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Preliminary Studies, Antimicrobial and Antioxidant Activity of Leaf, Stem Bark and Root Extracts of *Calotropis Procera* Plant

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Abstract

The phyto-components of leaf, root and Stembark of Calotropis procera L. Asclepiadaceae family species were screened by gas chromatography- mass spectroscopy (GC-MS) analysis. Ethanol extract was prepared by soxhlet apparatus from all the parts of C. procera. The present study aimed to determine the phytochemical screening, antimicrobial and anti-oxidant properties of the leaves, root and stem bark of the ethanol extracts of the C. procera plant. Phytochemical screening was carried out for all the three extracts obtained from the leaves, root and stem bark which revealed the presence of most secondary metabolites such as flavanol, phenol, alkaloids, saponins except glycosides in leaf, while alkaloids and saponins were absent in the root and glycosides absent in the stembark. From antibacterial result, the highest zone of inhibition of leave extract for E. coli was (14mm) and the lowest zone of inhibition was (2mm), the highest and lowest zone of inhibition for the leaf of Staph. A was (14mm and 4mm) respectively, Similarly, the highest zone of inhibition and the lowest zone of inhibition of leaves for Bacillus Cereus was (14mm) and (4mm). The highest zone of inhibition of Aspergillus favus was 12mm in the leaves extract and it was 14mm for Candida albicans in the leaves extract. The FTIR of F3 fraction of the leaf confirmed the presence of alkane, alkene, hydroxyl, amines and amides which showed

characteristics peaks at 3326cm^{-1}, 2945.07cm^{-1}, 2833.56 cm^{-1}, 1649.24 cm^{-1}, 1449.04 cm^{-1}, 1111.76 cm^{-1} and 1021cm-^{1}.The chemical component of the ethanolic extracts of the leaves of C.procera as revealed by GC-MS consist of mainly twenty one (21) compounds ranging from phenol, Terpenes and fatty acids The ferric reducing antioxidant power assay (FRAP) was determined and the leaves and the root was observed to have higher antioxidant potential as the observance was 1.690 and 2.453nm for leaf and 2.44 concentration of 30 and 40 respectively. This study show that the leaves extract of C.procera inhibits bacterial and fungal growth and anti-oxidant properties which is very important for medicinal uses. The present investigation deals with the process of determining the phytocomponents, antioxidant and antibacterial active.

Keywords: Preliminary, Antimicrobial, Antioxidant, Leaf, Stem Bark, Root, Extracts, Calotropis Procera

INTRODUCTION

Plants have been used for generations as sources of medicine in the treatment of human and animal diseases or illness. Different plants are used for this reason by different traditional healers without scientific evidence for their therapeutic values. Traditional healers have succeeded in the use of plants and its product as effective therapeutic tools to fight against disease and various common other health hazard. The crude use of natural plants as primary health remedies due to their pharmacological properties is very common in India, America, Africa, Asia and Ethiopia. Herbal medicine is becoming versatile all over the world than the allopathic medicine for medication. *Calotropis procera* is an important drug plant of Ayurveda, and it is known in India and Africa from the earliest time. The plant reported to have diverse pharmacological actions (Sharma *et al.*, 2022). It is widely used in India as traditional medicine system in the treatment of various kind of disease. *C. procera* is used by various tribes of the world as agent or curative agent for ailments such as skin disease, toothache, asthma, leprosy, and rheumatism. Different plant parts such as leaves, roots, stem bark, flower and latex of plant have been reported to possess many phytochemicals that contain pharmacological activities such as antimicrobial, anthelmintic, insecticidal, anti-inflammatory, anticancer, antidiarrheal and larvicidal activities. The plant is described as golden gift for humankind which contain cardio tonic agent such as *calotropin, calotoxin, uscharin, uscharidin, calotropagenin* used in the therapeutic treatment constituting different compounds such as flavonoids, alkaloids, sterols etc. and have made this plant of

scientific attraction for centuries. There are few results in the literature for antimicrobial and analgesic studies done on the plant species, nor are there reports found on the study of antioxidant properties (Hussain et al., 2021). Phenolic compounds are the most important antioxidant compounds of plant extracts. They neutralize or inactivate peroxide and hydroxide radicals through electron donation, which can cause cytotoxicity and cancer when they react with human body cells. In addition, plant extracts contain antimicrobial compounds that are a suitable alternative to antibiotics, which have developed resistance to a wide range of them in recent years (Behbahani et *al.,* 2017).

The total phenolic and flavonoids content in these plants are unknown and need to be determined, as these two chemical compounds are known to possess good antioxidant and antimicrobial properties. The traditional way of using herbal drugs has contributed a lot to human health especially in 21st century. Natural medicines from the ages past improves the inner immune systems of human body, hence due to no side effect the herbal drug acts more effectively than the modern medicines. Since ancient times, herbal drugs have been used as medicines for the treatment of various human disorder (Dixit *et al*., 2023).

Herbal medicines have become more versatile all over the world than the allopathic medicines for medication. Several medicinal plants have been screened and being under screening due to integrative approach on drug development. Amongst the plants, *calotropis* is a member of the plant family asclepiadaceae and has two common species as reported in literature which; are *Calotropis procera* and *Calotropis gigantea* (Linn) mentioned by ancient writers and can be interchanged because of similarity of their phytochemical compounds and can be used as substitute for one another because of their similar effects medicinally. *Calotropis procera* is a plant family of asclepiadaceae, a shrub about 6m high and is widely distributed in west Africa and other parts of the world such as India, Egypt, Ethiopia etc. The pharmacological evaluation and analysis of medicinal plants have promoted the use of herbal medicines through confirmation of their efficacy and safety. Research on antimicrobial and oxidative stress drugs from plants has gained much interest. This is because antibiotic have become ineffective to some degree since many microorganisms develop resistant against them. Therefore, more researchers are needed on the isolation and identification of bioactive compounds from different plant parts to develop new drugs with hopefully new mechanisms of action and better activity record. (Dixit *et al.,* 2023).

In Nigeria, traditional medicines, *C. procera* is either used alone or combined with other herbs to treat common, disease such as lever diseases, fever, cold, rheumatism, eczema, diarrhea, leprosy, pile, joint pain, ulcers, tumors, abdomen, snake bite, scorpion bite and spleen (Dixit *et al.,* 2023). The phytochemical, antioxidant and antimicrobial screening analysis of extract from this plant species can serve as baseline for identifying active compound responsible for this activities. The phytochemical compounds found in this plant are flavonoids, alkaloids, terpenoids, phenolic compounds, glycoside etc. that are responsible for different bioactive activities. This however, can be used as substrates in the formation of new antimicrobials and antioxidant agents. Validation of different species can also encourage the use of *C. procera* leaves, roots and stem bark extract which can benefit many communities because of their availability, simplicity and being cheap or low cost. The plant is erect, tall, much branched with milky latex throughout when broken or cut in any parts of the plant, it is large and ovate, sessile and perennial plant soft wooded evergreen. It has few stems or one, few branches, relatively few leaves, concentrated near tip. The bark is corky, light gray and furrowed, a copious white sap flows whenever and wherever stems or leaves and flowers are cut or pinched. Giant milkweed has a very deep, stout taproot with few or no near surface lateral roots. The milkweed roots were found to have few branches that reach depth of 1.6-3m. the opposite leaves are oblong obovate, to nearly orbicular, short-pointed to blunt at apex and have very short petioles bellow a nearly clasping heart-shaped base. The leaf blades are light to dark green with nearly white veins. (Widyaningrum *et al.,* 2020).

