IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

PHYTOCHEMICAL INVESTIGATIONS AND GREEN SYNTHESIS OF NANOPARTICLES FROM CALOTROPIS PROCERA

[1]Afeef Thasleem E S, [2] Dr. Umesh B T,[3]varsha Joseph [1] Student, [2] Head of department, ,[2]Department of Biosciences, MES college Marampally,[3] Student Aluva, India

Abstract: *Calotropis procera*, a medicinal plant known for its various pharmacological properties, has gained significant attention in recent years. This thesis presents a comprehensive study focused on the phytochemical characterization and green synthesis of silver nanoparticles (agnps) from *calotropis procera* and evaluates their potential applications in antimicrobial properties, *IN VITRO* citotoxicity in cancer cell and gc-ms analysis of the chloroform extract.The green synthesis of agnps from *calotropis procera* extracts represents an eco-friendly and sustainable approach that offers a novel route for nanoparticle production. The synthesized agnps were characterized using spectroscopic and microscopic techniques, including edx, and sem, ensuring their size, shape, and stability.This integrated study contributes to the development of novel therapeutic agents and highlights the ecological and medicinal significance of *calotropis procera* in modern science.

1.INTRODUCTION

Calotropis gigantia and *Calotropis procera* are the only two species of this widely dispersed plant. Both species are widespread, although the *Calotropis procera*, which has purple flowers while the *Calotropis gigantia* has white flowers, is more frequent. The primary physical difference between the two species that may be distinguished is the colour of the buds or blooms on their flowers. Since the plant doesn't have a blossom, it is difficult to identify the species. By merely testing the milky latex of the plants that is obtained from the cut area of the stem for pH, it is feasible to identify the species. This latex is releasing extremely slowly. The milky colour of the latex of many common plants, including Calotropis, is due to calcium oxalate crystals. The aim of the work conducts a comparative study of the leaf and flower characteristics of *Calotropis procera* and investigate their phytochemical composition and potential pharmacological activities. In this comparative study, various methods will be employed, including microscopic examination, phytochemical analysis, and biological assays. while scanning electron microscopy will provide detailed information on their surface structures. Additionally, phytochemical analysis will identify the metabolites present in both plant parts, thereby revealing their chemical diversity and potential medicinal properties. Furthermore, bioassays will be performed to evaluate the biological activities and potential therapeutic applications of these metabolites.This work will contribute to the current body of knowledge regarding *Calotropis procera* by providing a comprehensive comparative analysis of its leaf and flower characteristics. The findings of this study will not only enhance our understanding of the plant's biological features but also shed light on its potential applications in various fields, such as medicine, and environmental conservation.

2.Materials and Methods

2.1 Collection of samples

Fresh leaves and flowers of *Calotropis procera* were collected from the backside of MES College Marampally, Aluva. The collected plant materials were identified by Dr Umesh B T (HOD of Bioscience, MES College Marampally)

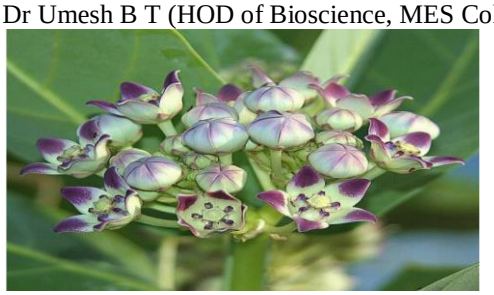


Figure 1 calatropis procera

2.2 Preparation of extracts

The collected plant materials were thoroughly washed (except flowers) to remove the soil and dirt and were shade-dried. The dried plant materials were chopped into small pieces blended to powder and then stored in an airtight container

2.2.1 Acetone extracts

20 g of the powder was weighed and mixed with 200 ml acetone. put this mixture in a rotatory shaker for 13 to 15 hours. Then filter the extract by using Whatman No1 filter paper. Then the extract was stored in glass bottles at 4^0 c for further use.

2.2.2 Chloroform extracts

20g of the powder was weighed and mixed with 200 ml chloroform. put this mixture in a rotatory shaker for 13 to 15 hours. Then filter the extract by using Whatman No1 filter paper. The extract was then stored in glass bottles at 4^0 c for further use.

2.2.3 Hot water extracts

20 g of the powder was weighed and mixed with distilled water. Keep the extract in a boiling water bath for 15 minutes. Allowed to cool and then filtered using Whatman No1 filter paper. Then the extract was stored in glass bottles at 4^0 c for further use.

