



Antimicrobial and Phytochemical Evaluation of *Calotropis procera* (“SODOM APPLE”) against Human Pathogens

Asoso, Oluwakemi Oyesola¹, Akharaiyi, Fred Coolborn^{1*},
Oladunmoye, Muftau Kolawole² and Makinwa, Bisola¹

¹Department of Biological Sciences, Afe Babalola University, P.M.B. 5454, Ado Ekiti, Ekiti State, Nigeria.

²Microbiology Department, Federal University of Technology, Akure, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors AOO and OMK designed the study. Author AFC performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and managed literature searches. Author MB managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the antimicrobial and phytochemical effects of acetone, ethanol, methanol and aqueous leaf extracts of *Calotropis procera* on human pathogens.

Study Design: Five pathogenic and two fungi species were obtained from the Department of Biological Sciences, Afe Babalola University, Ado-Ekiti and were evaluated in *in vitro* antibacterial testing.

Methodology: We studied the *in vitro* antimicrobial sensitivity of *C. procera* by well in agar diffusion method. Also studied was the extract durability to ascertain expiration after preparation and the phytochemical constituents of the extracts by chemical methods.

*Corresponding author: E-mail: akharaiyifc@gmail.com;

Results: The results revealed that acetone extract exhibited the highest antimicrobial properties on the test organisms followed by methanol, ethanol and aqueous extracts in that order. However, *Morganella morgani* was the most inhibited by the solvent extracts with zone of inhibition 45, 56, 59 and 43 mm by acetone, methanol, ethanol and aqueous extracts, respectively. The minimum inhibitory concentration (MIC) of acetone extract on bacteria species was between 25.0 and 100 mg/ml and between 25 and 50 mg/ml on the fungal species. Minimum bactericidal and fungicidal concentrations (MBC/MFC) of the extracts were valued at concentrations ranged from 50-100 mg/ml on the selected microorganisms. The durability study of the leaf extracts in consistent sensitivity pattern was potentially effective for 57-66 days. Phytochemical analysis of the leaf extract showed the presence of saponins, alkaloids, tannins, steroids, terpenoids, flavonoids, phenolics and carotenoids. The results provide a partial support for the use of *C. procera* in traditional medicine.

Keywords: Plant; microorganisms; antimicrobial and solvent extracts.

1. INTRODUCTION

The pharmacological treatment of disease began long ago with the use of herbs [1]. Methods of folk healing throughout the world commonly used herbs as part of their tradition [2]. The traditional use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as traditional herbal medicines. In many developing countries of the world, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons. Such products have become more widely available commercially, especially in developed countries. In this modern setting, ingredients are sometimes marketed for uses that were never contemplated in the traditional healing systems from which they emerged. An example is the use of ephedra (= Ma huang) for weight loss or athletic performance enhancement [2]. While in some countries, herbal medicines are subject to rigorous manufacturing standards, this is not so everywhere. In Germany, for example, where herbal products are sold as phytomedicines, they are subject to the same criteria for efficacy, safety and quality as are other drug products.

Calotropis procera or Sodom apple is a member of the plant family Asclepiadaceae, a shrub about 6m high and is widely distributed in West Africa and other parts of the tropics [3]. In some countries of the world where traditional medicine is practiced wide pride this plant has been used traditionally for the treatment of skin diseases, enlargements of abdominal viscera and intestinal

worms [4], cutaneous diseases such as ringworm, syphilitic sores and leprosy [5], fevers, rheumatism, indigestion, cold, eczema and diarrhea. In addition preparations from latex with honey are used as antirabies and also in the treatment of toothache and cough even in water treatment [5], as a nematicide *in vitro* and *in vivo*, water treatment and its ability to reduce total viable count have also been reported [6]. Because of the medicinal values *C. procera* plays in traditional medicine, this study therefore will investigate the antimicrobial effect of the solvent extractions against selected enteric bacteria and human pathogenic fungi. Inclusive in the investigation is also the presence or absence; and amount of phytochemicals (antinutrients) of this plant growing in the rain forest zone of Nigeria.