They are 7-17cm long and 5-12cm broad, slightly leathery, and have a fine coat of soft hairs that rub off when touched. The lower clusters are umbelliform cymes that grow at or near the end of twigs. The flowers are shallowly campanula with five sepals that is 4-5mm long, fleshly and variable in color from white to pink, often spotted with purple. The fruits are inflated, obliquely ovoid follicles that split and invert when mature to release flat, brown seeds with a tuft of white hairs at the end. The roots are very deeply and rarely grow in soils that are shallow over fractured rocks. Soil of all textures and derived from most parent materials are tolerated as well as soil with high sodium saturation. Competition with tall weeds, brush and especially grass weakens existing plants, and being over topped and shaded trees soon eliminate them. The plant is occasionally grown as an ornamental in dry or coastal areas because it is beautiful, convenient size and it is easy to propagate and manage. It is recommended as a host plant for butterflies. In the past, the silky hairs were

used to stuff pillows. *Calotropis procera* was tested as host for sandal wood, *Santalum Album L.,* a partial parasite extracts chopped leaves and latex have shown great promise as nematicide. (Dixit *et al.,* 2023).

In northern states and northwestern Nigeria, however, since *C. procera* is used by local communities for the treatment of infectious diseases. There is need to carryout investigation to ascertain the authenticity of this claim on medicinal properties of this specie (Dixit *et al.,* 2023).

Antimicrobial and antioxidant activity of *C. procera* have not been properly documented and there is need for better and safer antioxidants since current antioxidant drugs have safety concerns (Teopolina, 2016). The aims of this study is to evaluate the screening, antimicrobial, and antioxidant properties and activities of roots, leaves and stem bark extract of *Calotropis procera.*

The prevalence and spread of drug resistant microbes (microorganisms) and disease have threatened the activities of available drugs and remain the major cause of treatment failure. The burden of mortality and morbidity has been inclined towards developing countries due to high risk factors associated with economic transition. Antibiotics that were thought to be miracle cures are now unable to treat resistant bacteria. Recently the number of new approved antimicrobial medicine has dropped greatly and the supply of effective antimicrobial is in anticipation shortly. The capacity to use active substance derived from plants or their synthetic equivalents in medicine has improved with the development of phytochemistry and pharmacological chemistry. This is due to the fact that the medicinal plants have a greater variety and novelty of chemicals than any other sources. Therefore, it is of paramount significance to focus on antimicrobial and antioxidant screening of *Calotropis procera* extract as a result of bioactive phytochemicals for the mitigation of infectious disease and causative agent or organism. Thus, the main aim of this work is to detect the various bioactive components present in *Calotropis procera* to prove its use as antimicrobial agent. (Widyaningrum *et al.,* 2020).

This study aim to contribute to a better knowledge of the metabolic activities of C.*procera* in northeast part of Nigerian region, based on antimicrobial, antioxidant, FTIR and GC-MS spectral profile and phytochemical content analysis, in order to explain its efficient use in traditional medicine and to allow better control of the quality, efficiency and standardization of the plant raw material . To determine the yield of crude extract of leaf,

root and stem bark of *C. procera,* the percentage yield obtained from the plant parts was the amount of the crude extract recovered in mass compared with the initial amount of powdered plant materials used and it is presented. It is presented in percentage (%) and was determined for each sample while the solvent used was ethanol. The study focuses on the evaluation of *C. procera* in selected areas of Jablamba, Gerie Local Government Area of Adamawa state.

MATERIALS AND MTHODS

Sample Collection and Identification

The leaves, roots, and stem barks of *Calotropis procera* (Apple of Sodom) was collected from Jabulamba, Girei local government of Adamawa State. The leaves and the stem barks are healthy in which the plant materials were authenticated and identified in the Department of Plant science by a botanist in Modibbo Adama University, Adamawa state of Nigeria.

Preparation of plant samples C. procera

The collected plant parts of *C. procera* were separately washed thoroughly 3 times with tap water followed by once with sterile distilled water to remove debris and dust particles and cut into smaller sizes using a sterile knife. Then the leaves, roots and stem barks of plant were dried under shade on a paper towel for two weeks with occasional shifting at room temperature. The resulting dry parts were ground into fine powder with the help of suitable sterile grinder. (Singh *et al*., 2017)

Preparation of crude extracts using ethanol

The studies encompassed a range of methodologies including sample collection, extraction of bioactive constituents, culturing of microbial strains, and evaluation of antimicrobial activity through techniques such as disc diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays

The dried coarse powder material (200 g) was subjected to Soxhlet's extraction separately and successively with ethanol. The solvent was distilled under reduced pressure, controlled temperature (40-50°c) and the resulting semisolid mass was vacuum dried using rotary flash evaporator to yield a solid residue put in airtight container and stored in refrigerator. The following abbreviation has been used for each extract: C. of Leaves and root and SB. *C. procera*: Leaves (CPL) and root (CPR) and Stem bark respectively.

Two hundred grams (200.00g) of each of the coarsely powdered plant materials (leaves, roots and stem barks) of *C. procera* were suspended in 100ml of different solvents, i.e., ethanol (99.5%) and water (99.8%), separately in 250 ml conical flasks. As indicated by Cheesbrough the suspended plant materials were kept on a rotary shaker rotating at 190-220 rpm for 72 hrs. at room temperature (Cheesbrough, 2015). Muslin cloth was used to filter the plant residue, the filtrate thus obtained will be further purified by filtration through Whatman No.1 sterile filter paper, and the resulting filtrates were collected as sources of crude extracts (Newtons *et al.,* 2014). The filtrates were concentrated under reduced pressure in Rota vapor (STERILIN. Ltd., Stone Staffordshire, England) at 40°C and the gummy residue was further dried in a water bath at 40°C – 50°C for 24 hrs. until the solvents were removed (Amit *et al*., 2015). After the evaporation of solvents, the remaining crude extracts were weighed using a balance and the resulting weights recorded. These crude extracts were kept in sample vials with stoppers at 4°C until they were used against the test pathogens (Dewanjee *et al.,* 2017).

Percentage Yield Determination of Crude Extracts

The percentage yield obtained from the plant parts was the amount of the crude extract recovered in mass compared with the initial amount of powdered plant materials used. It is presented in percentage (%) and was determined for each extraction solvent used. The percentage yield was calculated as follows;

$$\% \text{ yield (W/W)} = \frac{\text{Extract Obtainted (in g)}}{\text{Grounded Plant Sample Taken (in g)}} \times 100$$

Preliminary phytochemical screening of leaf, root and stem bark extract of *Calotropis procera*

Phytochemical screening is an essential step in the analysis of plant extracts to identify and characterize various bioactive compounds. Different qualitative chemical test will be carried out for establishing the profile of given extract for its chemical composition. The extract will then be subjected to qualitative chemical test for various phytoconstituents. It involves the qualitative and quantitative assessment of secondary metabolites present in plant materials. In recent years, the interest in natural products as potential sources of therapeutic agents has led to an increased focus on phytochemical screening of medicinal plants. This literature review aims to explore the methods used for phytochemical screening of extracts from different parts of plants, including the leaf, root, and stem bark, and the identification

of their bioactive constituents. Phytochemical screening was carried out in order to analyze and detect the presence of plant constituent which are as follows; alkaloids, steroids, saponins, tannins, glycosides, phenolic compounds carbohydrates, steroids and terpenoids.

