School of Pharmacy

PHYTOCHEMICAL COMPOSITION, ANTIOXADANTa n d ANTIMICROBIAL OF *Calotropis procera (*AIT.) LEAVES.

This research project submitted in partial fulfillment for the requirement for the bachelor's degree (Honor Degree)

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بسم الله الرحمن الرحيم

قال تعالى

﴿فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَى إِلَيْكَ وَحْيُهُ وَقُلْ رَبِّ زِدْنِي عِلْمًا ﴾

صدق الله العظيم

سورة طه ، الآية 114

DEDICATION

We dedicate this thesis to my parents, wife, husban, dbrother and sisters for nursing me with affections and love and their dedicated partnership for success in my life

Acknowledgments

We begin with special acknowledgments to those who have made direct contributions to this paper. Foremost, we are

pleased to thank our professors, particularly, **Dr. Nahla Allahgabo** ,Phytochemist, department of pharmacognocy for the incredible support and Unmeasurable supervision, and we are very glad, to be honest, very lucky to have her as our supervisor.
We would also thank Medical Biochemistry Research Department Of Medicinal And Aromatic Plants Research Institute National Center For Research.

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Declaration

We hereby declare that the project report entitled by Calotropis procera ***(AIT.) LEAVES:***

(phytochemical composition ,ANTIOXADANT , ANTIMICROBIAL)
Submitted by us to Napta
Collage , School of Pharmacy for partial fulfillment of the
requirement for the award of the degree of bachelor from Napata collage , School of
Pharmacy. The record of project work had carried out by us under the supervision of **Dr**. **Nahla Allagabo**. We further declare that the work reported in this project have not been submitted and will not be submitted
either in part or in full, for the award ofany other degree or diploma in this
institute or any other institute or university.

Dr/ Nahla Allagabo

Omer Bashir Khlefa

Shaza Eltayeb Mohammed

Muzdalifah Abdellah Abdrazig

Abbreviation

picrylhydrazyl-2-diphenyl-1.1 ≡DPPH

MH A≡ Mueller Hinto nAgar

GC-MS ≡ Gas Chromatography-Mass Spectroscopy.

NCCLS ≡ National Committee for Clinical Laboratory Standards Guidelines

NIST ≡ National Institute of Standards and Technology .

C.P ≡ Calotropis procera

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Abstract

The world is fertile with natural and medicinal plants, which are now more focused than ever, because they have the capability of producing many benefits to society, indeed to mankind, especially in the line of medicine and pharmacology. The medicinal power of these plants lie in phytochemical constituents that cause definite pharmacological actions on the human body.

Calotropis procera (Ait.) leaves, from Asclepiadaceae family is a medicinal plant, which has been used for centuries as a traditional medicinal plant in different countries. This study aimed to the determination of chemical composition and evaluation of two pharmacological activities; antioxidant, and antimicrobial. Macerated methanolic extract was prepared.and then Gas Chromatography-Mass Spectroscopy profiling was done.GC-MS revealed a total of eleven compounds which were identified in the methanolic extract ranging from fatty acids and alkane compounds. The major constituents were– Phytol (26.60%), Tridecanoic acid (22.07%), and 9-Hexadecenoic acid(17.50%).

The antioxidant properties of the methanolic extract was screened using DPPH assay, and compared with Propyl gallate as standard. Methanolic extract showed significant scavenging activity (46±0.00), but revealed percentage inhibition lower than that obtained by Propyl gallate (92±0.01).

The disc diffusion method was employed to explore the antimicrobial activity of methanolic leaf extract agamicrobs ,the extract proved to be an efficient antimicrobial agent. The highest zone of inhibition observed was 19 mm obtained by P.aeruginosa ,whereas the less effective was against B.subtillis (12mm)at the concentration 10%,with the Staandard deviation(1.414)

The important role that C.procera plants play in folk medicine has led us to develop this study in order to investigate the major pharmacological activities, so this plant can be used in future as a valuable source of pharmacologically active antioxidants, antimicrobial agents.

Keywords: C.procera (Leaves); Gas Chromatography-Mass Spectrometry (GC-MS); Antioxidant Activity; Antioxidant Activity ,Ethnomedicine.

الملخص

إن العالم خصب بالنباتات الطبيعية والطبية ، والتي أصبحت الآن أكثر تركيزًا من أي وقت مضى ، لأن لديها القدرة على إنتاج العديد من الفوائد للمجتمع ، بل للبشرية ، خاصة في مجال الطب وعلم الأدوية. تكمن القوة الطبية لهذه النباتات في المكونات الكيميائية النباتية التي تسبب تأثيرات دوائية محددة على جسم الإنسان.

أوراق (.Calotropis procera (Ait ، من عائلة Asclepiadaceae هي نبات طبي ، تم استخدامه لعدة قرون كنبات طبي تقليدي في بلدان مختلفة. هدفت هذه الدراسة إلى تحديد التركيب الكيميائي وتقييم نشاطين دوائيين ؛ مضادات الأكسدة ومضادات الميكروبات. تم تحضير مستخلص الميثانول المتقرح ، ثم تم عمل تحليل كروماتوجرافيا الغاز - التحليل الطيفي الكتلي. كشف GC-MS عن إجمالي 11 مركبًا تم تحديدها في المستخلص الميثانولي تتراوح بين الأحماض الدهنية والألكان. المكونات الرئيسية هي فيتول (26.60٪) ، حمض التريديكانويك (22.07٪) ، 9-حمض هيكساديسينويك (17.50٪.(

تم فحص الخصائص المضادة للأكسدة للمستخلص الميثانولي باستخدام مقايسة DPPH ، ومقارنتها مع Propyl gallateكمعيار. أظهر المستخلص الميثانولي نشاط كسح معنوي (46 ± 0.00) ، لكنه أظهر نسبة تثبيط أقل من تلك التي حصل عليها.(92 ± 0.01) Propyl gallate

تم استخدام طريقة نشر القرص لاستكشاف النشاط المضاد للميكروبات لمستخلص أوراق الميثانول agamicrobs، وأثبت المستخلص أنه عامل فعال مضاد للميكروبات. كانت أعلى منطقة تثبيط تمت ملاحظتها هي 19 ملم التي تم الحصول عليها بواسطة P.aeruginosa ، بينما كانت أقل فعالية ضد.B

قادنا الدور المهم الذي تلعبه نباتات C.procera في الطب الشعبي إلى تطوير هذه الدراسة من أجل التحقيق في الأنشطة الدوائية الرئيسية ، لذلك يمكن استخدام هذا النبات في المستقبل كمصدر قيم لمضادات الأكسدة النشطة دوائيًا ، والعوامل المضادة للميكروبات.

الكلمات المفتاحية) C.procera :أوراق) ؛ اللوني للغاز - مطياف الكتلة (GC-MS) ؛

النشاط المضاد للأكسدة؛ نشاط مضادات الأكسدة ، الطب الإثني.

Chapter One

Introduction

1.1. Introduction

The world is fertile with natural and medicinal plants, which are now more focused than `ever, because they have the capability of producing many benefits to society, indeed to mankind, especially in the line of medicine and pharmacology. The medicinal power of these plants lie in phytochemical constituents that cause definite pharmacological actions on the human body.

Phytochemical, natural compounds occur in plants such as medicinal plants, vegetables, and fruits that work with nutrients and fibers to act against diseases or more specifically to protect from them (1).

Many countries in the world, that is, two-third of the world's population depends on herbal medicine for primary health care (2). The search for new compounds that may be useful as a source of medicine has aroused the interest of many researchers who study plants and biologically active compounds, especially plants that are used by people since ancient times and are perpetuated in different cultures (3). Calotropis procera is a member of Asclepiadaceae family. It is a soft-wooded, evergreen, perennial shrub. It has one or a few stems, few branches, and relatively few leaves, mostly concentrated near the growing tip. The bark is corky, furrowed, and light gray. A very toxic and viscous milk like liquid (latex) flows whenever stems or leaves are cut. It is widely distributed in Africa, Asia, and America (4). Because of the different activities of C.procera, it stands out now in ethno pharmacology as a major source of compounds that can be used in the future as new drugs (3).