2. MATERIALS AND METHODS

2.1 Collection and Processing of Plant Samples

Leaves of *C. procera* were collected from Igede, Ekiti State in September, 2013. Botanical identification was determined by the descriptions given by [7] and confirmation by native regular users of plant samples for traditional medicine at area of collection. A voucher specimen was preserved at the Department of Biological Sciences, Afe Babalola University, Ado Ekiti, Nigeria. The leaves were air dried under shade at ambient temperatures and blended into powder using an electric blender (Moulinex). The samples were stored in air tight containers and preserved with silica gel prior use.

2.2 Test Microorganisms

Five clinical bacteria species (*Pseudomonas aeruginosa*, *Morganella morgani*,

Aeromonas bestiarum, *Proteus mirabilis*, *Citrobacter youngae*) and two fungi (*Fusarium oxysporum* and *Candida albicans*) were obtained from the Department of Biological Sciences, Afe Babalola University, Ado-Ekiti. These microorganisms were sub-cultured on Nutrient agar and Potato dextrose agar respectively and stored at 4°C until required for study. The fungi species was confirmed by the criteria of [8] and bacteria species were studied through cultural, morphological, physiological and biochemical characteristics and were identified by comparison of results obtained with literature standards according to Bergey's Manual of Determinative Bacteriology [9,10] and Bergey's Manual of Systematic Bacteriology [11].

2.3 Preparation of Plant Extracts

Powdered leaves of *C. procera* were extracted with sterile water, ethanol, methanol and acetone by soaking 150 g in 750 ml of each solvent. The suspended solutions were left to stand for 2 days (48 hours). The aqueous extraction was done by subjecting to agitation in a shaker water bath. The extracts were filtered with oven sterilized Whatman No 1 filter paper. The filtrates from methanol, ethanol and acetone solvent extractions were concentrated to dryness in vacuo using rotary evaporator at temperature of 50°C while the aqueous extract was evaporated with water bath regulated at 55°C. All concentrated extracts were stored at 4°C until required for use.

2.4 Durability Study of Plant Extracts

At a regular interval of five days, the antibacterial activity of the extract was checked against the test bacteria species by well diffusion method for a period of 2 months. 5 mm diameter wells were bored in the pre seeded Mueller-Hinton agar plates using a cork borer and 0.55 ml of each extract was added in the well. The plates were incubated at 37°C and the inhibitory halos were measured after 24 hours. At every durability study, halos are compared with susceptibility results on day one to check the stability of the antibacterial activity of the extract [12].

2.5 Antimicrobial Test

The antimicrobial activities of the extracts were determined by agar well diffusion method as described by [13]. Mueller Hinton Agar culture plates were seeded with 10⁶ CFU/ml of the test

bacteria and allowed to stand for about 2 h for the organisms to be well established in the medium. The seeded agar plates were punched with a sterile cork borer (5 mm diameter) to make open wells. The open wells were filled with 0.05 ml of the extracts. The plates were incubated at 37°C for 24 h. For the fungi, the test was carried out on potato dextrose agar plates and incubated at 28±2°C for 72 h. Zones of inhibition were measured and recorded as degree of sensitivity.

2.6 Minimum Inhibitory Concentration (MIC)

Extract concentrations of 12, 25, 50, and 100 mg/ml of the leaf of *C. procera* were prepared. From 24 h 10⁶ CFU/ml of each test bacteria species broth culture, 1ml each were inoculate into a test tube containing 8ml of sterile nutrient broth and 1 ml of extract and incubated at 37°C for 24-48 h. 10³Spore/ml of Fungi were also inoculated in different tubes and incubated at 28±2°C for 72 h. The lowest concentration of the extracts that inhibited the growth of the test organisms was recorded as the minimum inhibitory concentration (MIC).