Figure 2 Extract

2.3 Phytochemical screening

Preliminary phytochemical screening (Harborne JB (1998) was carried out using the desired plant materials (root,stem,leaf,fruit,flower etc)

2.3.1 Test for coumarins

A small amount of extract was dissolved in methanol/ethanol and 3-4ml alcoholic KOH or NaOH was added. The formation of a yellow colour which disappears upon adding concentrated HCl indicated the presence of coumarins.

2.3.2 Test for tannins

Ferric chloride test few drops of ferric chloride were added to a small amount of the extract. The development of a green colour revealed the presence of tannin.

2.3.3 Test for carbohydrates

Molishs test -100 mg of the substance was dissolved in 1ml water and 2 drops of 1% alcoholic solution of alpha-naphthol were added 1ml of concentrated H_2So_4 was added along the sides of the test tube so that it formed a heavy layer at the bottom. A deep violet ring at the liquid junction confirmed the presence of carbohydrates.

2.3.4 Test for the detection of glycoside

Benedict's reagent test -the extract was added to Benedict's reagent in equal amounts and the mixture was heated for 2 minutes. The appearance of brown to red colour indicated the presence of glycoside.

2.3.5 Protein detection

Xanthoprotein test: A small amount of the extract was added to 0.5ml of concentrated HNO_3. The appearance of white or yellow precipitate revealed the presence of protein.

2.3.5 Reducing sugar detection test

Benedict’s reagent test extract was added to Benedict’s reagent in equal amounts and the mixture was heated for 2 minutes. The appearance of brown to red colour indicated the presence of reducing sugar.

2.3.6 Wagner's test

A few drops of Wagner's reagent were added to the extract. Alkaloids gave a brown flocculent precipitate with the reagent. Wagner's reagent- 1.2gm of Iodine and 2gm of KI were dissolved in 5 ml of distilled water and the solution was made up to 100ml with distilled water.

2.3.7 Detection of steroids

Salkowski test-A few drops of concentrated H_2SO_4 were added to a small amount of extract and shaken for a few minutes; the development of a red or brown colour indicated the presence of sterols.

2.3.8 Test for phenols

A few drops of alcoholic ferric chloride solution were added to the extract dissolved in alcohol or water. Formation of violet, bluish green or bluish-black colour indicated the presence of phenols..

2.3.9 Test for quinines

To the extract, sodium hydroxide was added. The formation of blue, green or red colours indicated the presence of quinines.

2.3.10 Test for anthroquinones

Bontrager's test extract is shaken with aqueous ammonia or caustic soda. The formation of pink, red or violet colors in the aqueous layer indicated the presence of anthraquinones.

2.4 Antimicrobial test

The culture plates seeded with test organisms were allowed to solidify and punched with a sterile cork borer (7.0 mm diameter) to make open wells. The empty wells were filled with 0.05 ml of the extract. The plates were incubated at 37^0 C for 48 hours. For the fungi, the test was carried out on SDA plates and incubated at 30^o C for 72 hours. The zones of inhibition were measured and recorded. Test organisms, seven microorganisms were used in this study as test organisms comprising clinical isolates of four bacteria (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia*) and three fungi (*Aspergillus niger, Aspergillus flavus, Rhizopus oryzae*). The typed cultures of bacteria and fungi were sub-cultured on Nutrient agar (Oxoid) and Saboraud dextrose agar (Oxoid) slants respectively and stored at 4^0 C until required for study

2.5 Nanoparticle synthesis

2.5.1 Preparation of aqueous extract

The dried plant materials were chopped into small pieces blended to powder and then stored in an airtight container (10 g), were put in double distilled water, and boiled in 100 ml of distilled water, at 60°C for 5 minutes. The extract was filtered through Whatman No1 filter paper. Then the extract was stored in amber glass bottles used for further experiments.

2.5.2 Preparation of 2mm $AgNo_3$

2mM Silver Nitrate solution was prepared by adding 0.339g of AgNO3 to 1L distilled water.Mixed the two solutions at equal volumes heated the solution at 60^0C for 3 hours and incubated in the dark for 24 hrs. at room temperature. After incubation centrifuged at 10,000 rpm for 10 minutes. The supernatant was removed and double distilled water was added. Centrifuged at 10,000 rpm for 10 minutes. The supernatant was removed and the pellet was dried. Stored nanoparticles in an air-free container for further experiments.

