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Calotropis Procera Plant Extract: Phytochemical Analysis, Antimicrobial Activity, and Coumarin Compound Identification

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Abstract

Phytochemical screening of *Calotropis procera* (Aakh, Milkweed) leaf and stem extracts was performed using solvents: hexane, benzene, dichloromethane, methanol and distilled water. zAlkaloids, carbohydrates, reducing sugar, tannins, phenolic compounds, gum and mucilage, flavonoids, quinines, and coumarin were all present. At a concentration of 20 g/mL, the methanolic extract of *Calotropis procera* demonstrated good antibacterial action against two bacteria: the gram-positive *Bacillus subtilis* and the gram-negative *Escherichia coli*, as well as fungi, *Candida albicans*. The therapeutic value of *Calotropis procera* is positively impacted by the zone of inhibition of bacteria and fungi. The extraction of coumarin from the plant extract was done using the Soxhlet extraction method. Preliminary tests were conducted to identify coumarin in plant extract. The tests include FTIR data collection and melting point determination concerning commercial coumarin. The melting point measurement was found to be 68±2˚C. The stretching band at 1702 cm^{-1} was attributed to >C=O functional group of coumarin. The plant extracts themselves can be applied in therapeutic use, and the pure form of coumarin can be utilized to create various derivatives, including acetyl coumarin, thiosemicarbazones, and metal complexes, which can be used to investigate antifungal, antibacterial, and anticancer properties.

Keywords: Antibacterial study, Calotropis procera, Coumarin, FTIR, Phytochemical

Introduction

Nepal, having a wide variety of biodiversity and geographical distribution, comprises a rich tradition of using medicinal plants. Aloe vera (*Aloe barbadensis miller*), Neem (*Azadirachta indica*), Turmeric (*Curcuma longa*), Ashwagandha (*Withania somnifera*), Kutki (*Picrorhiza kurrooa*), Satuwa (*Paris polyphylla*),

Chiraito (*Swertia chirayita*), Sarpagandha (*Rauvolfia serpentina*), Gokshura (*Tribulus terrestris*), Aak (*Calotropis procera*), Timur (*Zanthoxylum piperitum*), Tulshi (*Ocimum tenuiflorum*) etc. for treating various diseases. These plants are used to treat a wide range of ailments, including skin diseases, digestive disorders, cancer treatment, liver diseases, kidney problems, hypertension, insomnia, etc.

The evergreen, perennial shrub Calotropis procera is found throughout the tropics, as seen in Figure 1. Native to Africa, South Asia, and Nepal, the plant is erect, up to 5 meters tall, big, branching, and has large waxy leaves with milky latex throughout (Mansurat et al., 2020). It is commonly known as the Apple of Sodom, a name derived from the Hebrew tapuah Sodom (Adebayo et al*.,* 2015.). They are commonly known as Aakh in Nepal and are used in dyspepsia, paralysis, swelling, spraining, skin diseases, fever, diarrhea, etc. However, it is important to note that the plant is toxic and can cause skin irritation, vomiting, and even death if ingested in large volumes.

A widely accessible herb, *Calotropis procera* is typically used to treat infections of the external body. According to WHO, around 80% of the population depends on traditional medicine, especially in developing countries (Amini et al., 2021). However, only 50% of Western drugs contain plant bioactive compounds or their analogs as their active ingredients. The latex of *Calotropis procera* contains most hydrocarbon contents (Doshi et al*.,* 2012). Since ancient times, *Calotropis procera has been used for fiber, fuel, fodder, and other purposes* (Kaur et al., 2021). It has been used as a conventional bio-pesticide.

Figure 1

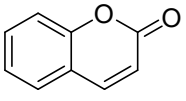
Calotropis Procera: (a) Growing Plants, (b) Flowers and Fruit

Phytochemical screening is a process that involves the extraction of different chemical compounds from a plant and the identification of the classes of compounds

present. The different classes of bioactive compounds that can be detected include alkaloids, flavonoids, terpenoids, saponins, tannins, glucosides, coumarin, anthraquinones, gum mucilage, etc. The phytochemicals are of different classes; some are nitrogen-containing compounds, polyphenolic compounds, isoprene units, benzene ring compressed compounds, etc., with a wide range of biological activities *viz.* analgesic, anti-inflammatory, antitumor effects, antioxidant, anticancer properties, antimicrobial activities, etc. Phytochemical screening can be done using various methods, including TLC, Infusion, Ultrasonic, HPLC, GC-MS, Soxhlet extraction, etc.

