Phytochemical Analysis, Volatile Components and Biological Evaluation of the leaf of *Calotropis Procera* (*Asclepiadaceace*)

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Abstract: **- The dried and powdered leaf of C. procera was exhaustively macerated in 98% methanol for 8days. The extract obtained was concentrated using the rotary evaporator to give a residue. Phytochemical qualitative analysis showed the presence of alkaloids, terpenoid, Saponins, tannins and flavoinoids. The plant was screened against the following microorganisms: Staphylococcus aureus, Bacillus cereus, Escherichia coli, Micrococcus luteus, Pseudomonas aeruginosa and Candida albicans using agar well diffusion method. The crude methanolic extract of the leaves of C.procera exhibited significant antimicrobial activity against the microorganisms tested with some degree of variations against the standard drug, penicillin. It was found that the higher the concentration of the crude extract of C. procera the stronger the activities against the organisms. The in vivo ant-malarial study of the crude extract was evaluated using the 4-day suppressive test. The results showed that the crude extract of this plant exhibited dose-dependent suppression of parasites growth in mice. The methanolic leaf extract of C. Procera caused 31.02%, 45.17% and 50.12% suppression in parasitaemia of P. berghei ANKA infected mice at 200, 400 and 800mg/kg body weight, respectively. The essential oil from the leaves was extracted through steam distillation using the Clevenger-type apparatus and was analyzed by GC-MS. About 49 compounds were revealed with known biological activities, they are essentially aliphatic hydrocarbon, long chain fatty alcohols, long chain fatty acids and their derivatives and some aromatic compounds including naphthalene, a thymol and a phthalate. The major chemical constituents present in the oil were 1-heptacosanol (9.11%), 6-octadecenoic acid (4.56%), 9-octadecenoic acid, methyl ester (oleic acid) (4.67%), octadecanoic acid, methyl ester (stearic acid) (3.86%), 1-nonadecene (6.79%) and eicosane (4.02%). The highest peak area was obtained for1-Heptacosanol ($C_{27}H_{56}O$) with retention time of 19.339 and peak area of 9.11% while the lowest peak was for 1, 6-dimethynaphthalene ($C_{12}H_{12}$) with retention time of 8.192 and peak area of 0.21%. This study highlights the presence of secondary metabolites in the leaf parts and chemical constituents in the essential oil of C. procera, and provide an overview of the different classes of molecules present that have led to their pharmacological activities which may account for the ethno medicinal uses of the plant, it is also confirmed that the essential oil could be used for the treatment of various diseases.**

Keywords: **Calotropis procera crude extract. Plasmodium berghei ANKA. Phytochemical, Antimicrobial activity, Antimalarial activity, GC-MS**

I. INTRODUCTION

*Calotropis procera (*Ait). Commonly known as Sodom apple or a wasteland weed belongs to the family Asclepiadaecae. It is an Ayurvedic plant with important medicinal properties. It grows up to a height of 1-3 metres and its leaves are 9–14 cm wide by 16–20 cm long. It is commonly referred to as ark, swallow-wart and it occurs frequently in Asia and Africa and is used in many traditional systems of medicine. It is found in most part of the world with a warm climate in dry, sandy and alkaline soils, it is found in waste lands and grows as a weed in cultivated areas, it also grows well on rubbish heaps, waste and fallow land, by the road side and in sand dunes. *C. procera* primarily harvested because it's distinctive medicinal properties. It is known as madar in Hindi and alarka in Sanskrit. In Nigeria, it is popularly known by the Yoruba as ewe Bomu Bomu and Tumifafiya by the Hausa. The plant has been reported to be effective in managing more than hundred human diseases. It is a lactifer plant and its endogenous production of latex is admiration. The latex of the plant has been used in leprosy, eczema, inflammation, cutaneous infections, syphilis, malarial and as abortifacient [1]. The literature of the plant revealed that various parts of the plant such as root bark, stem bark, leaf, flower, and latex and their extracts, fractions, and isolated compounds showed significant anticoagulant, antidiarrheal, anti-inflammatory, antioxidant, antiulcer, analgesic, cough-suppressing, hepatoprotective, smooth muscle-contracting, neuromuscular blocking, spermicidal, and wound healing activities. The previous reports have confirmed that this plant possesses various pharmacological properties that may be used for the treatment of inflammatory disorders such as arthritis, cancer, sepsis [2, 3]. The Antimicrobial activities of the ethanolic crude extract of the plant was reported by [4] while the Antiplasmodial activity (*Plasmodium falciparum*) of the ethanolic crude extract was reported by [5]. [6] Reported that Alkaloids, saponins, tannin, terpeniod, steroid and flavanoids were the main constituents present in leaf extract of plant and exhibited significant antimicrobial activity.