It has many important secondary metabolites including, cardenolides, steroids, tannins, glycosides, phenols, terpenoids, sugars, flavonoids, alkaloids, and saponins. According to many

reports, it has many pharmacological activities such as antimicrobial, anti-inflammatory, analgesic, anticancer, antidiabetic, antioxidant, hypolipidemic, (4) and cytotoxic activities (5).

Antioxidant refers to a molecule that inhibits oxidation (a chemical reaction that transfers electron or hydrogen from substance to an oxidizing agent) of other molecules (6) antimicrobial is destroying or inhibiting the growth of microorganisms and especially pathogenic microorganisms.

1.2. Justification:

Sudanese medicinal plants are divers and widely used in folk healing, but still there is need for systematic and deep studies about their biological activity and phyto constituents. Calatropis procera is a very common plant but despite of it wide applications in Sudanese folk medicine, there is still a paucity of information regarding its secondary metabolites contents. This fact has spurred us to initiate a comprehensive phytochemical and biological investigation of this plant.

.The lack of treatment of infectious diseases in developing countries is a tragic problem, and one of the most serious challenges, that the world must face at the beginning of this century. Despite progress made in the basic knowledge of many infectious diseases, many of these have continued to cause significant morbidity and mortality Shakya et al. (7)

However, over the past few decades, many of commonly used antibiotics and other drugs have become less and less effective against certain illnesses, not only because many of them produce toxic reactions, but also due to emergence of drug-resistant pathogenic microorganisms.

1.3. Literature Review

1.3.1. Sudanese medical plants

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesise hundreds of chemical compounds for functions including defence against insects, fungi, diseases, and herbivorous mammals. Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. Further, the phytochemical content and pharmacological actions, if any, of many plants having medicinal potential remain unassessed by rigorous scientific research to define efficacy and safety

1.3.2. Calotropis procera

C.procera is a member of the Asclepiadaceae family which is derived from Asclepius the Greek god of medicine . The family Asclepiadaceae (the milkweed family) is now considered a subfamily of the Apocynaceae which is one of the most medicinally diverse families in the plant kingdom, and is a rich source for drugs that have found use both traditionally and in conventional medicine. The medicinal activity of these plants was due to the presence of alkaloids which were either indoline alkaloids or steroidal alkaloids .

1.3.3. Taxonomic Classification

Photo (1) : dry leave Calotropis Procera

Kingdom: Plantae. Division: Magnoliophyta.

Class: Magnoliopsida.

Order: Gentianales. Family: Asclepiadaceae Genus: Calotropis. Species: procera (4) Arabic Name: العشر Common Names: Dead Sea Plant, Debaj, Oshar, Kisher; Calotrope, Dead Sea Fruit, Desert Wick, Giant Milkweed, Swallow-wort, Mudar Fibre, Rubber Bush, Rubber Tree, and Sodom Apple.

1.3.4. Geographic Distribution

Calotropis procera is a plant widely distributed in Asia, Africa, and America [4]. It is native to West Africa as far south as Angola, North and East Africa, Madagascar, India, Pakistan, Nepal, Afghanistan, Algeria, Iran, Iraq, Palestine, Kuwait, Oman, Saudi Arabia, United Arab Emirates, Yemen, Vietnam, Niger, Nigeria, Kenya, Zimbabwe, southern Asia, and Indochina to Malaysia. The species is now naturalized in Australia, many Pacific islands, Mexico, Central and South America, and the Caribbean islands .

1.3.5. Botanical description

Description Calotropis procera is a soft-wooded, evergreen, perennial shrub. It has one or a few stems, few branches, and relatively few leaves, mostly concentrated near the growing tip. The bark is corky, furrowed, and light gray. A copious white sap flows whenever stems or leaves are cut. Giant milkweed has a very deep, stout taproot with few or no near-surface lateral roots. Giant milkweed roots were found to have few branches and reach depths of 1.7 to 3.0 m in Indian sandy desert soils. The opposite leaves are oblongobovate to nearly orbicular, short-pointed to blunt at the apex and have very short petioles below a nearly clasping, heart-shaped base. The leaf blades are light to dark green with nearly white veins. They are 7 to 18 cm long and 5 to 13 cm broad, slightly leathery, and have a fine coat of soft hairs that rub off. The flower clusters are umbelliform cymes that grow at or near the ends of twigs. The flowers are shallowly campanulate with five sepals that are 4 to 5 mm long, fleshy and variable in color from white to pink, often spotted or tinged 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 one end.

1.3.6. Phytochemical Constituents

The preliminary phytochemical screening of leaf powder of C.procera showed that the leaves contained cardenolides, steroids, tannins, glycosides, phenols, terpenoids, sugars, flavonoids,alkaloids, and saponins. The leaves also contained bitter compound (mudarine)

and manyglycosides; calotropin, uscharin, calotoxin, and calactin, and procesterol; a new steroidal hydroxyl ketone was isolated from the fresh and undried flowers of C. procera. Both leaf and stem contain

volatile oils octadecenamide and its saturated form in appreciable amounts. Also it is characterized by the presence of long chain fatty acids, amides, sulfurate, halogen compounds, and carbonyls like ketones. The fresh C. procera leaves produced volatile organic compounds that included thioacetic acid (4)

1.3.7. Pharmacological Actions

Many reports proved that C. procera has anti-helminthic, anti-inflammatory, analgesic and antipyretic, anticancer, anti-angiogenic, immunological, antidiabetic, cardiovascular, hypolipidemic, gastroprotective, hepatic protective, renal protective, antidiarrheal, antioxidant,

anticonvulsant, cytotoxic, enhancement of wound healing, antifertility and smooth muscle relaxant effect. The inner bark of C.procera is used to make strong fibers called madar which are used in the manufacture of weave carpets, ropes, sewing thread and fishing nets (14)

1.3.8. Antioxidant

Antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electron or hydrogen from substances to an oxidizing agent.

Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions, when the chain reactions occur in a cell, it can cause damage or death to that cell. Your body can also be exposed to free radicals from a variety of environmental sources, such as cigarette smoke, air pollution, and sunlight. Free radicals can cause “oxidative stress” a process that can trigger cell damage. Oxidative stress is thought to play a role in a variety of diseases including cancer, cardiovascular diseases, diabetes, Alzheimer’s disease, Parkinson’s disease, and eye diseases such as cataracts and age-related macular degeneration. Examples of antioxidants include vitamins C and E, selenium, and carotenoids, such as beta-carotene, lycopene, lutein, and zeaxanthin (16). There are different types of methods to evaluate the antioxidant activities, like Hydrogen Atom Transfer methods, Electron Transfer methods (ET) including, Copper (II) reduction capacity and DPPH free radical scavenging (6).

1.3.9. Antimicrobial activity

The term microbe refers collectively to the microscopic organisms, including bacteria, fungi, protozoa, and viruses that are harmful to the human (8)

Bacteria are single called microorganism that lack a nuclear membrane are metabolically active and divide by binary fission. Medically they are the major cause of diseases. Because of differences in pathogenicity or the necessity to characterize a disease outbreak, strains of medical interest are often classified below the species level by stereotyping, enzyme typing, identification of toxins or other virulence factors, or characterization of plasmids, protein patterns, or nucleic acid According to gram stain test bacteria can be classified into gram positive bacteria which have a cell profile of the gram positive type; cells may be spherical, rods or filaments. On the other hand, gram negative bacteria are a heterogeneity group of bacteria that have a complex (gram negative type) cell envelop consisting of an outer membrane, an inner, thin peptidoglycan layer and cytoplasmic numbering. The cell shape may be spherical, oval, straight or curved rods or helical Staphylococcus

It is gram positive cocci that are facultative anaerobe. S. aureus is the causative agent of many infections such as skin infections, acute osteomyelitis, sinusitis, severe pneumonia, septicemia, food poisoning.

