Potential Anti-Inflammatory Properties of *Calotropis gigantea* Extracts: A Promising Alternative to Traditional Medicine

Rakhi Saxena, Jitendra Malviya, Sunita Singh

Abstract

Herbal treatments have shown potential in treating various chronic inflammatory diseases, such as rheumatoid arthritis and leprosy. The plant *Calotropis gigantea* from the *Asclepiadaceae* family has been traditionally used by healers in Asia and Africa to treat infectious disorders, as well as inflammatory illnesses including boils, gout, and leprosy.

Material and Method

This study aimed to investigate the anti-inflammatory and antioxidant properties of *C. gigantea* leaf extracts obtained using methanol, petroleum ether, and water. The Soxhlet extraction method was employed to extract the phytoconstituents from the leaves, and qualitative and quantitative analysis were used to determine their concentrations. The extracts were tested for their ability to scavenge free radicals, limit protein denaturation, inhibit enzymes causing tissue injury, stabilize membranes, and exert antioxidant potency.

Results

The results showed that the methanolic extract of *C. gigantea* had significantly higher anti-inflammatory and antioxidant activity than the aqueous and petroleum ether extracts. GC-MS analysis of the extracts revealed a diverse range of medicinal compounds.

Conclusion

In conclusion, this study suggests that the methanolic leaf extract of *C. gigantea* has potential for treating inflammatory diseases due to its strong anti-inflammatory and antioxidant properties.

Keywords:*C. gigantea's* methanolic extract, DPPH, FRAP, GC-MS

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Introduction

Inflammation is a fundamental immunological response that plays a vital role in the development and pathogenesis of chronic diseases. It is triggered by injurious stimuli, which results in discomfort, redness, edema, and heat. Inflammatory responses involve the enlargement of blood vessels, followed by the recruitment of neutrophils, macrophages, and lymphocytes at the site of injury. The release of arachidonic acid, a polyunsaturated fatty acid, occurs when the cell membrane is damaged, which is converted into eicosanoids by various enzymes. Eicosanoids serve as inflammatory mediators and precursor molecules to the COX and lipoxygenase pathways. These pathways produce hydroperoxyl fatty acids, leukotrienes, and prostaglandins, which contribute to various inflammatory disease progressions. Non-steroidal anti-inflammatory drugs (NSAIDs) treat the symptoms of inflammation by inhibiting COX-1 and COX-2 enzyme pathways or by reducing the production of PGs and TX. However, NSAIDs have negative side effects such as gastrointestinal mucosal damage, kidney failure, respiratory issues, liver damage, and cardiovascular problems.

Oxidative stress and inflammation are linked, and cellular oxidative stress can occur due to the overproduction of free radicals during chronic inflammation. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have advantageous and detrimental impacts on the organism. Plants containing polyphenolic compounds have the ability to scavenge free radicals and prevent cell damage and aging.

Herbal medicines have played a significant role in ancient medicine, and they are more effective, less poisonous, affordable, and distinctive. The World Health Organization (WHO) reports that 80% of people worldwide utilize natural therapies. Traditional medical systems such as Ayurveda, Unani, and Siddha use a variety of plant species to treat a wide range of maladies. The entire plant or any of its parts, such as the flowers, leaves, roots, bark, fruits, and seeds, are used to make herbal medications in phytomedicine.

Plant-based medicines are more potent and secure than synthetic ones. Plants produce various primary and secondary metabolites, including phytochemicals. Flavonoids, phenols, alkaloids, tannins, saponins, steroids, and carbohydrates are some of the bioactive components that serve a variety of biological purposes. These phytoconstituents have antioxidant, analgesic, anti-inflammatory, anti-arthritic, anti-cancerous, anti-fungal, anti-diabetic, anti-malarial, and immunomodulatory properties. Herbal extracts have been shown to lower the degree of NO synthase induction, and flavonoids and terpenoids, components of plants, have anti-inflammatory characteristics. *C. gigantea* is a plant species that has excellent medicinal characteristics in Siddha, Ayurveda, Unani, homeopathy, and Chinese medicine. The genus *C. gigantea*, a member of the Apocynaceae family, is a highly valued medicinal plant that is widely distributed across southern Asia and Africa, with a concentration in India. This xerophytic plant is capable of surviving in dry and saline environments, thanks to its thick wax-covered leaves and well-branched root system. The plant contains a variety of phytochemical substances, including cardiac glycosides, saponins, flavonoids, alkaloids, and tannins, which are found in various parts of the plant, including its seeds, leaves, and latex.

Traditional Ayurveda medicine has used *C. gigantea* for many years to treat various ailments, including asthma, snake bites, and rheumatic ailments, among others. Numerous studies have supported the traditional use of *C. gigantea* in treating various illnesses. The plant's extracts have shown promising anti-inflammatory and antioxidant properties, making it an excellent candidate for treating diseases such as rheumatoid arthritis, hepatitis, cancer, Alzheimer's, and Parkinson's diseases.

Herbal remedies are gaining popularity in recent years due to their easy accessibility, affordability, and low incidence of side effects. The crude extracts of *C. gigantea* were examined in this study using petroleum ether, methanol, and aqueous solvents to identify the plant's pure biological compounds. In vitro tests were performed to assess the plant's anti-inflammatory and antioxidant properties. GC-MS analysis was

conducted to determine the chemicals present in the leaf extracts.

The results of this study are promising, and further research is warranted to investigate the full potential of *C. gigantea* in treating various ailments. By conducting preclinical tests on in vivo models, the efficacy and safety of *C. gigantea* extracts can be established, leading to the development of new drugs for treating inflammatory diseases. The use of herbal remedies offers an alternative to chemically produced drugs and provides a natural way to alleviate symptoms with fewer side effects.

Material and Method

In the Madhya Pradesh province of India, fresh *C. gigantea* leaves were obtained on and near the Vidisha district. Dr. Ajay Bharadwaj, Department of Botany, Institute of Biotechnology IEHE, Bhopal, Madhya Pradesh, performed the identification and authenticity of fresh leaves of *C. gigantea*. Additionally, the plant's name has been confirmed in the online database of worldwide plant species recognized as *Calotropis gigantea* of Family *Asclepiadaceae*, Milkweed (http://www.worldfloraonline.org/).

Study Area and Sample site

The research problem was centered on *Calotropis gigantea*. The plant parts of these two species are antibacterial, larvicidal, laxative, and abortifacient. Cardenolides, heart-energizing and proteolytic compounds, are abundant. Besides being restorative, the species have phytochemical potential and could be used in biomethane.

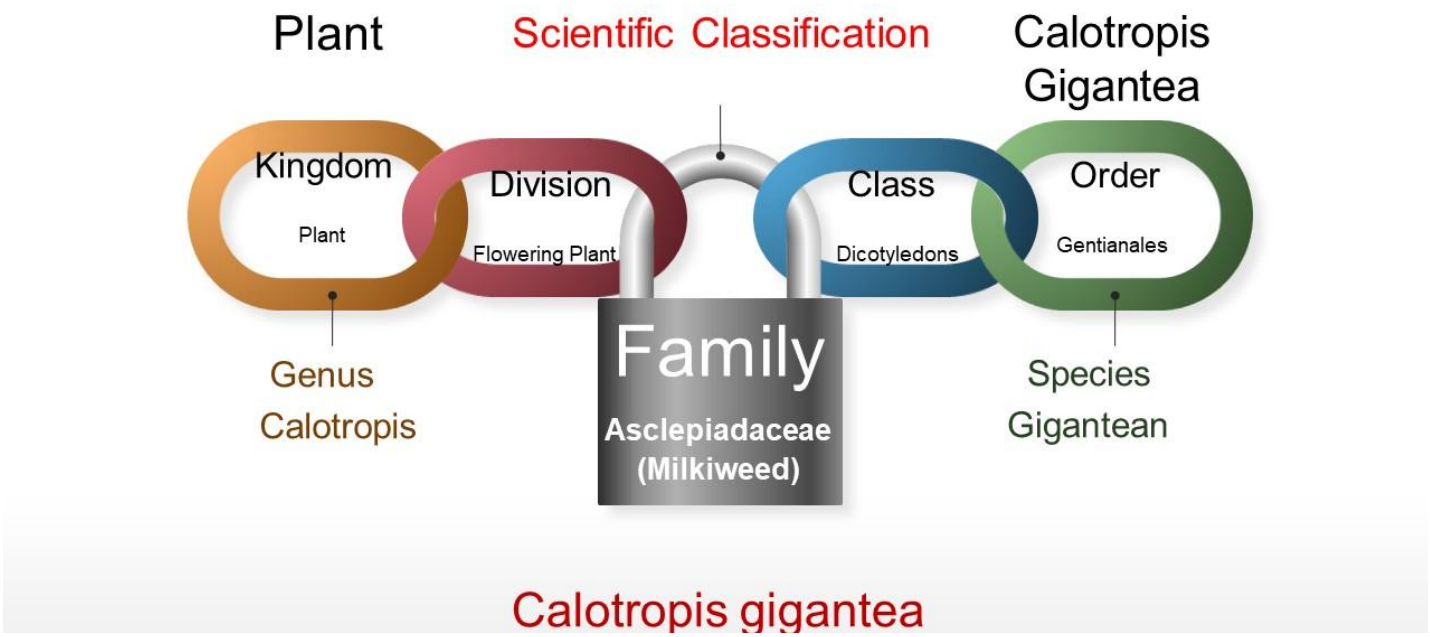


Figure 1. Classification of *Calotropis gigantea*

Calotropis gigantea

Milkweed or swallow wort, *Calotropis gigantea L (R.Br.) (Asclepiadaceae)*, is a characteristic no man's land weed. This native Indian plant grows wild up to 900m asl on varied soils in various climates. According to Ayurveda, dried plant is a good tonic, expectorant, depurative, anthelminthic, and root bark alternative for ipecacuanha species.(Fig 1)

Sample Collection

Fresh and healthy *Calotropis gigantea* plants were collected in sterile polythene bags from Vidisha (district), Madhya Pradesh. Regional floras identified plant components. Herbarium

sheets at IES University, Bhopal, confirmed specimens.