This study is design to evaluate and confirm the secondary metabolites, ant-malarial, antimicrobial activities of the methanolic extract of the leaves of the C. *procera* collected from Navy Estate Karshi, Nassarawa state, Nigeria. and GC-MS analysis of the essential oil of leaf of the plant with the

aim of providing lead compound(s) for the development of new, safe, effective and affordable ant malarial and antimicrobial agent(s). Thus, the plant was chosen based on its numerous pharmacological properties. On account of the drawbacks of the vast majority of antimalarial drugs available to date, the severity of the disease and the huge threat it poses to the health sector, educational sector as well as the social economic development of Nigeria,

II. MATERIAL AND METHOD

Plant Materials

The leaves of *C. procera* were collected from Navy Estate karshi, Abuja-Nigeria on 25th April 2018. It was botanically identified by Mr Lateef Akeem of National Institute for Pharmaceutical Research and Development (NIPRD) with a herbarium of NIPRD/H/6915. The leaves were washed thoroughly with water, sun dried for four weeks and powdered using hand mill to make a coarse powder and stored in well-closed light resistant container until further used.

Preparation of the leaves extract

347g of the dried and powdered leaf of *C. procera* was exhaustively macerated in two litters of 98% methanol for 8days [7]. The extract obtained was concentrated in a rotary evaporator to dryness. The yield of the extract obtained was 65.50g (18.2%).

Extraction of essential oil from fresh leaves using steam distillation

The collected fresh leaves of *C. procera* were washed to remove the dirt on the surface of the leaves. It is to make sure that no any other impurities stick to the leaves. The extraction was carried out using only fresh leaves. Fresh leaves of *C. procera* (166.66g) was mixed together with 500ml of distilled water in the round bottom flask connected to the Clevenger-type apparatus. The yield of the essential oil obtained is 0.017g (0.01%).

The extraction was performed using steam distillation Clevenger-type apparatus. The essential oil was then separated from the distillate with separating funnel by using dichloromethane as a solvent.

Phytochemical screening of methanolic extract

The methanolic extract of C. *procera* was prepared by soaking 52.25g of the dried powdered samples in 300ml of methanol for 3 days using qualitative methods as described by [8] which were used to analyse the bioactive components in the plant extract. The phytochemical tests carried out in this work include alkaloids, saponins, carbohydrate, tannins, steroid, terpenes and flavonoids, the extract is filtered by using whatman filter paper. The filtrate was used for phytochemical screening.

Test for Tannins: 0.5 g (powdered sample of C. *procera* dried extract) is boiled in 20 ml water in test tube. To its filtrate 0.1 % of Ferric Chloride was added. Brownish green colour was observed.

Test for Saponin: The filtrate (10 ml) of aqueous plant extract was taken and 5 ml of distilled water was added. This mixture now in the test tube is vigorously shaken for a while. A permanent froth was found. To the froth 3 drops of olive oil was added and shaken vigorously to give an emulsion.

Test for Flavonoids: A few drops of 1% NH_3 solution was added to the methanolic extract of the plant leaves in the test tube. A yellow coloration was observed.

Test for Steroids: One millilitre of the extracts was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence.

Test for Terpenoids: The aqueous filtrate of the plant extract was taken in a test tube and then 2 ml of chloroform was added and mixed well. The test tube was placed in a water container to keep cool. Now 3 ml of concentrated sulphuric acid was added from the side of the test tube. We observed a clear Reddish-brown colouration at the interface.