Escherchia coli

It is gram negative straight, rod, appears single or in pairs, it is motile by flagella, although some strains are non-motile. E. coli commonly cause the urinary tract infections and diarrhea in infants and in travelers, in adult also case meningitis, septicemia as well as sepsis in operation wound and sepsis .

Pseudomonas aeruginosa

A gram negative bacillus, non sporing rod, actively motile by a polar flagellum, gross well at 37-42OC. P. aeruginosa cultured from the respiratory tracts of patients with cystic fibrosis or other obstructive air ways disease. Produce infection of wounds and burns, meningitis and urinary tract infection .

2.2.8. Previous Studies

Study 1

Phytochemical screening of C.procera ethanolic leaves extract, revealed the presence of alkaloids, terpenoids, flavonoids, saponins, and reducing sugar. (Estabraq et al., 2019), Whereas the GC-MS analysis of the ethanolic extracts indicated the presence of camphene 6.22%, thebaine 7.59%,dodecanoic acid 19.15%, and linolenic acid ethyl ester 14.87%. On the other hand, 70% ethanolic leaves extract showed different constituents which belongs to hexa-hydro-farnesol 9.87%, gamolenic

acid 12.71%, and linolenic acid ethyl ester 6.83% (8).

Study 2

Another study evaluated the antioxidant activity of C.procera comparing with anti-diabetic drugs using DPPH (Bajpai et al., 2018). In which the scavenging activity of Petrolum ether, hydroethanolic and aqueous extracts of leaf, root and stem of C.procera were investigated comparing with insulin, metformin, and pioglitazone. The result proved that all extracts have an antioxidant activities and the highest radical scavenging activity obtained from the leaf extract of pet ether 78%comparing with 80%, 70%, 60% from pioglitazone, metformin, and insulin, respectively. (10)

Study 3

Antibacterial activity of C.procera methanolic leaves extract was evaluated using disc diffusion method (Alzahrani et al., 2017). The test was employed against methicillin resistant Staphylococcus aureus. The zone of inhibition was 18 mm after 48 h of treatment with 50 µl of C.procera. Thus, the Total extract proved to be an efficient antibacterial agent (9).

1.4. Objective:-

1.4.1. General objective

The aim of this study is to investigate the phytochemical screening, antioxidant, anti-microbial activity of C.procera leaves Methanolic extract.

1.4.2. Specific objective:-

To extract active constituent from the leaves by maceration technique.

To determine phytochemical composition using GC-MS technique.

To determine anti -oxidant activity using DPPH assay.

To determine anti -microbial activity using disc diffusion method.

Chapter Two

Material and Methods

2.1. Study Design

Experimental study design

2.1.1. Study Area

The research was done in National Center for Research Institute of Medical Plants Research Laboratory Chemistry (for extraction, qualitative phytochemical screening, and antioxidant activity) ،University of Medical Sciences and Technology UMST (for GC-MS)

2.1.2. Study Duration

The research was conducted between - June to December 2021

2.1.3. Data Collection

2.1.3.1. Primary Data Collection

From manual and computerized experiments and observations.

2.1.3.2. Secondary Data Collection

From references (journals, published articles, books, and websites).

2.1.4. Data Analysis

Computer log probity regression analysis

2.2. Collection of the Plant

Leaves of the C. procera were collected from outskirts of, Khartoum state in june2 020, The plant was recognized and authenticated by one taxonomist from the Institute of the Medicinal, Aromatic Plants, and Traditional Medicine Research (MAPTMRI), Khartoum.
The plant was identified visually through the characteristics of leaves and flowers. The leaves were cut, washed, and shade dried. Plant materials were then milled using mortar and pestle.

2.3. Extraction

500g of dried powdered C. procera leaves were macerated successively in2000ml of methanol for three days at room temperature and filtrated three times .Then the solvents were removed and the extracts were concentrated to dryness through the rotatory evaporating . the extract was kept at room temperature for 24 hours for complete drying. stored in close petri dish at room temperature until further study.

2.4. Phytochemical Screening

Qualitative phytochemical screening analysis of the chemical constituents were conducted. Separation of the main classes of chemical constituents was obtained through extraction with solvents so as to correlate between the nature of the chemical constituents and the biological activities. Following tests were carried out to investigate the chemical constituents.

2.4.1. Test for Alkaloids

1ml of each extract was added to a test tube, and 2-3 drops of the dragendorff's reagent was added to each tube. Then, the changed color of each extract was observed.

2.4.2. Test for Tannins

1ml of each extract was added to the test tube, and 2-3 drops of 1% ferric chloride solution was added to each tube. Then, the changed color of each extract was observed.

2.4.3. Test for Flavonoids

1ml of each extract was added to the test tube, and 2-3 drops of 1% potassium hydroxide was added to each tube. Then, the changed color of each extract was observed.

2.5. Gas Chromatography-Mass Spectroscopy (GC-MS)

The qualitative and quantitative analysis of the sample was carried out by using GM/MS technique model (GC-MS-QP2010-Ultra) from japans 'Simadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 mm×0.25µm). The sample (C.procera) was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60c with rate 10c/min to 300c as final temperature degree with 17 minutes hold time, the injection port temperature was 300c, the ion source temperature was 200c, and the interface temperature was 250c.The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio, and the total run time was 41 minutes. Identification of components for the sample was achieved by comparing their retention index and mass fragmentation patents with those available in the library (the National Institute of Standards and Technology (NIST), results were recorded.

2.6 . Antimicrobial Activity

2.6.1. Microbial strains and cultures

Culture of four pathogenic strains *Bacillus subtilis* (NCTC 8236) , *Staphylococcus aureus* (ATCC 25923), Escherichia coli (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) were provided by microbiology laboratory staff by national center of research. Nutrient agar broth was used as the media for the culturing of bacterial strains. Aliquot of the broth bacterial cultures were inoculated in the nutrient broth and incubated at 37º C for 24 hrs.

The fungal strain *Candida albicans* (ATCC7596) was grown in the laboratory. Loops full of fungi was inoculated in Sabouroud dextrose agar and incubated at 25 °C for 4 days.

2.6.2. Disc diffusion method

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (What man No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

2.7. Antioxidant activity

The DPPH radical scavenging was determined according to the method of shimadaet.al(1992),with some modification. In 96-wells plate, the test samples were allowed to react with 2, 2Di(4-tert-octylphenyl)-1-pieryl-hydrazyl stable free radical (DPPH) for half an hour at 37 C. The concentration of DPPH was kept as (300 micrometer). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiple reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

Chapter Three

Results

3: Results

3 .1. Yield percentage of the extract:

Total weight of the plant =500 gm

Total weight crude extract = 17.981 gm

Yield extract = (17.981/500) x 100

Yield extract = 3.5%

3.2 . Physical proprieties of the extract

Table 1: Yield percentage and Physical Characteristic of Methanolic Extract of *C.procera* Leaves

Types of Extract	Yield %	Physical State	Color	Texture
MeOH	3.5%	Solid	Olive color	Smooth

Extractable matter percentage is one of the parameters used for the characterization of crude drugs. The investigated extract was in the average of 3.5 %, with certain physical properties. The variation in yield may be due to the polarity of the solvents used in the extraction process. The colorand texture of all types of extracts were also reported (Table 1).