2.7 Minimum Bactericidal Concentration (MBC) and Minimum Fungal Concentration (MFC)

One milliliter of each positive MIC tubes was pour plated in freshly prepared nutrient agar and potato dextrose agar and incubated appropriately. Plates where test organisms grow were considered as bacteriostatic concentration of the extracts and where no growth observed were considered as bactericidal concentration of the extracts [14].

2.8 Phytochemical Analysis of the Extract

Specific qualitative tests were performed to identify bioactive compounds of pharmacological importance through standard methods. In brief, phytochemicals such as tannins, alkaloids, saponins, flavonoids, terpenoids, and phenols / polyphenols were qualitatively determined as following:

2.8.1 Test for alkaloids (Mayer's test)

Two milliliters of extract was measured in a test tube to which picric acid solution was added. The formation of orange coloration indicated the presence of alkaloids [15].

2.8.2 Test for phenolics

The extract mixed with 1% FeCl₃ will form a blue, violet, purple, green or red-brown. This indicates the presence of phenols [15].

2.8.3 Test for tannins

The extracts mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of Tannins.

2.8.4 Test for saponins

Froth test for saponins was used. 1 g of the sample was weighed into a conical flask in which 10 ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5 ml of the filtrate was added to 10 ml of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

2.8.5 Test for flavonoids

Five milliliters of dilute ammonia solution were added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated H₂SO₄. Formation of yellow color observed in each extract indicated the presence of flavonoids [15].

2.8.6 Test for steroids

One gram of the plant extracts was dissolved in a few drops of acetic acid. It was gently warmed

and cooled under the tap water and a drop of concentrated sulphuric acid was added along the sides of the test tube. Appearance of green colour indicates the presence of Steroid [16].

2.8.7 Test for terpenoids (Salkowski test)

5 ml of each plant part extract was mixed in 2 ml of chloroform, and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. Formation of reddish brown coloration at the interface shows the positive results for presence of terpenoids [15].

2.8.8 Test for carotenoids

Ten milliliters of the extract was filtered and 85% sulphuric acid was added. A blue colour at the interface showed the presence of Carotenoids.

3. RESULTS

The confirmatory test of bacteria species is presented in Table 1. From the durability study of the extracts for 72 days, acetone extract in consistent sensitivity profile on the test bacteria was potentially effectively for 63 days, methanol and ethanol extracts for 66 days; and aqueous extract was effective for 57 days at room temperature storage Fig. 1. All test organisms were susceptible to the solvent extracts of *C. procera* except *Fusarium oxysporum* and *Citrobacter youngae* that were resistant to aqueous extract. Among the solvent extractions, acetone extract was the most potent on the test organisms followed by ethanol, methanol and aqueous extracts.

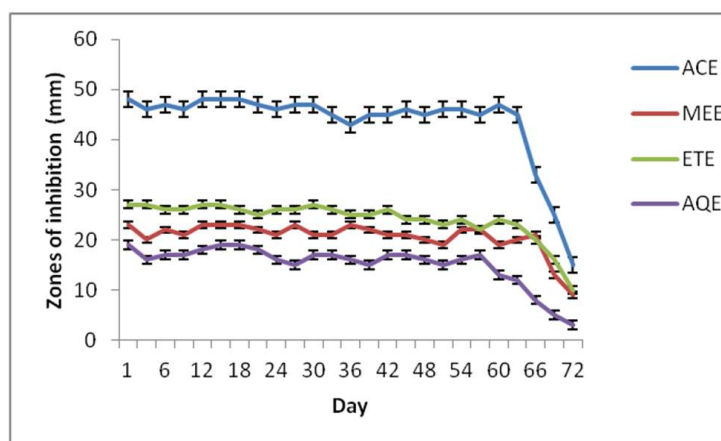


Fig. 1. Extract durability test

(The X axis represents the number of days after extract preparation and the Y represents zones of inhibition in mm); Legend: ACE = Acetone extract, MEE = Methanol extract, ETE = Ethanol extract and AQE = Aqueous extract