An antimicrobial is a substance that can destroy or hamper the growth of microbes/germs. Now, there are more classes of antimicrobial/antibacterial against which are numerous and effective. The purpose of antibiotics is to treat bacteria, while the purpose of antifungals is to treat fungi. They can also be categorized based on their function. Currently, the majority of pharmaceuticals used in medicine are synthetic. The rise of drug-resistant bacteria has generated a concerning clinical situation regarding microbial disease therapy. Multiple drug resistance has hindered the development of new synthetic antimicrobial medications, necessitating the quest for revolutionary antimicrobials from natural plant sources. In light of these factors, recent studies have concentrated on identifying natural chemicals found in plants that are vital for medicine to create innovative and potent drugs to treat microbial infections and illnesses (Hassan & Kazmi, 2015). Methanolic extract of *Calotropis procera* flavonoids showed significant antimicrobial activity against microorganisms *viz.* gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis) and gram-negative bacteria (*Pseudomonas aeruginosa and Salmonella enteritidis*) ranging the diameter of inhibition between 15.5 and 28.5 mm and *Candida albicans* with 30 mm.

Coumarin is a class of organic compounds that is found in many plants, such as tonka beans (*Dipteryx odorata*), sweet clover (*Melilotus*), *Calotropis procera*, turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), etc. Coumarin is a benzene ring fused to a pyrone ring (Luchini et al., 2008). Coumarin and its derivatives have a distinctive sweet scent and are used in producing perfumes, cosmetics, and flavorings. They are also used in medicine as anticoagulants, which means they help to prevent blood clots from forming. Some coumarin derivatives have been found to have anti-inflammatory and antitumor properties as well. However, coumarin can also be toxic in large amounts, so it is important to use it in moderation (Jain & Joshi, 2012).



Coumarin

Coumarin can be extracted or isolated from plants using various methods, including steam distillation, Soxhlet extraction, and liquid-liquid extraction. To isolate coumarin from plant extracts, a process called liquid-liquid extraction is typically used. This involves dissolving the plant material in a solvent, which is then separated from the extract using a separating funnel. The solvent is then evaporated to leave behind the coumarin compound (Niaz et al., 2020).

Soxhlet extraction is another best method that can be used to isolate coumarin from plant extracts. The plant material is placed in a thimble and inserted into a Soxhlet extractor during this process. The extractor is filled with a solvent, which is heated and allowed to evaporate and condense repeatedly, causing the solvent to circulate through the thimble and extract the coumarin. The solvent is then evaporated to leave behind the coumarin compound (Redfern et al., 2014). The coumarin can then be purified using column chromatography and recrystallization techniques. The specific extraction method will depend on the type of plant materials used and the properties of the extracted coumarin.

The difficulty of isolating bioactive chemicals from plant extracts is a research gap in plant extraction and compound isolation. Plant extracts frequently contain a variety of phytochemicals, or bioactive substances, with varying polarity. The separation of the pure form of the compound needs an advanced laboratory with various instrumentation facilities like FTIR, UV, NMR, TGA, EPR, CHNS, and ESI-MS spectrometry. The coumarin itself contains stable rings and acid amide group. To synthesize Schiff's base (thiosemicarbazones), a free carbonyl group needs to be synthesized by the acetylation process.

The continuation of this research work to introduce it in pharmacology needs purchasing additional chemicals, characterization by various spectroscopical techniques, and anticancer studies in other countries. These works may take time and a substantial budget. Therefore, it will be the best work only to the researchers who are planning to do their research for higher study and get grants from funding agencies like UGC, NAST, RECAST, etc.

The study aims to make extracts of *Calotropis procera* stems and leaves for antimicrobial study and coumarin identification using phytochemical screening and characterization of the compound by melting point determination and FTIR spectral studies.