3.2.1. Gas Chromatography-Mass Spectrometry (GC-MS)

In the present study, GC-MS procedure was applied for the identification of chemical profile of the methanolic extract. The relative retention times (Rt) and mass spectra of the extract components were compared with the standard mass spectra in the library and with data from the literature. Eleven compounds belonging to fatty acid and hydro alkane, were identified from the methanolic extract (Figure 1 and Table2). The major constituents were – Phytol (26.60%), Tridecanoic acid(22.07%), and 9-Hexadecenoic acid(17.50%)

Figure 1: GC-MS Chromatogram of *C. procera* Leaves Methanolic Extract

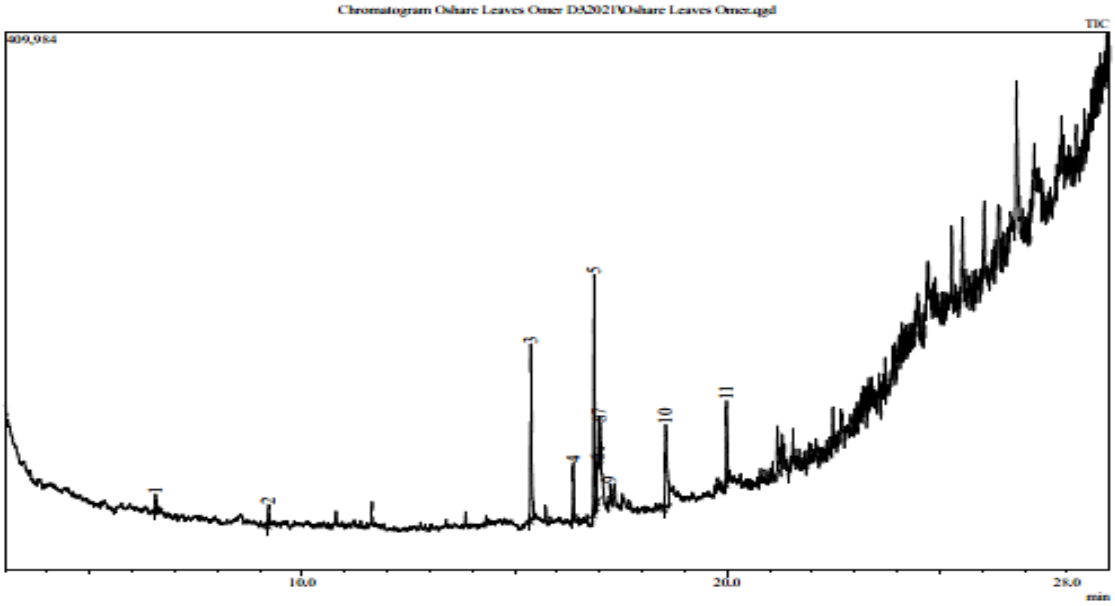


Table 2: Chemical Composition of *C. procera* Methanolic leaves Extract

Peak#	**Name**	**R.Time**	**Area**	**Area%**
1	3-Decyn-2-ol	6.544	18803	1.73
2	3-Trifluoroacetoxypentadecane	9.202	21047	1.94
3	Tridecanoic acid	15.377	239796	22.07
4	10-Oxocyclodec-2-enecarboxylic acid, methy	l ester16.374	66460	6.12
5	Phytol	16.862	289026	26.60
6	7-Oxabicyclo[4.1.0]heptane, 3-oxiranyl-	16.939	42148	3.88
7	cis,cis,cis-7,10,13-Hexadecatrienal	16.976	58117	5.35
8	Z,Z-8,10-Hexadecadien-1-ol	17.003	21773	2.00
9	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexy	l-, methyl este17.241	r 23034	2.12
10	9-Hexadecenoic acid	18.547	190148	17.50
11	Hexadecanoic acid, thanediy 1-(hydroxymethyl)-1,2- ester	19.980 l	116116	10.69

Figure 2: GC-MS Chromatograms of the Major Identified Components of *C. procera* Methanolic leaves Extract:-

Chromatogram of Decyn-2-ol:

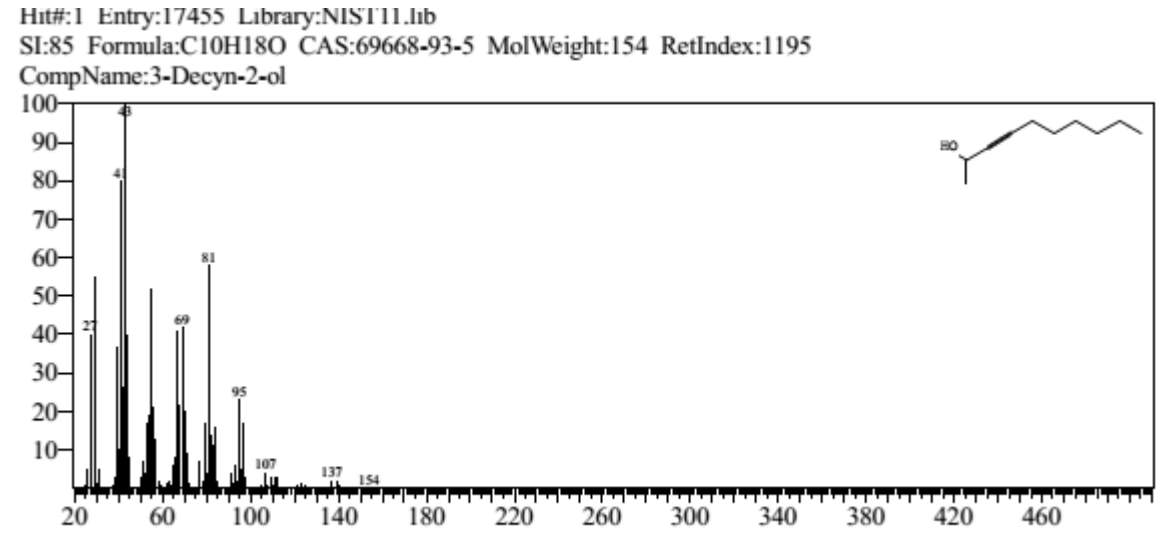


Figure 3: Chromatogram of Trifluoroacetoxypentadecane :