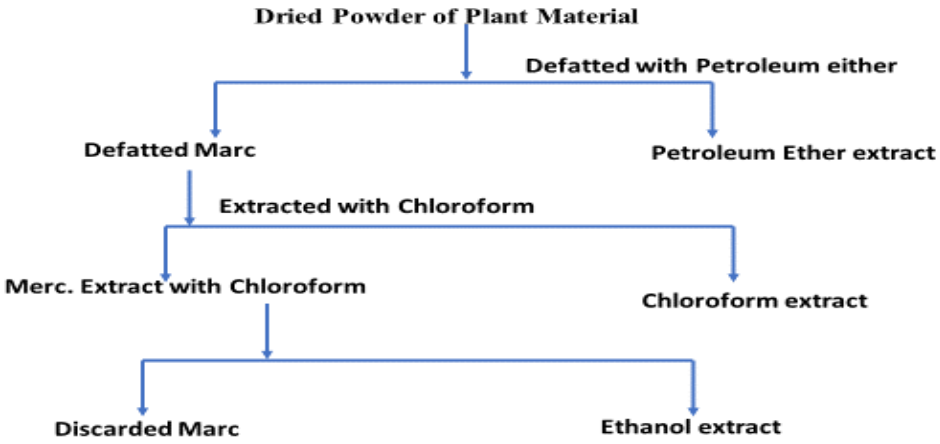
Solvent Extracts

Clean, nutritious plant materials are sliced into little pieces and shade-dried for three to four weeks. Electric grinders powdered the dried material. Extracting powder from desiccators. 5 grams of coarsely powdered plant material was extracted with 50 ml of solvent for 48 hours with gentle shaking. Ethanol, petroleum ether, and HPLC-grade methanol were employed to extract various chemicals from the sample. Room-temperature extraction was done. Extracts were filtered using Whatman No.1 paper and dried to concentrate. The leftover powder was weighed and redissolved in the solvents to reach 1mg/ml. Airtight containers refrigerated the powder. (Bhuiyan FR2020)

Soxhlet extraction

The modified Soxhlet extraction (Faizaan Ahmad, et al., 2019) takes advantage of reflux abstraction and permeation to continuously remove spice with new dissolvable. Soxhlet extraction is programmable, persistent, and faster than maceration or permeation. The Soxhlet's hightemperature and extended extraction time increase warm debasement's chances.

(Wei Q, et al., 2013), *Calotropis* Soxhlet extraction yielded 38.21 mg/g. Soxhlet extraction modification due to high extraction temperature. Soxhlet extraction at 70 °C reduced polyphenol and absolute alkaloid groups compared to maceration at 40 °C.



Phytochemistry

Qualitative Analysis: Alkaloids, Carbohydrates, Cardiac glycosides, Flavonoids, Phenols, Saponins, Tannins, Terpenoids, Quinones, and Proteins will be tested using conventional techniques. (Wakeel, A et a., 2019)

Quantitative Analysis

Alkaloids, Tannins, Phenols, Proteins, and Carbohydrates are quantified based on qualitative data. (Saddiq AA et al., 2022)

Stock preparation

After air-drying the *Calotropis gigantea* leaves, powder them. 1g of powdered in a tiny centrifuge tube was dissolved in 10 ml of toluene and vortex-mixed.

Preparation

Powdered dried *Calotropis gigantea*

Solution Preparation

Calotropis gigantea stock solutions were made by carefully weighing 1mg and dissolving them in toluene. 1ml of the mixture was dissolved in 10ml toluene. (Saddiq AA et al.,2022)

Methanol and HCL hydrolyzed TLC tests. Sigma Aldrich sugar standards examined several sugars. Ethyl acetic acid derivation was easy: Acetic Acid: Methanol: Water 30 minutes of

chromagraphic chamber drenching. Clear light allowed observation. Derivatizing reagent (p Anisaldehyde-sulphuric destructive) at 1100C for 10 mins made TLC.

HPTLC degraded steroids and terpenoids. Instrument details

Plates: Merck, Anchor research centres, Mumbai, India, aluminum sheet precoated with silica gel F254.

High-performance thin-layer chromatography
HPTLC uses solvent and stationary phase capillary action to separate chemicals in a sample combination. A thin coating of stationary phase adsorbent is coated on glass, aluminum foil, or plastic. The HPTLC system (CAMAG, Muttenz, Switzerland) had a Linomat 5 sample applicator with 100 µl syringes coupled to a nitrogen tank, an Automatic Developing Chamber 2 with twin trough chambers of 20 x 10 cm, TLC Plate Heater III, and TLC scanner 3 linked to win CATS software. (Kumar D, Kumar S, 2015)

Auto-sampler CAMAG

HPTLC lab production relies on it. Samples are applied as spots or bands/rectangles (0.5 to >50µl) utilizing contact transfer or spray on. It controls and monitors parts. Optimized resolutions help all TLC: Chromatography on conventional or HPTLC layers for qualitative or quantitative analysis, preparative separations. (Rashmi, K.P. Singh &Suchita Arya,2012)

Auto-Developing Chamber ADC-2
CAMAG ADC lets thin layer chromatography achieve jobs TLC cannot. The automatic developing chamber simplifies, protects, and reproduces isocratic development of 20X10 cm TLC and HPTLC plates and foils. Chromatogram development, the most important step of thin layer chromatography, can be pre-set and monitored using the ADC-2.

HPTLC Calotropis analysis

Sample application
After two toluene washes, syringe was wiped with tissue paper to absorb extra methanol. 50 µl of the standard was syringed onto linomate-5 for sample application on TLC plate (1-8µl). TLC plate had 8 conventional tracks. 8 mm band, 1-8 µl application volume, 150 nl/s gas flow. After loading the standard in TLC plate, the syringe was methanol-washed twice. After washing, syringe 20 µl 2 tracks of each sample applied 1-2 µl application volume 1st, 2nd, and 3rd samples. TLC plates have 14 sample and standard tracks.(Table 1)

Table1. Sample application

No.	Appl. position	Appl. Volume	Vial	Sample ID	Active
1	15.0 mm	1 µl	1	Standard	Yes
2	29.01mm	2 µl	1	Standard	Yes
3	43.2 mm	3 µl	1	Standard	Yes
4	57.3 mm	4 µl	1	Standard	Yes
5	71.4 mm	5 µl	1	Standard	Yes
6	85.5 mm	6 µl	1	Standard	Yes
7	99.6 mm	7 µl	1	Standard	Yes
8	113.0 mm	8 µl	1	Standard	Yes
9	127.0 mm	2 µl	2	Sample I	Yes
10	141.0 mm	4 µl	2	Sample I	Yes
11	156.0 mm	6 µl	2	Sample II	Yes
12	170.0 mm	4 µl	3	Sample II	Yes
13	184.0 mm	5 µl	3	Sample III	Yes
14	190.0mm	5 µl	3	Sample III	Yes

Result and Discussion

Study Area

Since civilization, medicinal plants have helped humanity. The current study region is Vidisha district in M.P. Ayurveda has many ideas for using plants as medicine, and Vidisha district's ethnobotanically important plants are a treasure. Tribal people have changed their ways of life, yet some of their natural health care practices are beneficial. Based on experience and forebears, they employed plants, trees, and plant items. Vidisha is rich in tribal plant medicine for numerous ailments. An ethnobotanical study was conducted to gather data for medicinal plant research in Vidisha, MP, which has a substantial tribal population. Tribal people have extensive plant knowledge. Vidisha, M.P., is between latitude 230,21' and 240,20' north and longitude 770,15'30" and 780,18' east. In eastern Malwa, the district is fertile. About 2 KM south of the district headquarters, the tropic of cancer crosses the district. North, south, and east are Guna, Raisen, and Sagar districts. Vidisha district has 1,458,875 people, 76.72% of whom reside in villages. Bheel, Sehariya, and Mongiya are the most common tribes. Vidisha has 261,816

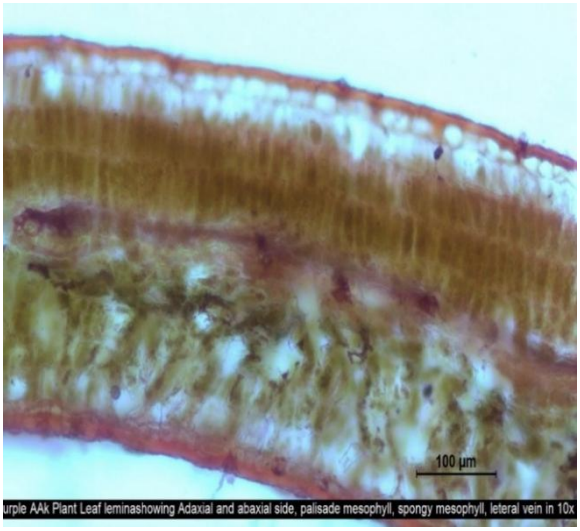
Sehariya.(Figure 2)

Figure 2. Geographical Location of study Site

Sample Details

Calotropis grows up to 900 meters (msl) nationwide (Tripathi BD, 2009) and prefers disturbed sandy soils with mean annual precipitation 300-400 mm. It quickly becomes a weed along roadside ditches, tidal pond edges, and overgrazed fields due to wind and animal seeds. It thrives in abandoned areas with sandy soils and low precipitation. It indicates overcultivation. (Zahid M, et., 2021)

Macroscopic root and Leaf



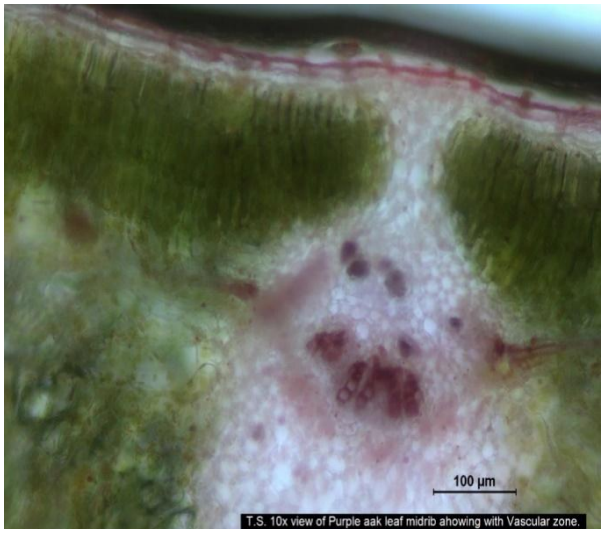


Figure 3. Microscopic structure root and leaf of *C.gigantea*

Root microstructure

Organoleptic examination revealed macroscopic characteristics such color, odor, taste, size, and shape. *C. gigantea* root was whitish grey in hue, slightly bitter, pungent scent, mucilaginous is flavor. Fresh roots are 0.5-20 cm in diameter and wrinkled showed in (Fig. 3)

Leaf microstructure

Epidermis of C. gigantea: Epidermal cells are tiny, polyhedral. Cuticle covers lengthy cells. Multicellular trichomes are formed. Collenchyrna: Both epidermis layers have 5-6 collenchymatous cells. Parenchyma: Large chloroplast-free cells. Large thin-walled cells. Vascular bundles: Crescent-shaped bi-collateral and open bundles. Xylem-phloem cambium. Lamina: Mesophyll is palisade and spongy. Palisade mesophyll tissue has three rows below the epidermis.

Small, polyhedral Cells were shown epidermal. Thick cuticle. Fewer trichomes. Epidermis: Small polyhedral epidermal cells. Cuticle covers lengthy cells. Multicellular trichomes are formed. Collenchyma: 3-5 layers on top and lower epidermis. Collenchyma: Both epidermis layers have 5-6 collenchymatous cells. Parenchyma: 4-8 layers of thin-walled, isodiametric to circular cells with intercellular gaps. Parenchyma: Large chloroplast-free cells. Large thin-walled cells. Vascular bundles: Crescent-shaped bi-collateral and open bundles. Xylem-phloem cambium. Vascular bundles: Crescent-shaped bi-collateral and open bundles. Xylem-phloem cambium. Lamina: Mesophyll is palisade and spongy. Palisade mesophyll tissue forms four rows below the epidermis. (Fig. 3)

Extracts Preparation

Soxhlet extraction was performed on 50-gram powdered *Calotropis gigantea* aeronautical portion and roots samples. For extreme phytochemical extraction, 100 ml of methanol, aqueous, petrol ether, and chloroform were used. Concentrates were extracted for now. The concentrates were focused using a rotating evaporator and stored at 4°C for further use.