Table 1. Characterization and identification of bacterial isolates

	<i>Citrobacter youngae</i>	<i>Pseudomonas aeruginosa</i>	<i>Aeromonas bestiarum</i>	<i>Proteus mirabilis</i>	<i>Morganella morgani</i>
Colour	Cream	Green	Cream	Grey	off white
Surface	Smooth	Smooth	Smooth	Smooth	smooth
Edge	Lobate	Entire	Entire	Entire	entire
Elevation	Flat	Flat	Raised	Raised	flat
Shape	Rod	Rod	Rod	Rod	Rod
Gram stain	-	-	-	-	-
Catalase	-	+	-	-	+
Oxidase	-	+	-	-	-
Starch hydrolysis	-	-	-	-	-
Motility	+	+	-	-	+
Nitrate reduction	+	-	-	+	-
Indole	-	-	-	+	+
Methyl red	+	-	-	+	+
Voges Proskauer	-	-	-	-	-
Citrate	+	-	-	-	-
Glucose	AG	A	A	AG	A
Lactose	AG	-	A	A	-
Mannitol	A	-	AG	-	-
Galactose	-	-	A	-	-
Fructose	-	-	-	-	-
Maltose	AG	-	-	A	A
Sorbitol	AG	-	A	A	AG
Sucrose	-	-	AG	-	-

Legend: AG = Acid and gas production, A= Acid production, + = Positive reaction, - = Negative reaction

However, *Morganella morgani* was the most inhibited by the solvent extracts where it was inhibited with 45, 56, 59 and 43mm of acetone, methanol, ethanol and aqueous extracts respectively, followed by *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Aeromonas bestiarum* and *Citrobacter youngae*. However, *Fusarium oxysporum* was inhibited by acetone, methanol and ethanol extracts with 48, 19 and 27 mm zones respectively while *Candida albicans* was inhibited by acetone and methanol extracts with inhibition zone of 20 mm each, ethanol and aqueous extracts with inhibition zones of 25 mm and 30.3 mm respectively Table 1. The inhibition exhibited on the test organisms by the solvent extract of *C. procera* is comparable to the inhibition exhibited by positive control (antibiotics) Table 1 and Fig. 2.

Fig. 1 expresses susceptibility of test bacteria to commercial antibiotic (positive control). Gentamycin among the commercial antibiotics inhibited all the test organisms followed by ofloxacin where *M. morgani* only was observed resistant. Nalidixic acid was the least potent among the antibiotics on the bacteria species. However all the test organisms were resistant to amoxicillin. *Pseudomonas aeruginosa* was

susceptible only to gentamycin and ofloxacin with zones of 11 and 29mm respectively; *M. morgani* was inhibited with 22, 20, 21 and 9 mm by nitrofurantoin, gentamycin, tetracycline and nalidixic acid respectively. *Aeromonas bestiarum* was inhibited by gentamycin, ofloxacin and cotrimozazole with zones of 2, 25 and 11 mm respectively. *Proteus mirabilis* was inhibited by nitrofurantoin, gentamycin, ofloxacin and tetracycline with zones of 9, 18, 26 and 8 mm respectively while *Citrobacter youngae* was inhibited by nitrofurantoin, gentamycin, ofloxacin, tetracycline, augmentin and cotrimozazole with zones of 8, 12, 18, 1, 12 and 14 mm respectively. However, all the test organisms were resistant to amoxicillin.

In comparison of the plant extract to commercial antibiotics, the test organisms in most cases were highly susceptible to the plant extracts than the positive control (antibiotics). The highest sensitivity exhibited on the test organism was 59mm by ethanolic extract of *C. procera* while the least inhibition was 12 mm by methanol extract. However, the highest inhibition exhibited by commercial antibiotics on test organisms was 29 mm by ofloxacin and least inhibition of 1mm by tetracycline Table 2 and Fig. 2.

