed dose dependent response of *E. coli* whereas, other two gram-negative bacteria (S. typhi and *P. aeruginosa*) were resistant with gram positive *S. aureus*.

In previous study done by, the aqueous extract of *Calotropis gigantea* showed remarkable antibacterial effect with ZOI of 13.3±1.15 mm for *S. aureus*, 16.0±1.73 mm for *P. aeruginosa*, and 17.6±1.15 mm for *E. coli*. The reported result varied from my study. This might be due to climatic variation, geographical condition, difference in solvent used for extraction, and chemical used might not be of high grade.

Conclusion

In recent years, ethnomedicinal studies received much attention as this brings to light the numerous little known and unknown medicinal virtues especially of plant origin. Pharmacological screenings of *C. gigantea* revealed that the plant has several phytochemical constituents like alkaloids, glycosides, tannins, flavonoids, phenols, quinones, coumarins, proteins, terpenoids, and carbohydrates. The aqueous extract of this plant is very potent with highest TPC and TFC value of 38.45±0.03 mg GAE/ml and 26.28±1.87

mg QE/ml. Further, the plant is a good antibiotic and antioxidant agent.

Thus, this plant has a great medicinal potential and can be a valuable medicinal plant. Besides the therapeutic value, *Calotropis gigantea* grows naturally and needs little care. So, this plant is convenient source of medicine. Moreover, nowadays researchers and pharmacologists are searching for some ways to involve natural sources in the allopathic medicines. So, they are emphasizing in development of modern medicine from this plant.

In addition, this study justified that this ethno-medicinal can be a vital component for the production of modern drugs with fewer side effects and that can treat serious health problems like cancer. Therefore, systemic research and development work should be undertaken for the conservation of *C. gigantea* for their better economic and therapeutic utilization

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