Figure 4. Study plant sample *C. gigantea*

Table 2. Physico-chemical Parameters of Calotropis plants

S.No	Parameters	Ariel Parts	Geomorphic Part (Roots)
z	Organic matter % w/w	0.18 ± 0.16	0.0189 ± 0.16
2	After drying loss % w/w	7 ± 0.28	10.2±0.99
3	Water Soluble ash % w/w	4.45± 0.13	5.75± 0.06
4	Acid insoluble ash % w/w	0.38±1.22	1.09±1.22
5	Water extractive	12.11± 0.41	7.36 ± 0.28
6	Alcohol extract	6.56± 0.83	1.75± 0.01
7	Foaming index	≤ 1	≤ 1
8	Arsenic	1.61±0.01	1.03±0.03
9	Mercury	0.051 ±0.03	0.056±0.05
10	pH	7.79 ± 0.28	6.43± 0.28

The table indicate the analysis of plants' ariel and root parts with in the permissible limit accordance with WHO (2002).

The details of physicochemical analysis of fine powder from *C. gigantea* leaves are given in (table 2 & figure 4) values of organic matter % w/w 0.18 ± 0.16 & 0.24 ± 0.01% w/w in *C. gigantea* and but in after drying loss % w/w were 7 ± 0.28% & 8.2 ± 0.88, different ashes namely, water soluble ash, acid insoluble ash were 4.45± 0.13 % w/w & 5.64± 0.11% w/w, 0.38±1.22% w/w. Moisture loss at 105°C. Foaming index (mL) was less than 100, swelling index 4.86 ± 0.03. 1% and 10% formulation solutions had pHs of 7.79 and 6.43, respectively, and 44.66 ± 0.88% w/w crude fibre content. Petroleum ether, chloroform, methanol, and water extracted 4.26%, 1.09%, 4.56%, 10.13% and 4.16%, 1.07%, 4.61, 10.11%, respectively. Thus, aqueous extract had maximal extractive value. Table2 and figures 4 showed the fluorescence analysis of *C. gigantea* fine powder and leaves under visible and UV light (254 nm and 365 nm).

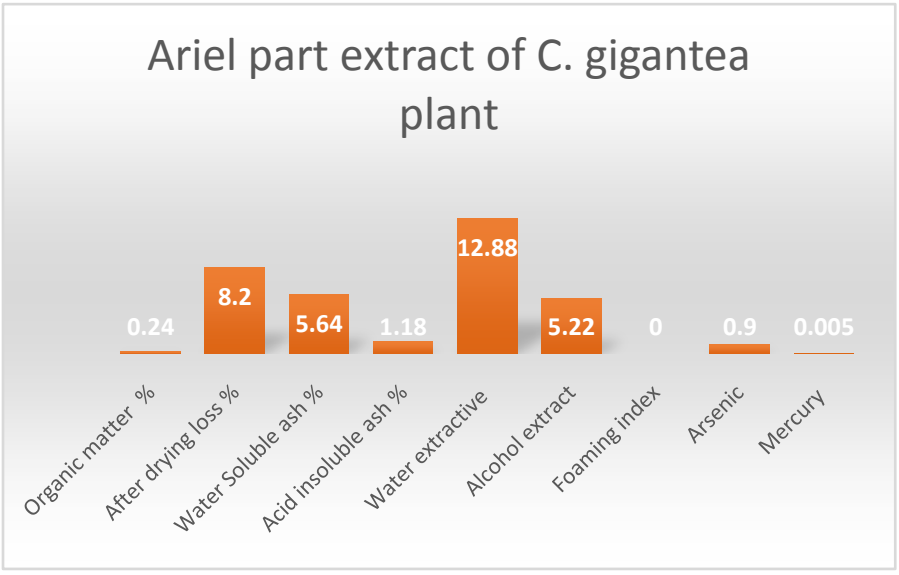


Figure 5. Ariel part extract of *C. gigantea*

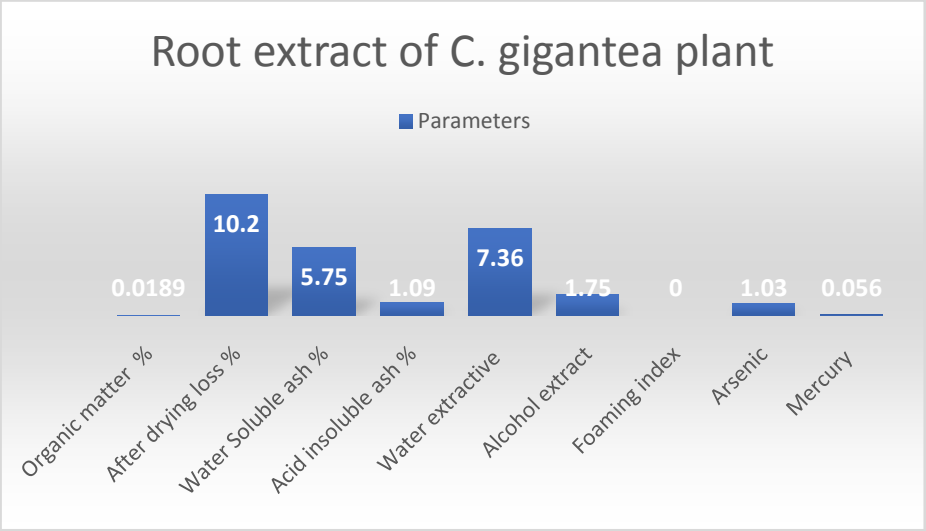


Figure 6.Root's part extract of *C. gigantea*

Table 3. The Extraction values of *Calotropis gigantea* plant powder by extraction method

S.No.	Extract character	Values (%w/w by extraction)
1	Petroleum ether	4.26
2	Chloroform	1.09
3	Methanol	4.56
4	Aqueous	10.13

Phytochemical analysis

Phytochemical compounds were partitioned in methanolic extract of ariel parts and root of plant of CG were designated. The preliminary phytochemical analysis was done qualitatively and quantitatively for total carbohydrate content, total protein content, total flavonoids content and total phenolic content. The aerial parts and roots extracts of *C. gigantea* and were analyzed showed in (figure 5&6 and table 3).

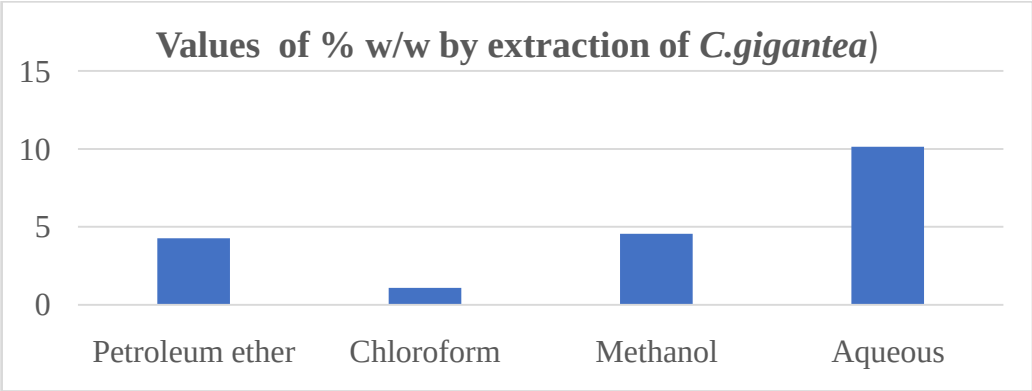


Figure 7. Value of percentage w/w by extraction of *C. gigantea*

Table 4. Phytochemical Screening of study plants.

Phytochemical screening	***Calotropis gigantea***		
	Flower	Leaves	Root
Tannins	-	-	+
Saponin	++	++	-
Flavonoids	++	++	-
Phenolics	++	++	++
Alkaloids	-	-	++
Cardiac glycosides	++	++	++
Terpenoids	++	++	++
Steroids	++	++	++
Protein	++	++	-
Carbohydrates	++	++	-

Qualitative Analysis

The primer phytochemical analysis of unrefined concentrates of CG plant parts revealed phytocomponent presence and absence. From the primer phytochemical analysis, carbohydrates, protein, flavonoids, steroids, and saponin were found throughout the plant sections of CG, while alkaloid and tannin were absent. However, basic phytochemical analysis of *Calotropis gigantea* revealed flavonoids, reducing sugars, phenolic combinations, and alkaloids showed in (Fig. 7 and Table 4) (Ullah A et al., 2020)

Only aerials had steroids and saponin. Only roots lack protein and carbohydrate. TLC of plant utilized for study demonstrated extensive array of bioactive and secondary metabolite in different stains.

All stem and leaf extracts had flavonoids, sterols, phenols, and terpenoids. The leaf and root extracts of all species except *C. gigantea* contained tannins and alkaloids. All plant leaf and stem extracts contained saponins except. (Fig. 7 and Table 4)

TLC

TLC and HPTLC analyzed all plant leaf and stem extracts. TLC investigations detected flavonoids, phenols, sterols, and terpenoids individually. Several solvent solutions were tested using flavonoids-detecting extract. NP/PEG reagent found a suitable solvent system. The best solvent system was aqueous (30:10:10:4). The phenolic extract was tested in several solvent systems. Spraying Folin's reagent and heating the plate. The highest number of spots and compound separation led to a better solvent system. Solvents For component separation, methanol: petroleum ether: chloroform (50:30:4) worked best. The extracted sterols were tested in several solvent systems. Showed in the (Fig 8)

Liebermann-Burchard reagent determined the optimal solvent system for separation. The mobile phase was hexane: diethyl ether: acetic acid (8:20:1) since it separated well. Toluene: Ethyl acetate: Formic acid was chosen for terpenoids. TLC selected a solvent system with optimal spot resolution for HPTLC analysis.

High performance thin layer chromatography (HPTLC)

The solvent system which showed the maximum resolution of spots was selected as the solvent system in HPTLC studies. In the HPTLC studies the number of compounds separated, the Rf values and their percentage were noted. HPTLC fingerprint profile was prepared for the leaf and stem extracts of plants. (Fig 10.)