Methods and Procedures

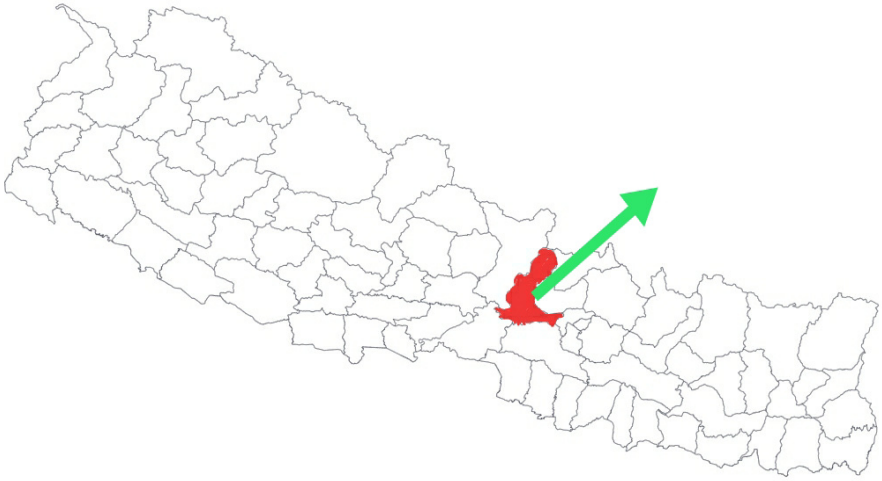
Sample Collection

Calotropis procera stems and leaves, as in Figure 2, were collected from

Khalte, Koshikhola, Neelkantha-5, Dhading, Nepal (Height-603 M, Latitute-27.84818˚ N, and longitude-84.98168˚ E).

Figure 2

Dhading District, Nepal, 45100, Where Samples were Collected



Rainwater and deionized water were used to clean the plant sample. After that, it was dried, grinded into tiny pieces, and crushed into powder with the help of a grinding machine. A variety of organic and aqueous solvents were used to dissolve the powdered materials. The sample extracts were diluted in organic solvents and water for phytochemical screening, and coumarin was extracted from the chloroform extract. All these steps involved in antimicrobial study, phytochemical screening, and the isolation of coumarin have been represented in Scheme 1.

Materials

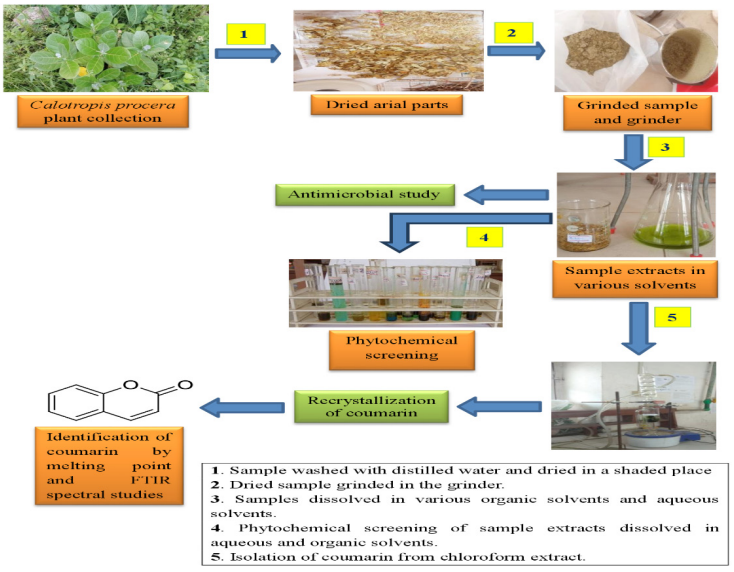
Distilled water, methanol, hexane, chloroform, benzene, DCM, (Fisher Scientific India Pvt. Ltd.) conc. H_2SO_4, conc. HNO_3, conc. HCl (35% extra pure, LABO CHEMIE Pvt. Ltd.), NaOH, KOH, ethyl acetate, acetone, Hager's reagent, Benedict solution, Fehling's solution, Mayer's reagent, Dregendroff's reagent, Wagner's reagent, etc. were used for the phytochemical screening of different solvent extracts of the plant.

Instruments

The melting point of isolated coumarin was determined using the Philip Harris melting point apparatus in the Central Department of Chemistry, Tribhuvan University (T.U.) Kirtipur, Nepal. Infrared spectra were measured from wave number 4000 to 400 cm^{-1} using PerkinElmer Spectrum IR Version 10.6.2 in the Department

of Chemistry, Amrit Campus, T. U., Nepal.

Methodology



Scheme 1. Methodology involved in preparing plant extract for antimicrobial studies, phytochemical screening, and isolation of coumarin

Preparation of *Calotropis procera* leaf and stem extract

Hexane extract: 10 g powder of stem/leaves was mixed with 80 mL of hexane. The solution was left for 1 day and filtered to get the extract.