Table 2. Zones of inhibition (mm) exhibited by *Calotropis procera* extracts on test bacterial species

S/n	Microorganisms	Acetone extract	Methanol extract	Ethanol extract	Aqueous extract
1	<i>Fusarium oxysporum</i>	48±3.0	19±1.5	27±7.0	–
2	<i>Candida albicans</i>	20±3.2	20±1.4	25±5.2	30±4.2
3	<i>Pseudomonas aeruginosa</i>	23±6.0	21±5.0	16±4.0	23±7.0
4	<i>Morganella morgani</i>	45±1.3	56±7.0	59±5.0	43±5.0
5	<i>Aeromonas bestiarum</i>	18±3.0	21±5.0	16±1.0	22±0.5
6	<i>Proteus mirabilis</i>	18±0.3	22±2.0	19±5.0	23±3.0
7	<i>Citrobacter youngae</i>	24±1.0	12±7.0	14±4.0	–

Value expressed as Mean ± SEM of Three replicates

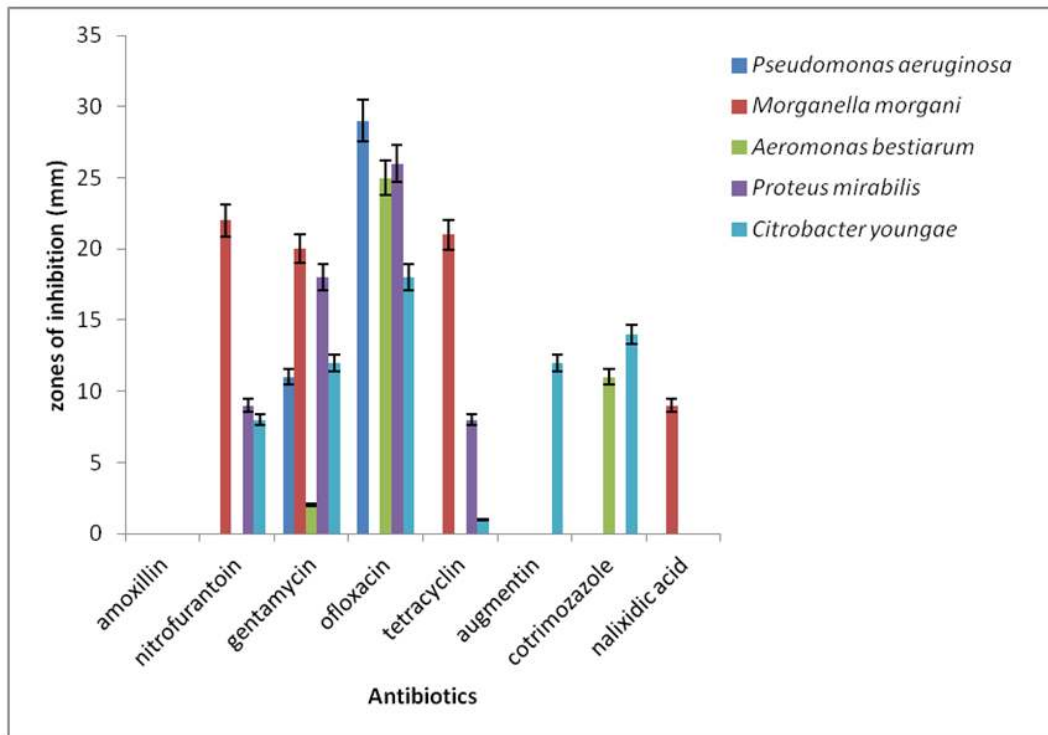


Fig. 2. Antibiotics sensitivity pattern of test bacteria species

The minimum inhibitory concentration of the plant extract was evaluated between 12.5 – 100 mg/ml. The result obtained ascertained 25-100 mg/ml as MIC value. The MIC value for acetone extract on *F. oxysporum* was at 25 mg/ml while methanol and ethanol was at 50 mg/ml. The MIC value for *P. aeruginosa* was 50 mg/ml each with acetone, methanol and ethanol extracts while it was at 25 mg/ml with aqueous extract. *M. morgani* MIC value was at 50 mg/ml each with methanol and aqueous extracts and 25 mg/ml with ethanol extract. *A. bestiarum* MIC was at 50 mg/ml each with methanol, ethanol and aqueous extracts and 100 mg/ml with acetone extract. The MIC value for *P. mirabilis*

was at 50 mg/ml each with acetone and methanol extracts. *C. youngae* had its MIC value at 25 mg/ml each with acetone and ethanol extracts and 50mg/ml with methanol extract Fig. 3.