Test of Alkaloid: Five millilitres of the extract were added to 2 ml of HCl. To this acidic medium, 1 ml of Dragendroff's reagent was added. An orange precipitate was observed immediately.

Test for Carbohydrates: (Molisch's test). To 2ml of the methanolic extract, two drops of alpha-napthol were added. The mixture was shaken well with few drops of concentrated sulphuric acid were added slowly along the sides of test tube. A violet ring was recorded.

Gas chromatography-mass spectrometry (GC-MS) analysis of steam distillate

For the determination of the chemical constituents contained in the essential oil of *C. procera* leaves, Gas Chromatography-Mass Spectrometry (GC-MS) machine used was, model Shimadzu GCMS - QP2010 plus Triple-Axis Selective Detector at Shimadzu training centre for analytical instruments(STC), Lagos. The operation involved a column of cross -linked 5% phenyl methyl silicone (DB-WAX, 30m X 0.25mm X 0.25µm) the carrier gas was helium at flow rate 0.90ml/min, injector temperature program was 250^{o}C and column temperature was 60^{0}C.

Antimicrobial screening

Test Organisms

The following microorganisms were used for the study. Clinical isolates of (*Staphylococcus aureus, Bacillus cereus, Escherichia coli, Micrococcus luteus, Pseudomonas aeruginosa* and *Candida albicans)*. Pure strains of the microorganisms were obtained from the laboratory stock of the Department of Microbiology, University of Abuja, Abuja, Nigeria, University of Abuja Teaching Hospital, Gwagwalada,

and Abuja. All isolates were of high purity and maintained on agar slants at 4^{o}C in the refrigerator.

Determination of antimicrobial activity

The antimicrobial activity of the crude extract of the *C. procera* against clinical pathogens was determined using agar well diffusion method based on the guidelines of the National Committee for Clinical Laboratory Standard [9, 10]. Sterile nutrient agar plates were prepared for bacterial stain inoculated by spread plate method under aseptic conditions. Sterilized nutrient agar (20ml) was poured into petri dished and allowed for solidifications. After solidification, 24 hours then the pathogenic cultures was swabbed on the respective agar plates using sterilized cotton swabs. Wells of 6mm diameter was punched over the agar plates using a sterile gel puncher or cork-borer. Different concentration of the crude extracts was added using sterile syringe into the wells and allowed to diffuse at room temperature for 1 hour and the plates will be incubated at 37^{0}C for 18- 24 hour for bacterial and fungi pathogen. After incubation, the diameter of inhibition zones formed around each wells will be measured and expressed in millimeter (mm) and recorded against the corresponding concentration to evaluate the antimicrobial activity. Positive control was set using the standard antibiotics drug while a negative control was set using extraction solvents.

In vivo ant-malarial screening of extract

The in-*vivo* ant-malarial screening was determine using peter's four days suppressive screening [11].Ten (10) weeks old Swiss albino mice weighing 20- 40g were obtained from National Vertinary Research Institute Vom, Jos, Nigeria. They were acclimatized and housed for 10 Days in plastic cages with saw dust as beddings; food and water were given. The mice were used in accordance with NIH Guide for the care and use of laboratory animals; NIH Publication revised (1985) NIPRD.

Sourcing of Parasite Plasomodium bergei ANKA

The parasite *Plasmodium berghei ANKA* was obtained from National Vertinary Research Institute, Vom, and Jos, Nigeria. The parasites were kept alive by continuous intraperitoneal inoculation of known amount of the parasite into Swiss mice.

Preparation of Parasite Plasomodium bergei ANKA

1ml of blood was taken from donor mice using 5ml syringe and diluted with 5ml phosphate buffer; such that 0.1ml contained standard inoculum of 2 ×10^{7} infected red blood cells in 0.2 ml suspension of 1 × 10^{8} infected red blood cells per ml [12].Thirty (30) healthy mice were selected and group into six groups as shown in Table I and twenty-five (25) were inoculated intraperitoneal from the same source to avoid variability in parasiteamia.