Methanol/benzene extract: 10 g powder was mixed with 80 mL of methanol and benzene separately, left for 1 week, and filtered to get the respective extract.

Aqueous extract: 10 g powder was mixed with 130 mL of distilled water, left for 1 day, and filtered to get the extract.

DCM extract: 10 g of powder was mixed with 80 mL of DCM, left for 5 days, and filtered to get the extract.

The dissolved solution was filtered using Whatman's no 1 filter paper, and the filtrate was taken to study different phytochemical constituents.

Shaikh and Patil (2020) have documented the various techniques of preparing reagents, including Million's reagent, Mayer's reagent, Hager's reagent, Fehling's solutions, Benedict's reagent, and Dragendroff's reagent.

Tests for Alkaloids

Dragendroff's test: Two drops of the reagent were applied to a test tube containing one millilitre of plant extract.

Mayer's test: Two drops of the Mayer's reagent were carefully placed along the side of a clean test tube containing one millilitre of aqueous extract.

Wagner's Test: A few drops of Wagner's reagents were applied to a test tube containing 1 millilitre of plant extract.

Tests for Carbohydrates

Molish's test: Two drops of alcoholic α-naphthol were added to two milliliters of plant extract. After giving the mixture a good shake, a few drops of concentrated sulphuric acid were gradually added along the test tube's sides.

Test for Starch

To 2 mL of filtrate plant extract, 5 mL of 5% potassium hydroxide solution was added.

Test for Reducing Sugar

Benedict's test: 1 mL of plant extract and 1 mL of Benedict's solution were taken in a test tube and placed for boiling in a water bath for 2 minutes.

Fehling's test: 2 mL of a mixture of Fehling's solutions 'A' & 'B' was mixed with 1 mL of plant extract and placed in a water bath for boil.

Test for Fixed Oils and Fats

Spot test: A small quantity of plant extract was pressed between two filter papers.

Saponification test: A drop of phenolphthalein and a few drops of 0.5 N alcoholic KOH solutions were added to a tiny extract. A water bath was used to heat the mixture for two hours.

Tests for Glycosides

Legal's test: 1 mL plant extract was dissolved in pyridine, 1-2 mL sodium nitroprusside was added, and 10% NaOH solution was added.

10% NaOH test: The mixture of 1 mL of plant extract and 1 mL of dilute H_2SO_4 was boiled for 15 minutes. The boiled solution was allowed to cold and neutralize (concentration) by adding 10% NaOH solution, and 1 mL of Fehling's solutions 'A' and 'B' were added.

Borntrager's test: 2 mL of plant extract and 3 mL of the chloroform mixed and agitated well, the separated chloroform layer was envisioned, and then 10% of ammonium solution was mixed.

Test for Cardiac Glucosides

Bromine water test: To 1 mL of plant extract, a few mL of bromine water was added.

Test for Cardenolides

A 20% NaOH solution, pyridine, and sodium nitroprusside were added to 1 millilitre of plant extract.

Tests for Phenolic Compounds

Ferric chloride: A few drops of a neutral 5% ferric chloride solution were added to 3 milliliters of plant extract.

Lead acetate test: 3 millilitres of a 10% lead acetate solution were combined with plant extract.

Iodine test: A few drops of diluted iodine solution were added to 1 mL of plant extract.

Tests for Gum and Mucilage

3 mL of plant extract was mixed with 2 mL of absolute alcohol, carried out on constant stirring.

Tests for Tannins

Braymer's test: 1 mL of plant extract, 3 mL of distilled water, and 3 drops of ferric chloride were mixed.

10% NaOH test: 10 mL of 10% NaOH was combined with 1 mL of plant extract and thoroughly shaken.

Bromine water test: To 5 mL of bromine water, 2-3 drops of plant extracts were mixed.

Tests for Proteins

Biuret test: 2 mL of the extract was mixed with 1 drop of 2% copper sulphate solution, 1 mL of 95% ethanol, and potassium hydroxide pellets.

Test of Coumarins

10% NaOH test: 1 mL of plant extract was taken in a test tube, 1 mL of 10% NaOH was mixed with it, and a little chloroform was added.

Test for Anthraquinones

Borntrager's test: The mixture of plant extract and 10 mL of 10% ammonia solution was shaken vigorously for a minute.