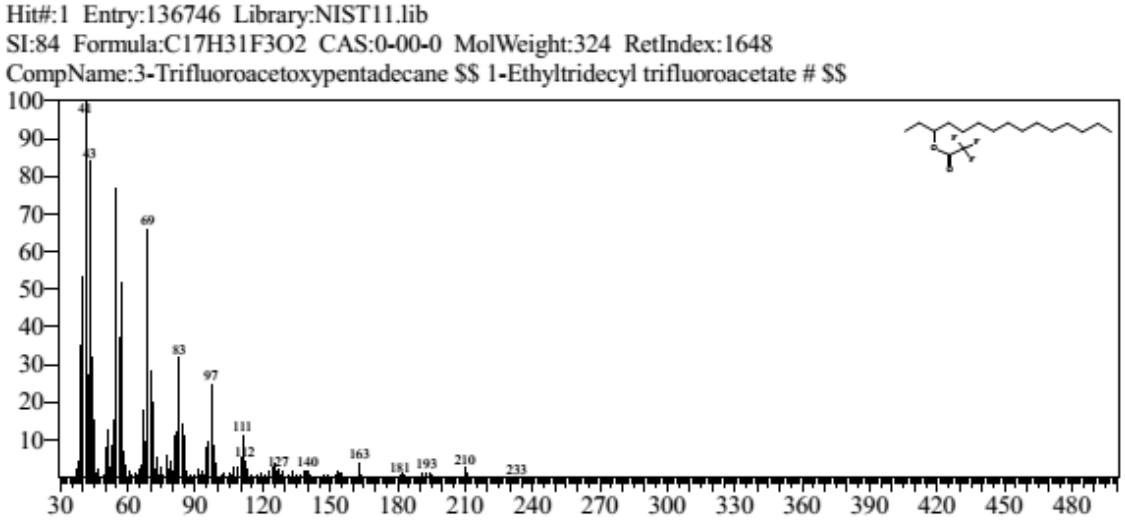


Figure 4: Chromatogram of Tridecanoicacid

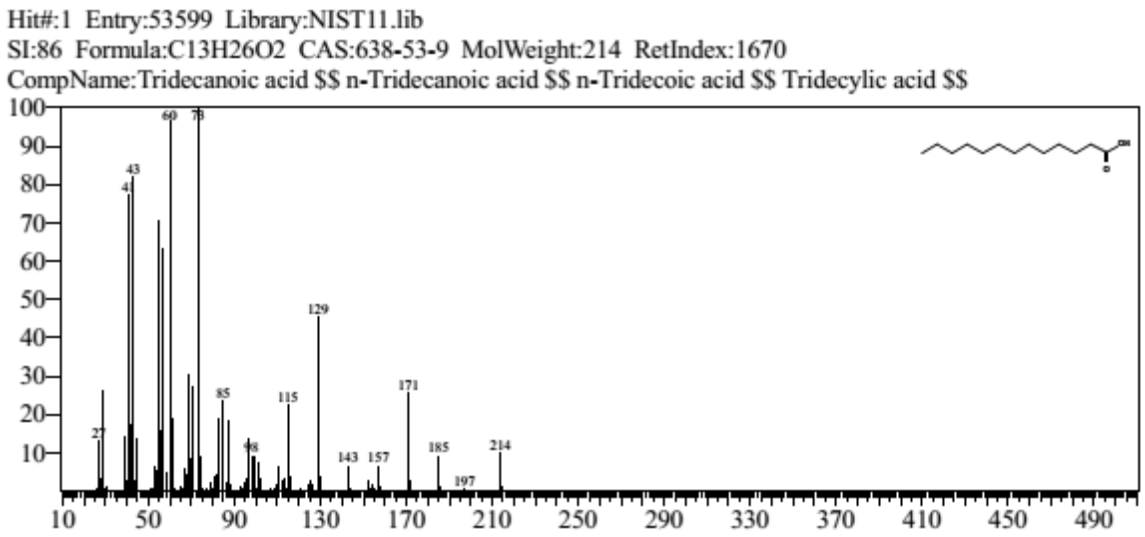


Figure 5: Chromatogram of 10-Oxocyclodec-2-enecarboxylic acid , methyl ester.

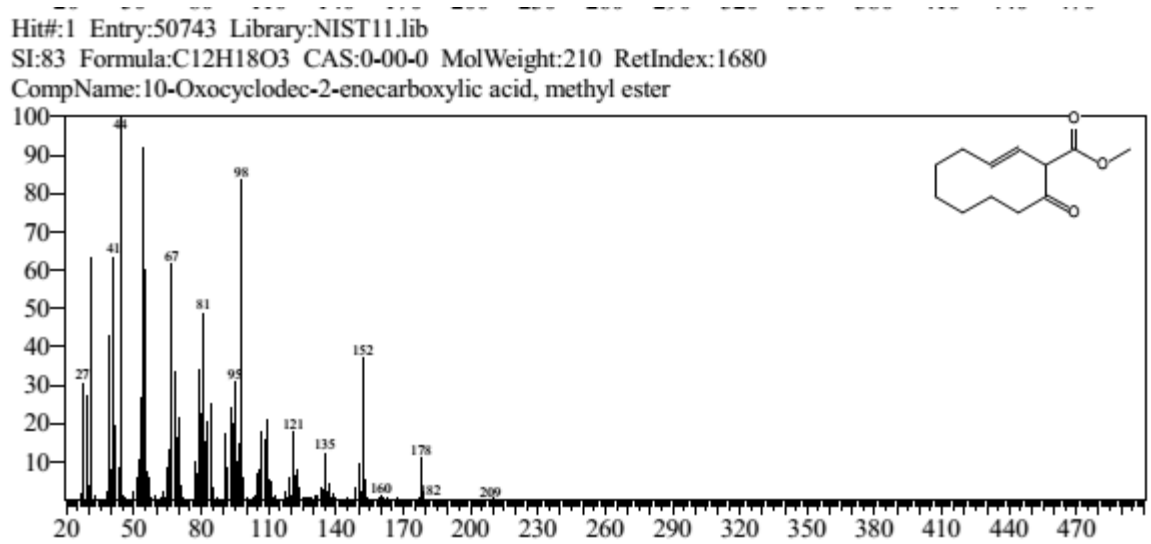


Figure 6: Chromatogram of Phytol

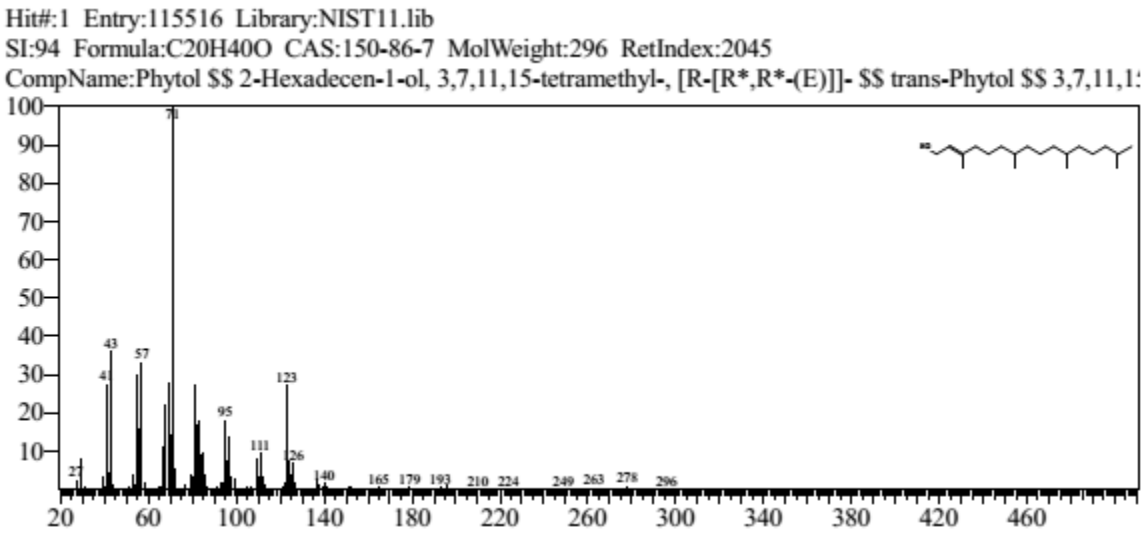
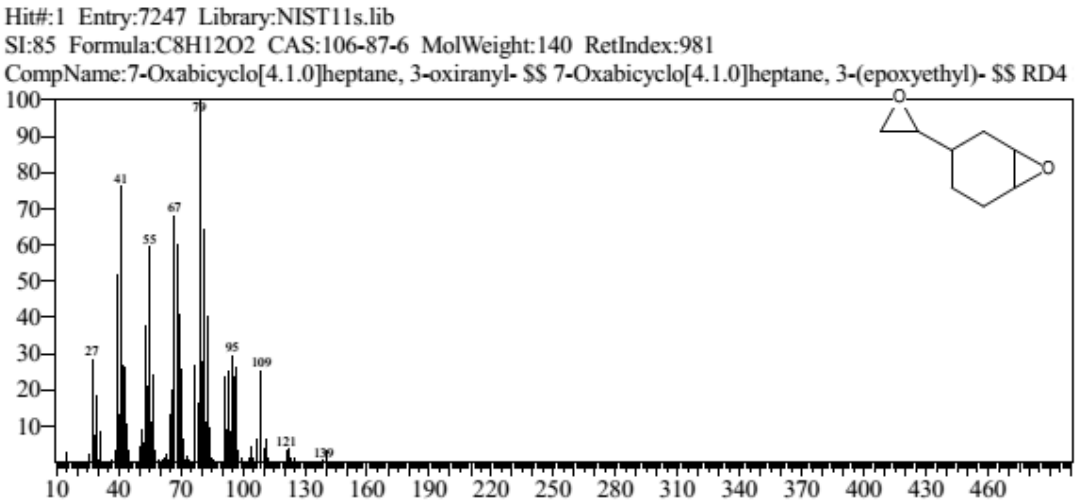


Figure 7: Chromatogram of 7-Oxobicyclo(4.1.0) heptane , 3-oxiranyl



3.3. Antioxidant activity

The free radical scavenging potential of the methanolic extract of *C.procera* leaves and percentage inhibition was determined *in-vitro* through the DPPH free radical-scavenging assay.

Results revealed that. the percentage inhibition of the Methanolic leaves extract of *C. procera* (46±0.0) compare to the percentage inhibition of the standard (92±0.01)(Table 3, and Figure 2).

Table 3: DPPH free radical scavenging activity of methanolic leaves extract of *C. procera*.