2.5.3 Nanoparticles Anticancer test- In vitro cytotoxicity test

The short-term In vitro cytotoxicity of the test chemical was investigated using Dalton's Lymphoma Ascites cells (DLA).Tumor cells were extracted from the peritoneal cavity of tumor-bearing animals and washed three times with PBS or a normal cell line. The trypan blue exclusion technique was used to measure cell viability. A viable cell suspension (1106 cells in 0.1ml) was added to tubes containing different concentrations of the test chemicals, and the volume was increased to 1ml using a phosphate buffered Saline (PBS). The only thing in the control tube was cell suspension. This test combination was incubated at 37 °C for 3 hours. The cell solution was then mixed with 0.1ml of 1% trypan blue for 2-3 minutes before being put into a haemocytometer. Dead cells absorb the blue colour of trypan blue, but healthy cells do not. The number of stained and unstained cells was separately counted. DMSO was used to dissolve the samples.

% cytotoxicity = No of dead cells /No of live cells + No of dead cells ×100

2.5.4 Scanning Electron Microscopy with Energy Dispersive X-ray spectroscopy (SEM/EDX)

Scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX) analysis was carried by using (SEM-EDX: Jeol 6390LA/ OXFORD XMX N) Accelerating voltage: 0.5 to 30 kV, Filament: Tungsten, Magnification x 300000, EDX resolution 136 eV, EDX detector area 30 mm2, Elemental Mapping

2.5.5 Nanoparticles Antimicrobial test

Test organisms- Clinical isolates of four bacteria (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia*) and three fungi (*Aspergillus niger, Aspergillus flavus, Rhizopus oryzae*) were employed as test organisms in this investigation.The culture plates seeded with test organisms were allowed to solidify and punched with a sterile cork borer (7.0 mm diameter) to make open wells. The empty wells were filled with 0.05 ml of the extract. The plates were incubated at 37^0 C for 48 hours. For the fungi, the test was carried out on SDA plates and incubated at 30^0 C for 72 hours. The zones of inhibition were measured and recorded.

2.6. GC-MS analysis

GC-MS analysis of chloroform extract of *Calotropis procera* leaf and flower by using Shimadzu Nexis GC- 2030 AUTO SAMPLER: AOC-30/20i (COLUMN SPECIFICATIONS Column Name: SH-I- 5Sil, MS Length: 30.0 m Inner Diameter: 0.25 mm, Film thickness: 0.25 µm, GCMS Software: GCMS Solutions, Libraries used: NIST 2) The interpretation of the unknown part's mass spectrum was compared to the spectrum of known components contained in the NIST library. The components of the test materials were discovered.

3.RESULT'S

3.1Phytochemical screening

Table 3.1.1 Leaf extracts

Test	**Hot water extract**	**Acetone extract**	**Chloroform Extract**
Coumarins	Negative	Negative	Negative
Tannis	Negative	Negative	Negative
Molish	Negative	Negative	Negative
Reducing Sugar	Negative	Negative	Negative
wagners	Negative	Negative	Negative
Xanthoprotein	Negative	Negative	Negative
Glycoside	Negative	Negative	Negative
Steroid	Positive	Positive	Positive
Phenol	Negative	Negative	Negative
Quinines	Positive	Positive	Positive
Anthroquinones	Positive	Negative	Negative

Table 3.1.2 Flower extracts

Test	**Hot water extract**	**Acetone extract**	**Chloroform Extract**
Coumarins	Positive	Positive	positive
Tannis	Negative	Negative	Negative
Molish	Negative	Negative	Negative
Reducing Sugar	Negative	Negative	Negative
wagners	Negative	Negative	Negative
Xanthoprotein	Negative	Positive	Positive
Glycoside	Negative	Negative	Negative
Steroid	Positive	Positive	Negative
Phenol	Negative	Negative	Negative
Quinines	Positive	Positive	Negative
Anthroquinones	Positive	Negative	Negative

3.2Antimicrobial test

Table 3.2.1 Leaf Extract

Microorganisms	Zone Formation in Extract		
	Hot water extract	Acetone extract	Chloroform Extract
Escherichia coli	0 mm	15 mm	10 mm
Staphylococcus aureus	0 mm	0 mm	10 mm
Pseudomonas aeruginosa	0 mm	0 mm	0 mm
Aspergillus Niger	0 mm	0 mm	0 mm
Klebsiella pneumonia	0 mm	0 mm	0 mm
Aspergillus flavus	0 mm	0 mm	0 mm
Rhizopus	0 mm	0 mm	0 mm

Table 3.2.2 Flower Extract

Microorganisms	Zone Formation in Extract		
	Hot water extract	Acetone extract	Chloroform Extract
Escherichia coli	0 mm	12 mm	11 mm
Staphylococcus aureus	0 mm	11 mm	0 mm
Pseudomonas aeruginosa	0 mm	12 mm	10 mm
Aspergillus niger	0 mm	0 mm	0 mm
Klebsiella pneumonia	0 mm	0 mm	0 mm
Aspergillus flavus	0 mm	0 mm	0 mm
Rhizopus	0 mm	0 mm	0 mm