TABLE I

GROUP I	GROUP II	GROUP III.	GROUP IV	GROUP V	GROUP VI
(Normal control) Healthy Swiss mice with distilled water.	(Negative control) mice infected with *P. berghei ANKA* received no treatment	(Positive control) = *P. berghei*+10 mg/kg b,wt of chloroquine (Standard drug)	*P. berghei* + 200mg/ kg b.wt crude extract.	P. *berghei* + 400mg/ kg b.wt crude extract.	*P. berghei* + 800mg/ kg b.wt crude extract.

Inoculation of parasite and administration of extract

The Peter's 4-day suppressive test against *P. berghei ANKA* infection in Swiss mice was used. Adult mice weighing between 20 to 40g were inoculated by intraperitoneal injection with standard inoculum of *Plasmodium berghei ANKA* with 2 × 10^{7} infected red blood cells in 0.2 ml suspension of 1 × 10^{8} infected red blood cells per ml.

The mice were divided into six groups as shown above and treated for 4 consecutive days with 10mg/kg.bwt of Chloroquine, 200, 400, and 800mg/kg body weight of *C. procera* extract orally daily. On day 5 of the experiment, blood was collected from the tail of each mouse and smeared onto microscope slide to make a film. The tail blood smear was fixed with absolute methanol (MeOH) and then stained with 10% Giemsa-stain in phosphate buffer (pH 7.2) for 20 min.

Determination of parasitemia and percentage suppression

The parasites were examined under microscope at ×100 oil immersion. Percentage parasitemia was determined by counting the infected erythrocytes in at least 1000 total erythrocytes (infected plus non-infected erythrocytes). The mean percentage suppression of parasiteamia for each extract was calculated by subtracting the mean parasiteamia in treated mice from the mean parasiteamia in negative control, dividing the value by the mean parasiteamia in the negative control then multiply by 100.

The mean parasiteamia was calculated for each group by comparing the parasiteamia in infected group (Group II) with those of control and those of treated mice.

III. RESULT AND DISCUSSION

Phytochemical screening

The dried powered leaf of *C. procera* was extracted with methanol to give the crude extract. The crude extract of the leaves of *C. Procera* was screened for the presence of secondary metabolites. In this study the results of the phytochemical screening (Table II) showed that the methanolic extract contained alkaloids, flavonoids, tannins,

steroids, terpenoids and saponin. The results also showed that carbohydrates were not detected, this maybe due to the low concentration of the extract used. The presence of steroids, terpenoids and saponins are in agreement with the work of [13], [14, and 15]. Previous studies have reported that naturally occurring alkaloids and their synthetic derivatives have analgesic, antispasmodic and bactericidal activities as reported by [16]. The use of some plants for medicinal purposes in the traditional treatment of diseases has been reported to be due to the presence of flavonoids and saponins [17, 18]. Hence the use of C. *procera* for the treatment of diarrhea, dysentery, colds and several other diseases by local herbalists or traditional healers is not surprising. Tannins in some medicinal plants have been found to be responsible for the antiviral and antibacterial activities exhibited by such plants [19, 20] Therefore, C. *procera* with tannin content could probably be a source of phytochemicals for the treatment of bacterial infections. Phenolic compounds like tannins present in plant cells are inhibitors of many enzymes (proteolytic and hydrolytic) used by plant pathogens. Other compounds such as saponins have antifungal properties [21, 22]. Therefore, these phytochemicals detected in this study may be responsible for the antimicrobial potency of the leaf extracts of C. *procera* and also lend credence to the claims of traditional application of the plant as remedies for various ailments.

