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PHYTOCHEMICAL SCREENING AND ANTI-MICROBIAL PROPERTIES OF *CALOTROPIS GIGANTEA*

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ABSTRACT
The present study was focused on the phytochemical screening and anti-microbial studies of *Calotropis gigantea* plant extracts (stem, leaf & flower). The crude extract of C. gigantea stems, leaves & flowers were prepared by using distilled water as solvent. The plant extract was subjected to the phytochemical tests and anti-microbial studies. The phytochemical screening of *C. gigantea* plant extracts showed the presence of alkaloids, glycosides, tannins, steroids and tri terpenoids, flavonoids, proteins and phenols etc. Anti-microbial studies showed results for *Pseudonomos aeruginosa*, *Klebsiella pneumonia* and *Aspergillus niger*. The results suggest that the chemical compounds present in the extracts of *C. gigantea* could further be exploited as effective antimicrobial agents.

KEYWORDS: *Calotropis gigantea*, anti-microbial, phytochemicals.

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.[1] This plant is referred as “shallow wort”, “giant milkweed” and “shallow wort”. They have typical thick, wide leaves and odourless, purplish-coloured flowers that make their identification easier. The white milky latex produce by this plant has cardiac glycosides calotropin, uscharin, calotoxin, calactin, and uscharidin and gigantin, are found to have great wound healing activity.[2] While the leaves are that to possess antimicrobial and anti-inflammatory properties.[3] 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. The extract of different parts of C. gigantea has been reported to have many promising primary and secondary metabolites and can be separated or combined for the development of effective drugs against various types of cancer.[4] Phytochemicals are biologically important molecules that play a critical role in the plant’s defence system. Many selective phytochemicals including primary and secondary metabolites have been developed as drug molecules. Carbohydrates, chlorophyll, proteins, and lipids form the primary metabolites, while the secondary metabolites include alkaloids, tannins, saponins, phenols, flavonoids, and terpenoids. 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.[5] While the leaves are that to possess antimicrobial and anti-inflammatory properties.[6] 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.[7]

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

Figure 1. Calotropis gigantea plant.

Figure 2. Calotropis gigantea flower.

Figure 3. Calotropis gigantea leaf.

Vegetative characters

Calotropis gigantea is a shurb or a small tree up to 2.5 m (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.[8] 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.[9]

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.[10] 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.

• Root and bark of Calotropis gigantea shows wound healing activity.[11]

• The cardenolide glycosides collected from the root Calotropis gigantea were reported to carry cytotoxic activity against several human and mouse cell lines.

• 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.[12]

• Methanol extract of roots shows good insecticidal activity.[13]

• Ethanol extract of Calotropis gigantea shows anti-inflammatory activity.

• Leaves of Calotropis gigantea carry profound amounts of antioxidants.

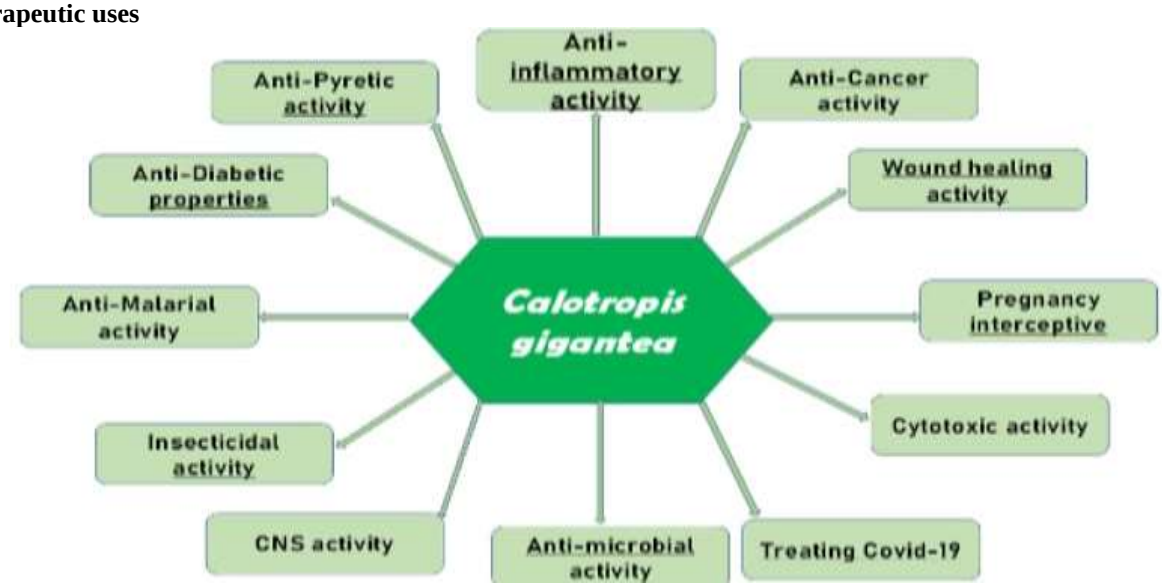
• Ethanol extract of stems has hepatoprotective activity.