NO.	Sample	%RSA±SD
1	*C. Procera*	46±0.00
Standard	Propyl gallate	92±0.01

3.4. Antimicrobial activity

Disc diffusion method was employed to explore the antimicrobial activity of methanolic leaves extract of *C. procera* against some pathogenic bacterial strains ;Gram positive bacterial strains(*S. aureus* and *B. subtilis*), Gram negative (*E. coli*, and *P. aeruginosa*).

Results exhibited significant antimicrobial activity; against all the tested pathogenic microorganisms; with the zone of inhibition ranged 12-19mm.

(Figure 3 and Table3) .Strong inhibitory activity could be observed against ***P.aeruginosa*** (19 mm) whereas the less effective was against ***B.subtillis*** (12mm)with the Standard deviation(1.414)

Table 4: Antibacterial activity of methanolic extract of *C. procera* leaves

Extract conc. (mg/ml)	**Zone of inhibition ZD(mm)**				
	S.aureus	***B.subtillis***	***P.aeruginosa***	***E.coli***	***C.albican***
Methanolic extract	**15.5**	**12**	**19**	**18**	**12.5**

(-) ≡ not detected, (*) ≡ the average zone of inhibition

Figure 8: Antimicrobial activity of methanolic leaves extract of *C.procera*

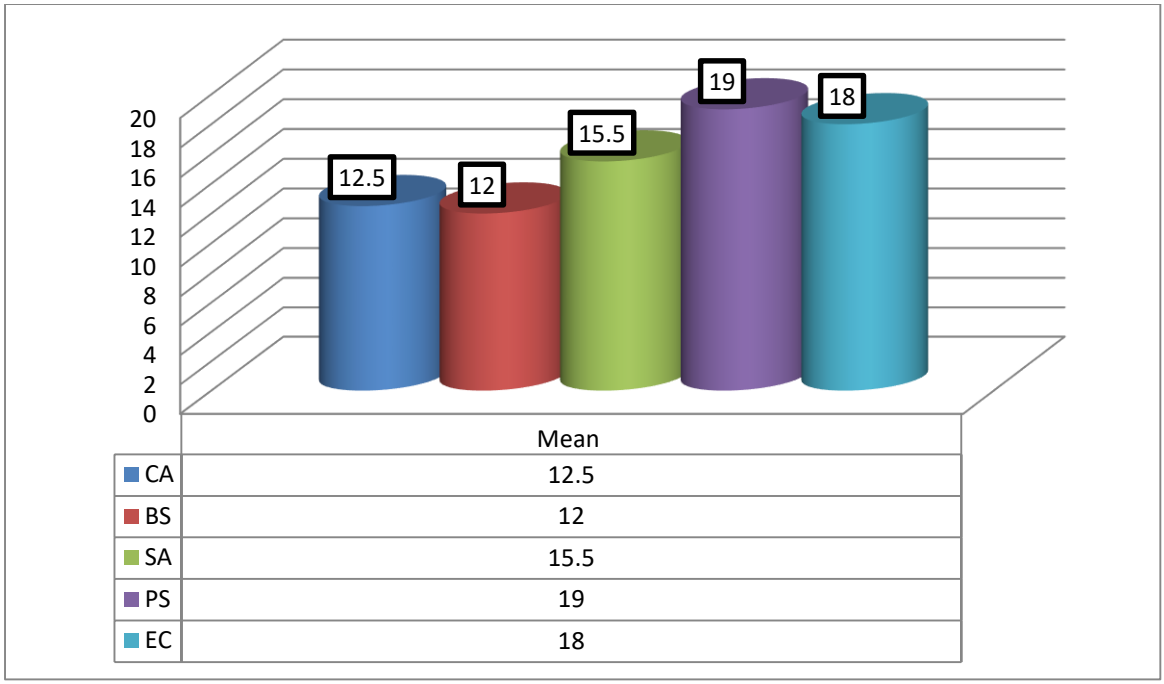


Table 5: Standard Deviation of the tested microorganisms

Statistics

	ZI_Ec	ZI_Ps	ZI_Sa	ZI_Bs	ZI_Ca
N valid	2	2	2	2	2
missing	0	0	0	0	0
mean	18.00	19.00	15.50	12.00	12.50
Std deviation	2.828	1.414	707	1.414	707

Chapter Four

Discussion and Conclusion

and Recommendations

4.1 Discussion

Plants belonging to the Asclepiadaceae family have a wide range of therapeutic activities. The genus Calotropis is used in traditional medicine for the treatment of leprosy, ulcers, and tumors, diseases of the spleen, liver, and piles. Also it acts as purgative, anthelmintic, antipyretic, analgesic, anti-inflammatory, and antimicrobial. The present study was conducted to determine the phytochemical composition, antioxidant activity, antimicrobial activities of methanolic extract of *C .procera* leaves.

The yield percentage of the extract was obtained3. 5 %. The color and texture of extract was also determined. Previous studies showed that variation in color, texture, and the number of bioactive phytochemicals produced by plants depends on the age of the plant, nature of the soil, and processing of plant material.

The analysis of methanolic extract, which occurred by using

GC-MS technique, displayed about eleven different compounds in the *C. procera* leaves extract.depending on the extraction method, the major are The major constituents were – Phytol (26.60%), Tridecanoic acid(22.07%), and 9-Hexadecenoic acid(17.50%) In contrast, Naser *et al.,* (12) demonstrated the presence of camphene 6.22%, thebaine 7.59%, dodecanoic acid 19.15%, and linolenic acid ethyl ester 14.87% in the methanolic extract.

The Major constituents in *Calotropis sp.* and presence of these phytochemicals sturdily acknowledge the medicinal property of this plant (15). In contrast (16), reported that, these bioactivities of the plants, are attributed to the presence of chemical components such as alkaloids, phenolic compounds, flavonoids, tannins, and others.

Methanolic xtract, showed significant scavenging activity, (46±0.00) but revealed percentage inhibition lower than that obtained by Propyl gallate (Standard). In line with this observation (20) reported that, the aqueous

methanol extract of *C.procera* leaves, exhibited a significant scavenging activity against DPPH radicals (64.7%) at concentration above 100 mg/ml. Previous studies demonstrated that, the antioxidant activity was mainly attributed to the phenolic content of extracts which could be obtained with methanol system. Also several studies have reported variations in the biological activities of extracts prepared using different extraction techniques (21).

On the other hand (22) reported that, factors like stereo selectivity of the radicals or the solubility of the extract in different testing systems affect the capacity of extracts to react and quench different radicals.

In The disc diffusion method was employed to explore the antimicrobial activity of methanolic leaf extract of *C. procera* against some bacterial pathogenic strains. Strong

inhibitory activity could be observed against ***P.aeruginosa*** (19 mm) whereas the less effective was against ***B.subtillis*** (12mm) with the Standard deviation (1.414).

Drug resisting microbes are of immense concern globally (17). The fact that new cases are emerging and the increase in demand for natural products with antimicrobial activity, is pushing the scientific community to identify natural compounds isolated from medicinally important plant (18). Total extract of *C. procera* proved significant in restricting the growth of some bacterial strains, as observed in our disc diffusion method.

Our preliminary data substantiates the importance of the methanolic leaf extract of *C. procera* as both antioxidant and antimicrobial agent. It effectively inhibited the growth of some microorganisms, which is a potential threat to humans due to its resistance to numerous antibiotics. This is not surprising, because all the leaves extracts tested positive for tannins which had been implicated in previous studies to be anti-microbial. It is very probable that these bioactive compounds might have played a significantly similar role in the observed activity (19) Furthermore, these results have partly justified some of the uses of the plant in ethno-medicine.

4.2. Conclusion

Plant products have played an important role in the discovery of new therapeutic agents since ancient times. *Cotropis procera* is widely distributed geographically has many pharmaceutical importance that needs to be exploited by researchers for new drug discoveries. Extracts from this plant, have many phytochemical constituents, which make it good candidates for drug development.