TABLE II PRELIMINARY PHYTOCHEMICAL SCREENING OF THE METHANOLIC LEAF EXTRACT OF *C. PROCERA.*

Chemical constituents	Test	Methanolic extracts
Alkaloids	Mayer's test	+
Saponin		+
Flavonoids		+
Terpenoids		+
Carbohydrates	Molish's test	ND
Steroids		+
Tannin	Ferric Chloride test	+

key :(+) = present; (ND) = Not-Detected

In-vitroantimicrobial screening

The Crude extract was tested against clinical isolates of *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Micrococcus luteus, Pseudomonas aeruginosa* and *Candida albicans*. Penicillin was used as a reference drug for the bacteria and fungi and the zones of inhibition are 2.9mm and 3.0mm respectively.It will be noted in this finding that the zones of inhibition of the crude extract shown in Table III increases as the concentrations of the crude extract of the plant increase, that is higher the concentration of the crude extract the stronger the activities on both bacteria and fugal organism.

The antimicrobial activities of the crude extracts of *C. procera* in Table III showed that the crude extract has a broad spectrum activity against the microorganisms tested. This activity could probably be correlated with presence of saponins for antifungal properties [21, 22], alkaloids for bactericidal activity [16, tannins for antibacterial properties [19, 20].Thus the phytochemicals revealed in Table I may be responsible for the antimicrobial potency of the leaf extracts of C. *procera* supporting to the claims of traditional application of the plant as remedies for various infections.

TABLE III ZONE OF INHIBITION (MM) OF THE CRUDE METHANOLIC EXTRACT AGAINST VARIOUS

Organism	Zone of inhibition(mm)/ **concentration (mg/ml**				Penicillin (mg/ml) 0.4
	100	**80**	**60**	**40**	
E.coli	24	20	14	10	29
S. aureus	25	21	16	09	30
P.aeruginosa	25	22	17	09	28
B.cereus	25	19	14	10	30
M. luteus	24	21	16	07	28
C.albicans	25	20	17	10	29

In-vivo antimalarial screening

In this study the *in vivo* antimalarial activity of the extracts of *C. procera* was evaluated using the peter's 4-day suppressive test. The results showed that the extracts of this plant species exhibited dose-dependent suppression of parasites growth in mice.

The methanolic leaf extract of C. *Procera* caused 31.02%, 45.17% and 50.12% suppression in parasitaemia of *P. berghei ANKA* infected mice at 200, 400 and 800mg/kg body weight respectively in (Table IV). In this finding it is observed that the greater the concentration of the methanolic extract, the higher the percentage suppression on the parasiteamia. The values from this study are slightly similar to that of the antiplasmodial activity (25%, 50% and 50% at 1000, 2000 and 5000ug/ml respectively) reported by [5] confirming the antimalarial activity of the plant. The presence of pharmacologically active phytochemicals like steroids, terpenoids, saponins, tannins, flavonoids, fats and oil in the extract has been shown (Table II). The presence of these secondary metabolites may be responsible for the ant-malarial activity. Anti-malarial activities of plant substance have been shown to be caused by alkaloids, terpenes and flavonoids [23, 24 and 25]. [26] And [27] showed clearly that Saponins, flavonoids and tannins have been suggested to act as primary antioxidant or free radical's scavengers that can counteract the oxidative damage induced by malaria parasite these compounds could be actingsingly or in synergy with one another to exert the antimalarial activity observed in this study. It is evident by these findings that *C. procera* possessed ant malarial activity supporting its usage in the management of malaria in Nigeria. Though the extracts demonstrated moderate antimalarial activities, the activities may be enhanced by testing their fractions and isolated compounds.

Chloroquine drug used in this study exerted 100% suppression at 10mg/kg body weight (Table IV). [28] Showed that when a standard anti-malarial drug is used in mice infected with *P. berghei*, it suppresses the parasiteamia to a non-detectable level which is in line with this study

TABLE IV SUPPRESSIVE TEST SHOWING THE EFFECT OF CHLOROQUINE AND METHANOLIC LEAF EXTRAC AGAINST *PLASMODIUM BERGHEI ANKA* INFECTED MICE

Treatment group	Dose of extract (mg/kg/day)	Mean percentage parasiteamia at day 4±SD (n=6)	Mean percentage (%)suppression of parasiteamia at day 4
Group I	NHC with Distilled water	NIL	NIL
Group II	NC	42.04±2.00	00.00
Group III	10 (PC)	NIL	100.00
Group IV	200	29.00±1.00	31.02
Group V	400	23.05±0.85	45.17
Group VI	800	20.97±3.27	50.12