Test for Alkaloids (Mayer`s Test and Dragendoff's Test**)**

1g of each of the ethanolic, methanolic and aqueous extract will be placed and /in weighed into two separate test tubes. To the first, 2-3 drops of Dragendoffs reagent will be added while 2-3 drops of Mayer's reagent will be added to the second test tube. The formation of an orange-red precipitate in the first with the Dragendoff's reagent and white precipitate in the second tube with the Mayer's reagent is the indication of the presence of alkaloids (Banu *et al.,* 2015).

Test for Glycosides

Add few drops of 10% NaOH to the extract to make it alkaline. Then freshly prepared sodium nitroprusside was added to make the solution. The formation of blue coloration confirms the presence of glycosides in the extract (Banu *et al.,* 2015).

Test for flavonoids

Alkaline reagent test

To the test solution few drops of sodium hydroxide solution will be added; the formation of intense yellow color, which turned to colorless on addition of few drops of dilute acid, confirm the presence of flavonoids (Banu *et al.,* 2015).

Test for phenolic compounds

Lead Acetate test

To the extract, 3m of 10% of lead acetate solution was added. A dark green color confirms the presence of phenolic compounds.

Ferric chloride test

To the extract, few drops of neutral 5%ferric chloride solution will be added. A dark green color confirms the presence of phenolic compounds (Banu *et al.,* 2015).

Test for carbohydrates

Molisch s test

To 2ml of extract, two drops of alcoholic solution of α-naphthol was added, the mixture will be shaking well and 1ml of concentrated H_2SO_4 will be added slowly along the sides of the test tube and allowed to stand. A violet ring confirms the presence of carbohydrates. (Banu *et al.,* 2015).

Test for tannins

Two grams of aqueous extract will be weighed and placed in a test tube. 2 drops of 5% ferric chloride solution will then be added. The appearance of dark or dark green color confirms the presence of tannins. The same procedure will be repeated using the ethanolic, and methanolic extracts (Banu *et al.,* 2015).

Test for steroids

1g of the ethanolic, extracts will be weighed and placed in a test tube. This will be dissolved in 2ml of acetic anhydride, followed by the addition of 4 drops of chloroform. Two drops of concentrated sulphuric acid will then add by means of a pipette at the side of the test tube. The development of a brownish ring color at the interface of the two liquids and the appearance of violet color in the supernatant layer is a confirmative presence of steroid glycosides (Banu *et al.,* 2015). The same procedure will be repeated using aqueous extract.

Methodologies for MIC Determination against bacterials

Minimum inhibitory/bactericidal concentration (MIC/MBC). The MIC and MBC were measured using the microdilution method according to the National Committee for Clinical Laboratory Standards (NCCLS). The method involved preparing a primary culture of pathogenic bacteria at a concentration of 1.5 × 108 CFU/mL and diluting the extract containing DMSO (up to 1 mg/mL) with MHB. Bacteria were added to each well of a 96-well plate and 125 µL of the extract was poured into each well. The plate was incubated for 24 h at 37 °C. Triphenyltetrazolium chloride (25 µL; 5 mg/mL) was added to each well and the formation of a dark red color, indicating the growth of microorganisms, was checked. The concentration of the extract in which no color change was observed was considered as the MIC. The contents of each well (100 µL) where no color change was observed were

cultured on MHA and incubated at 37 °C for 24 h. The minimum dilution that prevented growth was considered as MBC (Fowler *et al*., 2015; Proctor *et al*., 2016).

Broth dilution method

The broth dilution method is one of the most widely used techniques for MIC determination. It involves preparing a series of dilutions of the plant extract in a liquid growth medium, inoculating the test organism, and monitoring for visible growth. The lowest concentration that inhibits growth represents the MIC. This method offers quantitative results and allows for a range of concentrations to be tested (EUCAST, 2020).

Agar dilution method

Similar to the broth dilution method, the agar dilution method involves incorporating various concentrations of the plant extract into agar plates. The test organisms are then streaked onto the plates, and the MIC is determined by identifying the concentration at which there is no visible growth. This method is advantageous for assessing the effects of plant extracts on solid media (NCCLS, 2015).

RESULTS

The percentage yield was calculated as follows;

$$\% \text{ yield (W/W)} = \frac{\text{Extract Obtainted (in g)}}{\text{Grounded Plant Sample Taken (in g)}} \times 100$$

Plant Parts	Weight and Percentage Yield of the Extracts		
	Mass in gram	Percentage yield %	Extract Appearance
Leaf	195.26	65.63	**dark blue**
Root	182.37	70.63	**yellowish**
Stembark	164.48	69.76	**brown**

Qualitative phytochemical screening

The Phytochemical screening was done in other to find out the presence of phytochemical compounds or constituents in the plant which include flavonoids, tannins, saponins, phenolic, amino acids, glycosides, steroids, triterpenoids, reducing sugar using the methods adopted by Brain K.R and Tuner, T.D (2014). The phytochemical analysis showed the

presence of most of bioactive compounds tested as presented in table 2 below. The test for the presence of alkaloids, flavonoids, tannin, saponin, glycosides, was identified.

Table 2: Result Qualitative Analysis of ethanol extract of leaf, stembark and root of *Calotropis procera* plant

Phytochemical extract	Leaf extract	Root extract	Stembark
alkaloids	+	-	+
flavonoids	+	+	+
phenols	+	+	+
tannin	+	+	+
saponins	+	-	+
glycosides	-	+	-
amino acid	+	+	+
reducing sugar	-	-	-
terpenoids	+	+	+

KEY:

\+ Present

_ Absent

The phytochemical investigation is important as various significant elements are found in different plants part. The present study was carried out for the screening of phytochemicals in *Calotropis procera*. Due to higher metabolite changes and the bioactive compounds in plants, these compounds are helping to regulate growth of the plants. These phytochemicals have been described as preventing the growth of bacteria (Mayasari *et al*., 2023).

Flavonoids and phenols were higher in all parts of *Calotropis procera* due to their environmental factors and biological effects, however these compounds also have valuable in managing health and common human diseases and plants and they play a major role in anti-cancer and other diseases. These compounds are naturally found in almost all plants.

The various phytochemical screening (tannins, saponins, proteins, diterpenes, phytosterols flavonoids and phenols) were found in leaves and root and stem bark of *Calotropis procera*. The initial work of some researchers, has described the leaves extracts which contain bioactive molecules with a large number such as alkaloids, saponins, tannins, flavonoids,

proteins, phytosterols, diterpenes, carried out by other researchers using different solvent etc. The presence of the compounds in these species demonstrates that they might have some therapeutic capability. The phytochemical analysis by other scientists also confirmed the appearance of various phytochemicals. Various parts of the plants have a huge perspective to cure several disorders and their use in several medicinal or herbal preparations.

Phytochemically, the plants have been investigated for other activities, triterpenoids, triterpenes, alkaloid, cardenolides phytosterols and triterpenoid saponins from the latex of *Calotropis procera* exhibit diversity in their chemical constituents and secondary metabolites even within a different part of the plant may be responsible for the difference in their antioxidant potential and antimicrobial activities. Phenol is a necessary compound for the prevention of many ailments and diseases.