Our preliminary data substantiates the importance of the methanolic leaf extract of *C.procera* as both antioxidant and antimicrobial agent. So, we have clearly shown that, this extract displays these antioxidant, and antimicrobial activities, due to the presence of some hydroalkane and fatty acids contents which are detected by phytochemical analysis. The study also provides a deep information about the role of the Natural products which represent a rich source of antimicrobial agents with a low level of toxicity, a broad spectrum and sufficiently good pharmacokinetics to be clinically useful without chemical modification.

4.3. Recommendations

A**c**cording to the results obtained in the current study the following recommendations were suggested

1. Further studies are needed to confirm these biological properties of *C.alotropis procera* leaves.
2. Further research is therefore recommended to isolate, purify, and characterize these chemical constituents with a view to supplementing conventional drug development especially in developing countries.
3. The broad biological profile shown by this plant, should be operated by the pharmaceutical industry for the development of new drugs, so the therapeutic arsenal for many diseases could be extended to benefit humanity.
4. Further, *in vivo* studies are required to use the natural antimicrobial agents with new or modified mode of action to overcome the problem of microbial resistance to the chemical antibiotics.
5. More research on the toxicological properties of the identified compounds and in vivo confirmation of their side effects would be necessary for a full evaluation of their practical usefulness in the field of modern medicine.

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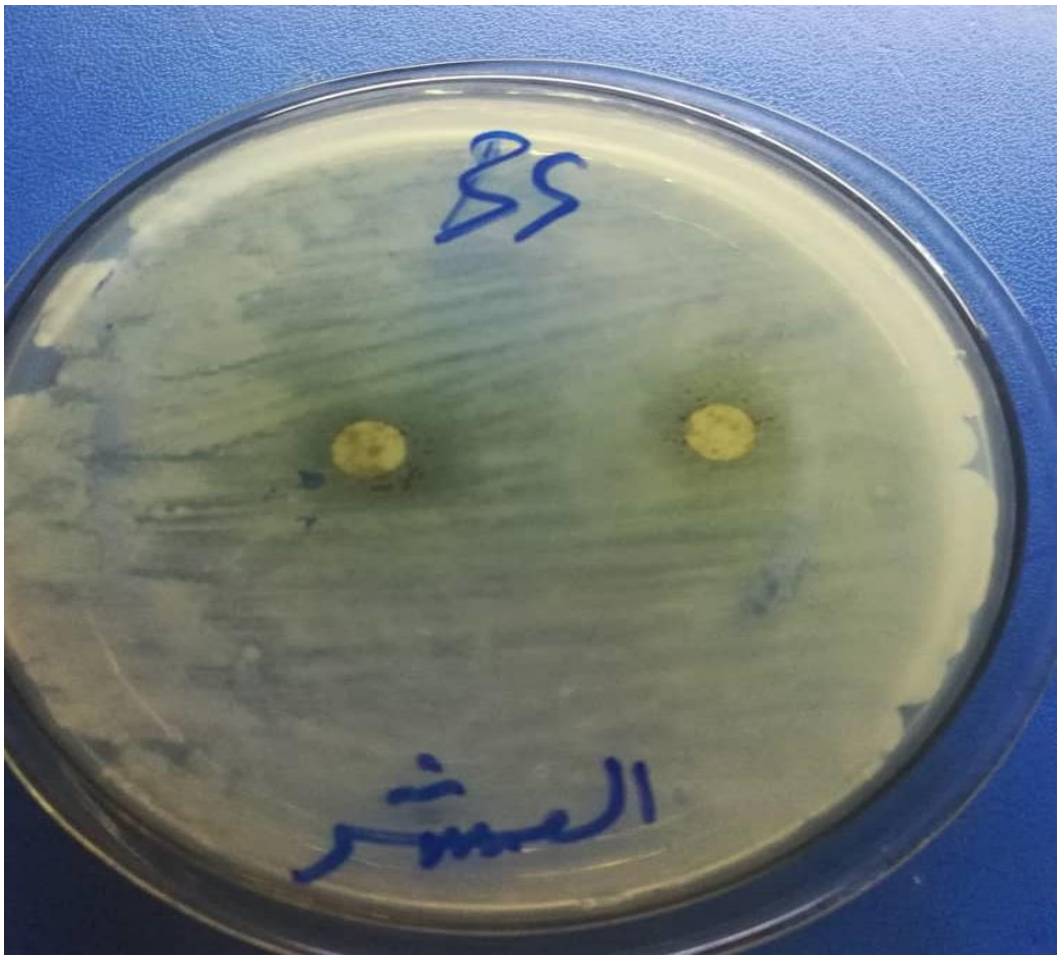
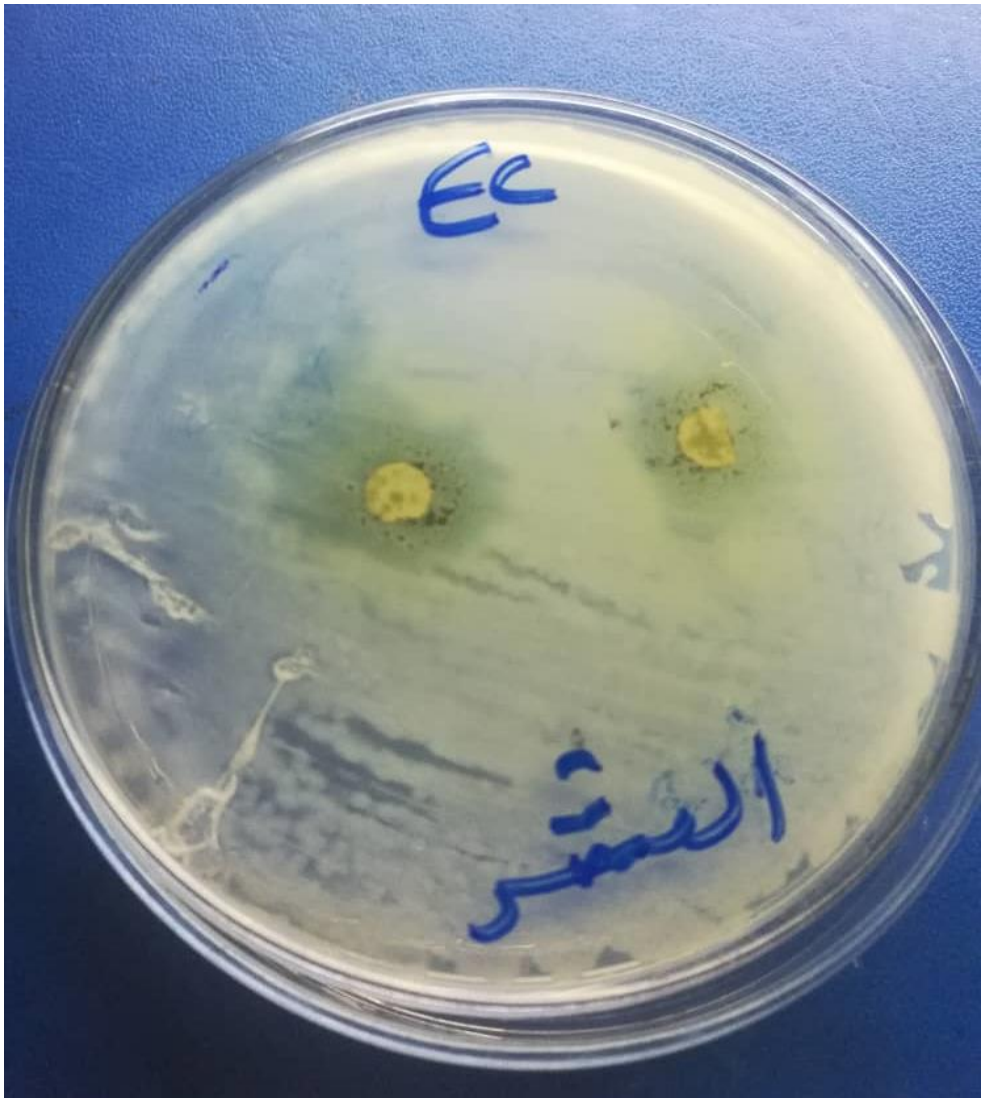
Appendix

Appendix 1: Maceration Extraction of *C.Procera* Leaves.