Table 3.2.3 Control

Microorganisms	Zone Formation of control	
	Acetone	Chloroform
Escherichia coli	0 mm	0 mm
Staphylococcus aureus	0 mm	0 mm
Pseudomonas aeruginosa	09 mm	7 mm
Aspergillus Niger	10 mm	0 mm
Klebsiella pneumonia	0 mm	0 mm
Aspergillus flavus	10 mm	6 mm
Rhizopus	0 mm	0 mm

3.3 Nano particle Synthesis

Nano particle was produced and then stored for further studies.

3.3.1 IN VITRO CYTOTOXICITY OF NANOPARTICLES

Table 3.3.1

Drug concentration (µl/mL)	% Cell Death	
	F	L
12.5	20.9±1.9	10.9±1.2
25	24.1±2	14.4±2.2
50	31.8±2.1	25.1±1.5
100	50.2±2.6	43±2.1
200	80.8±1.1	68.4±1.9

Control tube contains 5 dead cells

3.3.2 Antimicrobial Property of nano particle

Table 3.3.2 control

Microorganisms	**Zone Formation of control**	
	Acetone	**Chloroform**
Escherichia coli	0 mm	0 mm
Staphylococcus aureus	0 mm	0 mm
Pseudomonas aeruginosa	09 mm	7 mm
Aspergillus niger	10 mm	0 mm
Klebsiella pneumonia	0 mm	0 mm
Aspergillus flavus	10 mm	06 mm
Rhizopus	0 mm	0 mm

Table 3.3.2 Flower

Microorganisms	**Zone Formation of Extract**		
	Acetone extract	**Chloroform Extract**	**Hot Water Extract**
Escherichia coli	0 mm	12 mm	0 mm
Staphylococcus aureus	20 mm	20 mm	10 mm
Pseudomonas aeruginosa	12 mm	20 mm	18 mm
Aspergillus niger	16 mm	13 mm	0 mm
Klebsiella pneumonia	10 mm	20 mm	15 mm
Aspergillus flavus	26 mm	17 mm	0 mm
Rhizopus	22 mm	20 mm	0 mm

Table 3.3.3 Leaf.

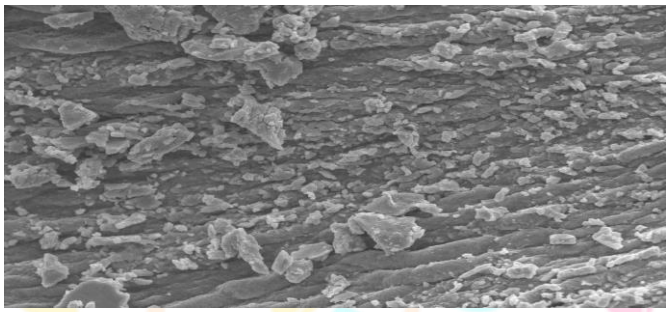
Microorganisms	**Zone Formation of Extract**		
	Acetone extract	**Chloroform Extract**	**Hot Water Extract**

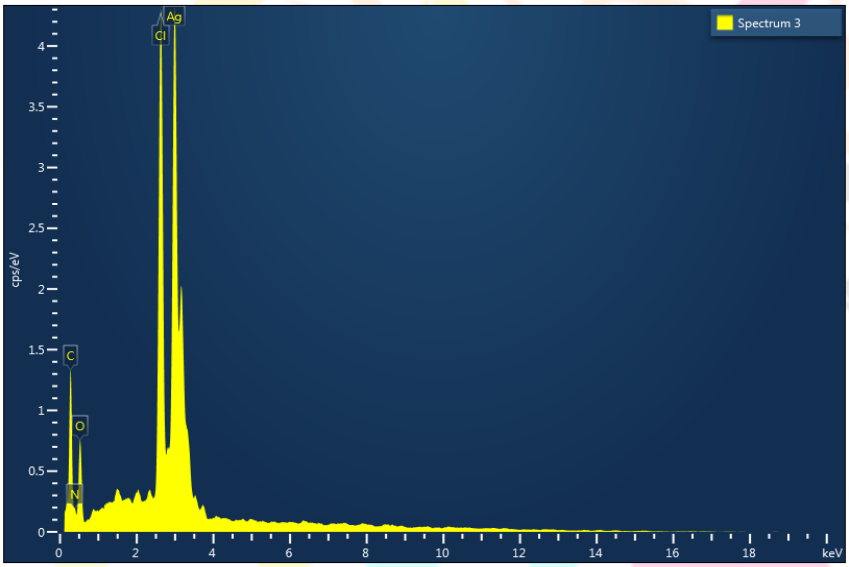
Escherichia coli	15 mm	18 mm	0 mm
Staphylococcus aureus	16 mm	23 mm	11mm
Pseudomonas aeruginosa	25 mm	22 mm	11mm
Aspergillus niger	24 mm	15 mm	0 mm
Klebsiella pneumonia	15 mm	16 mm	0 mm
Aspergillus flavus	15 mm	20 mm	0 mm
Rhizopus	25 mm	20 mm	0 mm