The minimum bactericidal concentration (MBC) of the plant leaf extract was evaluated between 50-100 mg/ml. The MBC value of aqueous extract on *P. aeruginosa* was at 50mg/ml while acetone, methanol and ethanol extract was at 100mg/ml each. The MBC value of *M. morgani* with ethanol extract was at 50 mg/ml while methanol and aqueous was at 100 mg/ml each. The MBC value for *A. bestiarum* was at

100 mg/ml each for ethanol, methanol and aqueous extracts. *P. mirabilis* had MBC value at 100 mg/ml with acetone and at 50 mg/ml with methanol extracts. *C. youngae* MBC value with methanol extract was at 100 mg/ml and 50 mg/ml with acetone and ethanol extracts respectively. The minimum fungicidal concentration (MFC) of the extract was 50 mg/ml each of acetone and ethanol extracts on *F. oxysporum* and at 100 mg/ml with methanol extract. Acetone and methanol extracts MFC was valued at (100 mg/ml each

while it was at 50 mg/ml each of ethanol and aqueous extracts on *Candida albicans* Fig. 4.

The phytochemicals quantitatively analyzed from the leaf of *C. procera* are saponins, alkaloids, tannins, steroids, terpenoids, flavonoids, phenols and carotenoids as shown in Table 3. The quantitative phytochemical analysis of carotenoids from *C. procera* leaf was 1623 ± 7.0 $\mu\text{g/ml}$ being the highest volume among the quantitatively analyzed phytochemicals.

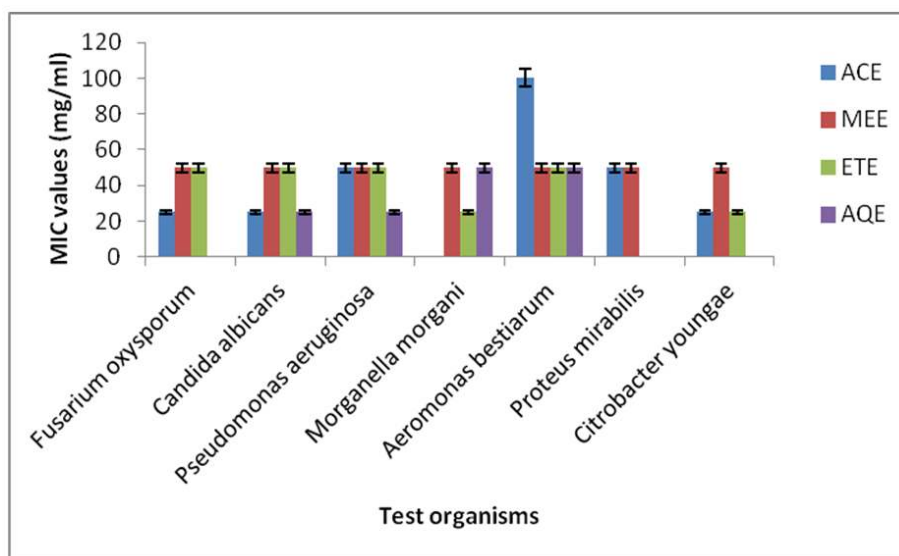


Fig. 3. Minimum Inhibitory Concentration (MIC) of *Calotropis procera*

Legend: ACE = Acetone extract, MEE =Methanol extract, ETE = Ethanol extract and AQE = Aqueous extract