NH_4OH test: The plant extract was dissolved in isopropyl alcohol and then 1 drop of conc. Ammonium hydroxide was added.

Tests for Flavonoids

Shinoda's test: Three milliliters of plant extract, magnesium turning, and a few drops of concentrated hydrochloric acid were put into a test tube.
Lead acetate test: 1-2 drops of 10% $Pb(C_2H_3O_2)_2$ were added to 1 milliliter of plant extract.

Ferric chloride test: A few drops of ferric chloride were added to a plant extract.

Conc. H_2SO_4 test: A few drops of conc. H_2SO_4 was put into a test tube containing 1 milliliter of plant extract.

Tests for Saponins

Foam test: 5 mL of extract was mixed with 10 mL of distilled water and shaken well.

Froath test: 0.5g of plant extract was mixed with 2 mL of distilled water.

$NaHCO_3$ test: 2 mL of plant extract, 2-3 mL of $NaHCO_{3,}$ and some volumes of distilled water were added and then shaken vigorously.

Tests for quinines

Sulphuric acid test: 1 mL of plant extract (isopropyl alcohol extract) was mixed with 1 mL of conc. $H_2SO_{4.}$

Conc. HCl test: One milliliter of concentrated hydrochloric acid was applied to one milliliter of plant extract.

Alc. KOH test: A few milliliters of Alc. KOH solution was combined with one milliliter of plant extract.

Isolation of Coumarin from *Calotropis procera* Extract

The powder sample of plants was weighed and placed in a thimble without any leakage and put inside the cleaned and dried Soxhlet extractor. The chloroform solvent was added to the RB flask, which was connected to a water condenser, and the flask was placed at a temperature of 40-50˚C. The solvent was evaporated and condensed with the condenser. The condensed solvent was dripped back into the thimble about 9-10 times to rotation, and the extracted sample was collected in a

beaker (Apu et al., 2018).

Isolation Process

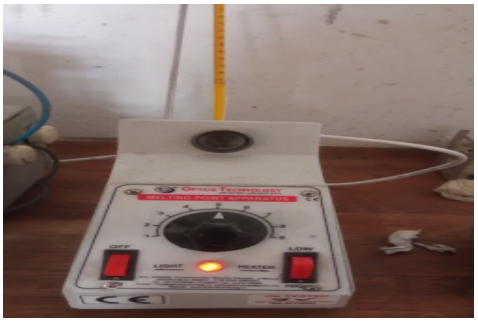
The plant extract in the RB flask was dried, and the dried extract was dissolved with 1N NaOH solution for refluxing at about 50˚C for 3 hours. The refluxed solution was placed for cooling for about half an hour and filtered by Watman's No. 1 filter paper. The yellow filtrate was taken in a beaker, and 1N HCl was added to it till acidic medium. The acidic solution was placed in a refrigerator for 24 hours to get coumarin crystals. The isolated solution was recrystallized to get a pure form of coumarin (Mustafa et al., 2018).

Recrystallization

There were two types of coumarin compounds: polar and non-polar. Non-polar compounds are highly soluble in organic solvents like ethanol and methanol but slightly soluble in water. Polar coumarin compounds are highly soluble in acetonitrile. Isolated coumarin compound was found to be non-polar and soluble in methanol. The solid sample was dissolved in 40% of aqueous methanol solvent, partially dissolved at room temperature but completely dissolved in a water bath at 40-50˚C. The dissolved hot solution was cooled at room temperature at 28˚C to get a crystalline form of the coumarin. Preliminary identification of coumarin was carried out by measuring the melting point (Figure 3) with reference to commercial coumarin.

Figure 3

Measuring the Melting Point



Antimicrobial Activity

Three microbial strains' active cultures were used in the investigation. Himalayan Research Institute of Biotechnology Pvt. Ltd. Radhe Radhe Bhaktapur supplied the microbial strains. Two bacteria and one fungus were included in the investigation. Gram-negative bacteria Escherichia coli ATCC 8739 and gram-positive bacteria Bacillus subtilis ATCC 6051 were among the microorganisms. It was

Candida albicans ATCC 2091, a fungal organism.