Key: NHC=Normal healthy control group, NC=Negative control, PC=Positive control. SD= Standard Deviation

Gas chromatography-mass spectrometry (GC-MS) analysis

The steam distillate from the fresh leaves of *C. procera* was subjected to GC-MS analysis and the results showed the corresponding characteristic volatile components in (Table V). The major chemical constituents present in the oil were 1-heptacosanol (9.11%), 6-octadecenoic acid (4.56%),9-octadecenoic acid, methyl ester (oleic acid) (4.67%), octadecanoic acid, methyl ester (stearic acid) (3.86%), 1-nonadecene (6.79%) and eicosane (4.02%).[29]have shown the essential oils of the fruit of *C. procera* to contain five main components, namely: lupenol (12.10%), n-hexadecanoic acid (12.07%), thymol (9.86%), tetratetracontane (6.88%) and linoleic acid (6.74%). The essentials oils obtained from the leaves of C. *procera* in this study showed low composition of n-hexadecanoic acid (2.69%) and thymol (1.27%) while tetradecane (3.37%), eicosane (4.03%), 1-heptacosanol (9.11%), behenic alcohol (2.07%) and hexadecane (3.37%) were higher in the leaves than in the fruit as reported by [29].

Thymol shows strong antimicrobial activities when used alone or with other biocides such ascarvacrol. Thymol can also reduce bacterial resistance to common drugs such as penicillin [30] and also exihibts antioxidant properties [31] Thymol and carvacrol reduce bacterial resistance to antibiotics through a synergistic effect [30] and thymol also act as an effective fungicide [30]. Thymol also exhibits strong antimutagenic effect [32]. In addition, it has antitumor properties [33]. The presence of thymol in the essential oil of the plant in this study also support the traditional use of the plant for treatment.

The essential oil has five main fatty acids namely: octadecanoic acid, methyl ester (stearic acid) (3.86%), n-hexadecanoic acid (2.66%) (palmtic acid), 9-octadecenoicacid, methylester (4.67%) (Oleic acid), 6-octadecenoic acid (2.17%) (petroselnic acid), octadecanoic acid and 2-(2-hydroxyethoxy) esther (3.08%) in Table 4.1.8. The oleic acid being the most abundant, its high content of oleic acid is of particular interest because it is used as a component in many foods and the fight against hypertension by reducing blood pressure [34]. It is a component of the normal human diet as a part of animal fats and vegetable oils. Other oils used as food additives have health promotive properties [35, 36], n-hexadeanoic acid also known as palmitic acid is the most common saturated fatty acid found in animals, plants and microorganism. According to world health organization the consumption of n-hexadeanoic acid increases the risk of developing cardiovascular disease [37] and [38].

There are five main fatty alcohols namely: Pentadecanol(0.98%),nNonadecanol(1.04%),Behenicalcohol1-docosanol (2.28%), 1-Heptacosanol (9.11%) and Hexadecen-1-ol (0.41%) (Table VII). Table VIII shows the long chain hydrocarbon and it derivatives namely: Tetradecane (1.44%), 3,3- dimethyl hexane (1.04 %), heptadecane (0.54%), 3-heptadecene, (Z) (0.60%), eicosane (0.94%), 1-nonadecene (6.55%), octadecane (0.56%), 9-heptadecane (2.27%), icosane (1.18%) and tetrapentacontane, -1,54-dibromo (1.08%). The chemical constituents in the oil are essentially aliphatic hydrocarbon, long chain fatty alcohols, long chain fatty acids and their derivatives and some aromatic compounds including naphthalene, a thymol and a phthalate.

This study highlights the presence of many secondary metabolites in the leaf parts and chemical constituents in the essential oil of C. *procera*, and provide an overview of the different classes of molecules present that have led to their pharmacological activities, it is also confirmed that the essential oil could be used for the treatment of various diseases.