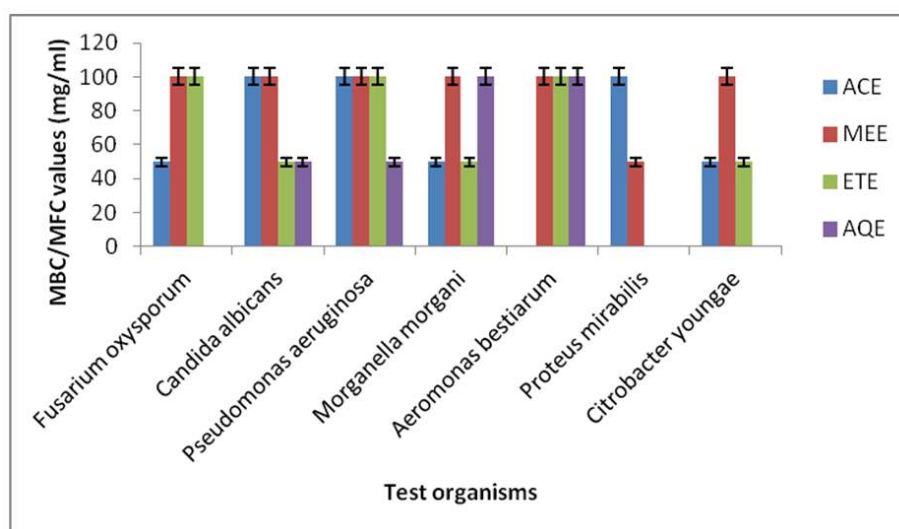


Fig. 4. Minimum bactericidal/fungicidal concentration (MBC/MFC) of plant extracts

Legend: ACE = Acetone extract, MEE =Methanol extract, ETE = Ethanol extract and AQE = Aqueous extract

Table 3. Phytochemical properties of *Calotropis procera* leaves

Parameters	Qualitative	Quantitative
Saponins (mg/100 g)	++	232±8.0
Alkaloids (mg/100 g)	+++	1343±1.7
Tannins (mg/100 g)	+++	353±2.0
Steroids (mg/100 g)	+	44±1.0
Terpenoids (mg/100 g)	++	667±8.0
Flavonoids (mg/100 g)	+++	943±1.2
Phenolics (GAE/100 g)	+	16±1.0
Carotenoids (µg/100 g)	+++	1643±7.0

**GAE = Gallic Acid Equivalent (Conventional unit for Phenolics)

** 1000 µg = 1 mg (µg/100 gis conventional unit for Carotenoids)

Alkaloids, terpenoids, flavonoids and saponins also were high in quantitative values in the leaf extract of *C. procera*. While steroids was of moderate quantity, phenolic content in the leaf extract of *C. procera* was as low as 16±1.0 Gallic acid equivalent per 100 g in the leaf extract of *C. procera* Table 3.

4. DISCUSSION

Natural products are having a great importance in ancient traditional medicine system and till date is being employed for validity in solving some health problems which orthodox medicine has failed to withstand. Herbs in innumerable ways have served as natural drugs [17] used to regain the alterations made in normal physiological system by pathogens or any malfunctioning in the body system by non microbial diseases.

From different literatures and reviews it has been seen that *C. procera* is a resuscitative plant due to its large number of medicinal properties which also have being proved as potential therapeutic in this study. The organic solvents used for the extraction of bioactive molecules from *C. procera* were able to extract valuable chemical substances sufficient enough for wide inhibition of the selected pathogenic microorganisms in this study. The increasing interest in natural products present in medicinal plants used in traditional medicine have placed medicinal plants on front line as one of the dependable sources of potential antimicrobial agents and possibly for discovery of novel drugs. So in this view, the study of antimicrobial activity of *C. procera* was based mainly on Gram negative enteric pathogens for their nuisance role in resistance to common antibiotics use in orthodox medicine. Gram-negative bacteria however, possess an outer membrane and unique periplasm space not found in Gram-positive bacteria [18]. The results

of this study highlights, the fact that the organic solvent extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and were extracted only through the organic solvent medium [19,20]. So the present observation suggests that the organic solvent extraction was suitable to verify the antimicrobial properties of *C. procera* which are also supported by many other investigators [21,22].