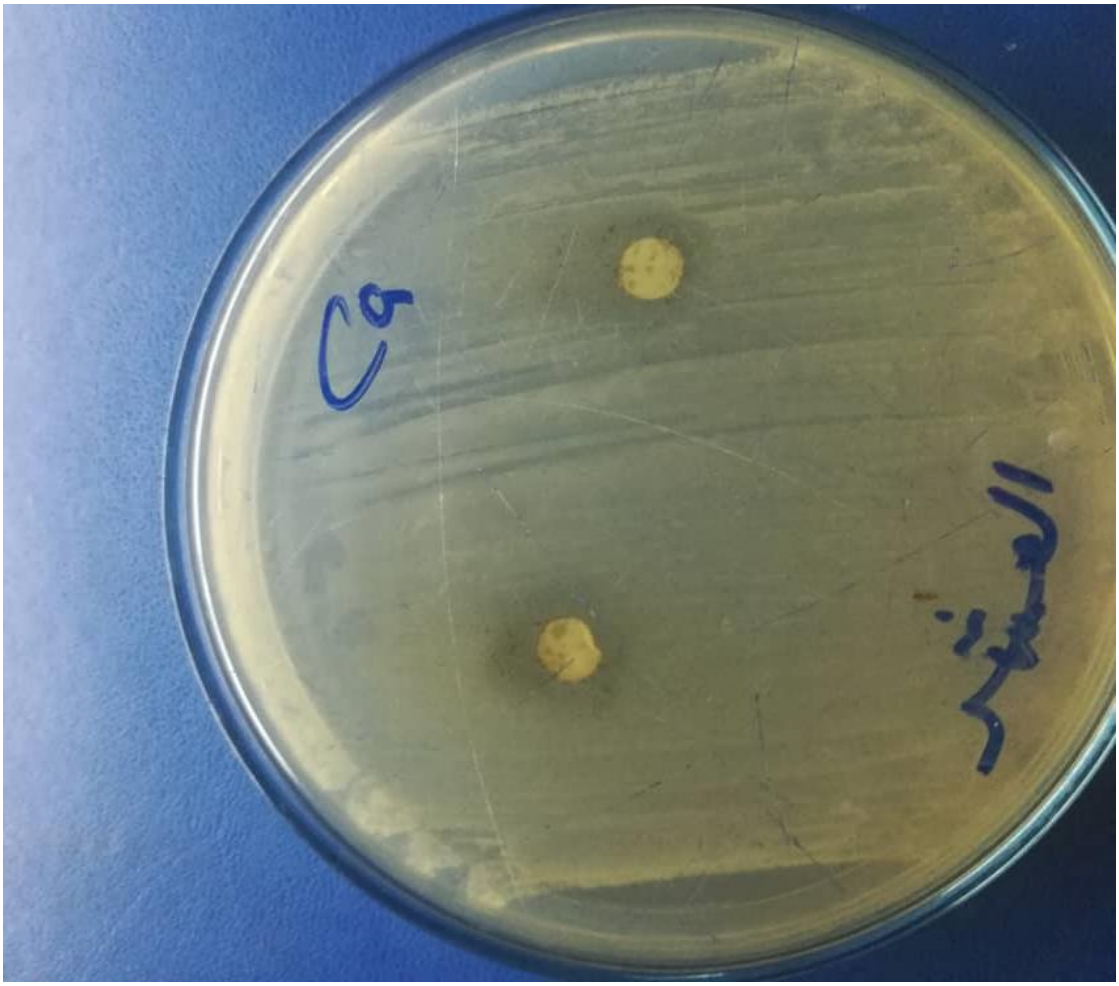
Appendix 2: *Bacillus Subtilis* In (Mha)



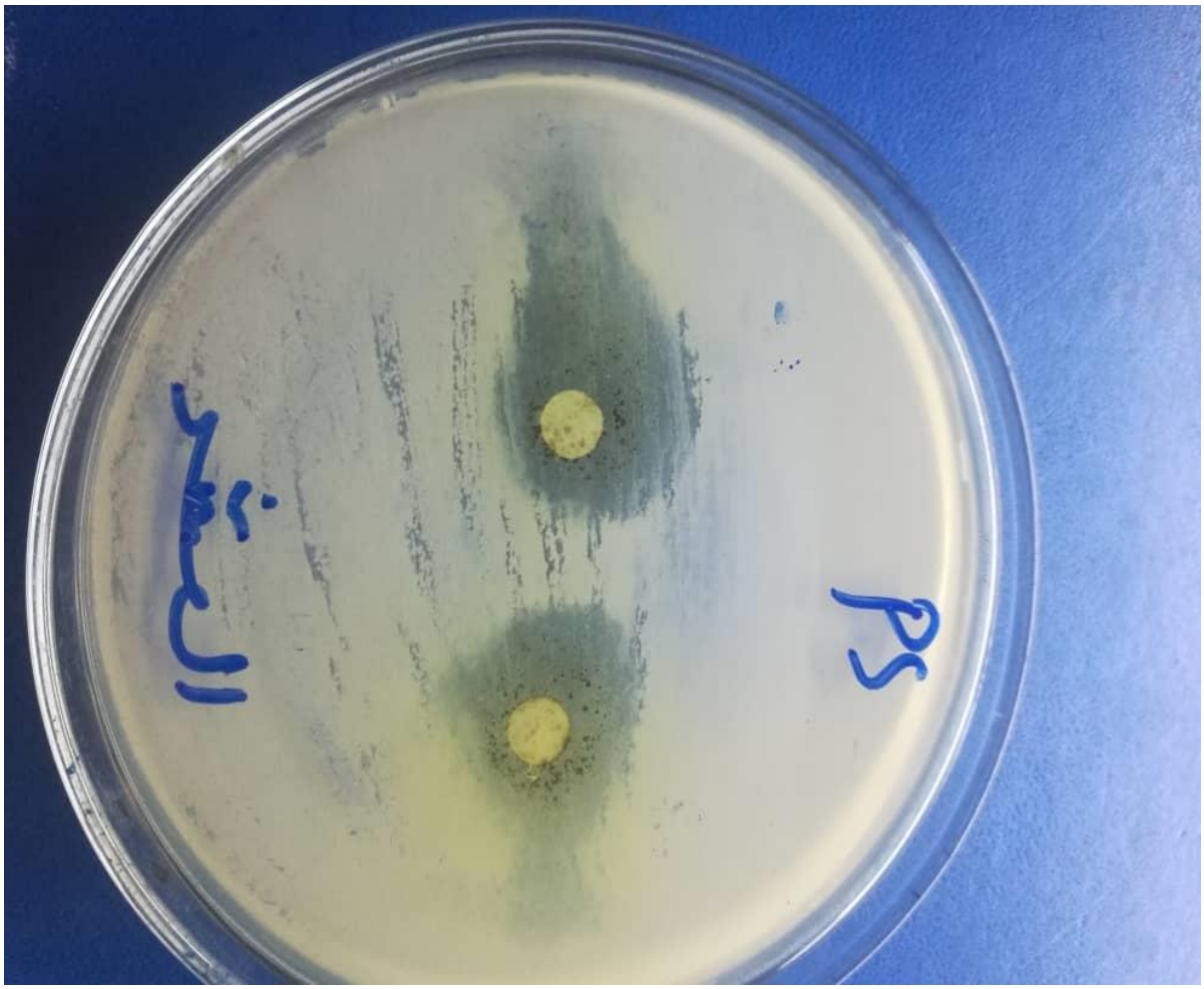
Appendix 3 : *Escherichia Coli* In (Mha)



Appenddix 4 : *Candida albicans* In Sabourud Dextrose Agar



Appenddix 5: *Pseudomonas aeruginosa* In (Mha).



Appenddix 6: *Staphylococcus aureus* In(Mha).