With reference to the Kanamycin A standard zone, the Bauer Agar Disc Diffusion method (Shandil et al., 2007) was used to screen the compound for antimicrobials using one species of gram-positive bacteria (Bacillus subtilis ATCC 6051), one species of gram-negative bacteria (Escherichia coli ATCC 8739), and one species of fungi (Candida albicans 2091). The antibacterial activity of the plant extract was estimated by measuring the average diameter of the zone of inhibition (ZOI) that plant extracts formed on the agar surface against specific pathogenic bacteria.

Microbes are cultivated on nutritional agar, a general-purpose nutrient medium. Because it includes many nutrients necessary for bacterial growth, it promotes the growth of various non-fastidious organisms.

In a conical flask of the proper size, nutrient agar was added to distilled water in a ratio of 28 g/L. The flask was then boiled while continuously shaken and autoclaved for 15 minutes at 121°C and 15 pounds of pressure. The sterilized media were allowed to cool to roughly 50°C. Aseptically, they were divided into 25 mL portions, with each plate on sterile Petri dishes having a 90 mm diameter and appropriately labeled. Plates were allowed to solidify in their current state.

The nutrient agar without the solidifying component, agar powder, is called nutrient broth. Microbial stocks are commonly preserved using liquid-form media that remain liquid at room temperature. The conical flask mixed 1.3 g of nutrient broth powder with 100 mL of distilled water. They were added and dissolved completely. It was autoclaved for 15 minutes at 121°C and 15 lb pressure to sterilize it. After letting it cool, 10 mL of it was transferred into a screwed bottle and sterilized once again.

Mueller Hinton Media (MHA) contains beef extract, acid hydrolysate for casein, starch, and agar. MHA was widely used to justify the regular testing of undemanding microorganisms using the Kirby-Bauer disk diffusion technique. To prepare the media, 3.8 g of the medium was suspended in 100 mL of distilled water and heated with frequent agitation. The mixture was boiled for a minute to ensure complete dissolution of the medium (Tille & Forbes, 2014). Then the medium was autoclaved at 15 lb. pressure and 121°C for 15 minutes and cooled to room temperature. Thus prepared Mueller Hinton Agar was poured into sterile Petri dishes in the ratio of 10 mL per plate on a level, horizontal surface to give uniform depth (Wang *et al.,* 2023*)* and allowed to cool to room temperature.

Potato Dextrose Agar was used for the cultivation of fungi. It was a general-purpose medium for yeasts and molds that could be supplemented with acid or antibiotics to inhibit bacterial growth. Fungi were cultivated on potato dextrose agar.

It was an all-purpose medium for molds and yeasts that could be enhanced with antibiotics or acid to prevent the growth of bacteria. Agar is added as the solidifying agent. 3.9g of commercial PDA powder was added to 100 mL of distilled water and boiled while mixing to dissolve. To sterilize the media, they were autoclaved for 15 minutes at 121°C and 15 lb of pressure. It was then chilled to roughly 50°C before being transferred onto 10 mL Petri dishes. For solidification, the plates were let to stand at room temperature.

The primary culture plates used to create it were described above, along with 5 µL of Kanamycin A, a common antibiotic was employed. Aseptically, the isolated bacterial colony was sub-cultured on nutritional agar plates using the inoculating loop, and it was then incubated for 12 hours at 37°C.

Results and Discussion

Phytochemical Analysis

To find the phytoconstituents, a phytochemical screening of Calotropis procera leaf and stem extract was carried out in various organic solvents. The phytochemical findings are reported in Tables 1 and 2, referencing Shaikh and Patil (2020).

Table 1

Phytochemicals Present in the Calotropis procera Leaf Extract in four Different Solvents: benzene, hexane, methanol, and water

S.N.	Phytochemicals.	Benzene Extract	Hexane Extract	Methanol Extract	Aqueous Extract
1.	Alkaloids	-	-	+++	+++
2.	Flavonoids	+	-	+	-
3.	Reducing Sugar	+	-	+	+
4.	Carbohydrates	+	+	-	+
5.	Tannins	+++	-	+++	+
6.	Quinines	++	+	++	-
7.	Saponins	-	-	++	++
8.	Glycosides	-	++	-	+
9.	Cardiac Glycoside	+	+	+	+
10.	Phenolic Compounds,	+	++	-	++
11.	Proteins and Amino Acids	-	+	-	-
12.	Anthraquinone	-	-	-	-
13.	Coumarin	+	+	+	+
14.	Gum and Mucilage	+	+	+	+
15.	Fixed Oils and Fats	-	-	-	-
-(Absence), + (presence), ++ (Presence in two test), +++ (Presence in three test)					