Antibacterial Activities of Crude Extracts (Leafs, Stem Stark and Root) of *Calotropis procera* Plant

The antimicrobial activity of leaf, root and stem bark extract and their inhibitory effect was evaluated and presented in table 3.3 bellow. The antagonistic effect of all the extracts against the microorganisms such *escharichia coli, Bacillus cereus*, *Staphylococcus aureus* and fungi such as *aspergillus flavus, candida albicans and aspergillus niger* were evaluated using agar well diffusion method of susceptibility test. Muella-Hinton agar and Sabouraud Dextrose agar plates were inoculated with 0.1ml of standardized inoculum of each bacterium in triplicates using 0.1ml pipette and spread uniformly with a sterilized swab sticks. Three wells of 6mm size were made with sterile cork borer into the inoculated agar plates. 0.1ml volume of the different concentrations; 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml,12.5 mg/ml and 6.25 mg/ml respectively using micropipette each of the crude extract were introduced into the wells of inoculated plates. The DMSO was used to dissolve the extracts. Water used as negative control and commercially available standard antibiotic chlorophenicol and was used as positive control with the extract. The prepared plate was then kept for 10 minutes at room temperature of 37 degrees Celsius. following the diffusion and the incubation at 37 degrees Celsius for 24 hours in an incubator and the diameter of zones inhibition were expressed and evaluated respectively. From table 3.3b below, the tested organisms were subjected to different concentrations which showed that the leaf showed high antimicrobial

activity on all the tested pathogens at minimum concentration of 12.5 mg/ml except S. aureous which showed growth at 12.5 mg/ml, and at 6.25 mg/ml all the bacterial strains were resistive to leaf, root and stem bark extract. *E. coli* showed resistance on root extract at 12.5 mg/ml as growth was observed while *B.cereus* showed growth at 25 mg/ml concentration.

However, *E. coli* was not able to survive at 100 mg/ml of leaf and root extract but it grew at 100 mg/ml of stem back extract nevertheless at 200 mg/ml there was inhibition of growth as a result of phytoconstituents such as flavonoids phenolic compounds etc.

Flavonoids and phenols were higher in all parts of *Calotropis procera* due to their environmental factors and biological effects. these compounds also have valuable bioactive functions in managing health and common human diseases and plants, nevertheless they play a major role in anti-cancer and other related diseases. These compounds are naturally found in almost all plants.

The findings show that the total phenolic compound is remarkably higher in leaves than in the root of the plant species. Comparatively, results show that the total phenolic compounds were higher in *Calotropis procera* as compared to *Calotropis gigantea*. The results are similar to (Pietta, *at el* 2015), who reported that the amount of total phenolic compounds varied widely with *Calotropis procera* specie whereby the differences could be attributed to ecological factors, such collection time, increasing population or environmental factors. According to Pietta et al., (2015) phenolic compounds are the strongest chain-breaking antioxidants, their information is described in many plant-related studies and it describes various antioxidant activities of the plants in which phenolic compounds are high. As reported by Randhir and Shetty (2015). Randhir and Shetty (2015) explained that the higher phenolic compounds and improved antioxidant activity may be involved in the growth and development process.

The multifaceted biological characteristics of *Calotropis procera* make it a medicinally and socio-economically important plant species. The study evaluates its expanding global distribution, ecological significance, applications in traditional and advanced fields and infestation as an environmental weed. Also, it is an attempt to recognize the lesser-explored aspects and knowledge gaps in ongoing research.

Although pharmacological and industrial applications of these plants have received due attention, their general biological and ecological attributes have not been well-investigated.

Evaluating the basic facets may improve its commercial utilization and pave the way for novel applications.

The current and potential spread of *C. procera* are required to be mapped to carry out their time management or containment. The spread of both species can be effectively controlled in the invaded ranges via mechanical, chemical, or biological methods, followed by constant monitoring. Recognizing the plant as an important environmental weed can supplement its management programs at research, legislative, stakeholder and local levels. Also, promoting its utilization at commercial and non-commercial scales can be an economically viable or better to say, economically beneficial way of its management.

The current study investigated the invitro antimicrobial activity against some pathogenic microorganisms of *C. procera* ethanolic extract. The evolving resistance of pathogenic microbes to currently existing antimicrobial agents requires new antimicrobial agents. The use of medicinal plants as a natural alternative is the primary research field for overcoming drug resistance to infectious agents. Scientists still need to assess medicinal plants' effectiveness against microbes (Dhama *et al.*, 2014).

Several activities have been attributed to *C. procera*, including antibacterial, antifungal, and antitumoral activity, (Vahidi, *et al.*, 2021), which indicate the pronounced biological potential of this genus. The plant extract's ability to destroy or inhibit the growth of pathogenic microbes with excellent efficiency indicates the presence of bioactive secondary metabolites that have been considered to be antimicrobial agents. Moreover, Thenmozhi *et al.* (2015) stated that the antibacterial activity of plants is due to the secondary metabolites they form for protection against pests, herbivores, and microbial infections. The distinct antimicrobial activity of *C. procera* could be attributed to the presence of phenolic compounds. Previous results supported our findings.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration MIC assay was adopted to evaluate the efficacy of the plant extracts that showed the significant antimicrobial activities in the test carried out. The method that was used was the tube modified dilution method. The plant extracts were serially diluted from 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml,12.5 mg/ml and 6.25 mg/ml respectively to obtained various concentration. Multiple dilutions of the extract were incorporated in Muller Hinton broth (Oxoid, UK) and Sabouraud Dextrose broth, and then inoculated with 0.1 ml each of standardized suspension of the test strains into

different test tube containing various concentrations. Another set of test tubes containing muller Hinton broth were used as negative control, and another test tube containing muller Hinton broth and test organisms were used as positive control. All the test tubes and control were then incubated at 37 degrees Celsius for 24 hours. The presence and absence of growth on each tube was observed after incubation period. The loop full from each tube was further sub cultured onto nutrient agar to confirm the growth of the bacterial isolate as it was inhibited and the result is presented in table 3 below.

Determination of Minimum Bacteriacidal Concentration (MBC)

The MBC and MFC were determined by removing 1ml of the broth culture from the tube used for MIC determination and sub culturing into fresh solid nutrient agar plates, and the results are shown in table 4 and 5 bellow. The plates were incubated at ambient temperature for 24 hours. The lest concentration that did not show any growth after incubation was regarded as the MBC and MFC.

Table 3 Zone of inhibition of Antibacterial activities of Root, Stem and Leaf Extracts

Organism	Extracts	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	+C	-c
E.coli	Root	14	11	08	06	02	00	24	00
	Stem	12	10	08	04	02	00	22	00
	Leaf	14	12	10	08	04	02	20	00
Bacillus cereus	R	12	10	08	04	00	00	20	00
	S	12	10	08	04	00	00	20	00
	L	14	12	10	08	04	00	18	00
Staphyloc ol	R	12	10	08	06	02	00	24	00
	S	14	12	08	06	04	00	20	00
	L	12	10	08	06	00	00	22	00

Table 4: Antibacterial activities of Root, Stem and Leaf Extracts

Organisms	Extracts	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	MIC
E.coli	Root	-	-	-	-	+	+	25
	Stem	-	-	-	-	-	+	12.5
	Leaf	-	-	-	-	-	+	12.5
B. cereus	Root	-	-	-	+	-	+	50
	Steam	-	-	-	-	-	+	12.5
	Leaf	-	-	-	-	-	+	25
S. aureus	Root	-	-	-	-	-	+	12
	Ste,	-	-	-	-	-	+	12.5
	Leaf	-	-	-	-	+	+	25

Table 5: Maximum bacteriacidal concentration (MBC)

Organisms	Extracts	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	MBC
E. coli	Root	-	-	+	+	+	+	100
	Steam	+	+	+	+	+	+	>200
	Leaf	-	-	+	+	+	+	100
B. cereus	Root	+	+	+	+	+	+	>200
	Stream	+	+	+	+	+	+	>200
	Leaf	+	+	+	+	+	+	>200
S. aureus	Root	-	+	+	+	+	+	200
	Stem		+	+	+	+	+	>200
	Leaf	+	+	+	+	+	+	>200

Key += cell growth (multiplication of bacteria)

++ = No cell growth

Antifungal activities of *C. procera*

The antifungal activities of all the three parts of the *Calotropis procera* was carried on three fungi which are *Aspergillus Niger, Candida Albicans and Aspergilus Flavus* and it was observed that at 200 mg/ml concentrations, all the fungi had their growth inhibited while at 50

mg/ml concentration, only candida albicans had its growth stopped by root extract of the plant which can be referred to as its minimum inhibition at 50 mg/ml.