3.4 NANO PARTICLE SEM EDX

3.4.1 flower

Figure 3.4.1 flower

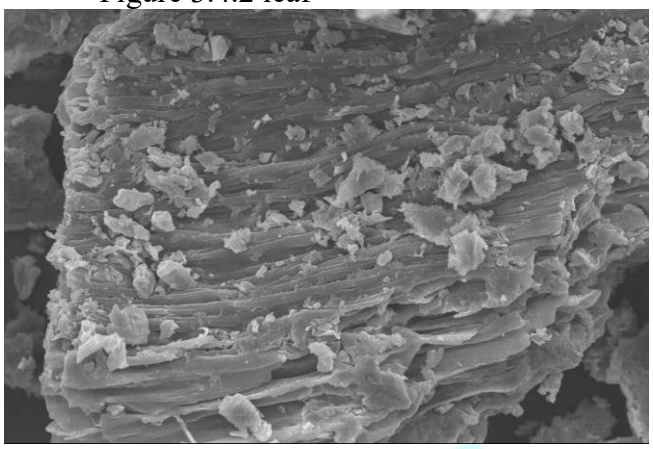




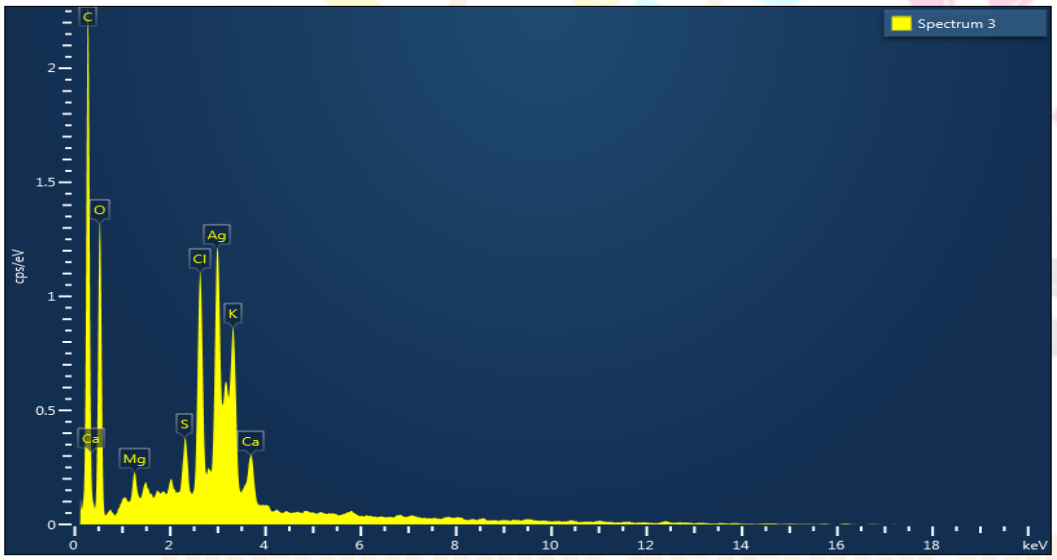
Element	Line Type	Wt%	Atomic %
C	K series	22.6	45.54
N	K series	9.05	15.63
O	K series	13.88	20.99
Cl	K series	12.24	8.36
Ag	L series	42.23	9.48
Total:		100	100

3.4.2 Leaf

Figure 3.4.2 leaf



While performing scanning electron microscopy, the mean diameter of the particles obtained is 50nm.