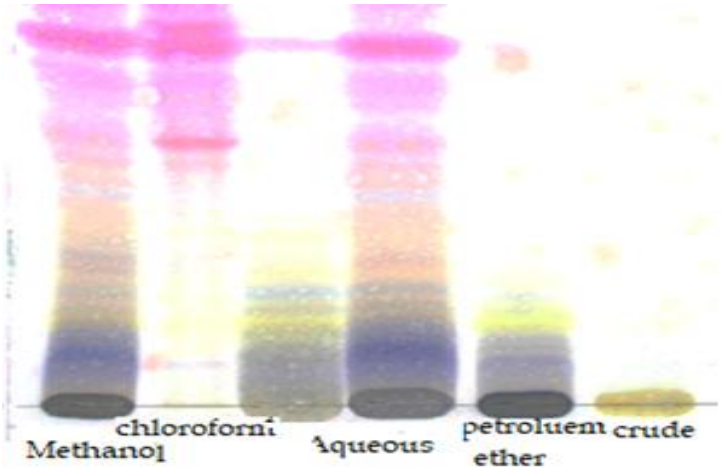


Figure 8. TLC of extract of *C. gigantea*

Table 5. Quantitative analysis of phytochemicals in CG.

Phytochemical screening	***Calotropis gigantea* (in mg)**		
	Flower	Leaves	Root
Protein	32	30	36
Phenol	80	90	90
Flavonoids	0.98	0.99	1.32

The quantitative analysis (table 4.6) showed that the aeronautical *Calotropis gigantea* had high total sugar and phenolic content. Plant foundations had high protein content and ariel parts low. *C. gigantea* root had more flavonoids than flying sections. roots have more phenolic and flavonoids than ethereal components. The initial phytochemical analysis of CG revealed that polyphenols like flavonoids, steroids, phenols, and terpenoids were shown in (Table 5).

Amino acid

Proteins require amino acids. The presence of various amino acids in ethereal and foundations of *C.gigantea* was confirmed by dainty layer chromatography with Sigma Aldrich amino acid standards. CG flying sections had 12 amino acids out of 20: Aspartic, Glutamine, Glutamic, Lysine, Phenylalanine, Tryptophan, Tyrosine, Methionine, Alanine, Leucine, Isoleucine, and Serine. Airborne parts had 13 amino acids, with Arginine and Proline expanding but Tryptophan not. CG foundation had 14 amino acids, while table 4.8 had 15 amino acids. One essential amino corrosive Histidine and one unimportant amino corrosive The species concentrations have no asparagine. Unlike its roots, ethereal sections had substantial proline content. It maintains water potential and osmoregulation in pressure-filled plants. This amino acid may help desert plants survive. In this study, Proline was identified in the plant's roots and upper part, which is typical

of semi-bone-dry plants shown in (Fig. 9 and Table 6)

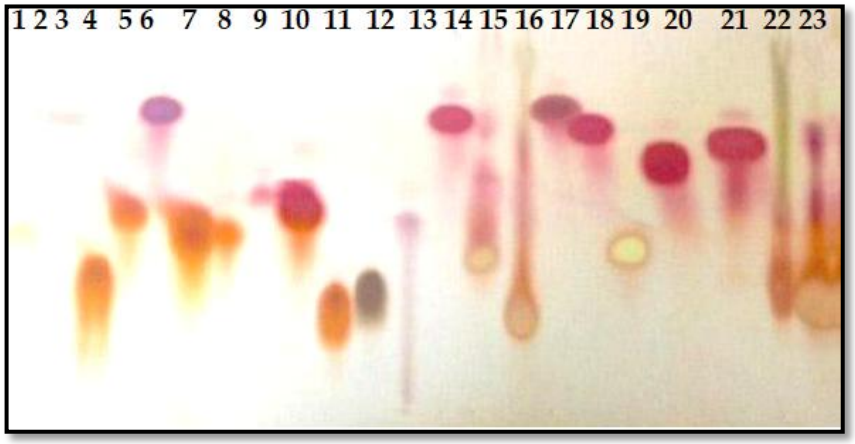


Figure 9.TLC of Amino acid from *C. gigantea*

Table 6. Amino acid present in plants

Amino Acid	*Calotropis gigantea*	
	Aerial Parts	Roots
Aspartic acid	+	-
Glutamine	+	-
Glutamic acid	+	+
Lysine	+	+
Phenylalanine	+	+
Tyrosine	+	+
Cysteine	+	+
Methionine	+	+
Proline	+	+
Glycine	-	+
Alanine	+	+
Valine	-	+
Leucine	+	+
Isoleucine	+	+
Serine	+	+
Threonine	-	+

Total Phenol Content

All methanol and ethanol-extracted stem, root, leaf, and fruit samples include phenolic chemicals. Compared to Gallic acid, fruit ethanol extract has the most phenol. Phenolic chemicals are aromatic, thus they all assimilate well in the UV range. Additionally, salt causes phenolic compounds' spectra to bathochromic. Thus, phenol identification and quantification require phantom approach. Two-dimensional paper chromatography analyzed the phenolic segments. It revealed vanillic and syringic corrosive in aplants all the parts. cis and trans ferulic corrosive in *C.gigantea* roots and flying parts. *C.gigantea* ethereal section and foundations contained o-coumaric corrosive. (Fig 10.)

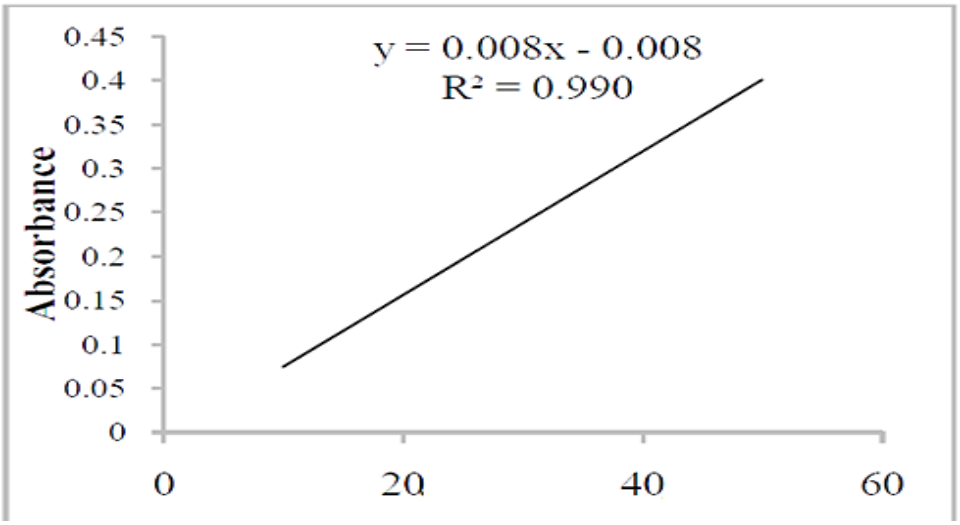


Figure 10Phenol content µg/ml

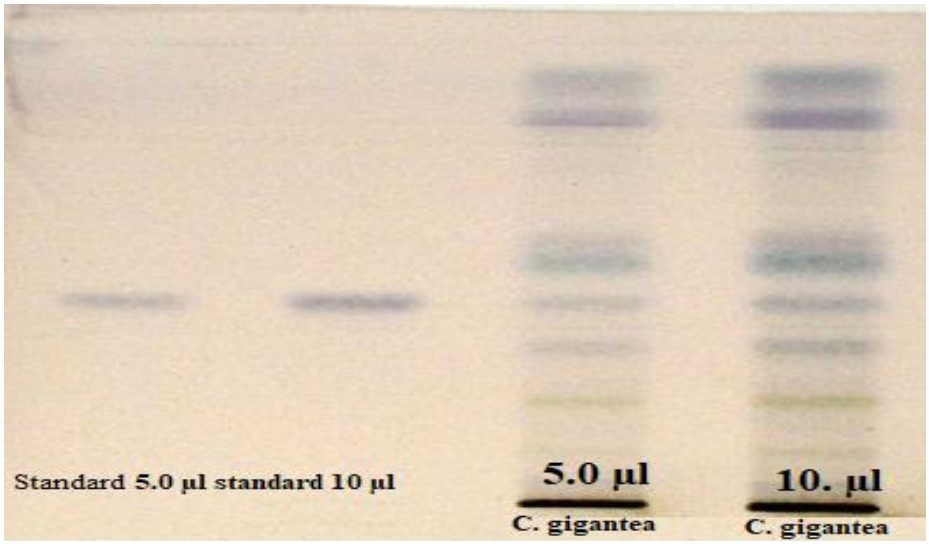


Figure 11. HPTLC fingerprint of *C. gigantea*

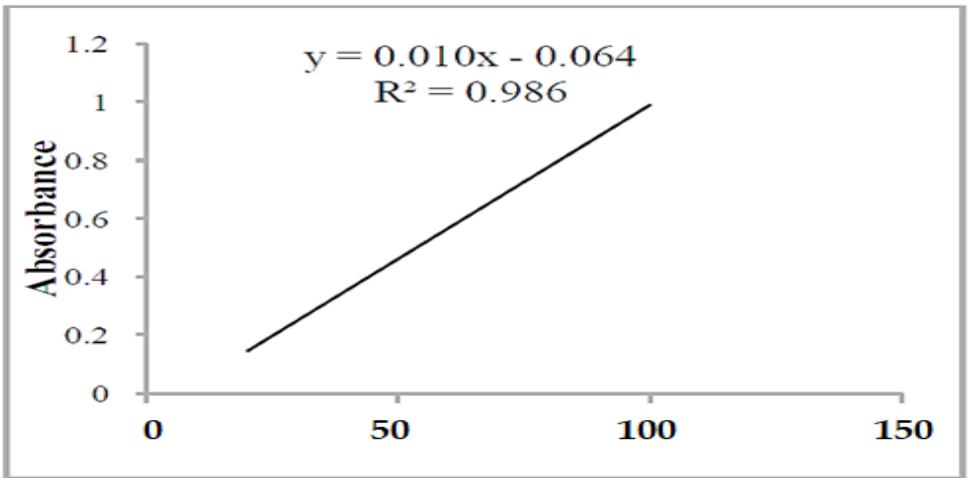


Figure 12. Gallic acid µg/ml

Flavonoids

Flavonoids—polyphenol phytonutrients—are famous. This group gives plants remarkable color variation. *Calotropis* flavonoids abound. Endemic plants also have good flavonoids in this study. UV spectroscopy identified *Kaempferol* and Quercetin in *Calotropis gigantea* aerial parts, but only *Quercetin* in C.gigantea root, stem, and leaves. HPTLC standards validated this. HPLC was used to detect components in the ethyl acetate fraction using a gradient flow mobile phase of A: methanol: B: 0.05 Trifluoroacetic. A=70% (0–5 min), A=40% (5–8 min), A=90% (8–15), HPLC column C18 – ODS (25cm * 4.6 mm). 1.0 ml/min flow rate and –280 nm detector. (Fig. 16 & 17)

HPTLC.