However, the maximum funicidal was 100 mg/ml concentration whereas at 100 mg/ml and 200 mg/ml only root extract worked against candida albicans, but aspergillus albicans, leaf extract neutralized its growth at 200 mg/ml and root and stem back extract could not stop the growth at all the remaining concentration.

Antifungal Activity of the root, stem and leaf extracts

From the table shown below, the root extract of *C. p, Candida albicans* was able to succumb to the active compound which inhibit its growth at 50 mg/ml concentration, while at 100ml concentration it exhibited the fungicidal activity on the test fungi and the leaf extract stopped the growth of *Aspergilus niger* at 100 mg/ml concentration. At concentration lower than 50 mg/ml all the fungi resisted to all the extract. Therefore, it is confirmed that the MIC value is 50 mg/ml concentration while the maximum concentration is 200 mg/ml.

Table 6: Antifungal activities of Root, Stem and Leaf Extracts

Organism	Extract	200 mg/ml	100 mg/ml	50 mg/ml	2.5 mg/ml	12.5 mg/ml	6.25 mg/ml	+c
Aspergillus Favus	R	10	06	04	05	00	00	24
	S	08	04	02	00	00	00	24
	L	12	08	06	00	00	00	22
Candida albicans	R	12	08	06	02	00	00	18
	S	10	06	02	00	00	00	26
	L	14	10	06	00	00	00	22

Table 7 minimum inhibitory concentration (MIC)

Organism	Extract	200 mg/ml	100 mg/ml	50 mg/ml	2.5 mg/ml	12.5 mg/ml	6.25mg/ml	+c
Aspergillus Flavus	R	-	-	+	+	+	+	100
	S	-	+	+	+	+	+	200
	L	-	+	+	+	+	+	200
Candida albicans	R	-	-	-	+	+	+	50
	S	-	+	+	+	+	+	200
	L	-	-	+	+	+	+	100

Table 8. Minimum fungicidal concentration (MFC)

Organism		200 mg/ml	100 mg/ml	150 mg/ml	2.5 mg/ml	12.5 mg/ml	6.25 mg/ml	+ c
Aspergillus Niger	R	+	+	+	+	+	+	>200
	S	+	+	+	+	+	+	>200
	L	+	+	+	+	+	+	>200
Candida albicans	R	-	-	+	+	+	+	200
	S	+	+	+	+	+	+	>200
	L	+	+	+	+	+	+	>200

Key; + Growth present

- No growth

Column Chromatography Analysis Qualitatively

The Column chromatography was carried out in other to identify the basic constituents embodied in the specific extract. The solvents system used were chloroform, n-hexane and methanol in the ratio of 1:2:2 and DMSO was used in the dissolution of the concentrated dried leaf sample of the crude extract. From the first antimicrobial activities of all the three extracted samples of the *Calotropis procera* plant which are root, leaf and stem bark extract,

leaf extract showed the most active and potent among them by inhibiting the growth of the bacterial and fungal isolates at lowest concentration as low as 12.5 mg/ml, and therefore, the leaf was used for the column chromatography and the analysis for separation or fractionation was obtained using the following procedures and TLC plate coupled with solvents suitable for the analysis at measurable ratios were used.

Thin layer chromatography uses a thin glass plate coated with either aluminum oxide and silica gel as the solid phase. The mobile phase is a solvent chosen suitable according to the properties of the components in the mixture. The principle of TLC is the distribution of a compound between a solid fixed phase applied to a glass or plastic plate and a liquid mobile phase, which is moving over the solid phase. A small amount of a compound or mixture is applied to a starting point just above the bottom of TLC plate. The plate is then developed in the developing chamber that has a shallow pool of solvent just below the level at which the sample was applied. The solvent is drawn up through the particles on the plate through the capillary action, and as the solvent moves over the mixture each compound will either remain with the solid phase or dissolve in the solvent and move up the plate. Whether the compound moves up the plate or stays behind depend on the physical properties of that individual compound presents and thus depend on its molecular structure, especially functional groups. The solubility rule "Like Dissolves Like" is followed. The more similar the physical properties of the compound to the mobile phase, the longer it will stay in the mobile phase. The mobile phase will carry the most soluble compounds the furthest up the TLC plate. The compounds that are less soluble in the mobile phase and have a higher affinity to the particles on the TLC plate will stay behind (Singhal *et al.*, 2019).

Antimicrobial activities of the fractions after column chromatography

From the result of column chromatography obtained in the isolation or identification of unknown compounds from the leaf sample, they were four (4) fractions obtained and all were indicated by coding as F1, F2, F3 and F4 respectively. The antimicrobial activities of all the four was later carried out again to determined which of the four samples was able to act against the growth of pathogens which might have been consistent in characteristic behavior of particular compound in prospect.

Table 9: Antibacterial Activities of the Fractions F1, F2, F3 and F4

E. coli	**F1**	**+**	**+**	**+**	**+**	**+**	**+**	**>200**
	F2	-	-	-	-	+	+	25
	F3	-	-	-	-	-	+	12.5
	F4	-	-	-	-	+	+	25
S. aureus	F1	+	+	+	+	+	+	>200
	F2	-	-	-	-	+	+	25
	F3	-	-	-	-	-	+	12.5
	F4	-	-	-	+	+	+	50
B. cereus	F1	+	+	+	+	+	+	>200
	F2	-	-	-	-	+	+	25
	F3	-	-	-	-	-	+	12.5
	F4	-	-	-	+	+	+	50

MIC = Minimum inhibitory Concentration

- = no growth

\+ = growth

Table 10: Antifungal Activities of the Fractions F1, F2, F3 and F4

Organism	**Fractions**	**200 mg/ml**	**100 mg/ml**	**50 mg/ml**	**25 mg/ml**	**12.5 mg/ml**	**6.25mg/ml**	**MIC**
A. flavus	F1	+	+	+	+	+	+	>200
	F2	-	-	+	+	+	+	100
	F3	-	-	-	+	+	+	50
	F4	-	-	+	+	+	+	100
A. niger	F1	-	-	+	+	+	+	>200
	F2	+	+	+	+	+	+	50
	F3	-	-	-	+	+	+	25
	F4	-	-	+	+	+	+	100
• ***Albicans***	F1	+	+	+	+	+	+	>200
	F2	-	+	+	+	+	+	200
	F3	-	-	+	+	+	+	100
	F4	+	+	+	+	+	+	200

	MFC							**MFC**
A. flavus	F2	+	+	+	+	+	+	>200
	F3	+	+		+	+	+	>200
	F4	-	+	+	+	+	+	>200
A. niger	F2	-	+	+	+	+	+	200
	F3	-	+	+	+	+	+	200
	F2	-	+	+	+	+	+	200
C. Albicans								
	F3	-	+	+	+	+	+	200
	F4	-	+	+	+	+	+	200

Measuring Rf- value

Using a TLC plate was developed in a beaker and small amount of solvent that that serve as a mobile phase in the container. A small of spot of solution containing the sample was applied to a plate, about 1cm from the base. The plate was later dipped into the solvents. The solvent moves up the plate by capillary action and meet the sample mixture, which is dissolved and is carried up the plate by the solvents. It was observed that, different compound in the sample mixture travel at different rates due to differences in their attraction to the stationary phase and also because of differences in solubility in the solvents

Measurements are often taken from the plate in order to help identify the compounds present. These measurements are the distance travelled by the solvent, and the distance travelled by individual spots. When the solvent front gets close to the top of the plate, the plate is removed from the beaker and the position of the solvent is marked with another line before it has a chance to evaporate.