• The leaves have anti-asthmatic property.

• 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.

• Aqueous extract of latex possesses antibacterial activity against S. aureus, E. coli, B. cereus, P. aeruginosa.

Therapeutic uses



MATERIALS AND METHODS

Identification and Collection of Plant

The medicinal plant *Calotropis gigantea* was collected in and around Coimbatore and Palakkad regions. The plant was authenticated by Dr. M. U. Sharief, Scientist 'F'& Head of Office, Government of India, Ministry of Environment, Forest & Climate Change, Botanical Survey of India.

Preparation of aqueous extracts

Maceration is one of the oldest and simplest extraction techniques.[14] The solid to be extracted, once introduced into the container, is completely covered by the solvent, minimizing contact with the air, so that the liquid can enrich itself as much as possible with the substances contained in the solid matrix. Maceration is an extraction procedure in which coarsely powdered drug material, either leaves or stem is placed inside a container; the menstrum is poured on top until completely covered the drug material. The container is then closed and kept for at least three days. The content is stirred periodically, and if placed inside bottle it should be shaken time to time to ensure complete extraction. At the end of extraction, the micelle is separated from marc by filtration or decantation. Subsequently, the micelle is then separated from the menstrum by evaporation in an oven or on top of water bath. This method is convenient and very suitable for thermolabile plant material. The plant was washed several times in running water. Then the parts were dried in shade place at room temperature for about 2-3 weeks. After drying the stems, leaves & flowers were grinded into fine powder using grinding machine. The powder was then sieved through sieve no.66 &100.The powder was extracted by maceration. The powdered crude drug of *Calotropis gigantea's* leaf, stem and flower was extracted using distilled water. The powder (3 g) was measured and mixed with 30 mL of distilled water[15] in a conical flask. For extraction, the conical flask was plugged with a cotton plug and was placed in a shaker at 28°C for 24 h at 150 rpm. The mixture was filtered through muslin cloth and Whatman (No. 1) filter paper to obtain the extract. The green sticky mass of 150 g extract was labelled and preserved in the refrigerator at 4°C until use.[16] Suspensions of the extract were freshly prepared for experimental use.

Phytochemical Screening

C. gigantea extracts (stem, leaf & flower) were subjected to successive standard phytochemical screening following the method of Harborne (1983) to identify alkaloids, glycosides, steroids, flavonoids, tannins, proteins and phenols.[17] All phytochemicals were carefully screened and identified by characteristic colour changes following standard procedures.

Anti-microbial Activity

Agar well diffusion method was used to screen the antibacterial and antifungal activities of the extracts. 70µl of fresh bacterial (*P. aeruginosa* and K. *pneumoniae*) and fungi (*A. niger* and *A. flavus*) culture was pipetted in the centre of sterile (sterilization at 121^0C for 15 minutes) petri dishes (39g of Mueller Hinton agar in 1000ml of distilled water for bacteria and 38g of malt agar in 1000 ml of distilled water for fungi) containing solidified media. Followed by spreaded with cotton swab and, wells were made using a sterile cork borer (6 mm in diameter). Then, 50 µl of each extract was added to respective wells and the plates were incubated (24 hrs at 37°C for bacteria and 5 days at 30^0C for fungi). Antimicrobial activity was detected by measuring the zone of inhibition (excluding the wells diameter) appeared after the incubation time. Vancomycin disc was used as positive control for bacteria and fluconazole was used for fungi.[18]

RESULTS AND DISCUSSION

The plant *Calotropis Gigantea* shows a lot of tremendous therapeutic effect and having a large economic value. In combination of past research and studies reports justify the use of *Calotropis gigantea* by traditional health care

for professional for the treatment of various pathological alterations. The plant having huge number of phytochemical ingredients and various active constituents and having various organic and inorganic salts including macro and micronutrients.