Ethyl acetate fraction flavonoid content was analyzed by HPTLC (Eike Reich/Switzerland) on silica gel GF254 plates in a mobile phase of chloroform: ethyl acetate: methanol: aqueous (70: 14: 14: 10) at 280 and 366 nm wavelength. Figure shows spectroscopic and chromatographic methods used to identify extracted flavonoids. MS: Shimadzu GCMS-QP2010 Ultra; IR: KBr disk, 4000-400 cm−1; HPTLC: As listed in material and procedure. (Kadiyala M, Ponnusankar S, Elango K, 2015) (Fig. 13)

HPLTC-exposed leaf extract quercetin

We created chromatograms of standard pure chemicals and plant extracts to determine the chemical composition of the separated components. Standard quercetin and flavonoid-enriched leaf and stem extracts were chromatographed here. Quercetin's chromatogram is displayed. Quercetin's Rf is 0.81 at 280 nm. The extracts included quercetin due to the appearance of a peak with the same Rf (0.82) at the same nmax of 280 nm and the apparent super impossibility. *Calotropis gigantea* leaf extracts contained quercetin. Superimposed graph. (Fig. 14 & 15))

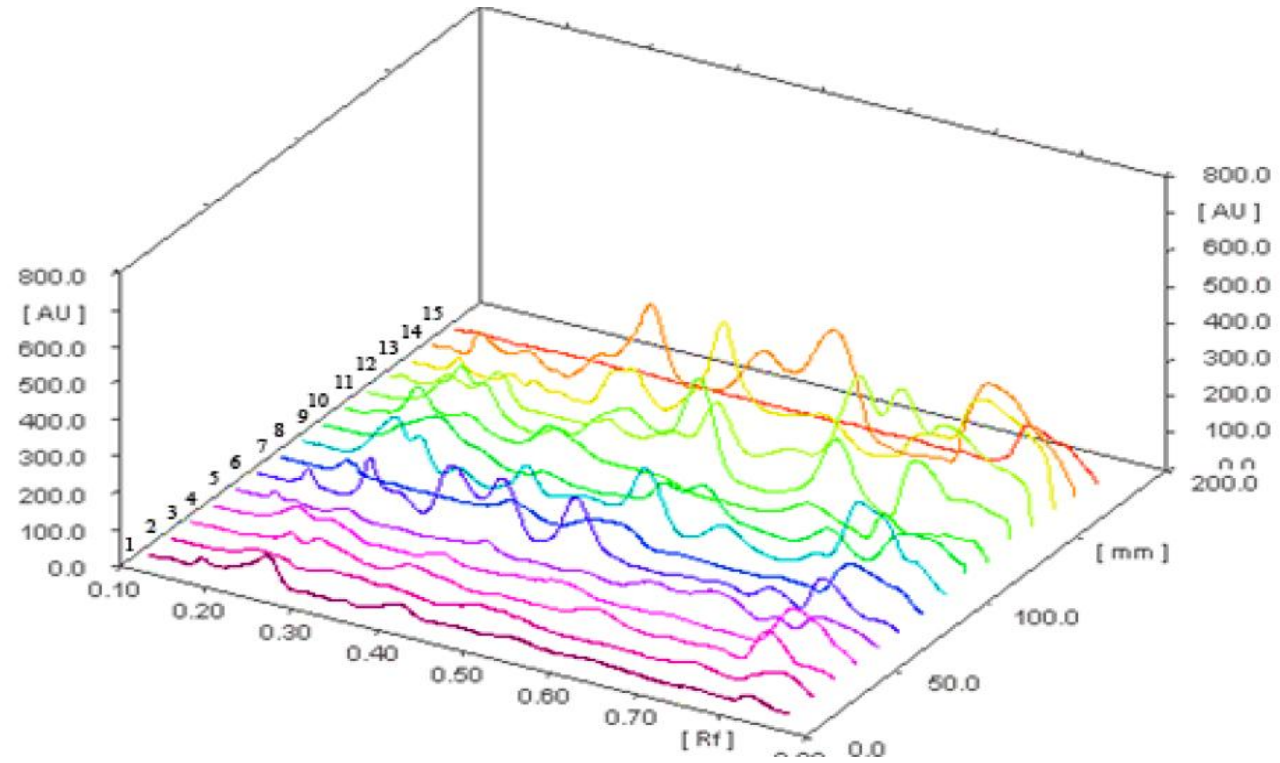


Figure 13. Overlying Chromatographical evaluation of leaf extract

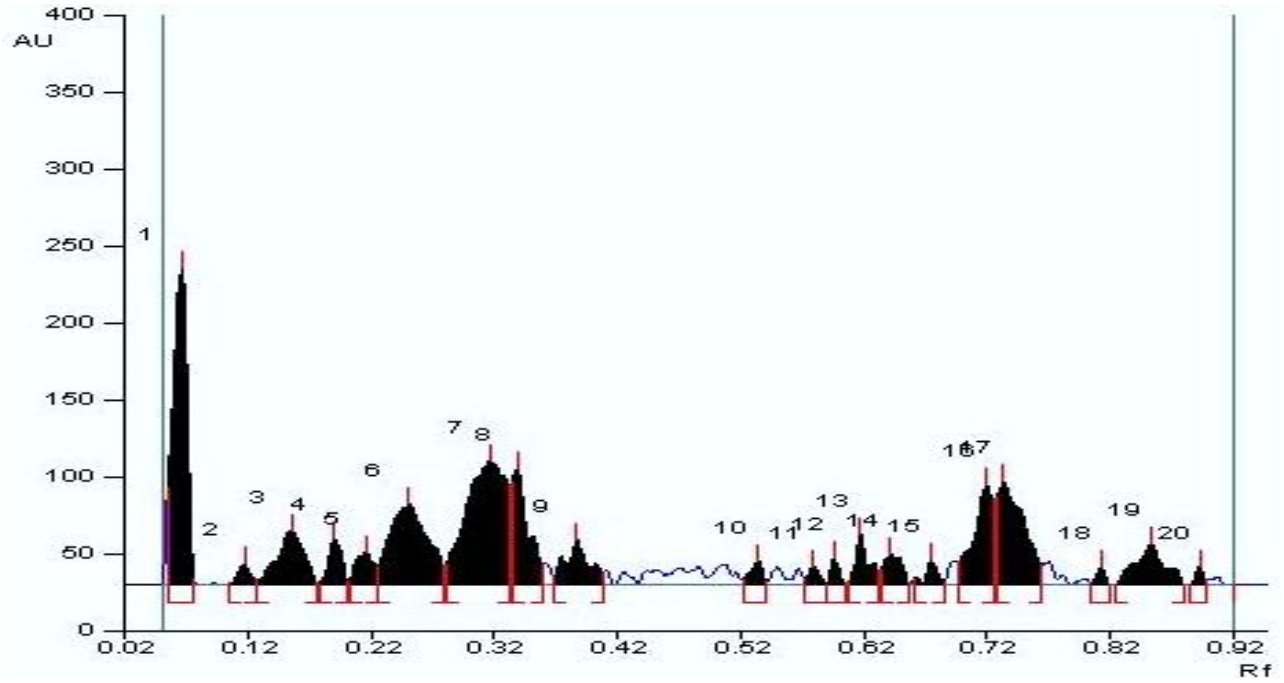


Figure 14. HPTLC fingerprint profile of methanolic leaf extracts

Peak	Start		Max			End		Area	
	Rf	H	Rf	H	%	Rf	H	F	%
1	0.06	0	0.07	205.30	24.63	0.08	0	2218.00	16.73
2	0.11	0	0.12	13.60	1.62	0.13	0	126.90	0.96
3	0.13	0	0.16	34.90	4.17	0.18	0	726.10	5.48
4	0.18	0	0.20	30.10	3.69	0.21	0	293.90	2.22
5	0.21	0	0.22	20.90	2.49	0.23	0	281.30	2.12
6	0.23	0	0.26	51.80	6.19	0.29	0	1519.00	11.46
7	0.29	0	0.32	80.00	9.55	0.34	0	2506.60	18.90
8	0.34	0	0.34	76.90	9.07	0.36	0	901.60	6.80
9	0.37	0	0.39	29.10	3.48	0.41	0	509.60	3.84
10	0.63	0	0.64	16.30	1.83	0.66	0	142.40	1.07
11	0.68	0	0.68	11.70	1.40	0.69	0	96.90	0.73
12	0.60	0	0.60	17.20	2.05	0.61	0	110.60	0.83
13	0.61	0	0.62	32.90	3.93	0.64	0	331.90	2.50
14	0.64	0	0.66	19.90	2.38	0.66	0	267.80	2.02
15	0.67	0	0.68	16.20	1.93	0.69	0	133.10	1.00
16	0.70	0	0.72	66.10	7.78	0.73	0	1004.80	7.58
17	0.73	0	0.74	67.10	8.02	0.77	0	1366.00	10.23
18	0.81	0	0.82	11.80	1.41	0.82	0	71.90	0.54
19	0.83	0	0.86	26.80	3.20	0.89	0	585.80	4.42
20	0.89	0	0.90	11.60	1.38	0.90	0	76.20	0.57
							Total area	13269.30	