However, the bioactive molecules extracted depend upon the type of solvent used in the extraction procedure [23]. These extracts are attributed to the inhibition of microbial growth by various mechanisms even as has been reported by [24]. It has been observed that many antimicrobial studies are based only on inhibition results without considering for how long it will be stored to maintain its inhibition potentials before expiration as seen in orthodox medicines. We however studied the expiration date of the extracts in this study and found that acetone, methanol and ethanol extracts were durable for effective antimicrobial therapy for a storage period of over 2 months and aqueous extract for over 1 month at room temperature. This illustrates that the inhibitory ingredients present in the crude solvent extracts of *C. procera* could be stored for a period of time at room temperature and yet be effective for therapeutic use.

In this study, Acetone extract of *C. procera* provided the most consistent antimicrobial potency on the selected pathogens. However, methanol and ethanol extracts were also effective on all the microorganisms susceptible than aqueous extract which was not active on *Fusarium oxysporum* and *Citrobacter youngae*. From this observation, it is evidenced that acetone, methanol and ethanol extracts contained more potent phytoconstituents that

enables the plant to possess its antimicrobial property than aqueous extract. Though in most cases the plant extracts were more active than commercial antibiotics, there is a correlation that the solvents leaf extracts of *C. procera* are comparable in inhibitory values with commercial antibiotics commonly used in treatment of several diseases in modern medicine. Many literatures have reported Multiple Drug Resistance (MDR) development due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. Adding to this problem, antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immune suppression and allergic response [25]. Measures to overcome such situations have also led to this study of antibacterial potency of *C. procera* for possible clue as an alternative to replacing some antibiotics known for their side and inconsistent effects in the purposes they were manufactured.

Owing to the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have started paying attention to medicinal plants and biologically active compounds isolated from plant species used in herbal medicine. As a contributive factor to these problems, Nigerian botanical wealth could be utilized to achieve major goal for solutions to health care problems hence medicinal plants are being increasingly reported from different parts of the world for lasting solutions to combating diseases as alternative medicine.

Despite the fact that the plant extracts were used in crude form, their MIC values were not higher than 50 mg/ml and MBC not exceeding 100 mg/ml. This plant extract if purified could work better than the present results obtained because it has been reputed that purified plant extracts are more potent than crude extracts in antimicrobial activity [26].

C. procera antimicrobial activity obtained in this study is in agreement with [27-29].

Previous phytochemical study of *C. procera* has revealed the presence of alkaloids, saponins, tannin, steroids, flavonoids and phenols [30]. Also, in the phytochemical tests of *C. procera* leaves, saponins, alkaloids, tannin, steroids, terpenoid, flavonoids, phenolics and carotenoids were observed in this study. The presence of these plant chemicals could be responsible for

the antimicrobial activity observed with the plant extracts, hence antimicrobial potency of plant is believed to be due to tannins, saponins, phenolic compounds and flavonoids [31,32].

Herbal medicines are generally believed as safe, however, it is important to evaluate their biological safety before use to avoid fatal consequences [33]. There is no doubt in pharmacological properties of *C. procera* but its toxicological assessment is also indispensable. In view of this, further work on its toxicity will be evaluated.

5. CONCLUSIONS

The present study provides the scientific rationale for medicinal use of *C. procera* as substitute antimicrobial agent in therapeutics. Further investigation is necessary to separate the active components and evaluate the antimicrobial activity of each component so as to confirm the bioactive nature of the compounds identified in this study. Also, the toxic effect of this plant extract is necessary for validation in the plant's use in therapeutics. Hence potential bioactive molecules such as phenols, steroids, flavonoids, tannins and saponins were isolated from this plant; composition for development of new drugs for the therapy of infectious diseases could be possible with *C. procera*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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