Element	Line Type	Wt%	Atomic %
C	K series	44.24	61.4
O	K series	29.99	31.25
Mg	K series	0.59	0.41
S	K series	0.95	0.49
Cl	K series	4.11	1.93
K	K series	3.64	1.55
Ca	K series	1.62	0.67
Ag	L series	14.86	2.3
Total:		100	100

3.5 GC-MS ANALYSIS

Figure 3.5.1 flower

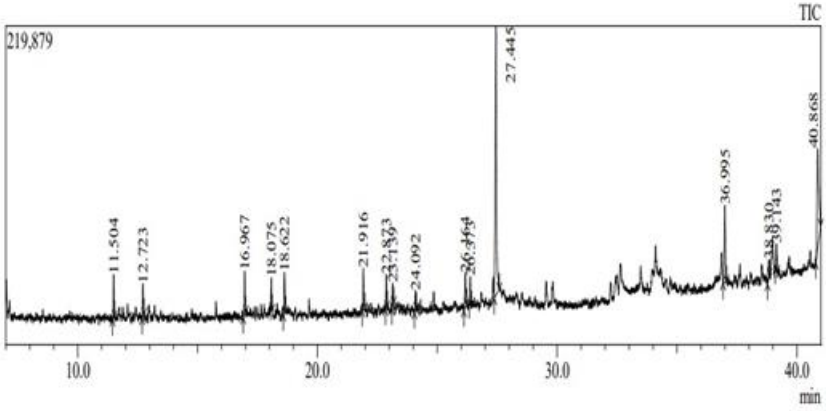


Table 3.5.1 Flower

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	11.504	66460	3.52	29948	5.06	2.22	MI	Dodecane, 4,6-dimethyl-
2	12.723	53966	2.86	22512	3.80	2.40	MI	3-Ethyl-2,6,10-trimethylundecane
3	16.967	66941	3.55	29826	5.04	2.24	MI	Eicosane
4	18.075	39382	2.09	20289	3.43	1.94	MI	Docosane
5	18.622	67233	3.56	28300	4.78	2.38	MI	Dodecanoic acid
6	21.916	72272	3.83	28427	4.80	2.54	MI	Hexacosane, 1-iodo-
7	22.873	57564	3.05	22791	3.85	2.53	MI	Heptadecane, 8-methyl-
8	23.139	37826	2.00	15706	2.65	2.41	MI	Myristic
9	24.092	33940	1.80	13862	2.34	2.45	MI	Heneicosane
10	26.164	69564	3.69	23802	4.02	2.92	MI	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-di
11	26.373	41667	2.21	18656	3.15	2.23	MI	Hexacosane, 1-iodo-
12	27.445	665537	35.26	185542	31.34	3.59	MI	Palmitic acid
13	36.995	173225	9.18	53005	8.95	3.27	MI	Dotriacontane
14	38.830	38831	2.06	12915	2.18	3.01	MI	Stearic acid, butyl ester
15	39.143	42139	2.23	17666	2.98	2.39	MI	Tetracontane
16	40.868	360931	19.12	68710	11.61	5.25	MI	Octacosanol
		1887478	100.00	591957	100.00			

Figure 3.5.2 Leaf

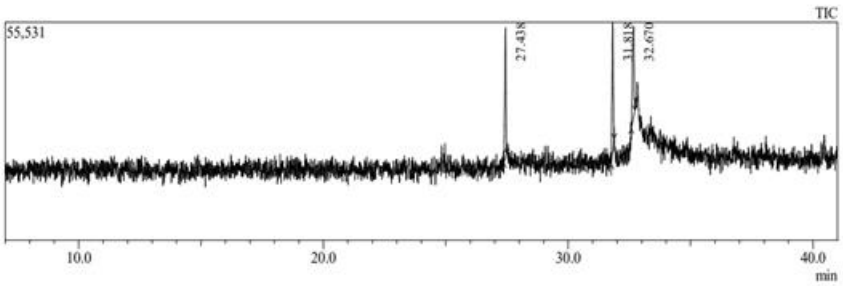


Table 3.5.2 Leaf

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	27.438	113800	33.66	33177	37.19	3.43	MI	n-Hexadecanoic acid
2	31.818	116939	34.59	32961	36.94	3.55	MI	Phytol
3	32.670	107360	31.75	23082	25.87	4.65	MI	cis-Vaccenic acid
		338099	100.00	89220	100.00			

4. Discussion

4.1 Phytochemical screening-

Steroids and quinines were discovered in all three solvent extracts—hot water, acetone, and chloroform—after phytochemical investigations of leaf extracts from Calotropis procera. This suggests that these chemicals are probably abundant and dispersed uniformly throughout the plant's leaves. The biological effects of steroids are well known, including their capacity to lower inflammation and alter the immune system. Quinines, on the other hand, are well known for their antimicrobial properties .It's interesting that only the hot water extract included anthraquinones, a specific quinones subtype known for its laxative properties. This study suggests that hot water, rather than acetone or chloroform, may be a more effective solvent for anthraquinones.