Phytochemical Screening

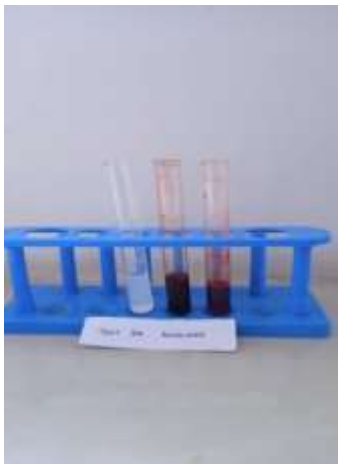
The value of the physiochemical parameters of the plant can be used as indicator in authentication and for assuring the quality of the powder form so that possibility of substitution and adulteration could be avoided. The results of preliminary phytochemical screening of stem, flower, leaf extracts of *Calotropis gigantea* showed presence of alkaloids, glycosides, flavonoids, proteins, phenol, steroids and triterpenoids and tannins.

Test for Alkaloids

(a) ***Dragendorff's test***. 1mL of extract was taken and placed into a test tube. Then 1mL of potassium bismuth iodide solution (Dragendorff's reagent) was added and shaken. An orange red precipitate formed indicates the presence of alkaloids.

(b) ***Wagner's test.*** 1mL of extract was taken and placed into a test tube. Then 1mL of potassium iodide (Wagner's reagent) was added and shaken. Appearance of reddish-brown precipitate signifies the existence of alkaloids.

(c) ***Mayer's test***. 1mL of extract was taken and placed into a test tube. Then 1mL of potassium mercuric iodide solution (Mayer's reagent) was added and shaken. Emergence of whitish or cream precipitate implies the presence of alkaloids.



Test for Glycosides

(a) ***Bontrager's test (modified).*** One gram of the crude extract was first weighed, placed into a test tube, and dissolved in 5mL of dilute hydrochloric acid. Then 5mL of ferric chloride (5%) solution was added. The mixture was shaken and placed over water bath. Then the mixture was allowed to boil for 10min, cooled, and filtered. Afterward, the mixture was then extracted again with benzene. Finally, equal volume of ammonia solution was added to benzene layer. Appearance of pink color indicates the presence of anthraquinone glycosides.

(b) ***Legals test.*** 1mL of an extract was taken, and then an equal volume of sodium nitroprusside was added followed by few quantities of sodium hydroxide solution and shaken. Formation of pink-to-blood-red precipitate signifies the existence of cardiac glycoside.

(c) ***Keller–Killiani test.*** 2mL of the extract was taken and diluted with equal volume of water. Then 0.5mL of lead acetate was added, shaken, and filtered. Again, the mixture was extracted with equal volume of chloroform, evaporated, and dissolved the residue in glacial acetic acid. Then few drops of ferric chloride were added. Again, the whole mixture was placed into a test tube containing 2mL of sulfuric acid. Emergence of reddish-brown layer that turns bluish green implies the presence of digitoxose.

Test for Steroids and Triterpenoids

(a) Salkowski's test. 1mL solution of the extract was taken and 2mL of chloroform was added, shaken, and filtered. Few drops of concentrated sulfuric acid were added to filtrate, shaken, and allowed to stand. Development of golden-yellow precipitate indicates the presence of triterpenes.

Test for Tannins

(a) ***Gelatin's test.*** 1mL of extract was taken and placed in a test tube. Then 1% gelatin solution containing sodium chloride added and shaken. Appearance of white precipitate indicates the presence of tannins.

Test for Flavonoids

(a) ***Shinoda's test.*** 1mL of extract was taken and placed into a test tube. Then, few drops of concentrated hydrochloric acid was added followed by 0.5mg of mRimandoium turnings and shaken. Emergence of pink coloration indicates the presence of flavonoids.

(b) ***Lead acetate test***. To detect the presence of flavonoids, 1mL of extract was taken and placed into a test tube. Then few drops of lead acetate added and shaken. Formation of yellow precipitate signifies the presence of flavonoids.

(c) ***Alkaline reagent test.*** 1mL of extract was taken and placed into a test tube. Then few drops of sodium hydroxide solution were added and shaken. Emergence of intense yellow color that turns to colorless after adding dilute acid implies the existence of flavonoids.

Test for Phenols

a. Ferric chloride test. 1mL solution of an extract was taken and placed into a test tube. Then 1% gelatin solution containing sodium chloride was added and shaken. Formation of bluish-black color indicates the presence of phenols.

Test for Proteins

(a) ***Biuret test.*** Some quantity of an extract was taken and 4% sodium hydroxide solution of the drug was produced. This is followed by the addition of 1 % copper sulfite. Appearance of violet color implies the existence of peptide linkage.