Table 2

Phytochemicals Present in the Calotropis Procera Stem Extract in four Different Solvents: hexane, DCM, methanol, and water

S.N.	Phytochemicals	Hexane Extract	DCM Extract	Methanol Extract	Aqueous Extract
1.	Alkaloids	-	++	+++	-
2	Tannins	+++	+++	++	+
3.	Carbohydrates	++	-	+	++
4	Flavonoids	-	+	-	-
5.	Reducing Sugar	-	-	+	-
6.	Cardiac Glycosides	-	-	+	-
7.	Glycosides	+	+	-	-
8.	Protein and Amino Acids	+	-	-	-
9.	Phenolic Compounds	-	+	++	-
10.	Saponins	++	-	-	++
11.	Quinines	-	++	++	-
12.	Anthraquinone	-	-	-	-
13.	Coumarin	+	+	++	+
14.	Gum and Mucilage	+	+	+	+
15.	Fixed Oils and Fats	+	-	-	+
-(Absence), + (presence), ++ (Presence in two test), +++ (Presence in three test)					

Antimicrobial results of methanol extract of plant

Agar Well diffusion methods were used to mount the antibacterial activity of the Calotropis procera plant extract in a methanolic solution against two American Type Culture Collection (ATCC) and a fungus. The zone of inhabitation (ZOI) by various bacteria and fungi was mentioned in Figure 4, and the results are presented in Table 3.

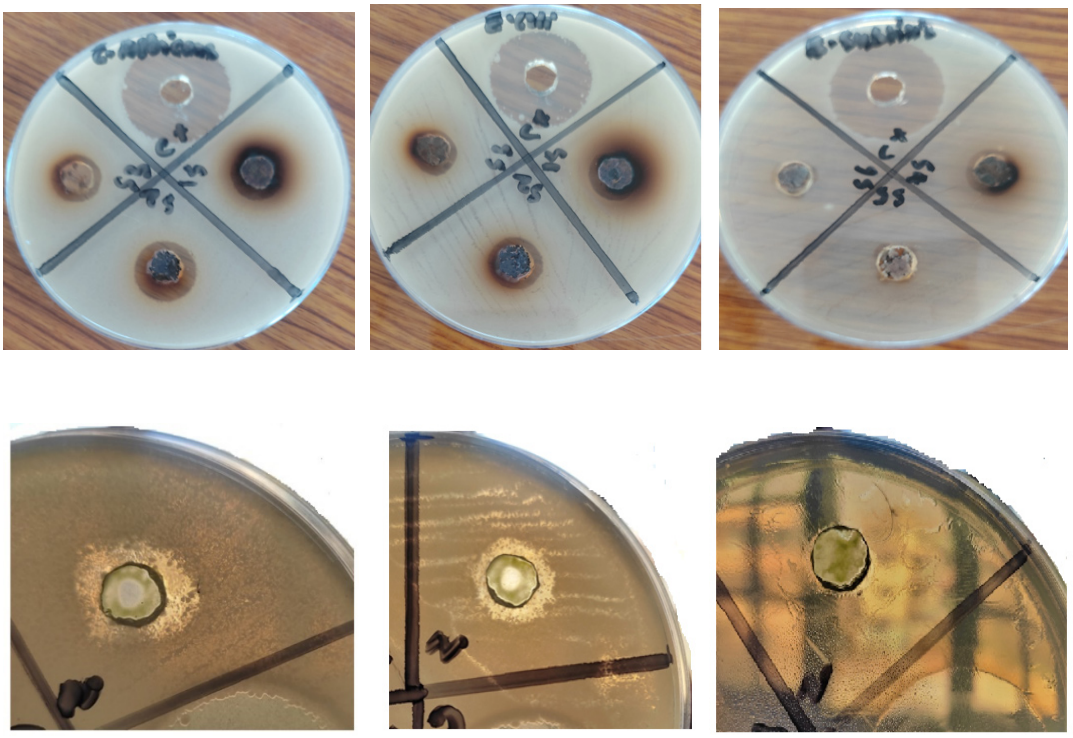
Table 3

Antimicrobial Study of Methanol Extract of C. procera

Microorganisms test organism (ATCC)	Reference culture	Type	Methanolic extract zone of inhibition (mm)	The standard value of zone Kanamycin A (mm)
Candida albicans (Fungus)	ATCC 2091	-	4	10
Bacillus subtilis (Bacteria)	ATCC 6051	Gram-positive	2	10
Escherichia coli (Bacteria)	ATCC 8739	Gram-negative	3	10