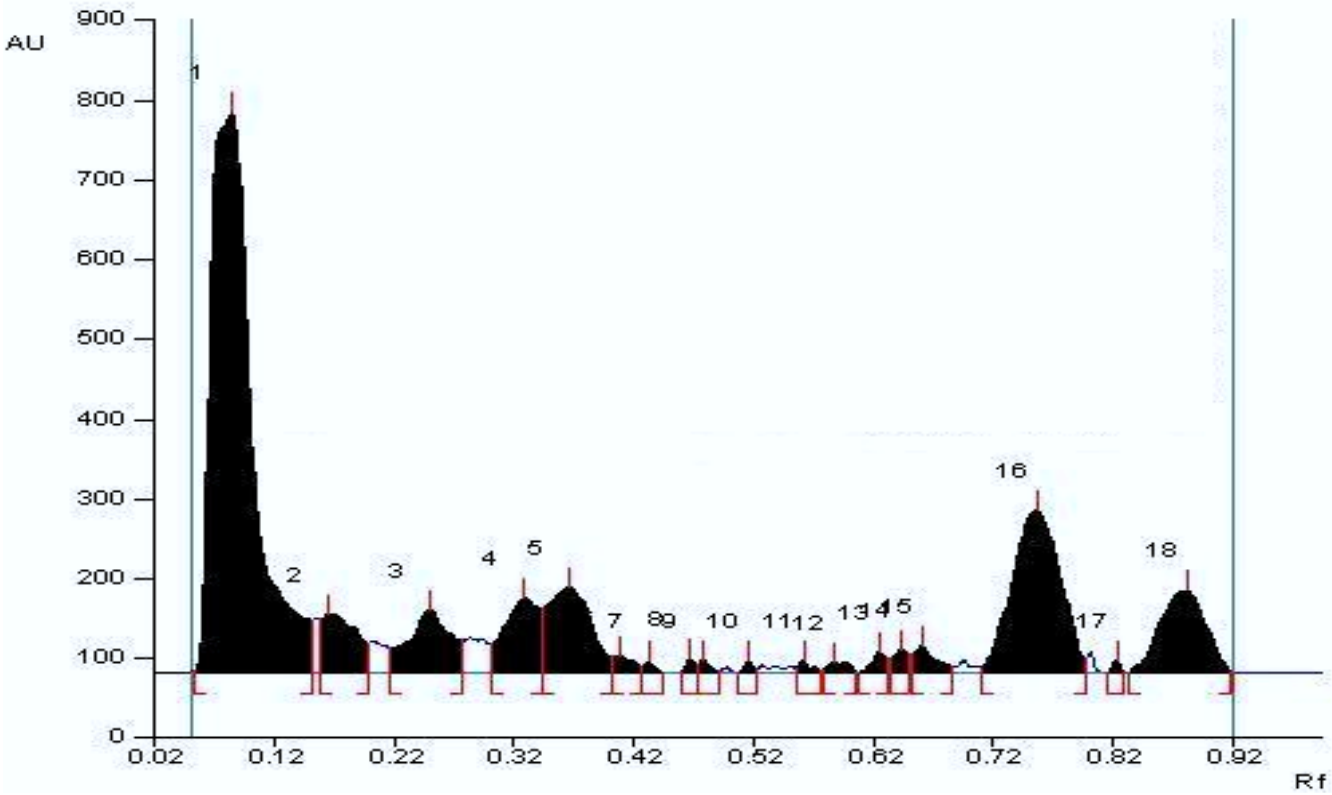


Figure 15. HPTLC profile of methanolic stem extracts

Peak	Start		Max			End		Area	
	Rf	H	Rf	H	%	Rf	H	F	%
1	0.06	0	0.09	702.80	43.09	0.16	0	23206.90	46.39
2	0.16	0	0.17	74.10	4.64	0.20	0	2084.20	4.17
3	0.22	0	0.26	79.80	4.89	0.28	0	2637.00	5.07
4	0.31	0	0.33	93.90	5.76	0.36	0	2623.90	5.06
5	0.36	0	0.37	107.80	6.61	0.41	0	3647.00	7.29
6	0.41	0	0.41	21.70	1.33	0.43	0	333.40	0.67
7	0.43	0	0.44	14.70	0.90	0.46	0	117.40	0.23
8	0.46	0	0.47	17.10	1.05	0.48	0	122.00	0.24
9	0.48	0	0.48	16.30	1.00	0.50	0	138.20	0.28
10	0.51	0	0.52	15.00	0.92	0.53	0	108.10	0.22
11	0.56	0	0.57	16.20	0.99	0.58	0	153.60	0.31
12	0.58	0	0.59	14.00	0.86	0.61	0	224.70	0.45
13	0.61	0	0.63	26.20	1.64	0.64	0	269.40	0.54
14	0.64	0	0.65	29.70	1.82	0.65	0	368.60	0.74
15	0.66	0	0.67	34.10	2.09	0.69	0	582.90	1.17
16	0.72	0	0.76	203.20	12.46	0.80	0	7794.90	15.58
17	0.82	0	0.83	15.30	0.94	0.84	0	93.80	0.19
18	0.84	0	0.89	116.60	7.14	0.93	0	4662.30	9.32

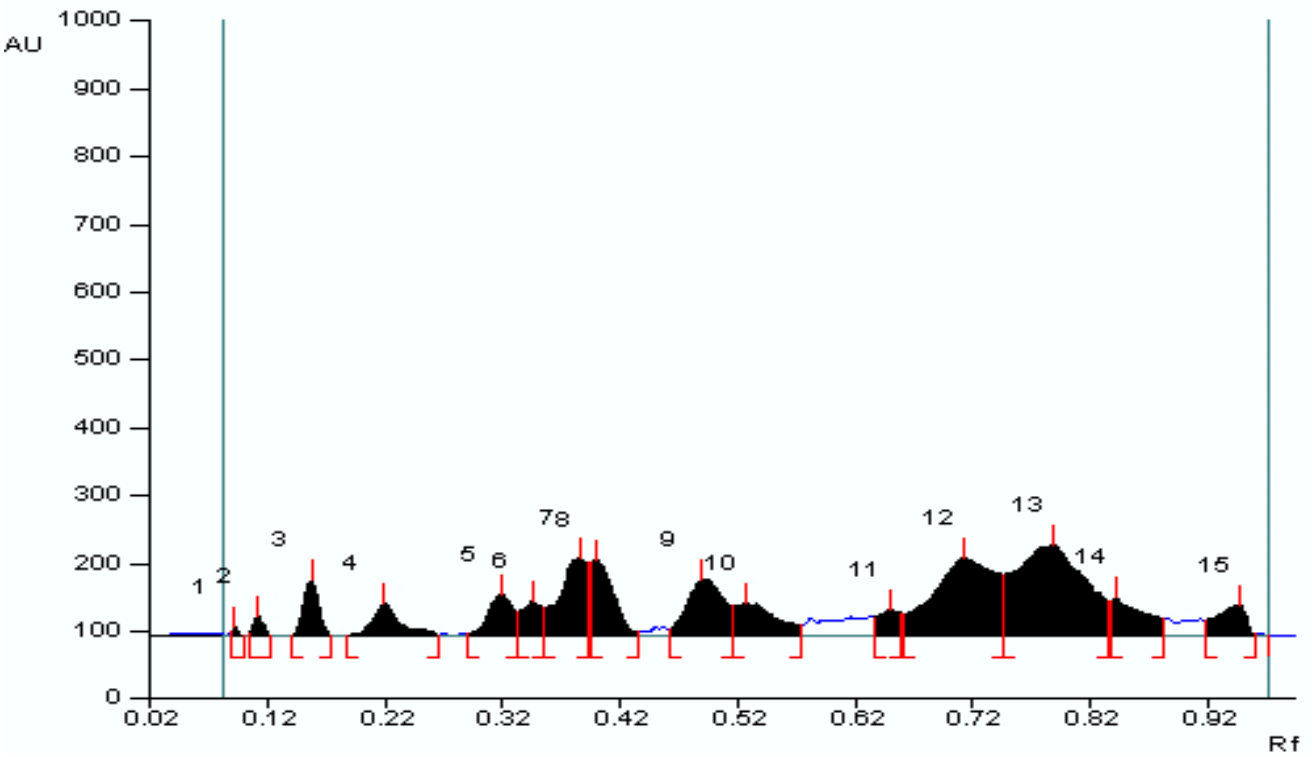


Figure 16.HPTLC profile of methanolic leaf extracts

Peak	Start		Max			End		Area	
	Rf	H	Rf	H	%	Rf	H	F	%
1	0.73	0	0.80	150.20	100.00	0.85	0	9395.50	100.00

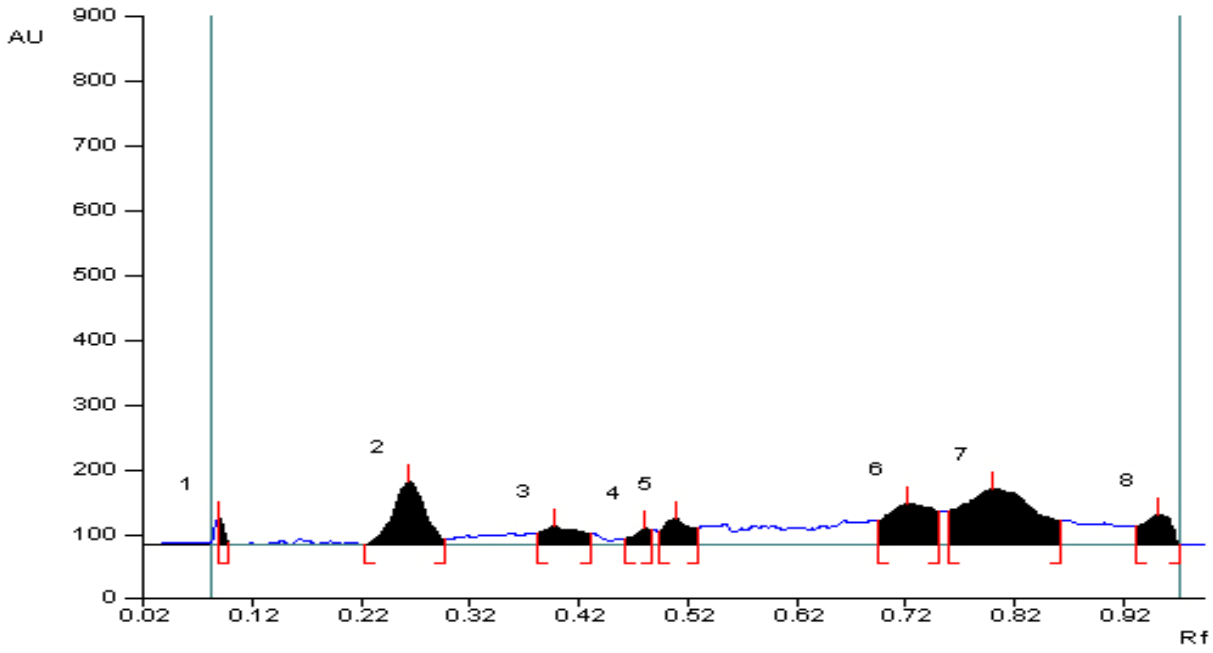


Figure 17. HPTLC profile of methanolic stem extracts

Peak	Start		Max			End		Area	
	Rf	H	Rf	H	%	Rf	H	F	%
1	0.09	0	0.09	75.70	15.74	0.10	0	678.50	4.38
2	0.23	0	0.27	98.40	20.48	0.30	0	2676.00	17.26
3	0.39	0	0.40	30.20	6.28	0.43	0	966.50	6.23
4	0.47	0	0.49	26.60	5.53	0.49	0	423.60	2.73
5	0.50	0	0.51	42.00	8.74	0.53	0	980.60	6.32
6	0.70	0	0.72	65.80	13.69	0.75	0	2665.10	17.19
7	0.76	0	0.80	90.50	18.84	0.86	0	5807.40	37.46
8	0.93	0	0.95	51.40	10.69	0.98	0	1306.20	8.43
						Total Area =		15503.90	

Antibacterial activity

Calotropis gigantea root bark treats uncleanliness, piles, wounds, tumors, parasite disorders, and loose bowels. In pale-skinned rodents, *Calotropis gigantea* root alcohol had pain-relieving, anticonvulsant, anxiolytic, and narcotic effects. *Calotropis gigantea* has many medicinal uses (Patial B et al., 2022). This plant's smooth white latex disrupts mucous coatings and causes scorching reactions when applied adjacent or coincidentally. Stems could provide fungus-fighting chemotherapeutics for *Epidermophytonflucosum* and *Tgypseum*. Root ethanolic concentration 100% subterranean insect implantation at 250mg/kg. Mortality and unique natural component of stored grain bugs by *Calotropis gigantea* extract were studied (Soni P et al., 2021) with significant results. *Calotropis* was found to be effective in preventing *Cryptolestus pusillus* from maturing.