(b) ***Ninhydrin test.*** 1mL of an extract was taken and placed into a test tube. Then 0.25% of ninhydrin reagent was added and shaken. The mixture was then boiled for few minutes. Formation of blue color signifies the presence of protein.

(c) ***Xanthoproteic test***. 1mL of the extract was taken and placed it into a test tube. Then few drops of nitric acid were added and shaken. Emergence of yellow-color indicates presence amino acids containing an aromatic nucleus (tyrosine, tryptophan and phenylalanine) in a protein solution which gives yellow color nitro derivatives on heating with conc. HNO3.

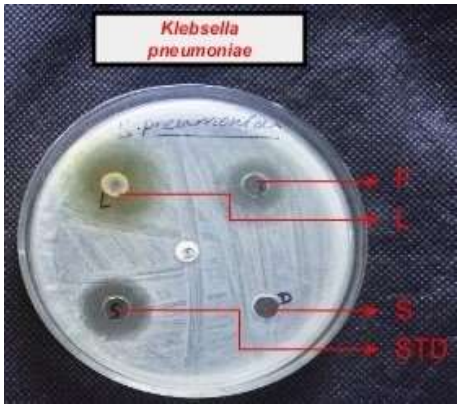
Table 1: The phytochemical tests of *Calotropis gigantea* extracts (stem, leave & flower).

Phytochemical test	Stem	Leaf	Flower
Dragondroffs test	+	+	+
Wagner's test	+	-	-
Mayers test	_	+	-
Bontrager's test	_	+	+
Legals test	+	+	+
Killer-kellani test	+	-	-
Salkowski test	-	+	-
Gelatin test	-	+	+
Shinoda test	+	+	-
Lead acetate test	+	+	-

Alkaline test	+	-	-
Ferric chloride test	+	+	-
Lead acetate test	_	+	+
Biurette test	_	+	-

Anti-microbial Activity

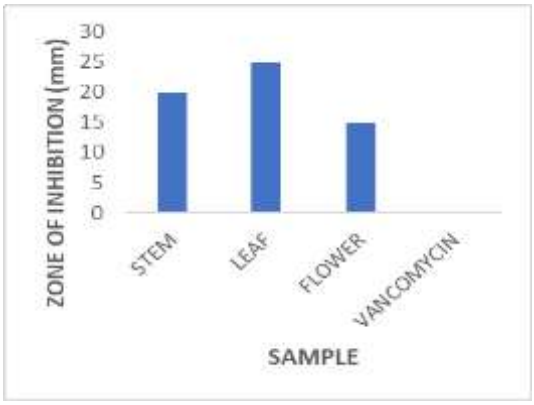
The presence of anti-bacterial and anti- fungal activity in the shade dry stem, leaf & flower extracts of *Calotropis gigantea* was obtained against human pathogenic organisms. The extracted was tested against bacterial pathogen such as *Pseudomonas aeruginosa* & *Klebsiella* pneumonia and fungal pathogen such as *Aspergillus flavus* & *Aspergillus niger.* The antimicrobial screening was done by agar well diffusion method. The efficiency of plant was determined by measuring zone inhibition. *Calotropis gigantea* extracts of (stem, leaf & flower) showed antibacterial activity. Anti-fungal activity was presence only for the extracts of leaf & flower against Aspergillus niger.



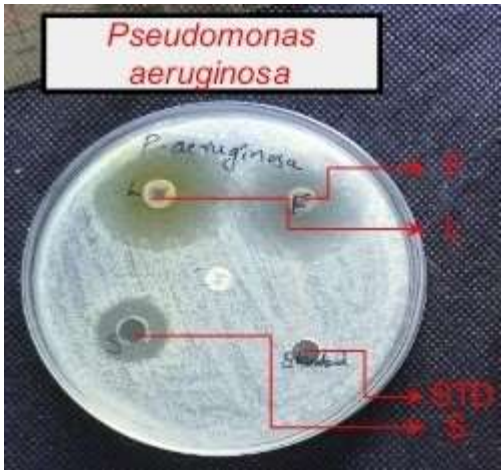
***Klebsiella* pneumonia**

Table 2: Comparative studies of sample (stem, leaf and flower extracts).