Figure 4

Antimicrobial Study against (a) Candida albicans, (b) E. coli, and (c) Bacillus subtilis



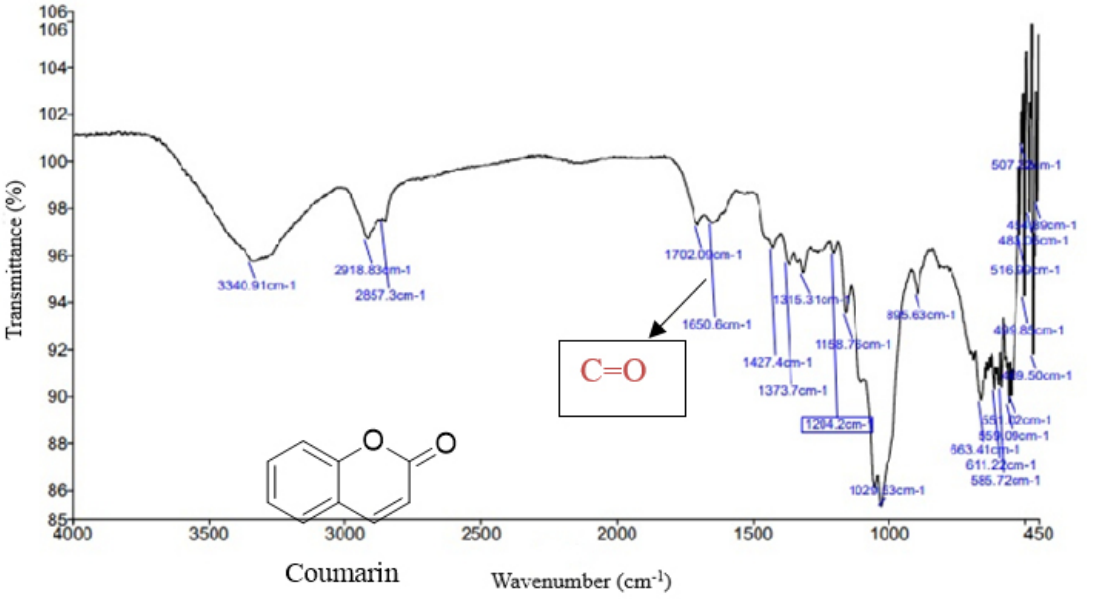
(a) **(b)** **(c)**

Identification of Coumarin

Compared with commercial coumarin, the melting point, 68±2˚C, and stretching vibration due to >C=O functional group present at 1702 cm^{-1} show the identification of coumarin in the extract, as shown in Figure 5. Thiosemicarbazones of heterocyclic compounds and their copper (II) complexes have been found to exhibit significant anticancer potency, so a pure form of isolated coumarin may be used to form its thiosemicarbazones and copper complexes for their test in biological assay (Singh & Yadav, 2021; Singh et al., 2022).

Figure 5

FTIR Spectra of Coumarin



Conclusion

The screening of *Calotropis procera* stem and leaf extracts for phytochemicals revealed the presence of phenolic compounds, alkaloids, carbohydrates, reducing sugar, flavonoids, quinines, coumarin, gum and mucilage, tannins, and saponins. The antimicrobial studies of the methanol extract using the ATCC technique revealed antimicrobial activity hostile to two bacteria, *viz.* gram-positive bacteria *Bacillus subtilis*, which can inhibit 2 mm of diameter, and gram-negative bacteria *Escherichia coli*, which can inhibit 3 mm, and fungi *Candida albicans* can inhibit 4 mm on the concentration 20 g/mL. The extracts exhibited good antimicrobial activities. The organic compound coumarin, isolated using the Soxhlet extraction of chloroform extract, was identified by determining its melting point and FTIR spectral analysis.

The pure form of coumarin can be isolated using column chromatography, and its derivatives, such as acetyl coumarin, acetyl coumarin thiosemicarbazones, and their metal complexes can be synthesized for their biological studies like antimicrobial, anticancer, etc.

Abbreviations

ATCC (American Type Culture Collection), DCM (Dichloromethane), g/L (grams

per liter), lb (pound), VF (Volumetric flask)

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