Table 11: Retention Factor

Rf	**Values in cm**
Rf1	0.80
Rf2	0.74
Rf3	0.63
Rf4	0.45

For example, if the yellow component travelled 1.7 cm from the base line while the solvent had travelled 5.0 cm, then the Rf value for the color is calculated.

If we could repeat this experiment under exactly the same conditions, then the Rf values for each would always be variable. For example, the Rf value for the yellow color would always be similar.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis Identification of components

The GC-MS mass spectrum was interpreted using NIST's massive database of spectral patterns (including over 4,000 peaks). Mass spectra from the NIST08 and Wiley08 libraries were used to cross-reference with the unknown component's spectra. The mass spectra of the various components were compared in order to determine their identities. Compounds were identified in electronic signals when they eluted from the column and were separated. Compounds were broken down into their constituent ions as they passed down the gas chromatographic column and into the electron ionization detector. The pieces turned out to be charged ions of a certain mass. The resultant graph, known as the mass spectrum graph, serves as a calibrator for the m/z ratio as it represents the molecular fingerprint

the main characteristics signals of first derivative FTIR spectra were shown in Figure 1A, and is one of the most sophisticated tools used to identify chemical functional groups present in a compound which is the most sensitive, rapid, selective and authentic method to characterize and identify functional groups of phytoconstituents.

Nevertheless, Fourier transform infrared spectroscopy is the most powerful among the analytical tools for identifying the types of bonds (functional groups) present in compounds. The wavelength of light absorbed is the characteristics of the chemical bond based on the annotated spectrum. However, by interpreting infrared absorption spectrum, the chemical bonds in a molecule can be understood. Dried fractions of the ethanolic extract of 0.11g was encapsulated in 100mg of KBr pellet, in order to prepare sample discs. The sample of the plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a scan range from 4000mc-1 to 550mc-1. The table in figue1A Bellow shows the spectrum of the FTIR Spectroscopy.

Figure 1A

PerkinElmer Spectrum Version 10.4.3

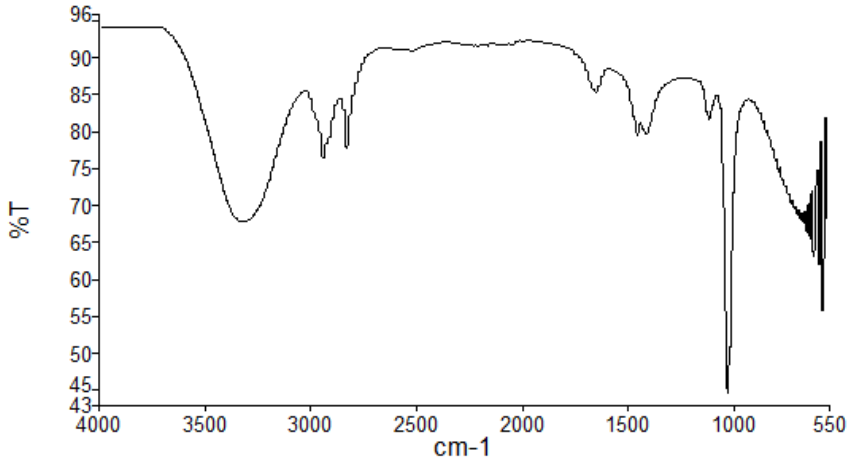
Friday, March 1, 2024 12:34 PM

Sample Details

Sample Name	01
Sample Description	Sample F3, Date Thursday, February 29 2024
Analyst	Administrator
Creation Date	3/1/2024 12:24:12 PM
X-Axis Units	cm-1
Y-Axis Units	%T

Spectrum

Figure 1B



Name	Description
___ 01	Sample F3, Date Thursday, February 29 2024

Figure 1C

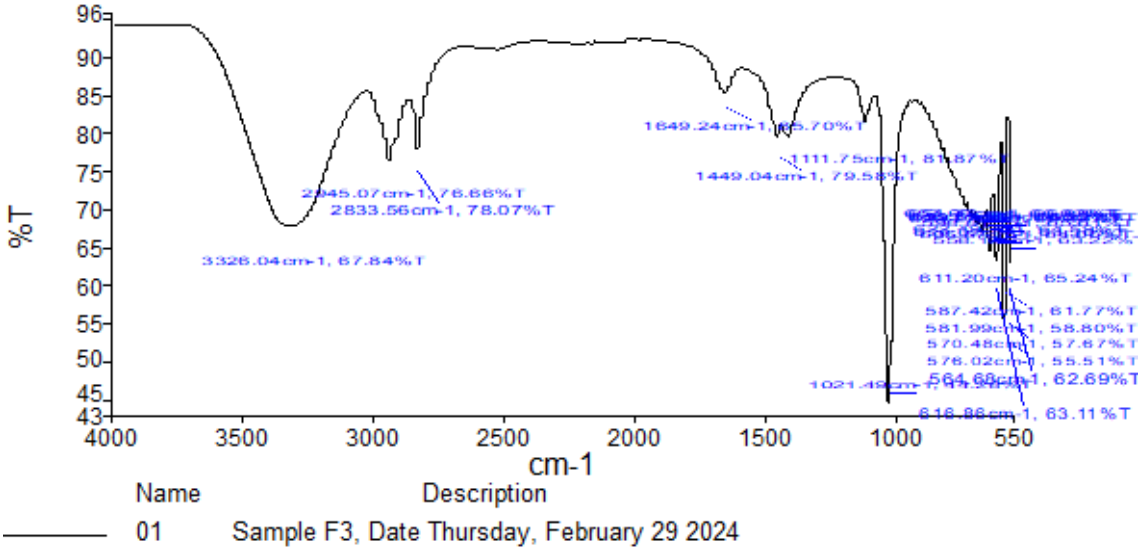


Table 12: Peak Table

Peak. No.	X (cm-1)	Y (%T)	Peak No.	X (cm-1)	Y (%T)	Peak No.	X (cm-1)	Y (%T)
1	3326.04	67.84	2	2945.07	76.66	3	2833.56	78.07
4	1649.24	85.70	5	1449.04	79.58	6	1111.7	81.87
7	1021.49	44.28	8	657.08	66.82	9	651.77	66.88
10	645.71	66.32	11	639.27	65.89	12	633.69	66.37
13	628.02	64.30	14	622.49	64.59	15	616.86	63.11
16	611.20	65.24	17	605.67	64.06	18	598.84	65.61
19	593.14	66.27	20	587.42	61.77	21	581.99	58.8
22	576.02	55.51	23	570.48	57.67	24	564.68	62.69
25	558.16	63.22	26	552.23	62.81			

The chemical bonds in the extract compounds were analyzed using FTIR as shown in the table 1B above which showed 26 different peaks. Peaks in the range of 3000–3500 cm−1 are related to the stretching vibrations of hydroxyl (O–H) groups, which may be associated

with alcohol groups or carboxylic acids (Samani *et al.,* 2022).The first peak has a signal at a wavelength of 3326.04cm-1 and the stretching vibrations of OH groups in alcohol and carboxylic acid were identified at a peak of 3326.04cm-1, and the peak next to it has absorption at wavelength of 2945.07cm-1 in the spectrum and its functional group is C-H stretching which is in the class of alkene as a compound, and it is medium. The subsequent peak is having absorption at a wavelength of 2833.5cm-1 in the spectrum which represent a particular compound which is N-H stretching and the class of the compound is amines salt being identified which has appearance of strong broad. The fourth peak has absorption at 1649.24cm-1 and it is C=C stretching and alkene which is in class of vinylidene. The fifth peak has absorption at 1449.04 cm-1 and it is C-H bending being an alkane with a class of methyl group which has medium appearance. The sixth peak has absorption at 1111.75cm-1 which is C-O stretching indicating secondary alcohol having strong appearance, and the last peak has absorption at 1021.49cm-1 which has N-H stretching indicating amine that has medium appearance, the stretching vibration of CH2 groups in alkane were identified at a peak of 570.45cm-1 and 552.23cm-1 in fingerprint regions. The table below different spectrum with their compounds and functional groups.