SAMPLE	ZONE OF INHIBITION
Stem	20mm
Leaf	25mm
Flower	15mm
Vancomycin	0mm



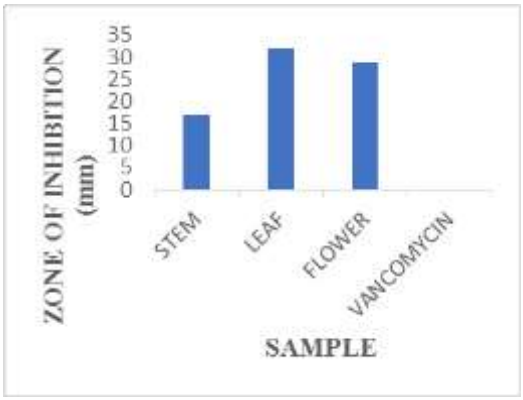
Graph 1: Comparative studies of sample, (stem, leaf and flower extracts).



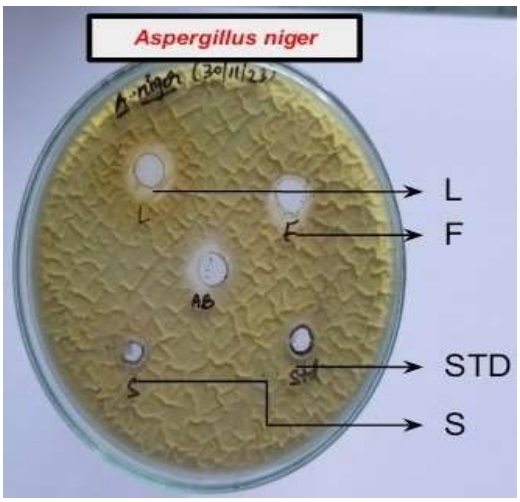
Pseudomonas aeruginosa

Table 3: Comparative studies of sample, (stem, leaf and flower extracts).

SAMPLE	ZONE OF INHIBITION
Stem	17mm
Leaf	32mm
Flower	29mm
Vancomycin	0mm



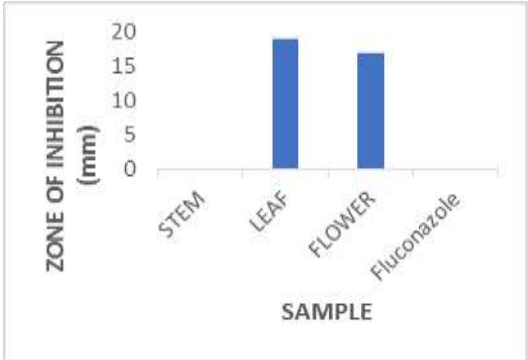
Graph 2: Comparative studies of sample, (stem, leaf and flower extracts).



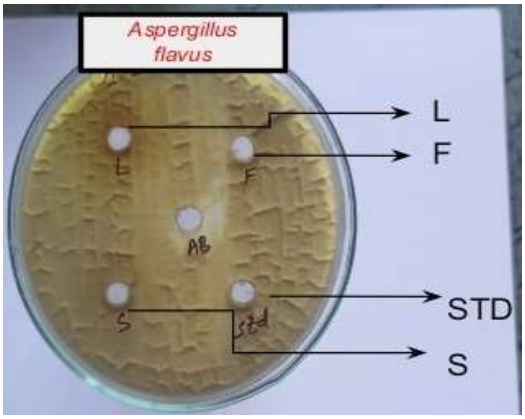
Aspergillus niger

Table 4: Comparative studies of sample, (stem, leaf and flower extracts).

SAMPLE	ZONE OF INHIBITION
Stem	0mm
Leaf	19mm
Flower	17mm
Fluconazole	0mm



Graph 3: Comparative studies of sample (stem, leaf and flower extracts).



Aspergillus flavus

Table 5: Comparative studies of sample (stem, leaf and flower extracts)

SAMPLE	ZONE OF INHIBITION
Stem	0mm
Leaf	0mm
Flower	0mm
Fluconazole	0mm

CONCLUSION

This study was screened for the phytochemical constituents and anti-microbial effects of the aqueous extracts *C. gigantea* plant (stem, leaf & bark) extracts. The presence of phytochemicals as major constituents in *C. gigantea* may uphold the medicinal property of this plant. The plant extracts also expressed anti-microbial activity. The aqueous extracts of the plant were found to be effective against *Pseudonomos aeruginosa*, *Klebsiella pneumonia* and *Aspergillus niger* during antimicrobial screening. The results suggest that the chemical compounds present in the extracts of *C. gigantea* could further be exploited as effective antimicrobial agents.

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