TABLE V: VOLATILE COMPONENTS OF THE LEAVES OF *CALOTROPIS PROCERA*

NAME OF COMPOUNDS	RT	MF	PEAK AREA %
Thymol	6.657	$C_{10}H_{14}O$	1.27
Naphthalene	6.740	$C_{11}H_{10}$	0.33
1,3-Cyclopentanediol,	7.367	$C_5H_{10}O_2$	0.24
Pentadecanol	7.612	$C_{15}H_{32}O$	0.98
Tetradecane,	7.703	$C_{14}H_{30}$	1.44
Chloroacetic acid,	7.908	$C_{12}H_{23}ClO_2$	0.26
Naphthalene 1,8-dimethyl	7.992	$C_{12}H_{12}$	0.36
Naphthalene 1,6-dimethyl	8.192	$C_{12}H_{12}$	0.21
n-octyl cyclohexane,	8.408	$C_{14}H_{28}$	0.34
3,3- dimethyl hexane	8.533	C_8H_{18}	0.22

Heptadecane	9.092	$C_{17}H_{36}$	0.56
Dodecanoic acid	10.058	$C_{12}H_{24}O_2$	1.30
1-Nonadecene	10.433	$C_{19}H_{38}$	3.29
Hexadecane	10.52	$C_{17}H_{36}$	3.05
Dichloroacetic acid	10.783	$C_{18}H_{34}Cl_2O_2$	0.43
1,2-Benzisothiazol-3 one	11.117	$C_8H_7N0_3S$	0.29
Cyclohexane	11.392	$C_{17}H_{34}$	0.66
3-Heptadecene, (Z)-	11.642	$C_{17}H_{34}$	0.60
Hexadecen-1-ol	11.842	$C_{16}H_{32}O$	0.41
Eicosane	11.908	$C_{20}H_{42}$	0.94
Benzoic acid, 2-ethyl ester	12.050	$C_{15}H_{22}O_2$	0.73
Eicosanoic acid)	12.767	$C_{20}H_{40}O_2$	0.72
1-Nonadecene	13.125	$C_{19}H_{38}$	6.55
Eicosane	13.208	$C_{20}H_{42}$	4.97
Octadecane	13.208	$C_{18}H_{38}$	0.56
1-Nonadecene	13.433	$C_{19}H_{38}$	0.87
Dodecylcylohexane	14.042	$C_{18}H_{36}$	0.84
9-Heptadecane	14.125	$C_{17}H_{34}O$	2.27
n-Nonadecanol	14.192	$C_{19}H_{40}O$	1.04
Icosane	14.408	$C_{20}H_{42}$	1.18
Octacosanoic acid,	14.717	$C_{29}H_{58}O_2$	0.94
n-hexadeanoic acid	15.142	$C_{16}H_{32}O_2$	2.66
1-Nonadecene	15.450	$C_{19}H_{38}$	7.53
Eicosane	15.517	$C_{20}H_{42}$	5.13
9-Hexacosene	15.725	$C_{26}H_{52}$	0.94
Oxirane heptadecyl	16.275	$C_{19}H_{38}O$	1.10
Behenic alcohol 1-docosanol	16.424	$C_{22}H_{46}O$	2.28
9-Octadecenoic acid)-, methyl ester	16.617	$C_{19}H_{36}O$	4.67
16-methyl-heptadecanoate	16.850	$C_{19}H_{38}O_2$	3.78
6-Octadecenoic acid	17.025	$C_{18}H_{34}O_2$	2.17
Octadecanoic acid 2-(2-hydroxyethoxy)ethyl ester	17.242	$C_{22}H_{44}O_4$	2.36
1-Heptacosanol	17.500	$C_{27}H_{56}O$	7.34
9-Hexacosene (9E)-9-Hexacosene	17.758	$C_{26}H_{52}$	1.04
Eicosane, 2-cyclohexyl	18.367	$C_{26}H_{52}$	0.66
Tetrapentacontane, -1,54-dibromo	18.483	$C_{54}H_{108}Br_2$	1.08
1-Heptacosanol	19.333	$C_{27}H_{56}O$	6.13
Heptacosyl heptafluorobutyrate	19.575	$C_{31}H_{55}F_7O_2$	0.82
Tetrapentacontane	20.233	$C_{54}H_{110}$	1.13
1,2 benzenedicarboxylic acid,disoocytl ester	20.725	$C_{24}H_{38}O_4$	10.70