Further research may be warranted given the unusual presence of anthraquinones in the hot water extract, particularly in light of Calotropis procera's long history of usage as a medicine.In conclusion, our studies of the phytochemistry of leaf extracts from Calotropis procera showed that all three extracts included steroids and quinines, with the exception of the hot water extract, which only contained anthraquinones. To further understand the therapeutic effects of Calotropis procera, additional research is required to isolate, characterise, and evaluate these compounds' potential pharmacological activity.

The results of this phytochemical research highlight the broad range of bioactive compounds present in floral extracts of Calotropis procera. In particular, coumarins were found in each extract, proving that this plant species is abound in them. Because of their anti-inflammatory and antioxidant capabilities, these compounds are widely recognised for their potential as medicines.

It's interesting that anthraquinones are only present in the hot water extract. The ancient usage of C. procera in digestive medicines may be explained by the fact that these chemicals are frequently linked to laxative qualities. Quinones, which have been found in both acetone and hot water extracts, suggest that the plant may have antiviral and antibiotic activities, which are consistent with its supposedly antibacterial benefits.

Steroids in acetone and hot water extracts may have immunomodulatory and anti-inflammatory effects, which might be of great importance in the creation of medicines or natural treatments. Last but not least, concerning the role xanthoproteins played in the extracts' colour have been raised by their discovery in acetone and chloroform extracts. It is necessary to conduct more research on their possible bioactivity and importance. This phytochemical investigation of Calotropis procera flower extracts showed the existence of a number of bioactive substances, each with distinct therapeutic potential. The pharmacological potential of this plant species for upcoming study and the creation of natural treatments is underscored by the disparities in compound distribution across the extracts, which emphasise the significance of solvent selection in plant extraction.

4.2 Antimicrobial test

According to the findings, the antibacterial activity differs according to the kind of extract (acetone, chloroform), as well as the microorganism being studied. While some extracts exhibited little to no effect on particular bacteria, others had modest action. Lack of Antimicrobial action in Hot Water Extracts: Hot water extracts from both leaves and flowers did not exhibit any discernible antimicrobial action, suggesting that the inhibitory chemicals may not be water-soluble.

Escherichia coli Activity: Both the leaf and flower extracts from acetone and chloroform shown some amount of activity against Escherichia coli. This implies that the plant may contain antibacterial substances. The acetone extract from the flower showed substantial effectiveness against Aspergillus flavus, however there was little antibacterial activity. This suggests the plant extract could have antifungal characteristics. For prospective future applications, it is crucial to select the proper extraction technique and comprehend the target microbe due to the variety in antimicrobial activity among various extracts and bacteria.

4.3 NANOPARTICLES SYNTHESIS& ITS ANTI MICROBIAL TEST

Antibacterial studies employing silver nanoparticles created from leaf and floral extracts of Calotropis procera revealed diverse levels of antimicrobial activity against various microorganisms.

Acetone extraction: Extract of a flower: With regard to Escherichia coli, Aspergillus niger, Klebsiella pneumonia, and Rhizopus, the acetone extract from the flower of Calotropis procera exhibits no zone development. However, it has notable effectiveness against Aspergillus flavus, Pseudomonas aeruginosa, and Staphylococcus aureus.

Leaf Extract: The leaf's acetone extract exhibits no anti-Rhizopus or anti-Aspergillus niger action. Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, and Aspergillus flavus are moderately resistant to it, though. Flower Extract in Chloroform: The flower extract in chloroform is poor antibacterial activity against Escherichia coli and Pseudomonas aeruginosa. It shows strong action against Klebsiella pneumonia and Staphylococcus aureus. Aspergillus niger, Aspergillus flavus, and Rhizopus are all unaffected.

Leaf Extract: The leaf extract in chloroform has a similar pattern of action. Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, and Aspergillus flavus are among the organisms it is effective against. However, it is ineffective against Rhizopus and Aspergillus niger.

Hot water extract Flower Extract: The hot water extract from the flower has minimal effect on Pseudomonas aeruginosa and Escherichia coli. It shows a moderate zone of inhibition against Klebsiella pneumonia and Staphylococcus aureus. There is no evidence of any action against Rhizopus, Aspergillus flavus, or Aspergillus niger.