Table 7. Antibacterial activity of *C. gigantea* extract

Bacterial culture	**Zone of inhibition in mm**					
	Water extract	**Methanol**	**Petroleum ether**	**Ethanolic extract**	**Flavonoids**	**Tetracycline (+ve control)**
E.coli	-	-	13.0±1.04	11.0±0.2	-	21.0±1.04
Staphylococcus aureus	11.0±1.15	19.0±1.18	14.0±1.04	12.0±0.3	15.0±1.91	26±1.41
Klebsiella pneumonia	-	16±1.18	19.0±0.13	11.0±0.5	11.0±1.04	20.0±1.02
Salmonella typhymurium	-	12.0±1.01	12.0±1.01	-	-	20.0±1.16
Bacillus subtillis	-	15.0±1.02	12.0±1.04	15.0 ±1.09	12.0±1.01	28±1.81

Table 7 illustrate the zones of inhibition of antibacterial activity of solvent extracts of *Calotropis gigantea R. Br. (Linn)* stem, leaf, and root. Antimicrobial specialists kill or halt bacteria. Microbial pathogens can cause disease forever. They shift drastically to pollute a host,

dodge its immune reactions, rehash inside the host, and escape to another host. Microorganisms can be shown or suggested. Concentrates of plant parts have shown antibacterial effectiveness against numerous microbes, including organisms and developments. (Fig. 18)

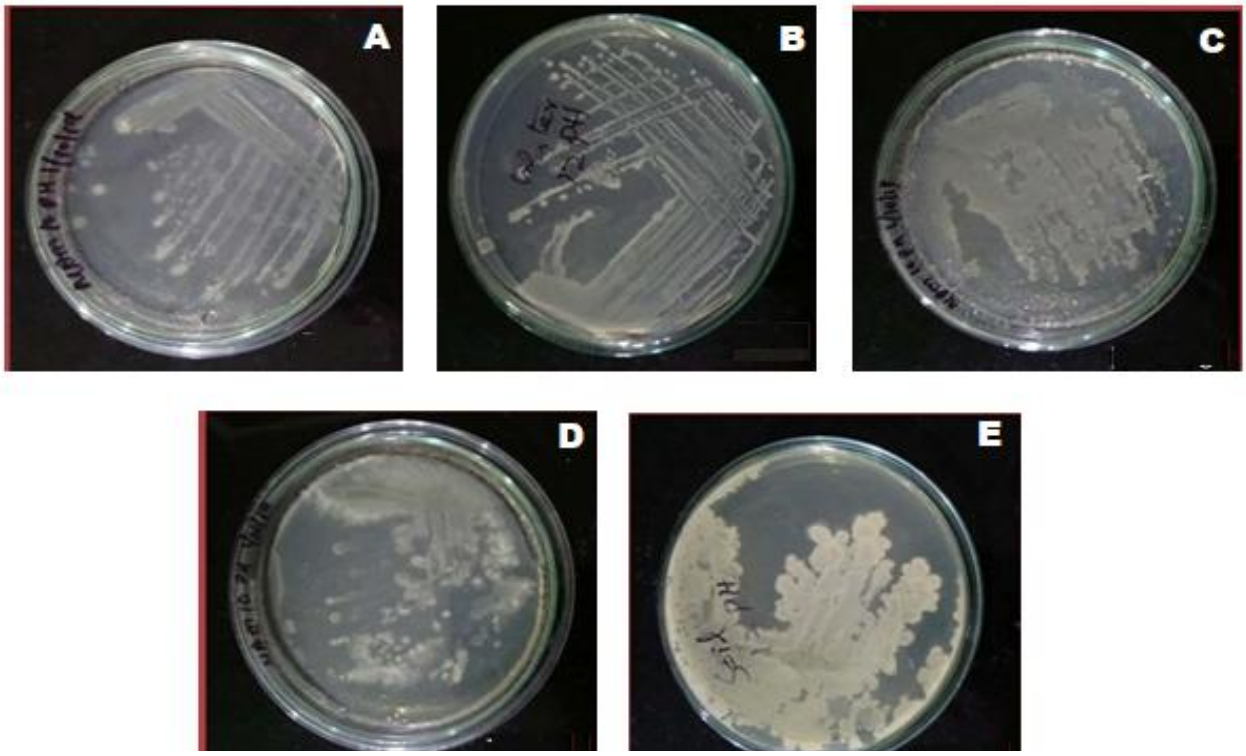


Figure 18. Culture Plates of Microorganism

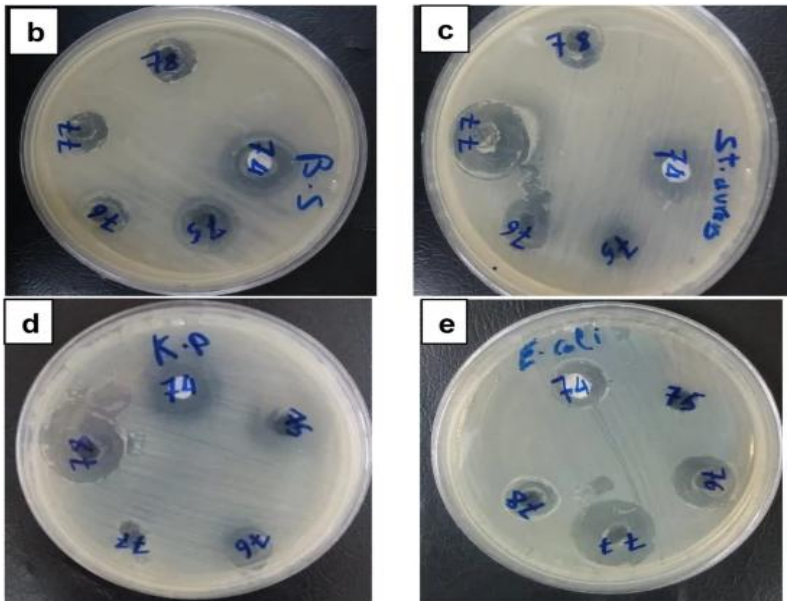


Figure 19. Screening for antibacterial activity of *Calotropis gigantea* plant extractCG Leaf Extract

Antimicrobial Activity

Antimicrobial activity of leaf, stem, and root plant parts of *Calotropis gigantea R. Br. (Linn)* produced in methanol, ethanol, isopropanol, and hexane (500 µg/ml) was tested against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*. (Fig. 19) The methanol-prepared stem extract demonstrated considerably stronger antibacterial activity than leaf and root extracts of *Calotropis gigantea R. Br. (Linn)* ($p<0.05$; n=3). (* $p<0.05$, for statistically significant comparisons between stem and leaf extracts in respective groups and between stem and root extracts) Accordingly, stem extract was made in different doses in methanol, ethanol, petroleum ether, and aqueous and tested against five clinical pathogens: *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa.* Figure 4.31 reveals that all methanolic extract (CGSME) doses had considerably stronger antibacterial activity than other solvent extracts against all tested microorganisms ($p<0.05$; n=3). Antibacterial activity increased dose-dependently against all species ($p<0.05$; n=3).Also, The MIC values of CGSME against *E.* coli and *S. aureus* were the highest (27±0.4 mm and 27±0.7 mm respectively), at 500 µg/ml concentration. (* $p<0.05$, for statistically significant comparison between concentration 62.5 µg/ml of all solvents, $p<0.05$ for comparison between concentrations 125 µg/ml, $p<0.05$ for comparison between concentrations 250 µg/ml, and + $p<0.05$ for comparison between concentration 500 µg/ml) Antimicrobial activity of leaf, stem and root plant parts of *Calotropis gigantea* R. Br. (Linn) were analysed against four pathogens namely *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* against standard antibiotics namely Ampicillin, Tetracycline and Vancomycin (Table 8 and Figure 20). The above analysis demonstrated that the CGSME has significant antimicrobial activity, and could be a potentially effective drug against clinically relevant pathogens. Further in our study, the methanolic stem extract of *Calotropis gigantea* R. Br. (Linn) was taken up for all other analyses. (Ramasar R, et al., 2022)

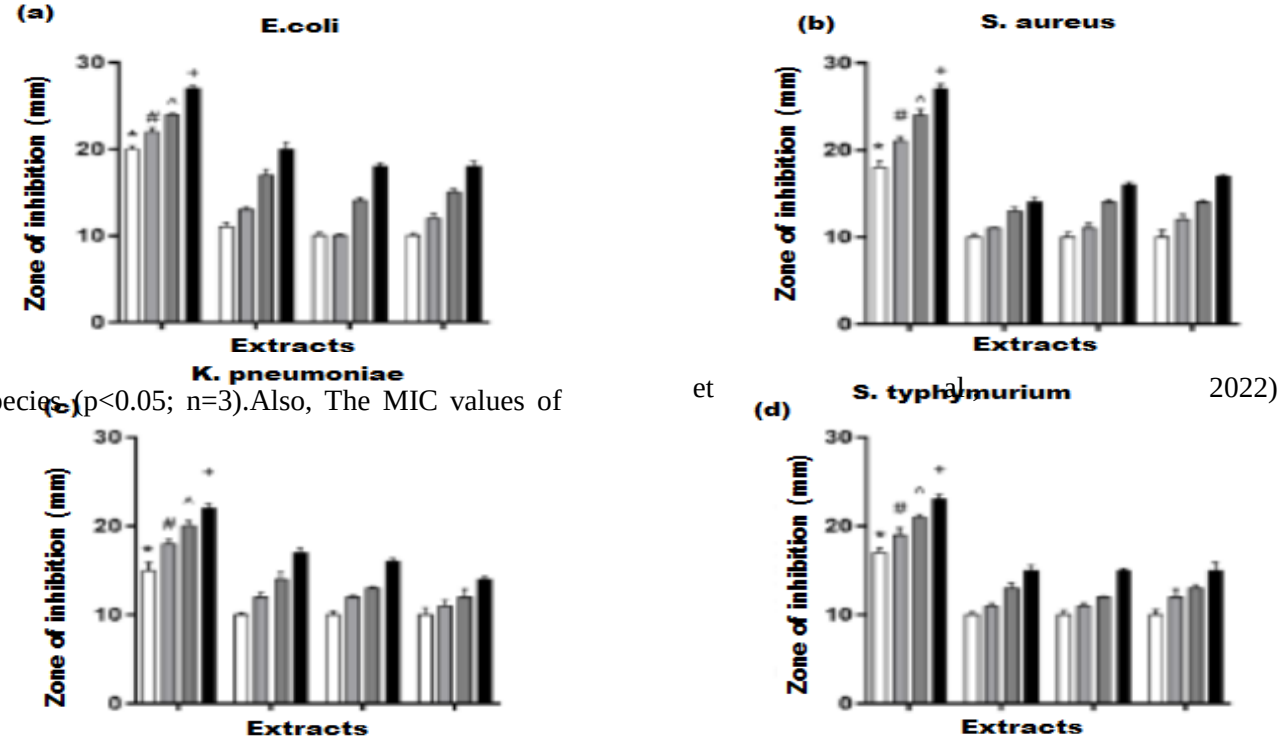


Figure 20. Comparison of antimicrobial activity of stem, leaf and root extracts in different solvents against (a) *E. coli* and (b) *S. aureus.*