Key: RT = Retention time, MF = Molecular formula

TABLE VI FATTY ACID PROFILE OF THE LEAVES OF *C. PROCERA*

Name of Compound	Retention Time	Molecular Formular	Peak Area %
Octacosanoic acid, (Montanic acid)	14.717	$C_{29}H_{58}O_2$	0.94
Eicosanoic acid (Arachitic acid)	12.767	$C_{20}H_{40}O_2$	0.72
n-hexadecanoic acid (Palmtic acid)	15.142	$C_{16}H_{32}O_2$	2.66
9-Octadecenoicacid, methylester (Oleic acid)	16.617	$C_{19}H_{36}O$	4.67
Octadecenoic acid methylester(Stearic acid)	16.925	$C_{19}H_{36}O_2$	3.86
6-Octadecenoic acid (Petroselnic acid)	17.025	$C_{18}H_{34}O_2$	2.17

TABLE VII FATTY ALCOHOL PROFILE OF THE LEAF OF *C. PROCERA*

Name Of Compound	Retention Time	Molecular Formular	Peak Area %
Pentadecanol	7.612	$C_{15}H_{32}O$	0.98
n-Nonadecanol	14. 192	$C_{19}H_{40}O$	1.04
Behenic alcohol (1-docosanol)	16.424	$C_{22}H_{46}O$	2.28
1-Heptacosanol	19.339	$C_{27}H_{56}O$	9.11
Hexadecen-1-ol	11.842	$C_{16}H_{32}O$	0.41

TABLE VIII: LONG CHAIN HYDROCARBON PROFILE OF THE LEAVES OF C.PROCERA

Nmae of Compound	RT	MF	PA%
Tetradecane	7.703	$C_{14}H_{30}$	1.44
3,3- dimethyl hexane	8.533	$C_{19}H_{40}O$	1.04
Heptadecane	9.092	$C_{17}H_{36}$	0.56
Hexadecane 3-Heptadecene, (Z)- Eicosane	10.152 11.642 11.908	$C_{17}H_{36}$ $C_{17}H_{34}$ $C_{20}H_{42}$	3.05 0.60 0.94
1-Nonadecene	13.125	$C_{19}H_{38}$	6.55
Octadecane	13.208	$C_{18}H_{38}$	0.56
9-Heptadecane	14.125	$C_{17}H_{34}O$	2.27
Icosane	14.408	$C_{20}H_{42}$	1.18
Tetrapentacontane, -1,54-dibromo	18.483	$C_{54}H_{108}Br_2$	1.08

Key: RT=Retention time, MF= Molecular Formular, PA = Peak Area

IV. CONCLUSION

In this study the phytochemical screening of the methanol extract of the leaves showed the presence of secondary metabolites alkaloids, flavonoids, tannins, steroids, terpenoids and saponins. These constituents may be responsible for the antimicrobial and antimalarial activities of the *C. procera*. Investigation of the volatile components using GC-MS has revealed the presence of components in the essential oil which may be of medicinal value. The leaves of *C. procera* from Navy Estate, Karshi, Abuja, Nigeria contained essentially aliphatic hydrocarbon, fatty alcohol, long chain fatty acids and their derivatives and some aromatic compounds including naphthalene, a thymol, phenol, carvacol and a phthalate. From the characteristics of the essential oil it showed that C. *procera* is reach in oleic acid which could be used as a component in many foods and the fight against hypertension by reducing blood pressure. These finding support the ethno medicinal uses of the leaves of *C. procera*. The preliminary phytochemical investigation of the extract which revealed the presence of alkaloids, flavonoids, steroids, tannins, terpenoids and saponins in the methanol leaf extract may contribute to the biological activity of the leaf.

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