Table 13: Results of the FTIR analyses of the crude extracts of *Calotropis procera* Leaf.

Frequency Range	Absorption (cm-1)	Appearance	Group	Class of compounds	Comments
4000- 3000	3700-3200	Strong Broad	O-H, Stretching	Alcohol	Intermolecular Bonded
	3100-3000	Medium	C-H Stretching	Alkane	
	3000-2800	Strong Broad	N-H Stretching	Amine Salt	
	1658-1648	Medium	C=C Stretching	Alkene	vinylidene
	1465	Medium	C-H	Alkene	Methyl
	1124-1087	Strong	C-O Stretching	Secondary Alcohol	
	1250-1020	Medium	C-N Stretching	Amine	

Identification of phytocomponents of ethanolic extract of *C. procera* Fraction using GC– MS analysis.

Gas chromatography–Mass spectroscopy (GC-MS) were proved to be a sophisticated tool for analysis of different compounds which are stable enough to withstand high

temperatures required for gas chromatographic separations. After ionization the ionized fragments are led through mass filters to the detector. It is clear that GC-MS makes use of an inert gas as a carrier whereas LC-MS uses a mixture of liquids with or without buffers or additives as a carrier phase. GC-MS is suitable for analysis of samples of alcohol, phenol, phosphonic acid, esters, carboxylic acids, ketones, fatty acids, terpense and gases.

Table 14: Results of some functional group of other organic compound with their vibrational peaks and type of bond

Peaks	Bond types	Assigned functional groups
3326.04	O-H	phenols, alcohols,
2945.07	C-H	alkanes, ketones, aldehydes, esters,
2833.56	N-H	amines, amides
1649.24	C=C	alkenes
1449.04	C-H	alkanes, ketones, aldehydes
1111.75	C-O	esters
1021.49	C-N	amines

Table 15: Compounds identified in methanol, chloroform and n-hexane in ratio 2:1:2 Crude extract of *C. procera leaf*

Numbers	Name of compounds	Mol. Formula	Mol. Weight (g/mol)	Retention Time (mins)
1	2-Hexano,2,5-dimethyl-,(S)-	$C_8H_{18}O$	130	1.429
2	(5H)-Furanone,3-chloro 5(dimethylamino)Methyl)4,5dimethyl	$C_9H_{14}CINO_2$	18.409203	2.022
3	2-tetradecanone	$C_{14}H_{28}O$	212	3.632
4	Phenol	C_6H_6O	94	3.633
5	2,3-Dehydro-4-oxo-β-ionone	$C_{13}H_{16}O_2$	204	14.931
6	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	18.409
7	9,15-Octadecadienoic acid, methyl Ester (Z, Z)	$C_{19}H_{34}O_2$	294	20.73
8	11-Octadecenoic acid, methylester	$C_{19}H_{36}O_2$	296	20.803
9	Methyl stearate	$C_{19}H_{38}O_2$	298	21.125
10	Trimethyl [4-(2-methyl-4 oxo-2-Pentyl)phenoxy]silane	$C_{15}H_{24}O_2$		26.199

Antioxidant Activity

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was used to measure the extract's antioxidant power. A solution containing 0.2 M phosphate buffer (2.5 mL; pH 6.6) and potassium ferricyanide (2.5 mL; 1% w/v) was prepared and charged with the extract and incubated at 50 °C for 20 min. The reaction was stopped by adding trichloroacetic acid (2.5 mL; 10% w/v) and centrifugation (3000rpm for 10 min) was then conducted. A mixture

consisting of 2.5 mL of supernatant, 2.5 mL of deionized water, and 0.5 mL of 0.1% chloride was prepared and after 30 min, the absorbance of the sample was read at 700 nm. The FRAP of the extract was reported in terms of ascorbic acid equivalent (mg AAE/g of extract) (Yomakou *et al.*, 2021). Vitamin C and water will be used as positive and negative controls respectively (Labiad *et al.*, 2017). The results Were presented in table 1 and 2 below and leaf and root were observed to have higher antioxidant potentials as the observance was 1.690 and 2.453 nm for leaf and 2.44 at concentrations of 30 and 40 respectively. The results in table 2 shows the color changes at different concentrations and the leaf has deep blue followed by root sample while ascorbic acid had mild color changes and it has higher antioxidant potential compared to stem bark sample.

Result of Ferric Reducing Antioxidant Power (Frap) Activities as Showing in The Tables Bellow

Table 16: The wavelength was at 700nm and the final sample in curvet is 3ml

Concentration	Leaf	Root	Stembark	Ascorbic acid positive control	Negative control water
10	1.361	2.231	1.241	1.331	0.0
20	1.611	2.241	1.501	2.492	0.0
30	1.690	2.241	1.591	2.852	0.0
40	2.453	2.244	2.361	2.965	0.0

Table 17: Concentrations and absorbance

Concentrations	Leaf	Root	Stembark	Ascorbic acid
10	+	+	+	+
20	++	+++	++	++
30	+++	++	++	+
40	++++	++++	++	+++

DISCUSSION

The C. procera extract was obtained using solvent extraction, as its main compounds are, polyphenolic, flavonoid compounds, saponins and alkaloids. Sulaibi et al., (2020), reported that p-coumaric acid is one of the main constituents of C. procera. Another study detected kaempferol-3-O-rutinoside flavonoids (41.3 mg), isorhamnetin-3-O-rutinoside (27.4 mg), and quercetin-3-O-rutinoside (18.6 mg) in the C. procera extract, with kaempferol being identified as the main component of the extract. The presence of rutin, kaempferol, and quercetin has also been reported in the organic extract of C. procera (Oraibi et al., 2018). Previous studies have shown that flavonoids and polyphenols are the main constituents of C. procera. This difference in compounds could be due to various factors such as climatic conditions, cultivation area and methods, plant species, and extraction methods. Also, the presence of the phenolic acids (caffeic acid and p-coumaric acid), and flavonoids (catechin, rutin, quercetin, and kaempferol) have been reported in Piper betle L., Amaranthus gangeticus, and Lepidium draba extracts (Sarker *et al.,* 2020). According to the type of bonds identified in the FTIR spectrum of the sample results, the presence of phenolic, carboxyl, benzene ring, propyl, aldehyde, cyclohexene, terpenoids (monoterpenes, sesquiterpenes and oxygenated derivatives), and phenyl properties; they can chelate redox-active metal ions and inhibit free radical chain reactions by preventing the conversion of hydro peroxides to reactive ox radicals, which can then inactivate free radicals. Flavonoids are also known for their ability to scavenge free radicals by forming complexes with metal ions.