Table 8. Test organism tested by antibiotic

Test Organism	Zone of inhibition of standard antibiotics		
	Ampicillin	**Tetracycline**	**Vancomycin**
E.coli	18	24	13
Staphylococcus aureus	17	15	20
Klebsiella pneumonia	14	16	16
Salmonella typhymurium	13	15	17
Bacillus subtillis	15	16	16

Antioxidants

The plant had DPPH free radical scavenging activity,quenched DPPH radicals in the DPPH radical scavenging model, indicating anti-oxidant action. Alcohol outperformed aqueous and ethyl acetate extracts. Compared to the other extract, the plant exhibits good hydrogen-donating activities with an IC50 value of 50–60 μg/ml. Due to odd electrons, this assay shows how chemicals react with stable free radicals. DPPH had a prominent visible spectrum absorption band at 517 mm. Table 9 & 10)

The extract reduced DPPH radical to hydrazine by transferring unpaired electrons to paired ones. Antioxidants couple electrons. The absorption disappears and the discoloration matches the electrons taken up. DPPH absorption bleaching shows the test medicines' free radical scavenging ability. The extract decreased absorbance and exhibited modest activity. The reaction between plant extract and radical developed, scavenging radicals by hydrogen donation. *C. gigantea* methanol extract has the highest DPPH assay free radical scavenging activity (57.31%). The methanol extract scavenged 43.18% DPPH free radicals. *C. gigantea* aqueous extract demonstrated 34.45% and 30.55% free radical activity. *C. gigantea* (36.36%) has higher DPPH free radical scavenging activity than (30.84%). Aqueous and petroleum ether plant extracts inhibited less than methanol. Plant extracts' antioxidant and reducing abilities are linked. (Malviya J, 2016)

Plant extracts reducing the standard. The aqueous (44.82%) and methanol (42.01%) extracts of *C. gigantea* have the highest reducing power. *C. gigantea* petroleum ether extracts (31.55%) and (25.09%) scavenged H_2O_2 poorly. Aqueous emulsion linoleate free radical destroyed β-carotene in the test. Plant extracts with spectrophotometer-measured antioxidant components reduce this breakdown rate. water (59.04%) and *C. gigantea* methanol (57.02%) had the strongest inhibitory activity. (Jahan, N., Mushir, A., Ahmed, A. 2016)

Plant antioxidant potential may be indicated by reducing power. Each compound's reducing capability changed the test solution's yellow color from green to blue. *C. gigantea* methanol extract had the highest activity (1.042 ± 0.002) (Table 11 & 14)

Statistics Analysis

Using correlation coefficient, phytochemical content and antioxidant activity were analyzed. substances and intercorrelation between the studied borders confirmed the relationship between the quantity of phenolic bunches linked to the essential design of flavonoids and their cell reinforcement movement. Using the chi-squared test and the intercorrelations lattice, an acceptable correlation coefficient (r2=0.5678; r=0.7536) between flavonoids' design and their action was found, confirming the cancer prevention agent movement's relationship with phenolic groupings. (Fig. 21 & 22)

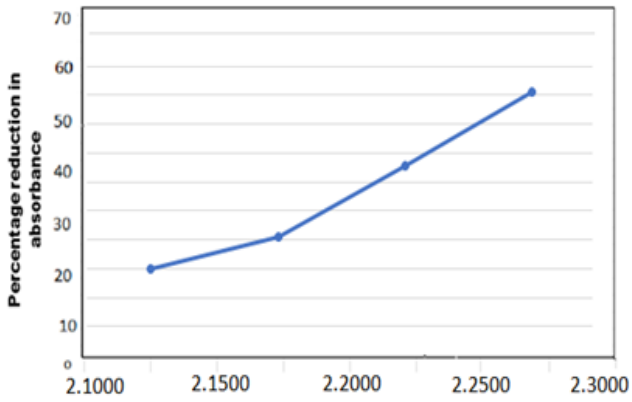


Figure 21. Concentration of test solution

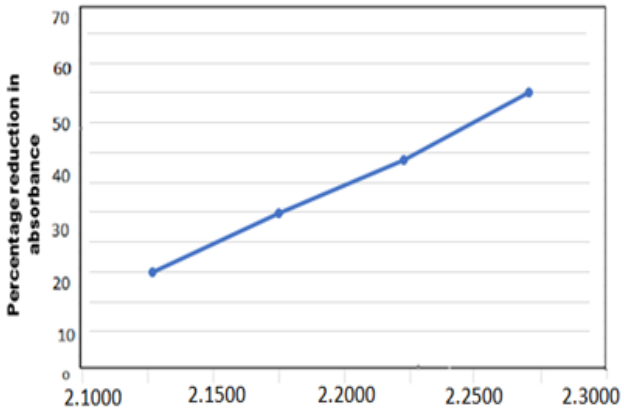


Figure 22. Concentration of test solution

In the table 8 showed the antibiotics Ampicillin, Tetracycline, Vancomycin test against bacterial culture *viz. E. coli, Staphylococcus aureus, Klebsiella pneumonia, Salmonella typhymurium and Bacillus subtills.* The zone of inhibition in (mm) showed in (Fig.23) ranges lies between 17 to 23 mm against the test organisms. (J. Malviya et al., 2012)

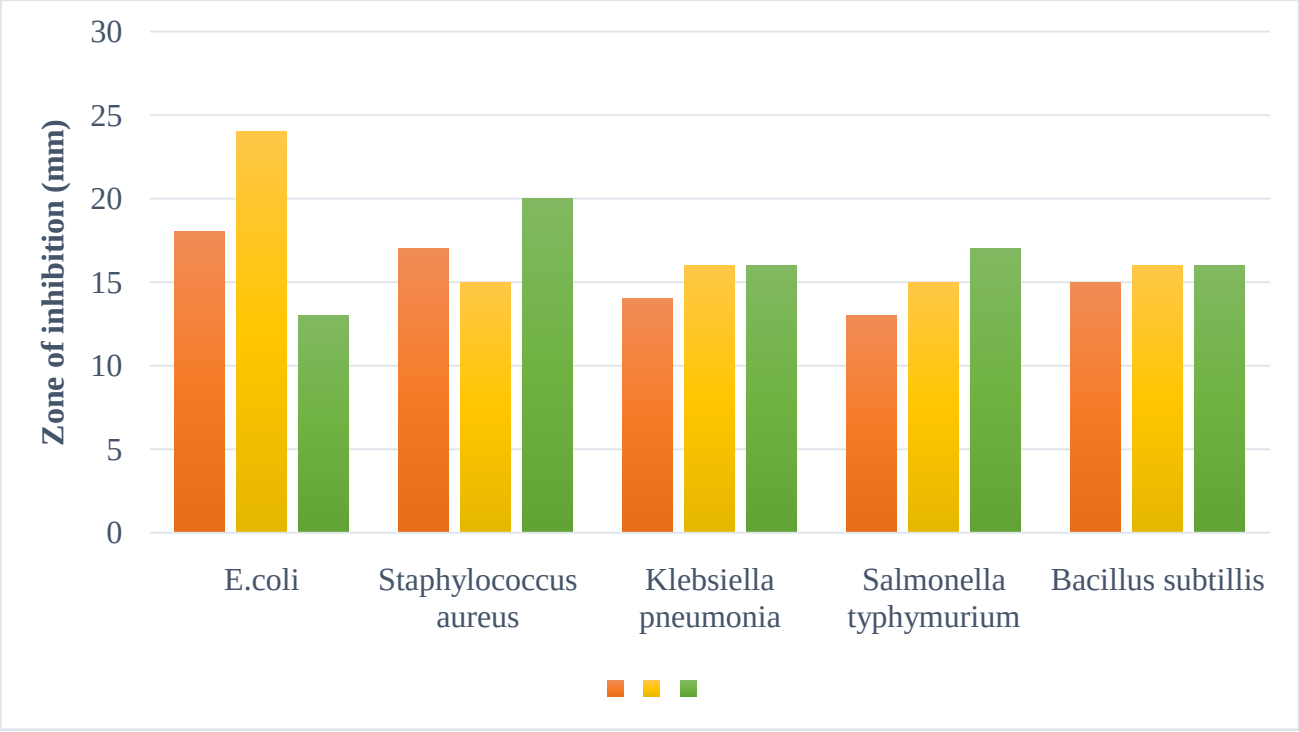


Figure 23. Antibiotics zone of inhibition of standards

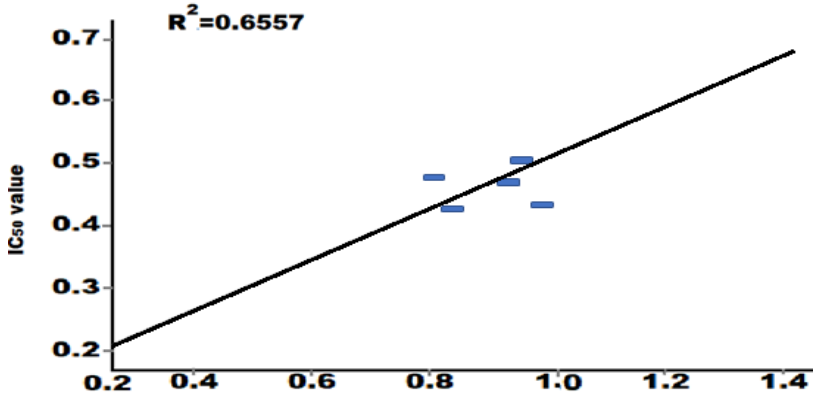


Figure 24. Correlation coefficient (R^2) of antioxidant capacity and total polyphenol content

TPC estimation and free radical scavenging activity were carried out to evaluate the antioxidant potential of the plant by using various antioxidant assays. TPC is generally used to estimate the antioxidant potential of plant (Shukla, S. *et al.*,2016). TPC was calculated from the standard curve of gallic acid and expressed in µg of GAE/mg of plant extract. Methanol extracts of plants remarkably showed high phenolic content as compared to the other extracts. TPC of plants ranged from 18.66 ± 0.57 to 63.33 ± 0.57 µg of GAE/mg of dried plant extracts (Fig. 24& 25 and Table 12 & 13).

Plants contain bioactive substances which have a vast array of therapeutic effects. In this investigation anti-inflammatory properties of *C. gigantea* leaf methanol, petroleum ether and aqueous extracts were compared. The phytoconstituents were derived from *C. gigantea* plant leaves using Soxhlet extraction utilizing several solvents. Several variables, including extraction process, heating rate, duration and solvents influence the extraction's effectiveness. Solvent plays a vital role among

Conclusion

The findings of this investigation indicate that the *C. gigantea* aqueous, methanol and petroleum ether extracts exhibited anti-inflammatory and antioxidant properties in a dose-dependent manner. From the obtained results, *C. gigantea* methanolic extract exhibited a prominent effect in all the experiments. The GC-MS analysis reveals that the highest peak area was observed in methanolic extract which shows the presence of Phytol. This phytochemical is a secondary metabolite that possesses.

Conflicts of Interest and Sources of Funding

The author has no conflict of Interest and there is not any funding source has been available.

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