Leaf Extract: The leaf's hot water extract has only weak antibacterial effects on Staphylococcus aureus and moderate antibacterial effects on Klebsiella pneumonia. Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger, Aspergillus flavus, and Rhizopus are all unaffected. According to the findings, Calotropis procera's flower and leaf chloroform extracts had the strongest antibacterial effects, mostly against Staphylococcus aureus and Klebsiella pneumonia. The hot water extract is often less effective, however the acetone extract from the flower and leaf exhibits modest efficacy against certain bacteria. It's important to know that silver nanoparticles made from various plant parts and extraction techniques can have variable antibacterial effects. This could be because the chemical makeup of the extracts varies. To pinpoint the precise bioactive substances in charge of the observed antimicrobial actions and to pinpoint the mechanisms of action, more research and analysis are required. These results suggest that further study into the potential of Calotropis procera extracts, particularly the chloroform extract, as natural antibacterial agents,

particularly against Gram-positive bacteria like Staphylococcus aureus, is required. However, more research is required to optimize and understand their applications in various settings.

4.4 IN VITRO CYTOTOXICITY OF NANOPARTICLES

These results suggest a dose-dependent relationship between the concentration of silver nanoparticles (AgNPs) and their cytotoxicity. As the concentration of AgNPs increases, the percentage of cell death also increases, indicating that AgNPs become more toxic to the cells at higher concentrations. The standard deviations provide information about the variability in the measurements, and in most cases, they are relatively small, suggesting that the results are fairly consistent.

It's essential to interpret these results in the context of the specific experiment and research objectives to draw meaningful conclusions about the cytotoxic effects of AgNPs on the tested cells. Additionally, further statistical analysis, such as a dose-response curve, could be performed to better understand the relationship between concentration and cell death.

To compare the cytotoxicity of flower nanoparticles, have more cytotoxicity than leaf nano particles.

5 Conclusion

In conclusion, the research on phytochemical investigations and green synthesis of silver nanoparticles from *Calotropis procera* has yielded promising results across multiple facets of scientific inquiry. The study began by successfully synthesizing silver nanoparticles using an eco-friendly approach, which not only showcases the potential of green nanotechnology but also aligns with sustainable practices in material science.

The research also underscored the importance of investigating the anti-microbial properties of the synthesized nanoparticles. The evaluation of their anti-microbial activity may pave the way for their use in various medical and healthcare applications, such as wound dressings and antibacterial coatings.

The comprehensive investigation of *Calotropis procera* derived silver nanoparticles has not only contributed to the growing body of knowledge in the fields of nanotechnology, cancer research, and antimicrobial agents but has also demonstrated the potential for eco-friendly, sustainable synthesis methods in the creation of nanoparticles with multifaceted applications. This research offers a promising foundation for future studies and applications in the fields of medicine, materials science, and beyond

7. REFERENCES

- **A O Kalu, E C Egwim, A A Jigam and H L Muhammed.(2022). Green synthesis of magnetite nanoparticles using calotropis procera leaf extract and evaluation of its antimicrobial.Nano Express.vol.3 .doi: 10.1088/2632-959X/aca925.**
- **Abdel-Farid, I.; El-Sayed, M.; Mohamed, E. Allelopathic potential of calotropis procera and Morettia philaeana.2013.International journal of agriculture and biology.ISSN: 1814-9596.**
- **Amarpret kaur, Daizy.R.batish,Shalinder kaur, Bhagirath.s.Chauhan.(2021) An Overview of the Characteristics and Potential of Calotropis procera From Botanical, Ecological, and Economic Perspectives. Front. Plant Science Volume 12 - 2021 | https://doi.org/10.3389/fpls.2021.690806,1-5**
- **Harborne, A. J. (1998). *Phytochemical methods a guide to modern techniques of plant analysis*. springer science & business media.**
- **•Hitesh Vashrambhai Patel, Jatin D. Patel, Bhautik Pate; comparative efficacy of phytochemical analysis and antioxidant activity of methanolic extract of calotropis gigantea and calotropis procera**
- **Jabeen, K.; Waheed, N.; Iqbal, S. Antifungal potential of Calotropis procera against Macrophomina phaseolina. *Life Sci. J.*, 2013, *10*, 572-576.. ISSN:1097-8135**
- **Jyoti verma(2021) Anticancer Potential of Calotropis procera: An Overview. Journal of Natural Products Research and Ethnopharmacology. https://doi.org/10.15562/phytomedicine.2021.165**
- **Varsha G Shetty, Meenakshi G Patil, Ambika Dound·(2015). Evaluation of phytochemical and antibacterial properties of *CALOTROPIS PROCERA* (AIT) R. BR. LEAVES.vol.7, Int J Pharm Pharm Science.**
- **Verma, V. N. (2014). The chemical study of Calotropis. *International Letters of Chemistry, Physics and Astronomy*, *1*, 74-90.**
- **Walied M. Alarif and Mohamed R. Rizgallah.(2017) Anticancer and Antibacterial Activity of Calotropis procera Leaf Extract.VOL.7.18-25.semantic scholar.**