The antimicrobial activity of a plant extract is generally attributed to the chemical compounds present in the plant mixture of calotropis procera. The maximum effectiveness of a medicinal plant may result from the interaction of several different constituents rather than from the presence of just one main active compound. The main reason for the

differences in antimicrobial activity is the variability in the amounts of secondary metabolites in the plant, which can be affected by many factors such as species and extraction methods (Behbahani *et al.,* 2019).

The extract caused significant changes in the structure of E. coli and S. aureus. Similar observations were made by Moghayedi et al. (2017), who found that treated E. coli had an incomplete and deformed shape with an absent cell wall under the effect of extract.

Much as the pharmaceutical industry is consistently producing new antibiotics, the number of microorganisms that are resistant to chemical antimicrobial drugs poses a harmful threat to the management of infectious diseases (Ghadimi et al., 2017). As a result, new, highly resistant bacterial strains appear, which is extremely dangerous, especially for people with weakened immune systems. In order to create bioactive antimicrobial agents with low toxicity, a wide spectrum, and good pharmacokinetics that can be used in clinical settings without requiring any chemical changes, natural plant products serve as a constant source of impression (Dogara A. 2023). Recently, there has been an effort to promote the use of plants as complementary medicines for treating and mitigating infectious diseases. The C. procera plants can serve as suitable substitute for preservatives in the industry, reducing both their toxic effects on the body and production costs. The antagonistic effect of all the extracts against the microorganisms such *escharichia coli, Bacillus cereus*, *Staphylococcus aureus* and fungi such as *aspergillus flavus, candida albicans and aspergillus niger* were evaluated using agar well diffusion method of susceptibility test. Muella-Hinton agar and Sabouraud Dextrose agar plates were inoculated with 0.1ml of standardized inoculum of each bacterium in triplicates using 0.1ml pipette and spread uniformly with a sterilized swab sticks. Three wells of 6mm size were made with sterile cork borer into the inoculated agar plates. 0.1ml volume of the different concentrations; 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml,12.5 mg/ml and 6.25 mg/ml respectively using micropipette each of the crude extract were introduced into the wells of inoculated plates. The DMSO was used to dissolve the extracts. Water was used as negative control and commercially available standard antibiotic chlorophenicol and was used as positive control with the extract. The prepared plate was then kept for 10 minutes at room temperature of 37 degrees Celsius. following the diffusion and the incubation at 37 degrees Celsius for 24 hours in an incubator and the diameter of zones inhibition were expressed and evaluated respectively. From table 3.3b, the tested organisms were subjected to different concentrations which showed that the leaf showed high antimicrobial activity on all the tested pathogens at

minimum concentration of 12.5 mg/ml except S. aureus which showed growth at 12.5 mg/ml, and at 6.25 mg/ml all the bacterial strains were resistive to leaf, root and stem bark extract. *E. coli* showed resistance on root extract at 12.5 mg/ml as growth was observed while *B.cereus* showed growth at 25 mg/ml concentration.

However, *E. coli* was not able to survive at 100 mg/ml of leaf and root extract but it grew at 100 mg/ml of stem back extract nevertheless at 200 mg/ml there was inhibition of growth as a result of phytoconstituents such as flavonoids phenolic compounds etc.

It has also been reported that the zone of inhibition created by C. procera extract against bacteria ranged from 8.5 to 28.5 and from 10.5 to 30 mm against fungal strains. They found that the extract of C. procera had better antimicrobial activity than other samples, with a zone of inhibition ranging from 9.5 to 22.5 mm, while ether and chloroform petroleum extracts showed no antibacterial activity in some cases. Kareem et al. (2018) reported that ethanol extracts of C. procera leaves and latex had moderate antimicrobial effects against E. coli bacteria, with a zone of inhibition 14.1 mm.

Column chromatography was run using crude extract of leaf and four fractions were obtained and coded F1, F2, F3 and F4. Antimicrobial activity of the leaf extract of the fractions was carried and fraction coded F3 was found the most active and sensitive and it was used for Fourier Transform Infrared Spectroscopy (FTIR) analysis in other to determine the functional groups present based on number of vibrational peaks.

The GC-MS **spectroscopic** analysis on F3 fraction of the leaf extract showed the following characteristic peaks at 3326.04, 2945.07, 2833.56, 1649.24, 1449.04, 1111.76, 1021.49 and 657.08 respectively with those peaks that fall under finger print region. The GC-MS analysis carried reveal the functional groups that are consistent in the spectrum. The peak at 3326.04cm-1 was assigned to hydroxyl vibration in alcohol while the band at 2945.07cm-1 was attributed to alkane. the peak at 2833.56cm-1 and 1649.24cm-1 were assigned to amine salt and alkene. The band at 1449.04cm-1 and 1111.75cm-1 were assigned to alkane with C-H bending which signify methyl group and C-O stretching indicating secondary alcohol. And the bands at 1021.49cm-1 and 657.08cm-1 to lower were C-N and assigned to amides and those in finger print regions whereby elements having atomic number less than 19 falls.

The chemical components of the ethanolic extract of the leaves of C. procera which identified by GC- MS are tabulated in Table 3. The 100% ethanolic extracts consist mainly

of twenty-two compounds ranging from phenol, terpenes, fatty acids, 2-Hexano,2,5-dimethyl-,(S)-

Trimethyl[4-(2-methyl-4-oxo-2-Pentyl) phenoxy]silane, Methyl stearate, 11-Octadecenoic acid, methylester, Hexadecanoic acid, methyl ester, 2-tetradecanone, steroids, alkaloids, phenolic compounds, esters, 2,3-Dehydro-4-oxo-β-ionone and others. The predominant constituents were phenolics, 1-Eicosene, 4-Hydroxy-β-ionone, phytol, n-hexadecanoic acid, octadecanoic acid, dodecanoic acid, methyl, hexa-hydro-farnesol, acid ethyl ester, nonadecanol, coniferol, tetrahydro-spirilloxanthin. Most of the compounds are alkaloids according to their structures and that what appear in the phytochemical study.

CONCLUSION

The study investigated the potential of C. procera leaf, root and stem bark extract in terms of its phytochemical compounds, antioxidant, and antimicrobial properties.

The phytochemical screening indicated that alkaloids and phenolic compounds were present in large quantities. The leaves of *C. procera* can be useful for the treatment of cancer due to the antioxidant activity of phenolic compounds.

The presence of so many phytocomponents in the extracts lends its credence to its importance by the local community as plant with medicinal properties which holds its promise for the production of novel pharmaceuticals. To further understand the synergistic effect of the compounds for therapeutic applications, it would be significant to further isolate the compounds and establish their specific activity.

Isolation and identification of active compounds are important to discover new drug from this plant because little articles appear on this side. Further research is required for these parts of the plant, GC-MS is a highly reliable as it can extract compounds in their pure form which can open a big platform for pharmacological companies to formulate different drugs from plants that can be good sources of these drugs, however, we required in turn care and conservation for these plants. The leaf ethanolic extract showed significant antimicrobial activity against Escherichia coli, *Staphylococcus aureus* and *Bacillus cereus* at all concentrations. This work has enhanced understanding about the phytoconstituents of the plant. Based on the major chemical compounds that are present in the leaves further study on the isolation, chemical characterization and clinical activities of the plant need to be

carried out. This is the first report of a single solvent extraction of the leaf of *Calotropis procera*. Twenty-six compounds were extracted based on the spectroscopic analysis and 10 components of the compounds consistent to functional groups were tentatively identified.

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