PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT EVALUATION OF ETHANOL LEAF EXTRACT OF CALOTROPIS PROCERA (BOMUBOMU)

BY

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CERIFICATION

This is to certify that this project work is the original work carried out and reported by TITILOYE ANIFAT OLAITAN with Matriculation Number HND/23/SLT/FT/0947 of the Department of Science Laboratory technology, Institute of Applied Sciences (IAS) Kwara State Polytechnic, Ilorin and it has been Approved In Partial fulfilment of The Requirements of the Award of Higher National Diploma (HND) in Science Laboratory Technology (**BIOCHEMISTRY**)

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DEDICATION

This research work is dedicated to the Almighty Allah, the creator of heaven and earth. I also dedicate it to my beloved parent **MR. AND MRS. TITILOYE**

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ABSTRACT

The present study investigates the phytochemical composition and antioxidant activities of ethanol extracts derived from the leaves of Calotropis procera (commonly known as Bomubomu), a medicinal plant widely recognized in ethnobotanical applications. Qualitative phytochemical screening was conducted to identify the presence of bioactive compounds such as alkaloids, flavonoids, tannins, phenolics, saponins, glycosides, and terpenoids. Quantitative assays were also performed to estimate total phenolic content (TPC) and total flavonoid content (TFC), using gallic acid and quercetin as standards, respectively. The antioxidant potential of the ethanol extract was evaluated through multiple in vitro assays, including 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and ferric reducing antioxidant power (FRAP). Results revealed a rich phytochemical profile and significant antioxidant capacity, which suggests that C. procera leaf extract possesses considerable free radical scavenging abilities, potentially attributable to its phenolic and flavonoid constituents. These findings support the traditional use of Calotropis procera in herbal medicine and underscore its promise as a natural source of antioxidant agents for pharmaceutical and nutraceutical applications. Further investigations involving compound isolation and in vivo efficacy studies are recommended to validate and expand upon these results.

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CHAPTER ONE

1.0. INTRODUCTION

1.1 Background to the Study

Medicinal plants have been a vital source of therapeutic agents for centuries, providing remedies for various ailments. Among these, *Calotropis procera* (commonly referred to as "Bomubomu" in Southwestern Nigeria) is a traditional plant with recognized ethnopharmacological uses. It belongs to the family Apocynaceae and has been used in traditional medicine for the treatment of inflammation, pain, skin diseases, and respiratory disorders. The bioactive compounds in plants such as flavonoids, alkaloids, tannins, and phenolic compounds are known to exert antioxidant activities that help combat oxidative stress-related diseases.

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and endogenous antioxidants, has been implicated in several pathological conditions including cancer, aging, cardiovascular, and neurodegenerative diseases. The search for natural antioxidants is therefore of paramount importance. This study aims to evaluate the phytochemical constituents and antioxidant potential of ethanol extract of *Calotropis procera* leaves using standard qualitative and quantitative methods. Calotropis procera belongs to the family Asclepladaceae which is an important plant with medicinal properties. It is known by various synonyms in English, calotrope, calotropis, dead sea fruit, desert wick, giant milk weed, swallow wort, mudar fiber, rubber bush, rubber tree, Sodom apple. (Gupta S. et al, 2012). French (pomme de sodome, algodon de seda, arbre a soie, cotton soie, arbe a soie du Senegal).

In Nigeria, it is known by various name e.g. in Hausa (Tumfafia), Kanuri (Kayou), Igbo (Kausa), Yoruba (bomubomu), Hindi (madar, akada, akdo, aak), Italian (calotropo), Mandinka (kipapa), Sanskrit (alarka) Somali (boah), Spanish (bomba, aladon extrajero, cazuela), Swahili (mpamba mwitu), Tamil (vellerukku), Tgrigna (dinda, ghindae, akalo), Wolof (faftan). It is found in most part of the world, especially in warm climate usually growing in dry, sandy and alkaline soil. Calotropis is primarily harvested because of its distinctive medicinal properties.

Calotropis procera is a draught resistant, salt tolerant weed found along degraded road sides, lagoon edges and over grazed pastures native to tropical Africa including Nigeria, Asia and Latin American where the plant is of high socio-economic value. Calotropis procera extract are reported to have antioxidant, antimicrobial and cytostatic properties (Kumar VL, Aryas, 2006). Calotropis procera is a perennial xerophytic woody shrub which is able to thrive in harsh conditions of heat, drought and poor soils. Also, it has captured the attention since and on the other hand, it has a great value as a medicinal plant with notable importance in folk medicine, its cardiac glycoside content and the ability to act as a pesticide. Researches have discussed its potentials to be source of renewable energy and hydrocarbon. (Erdman MD, Erdman BA).The plant is known generally to tolerate habitats with annual precipitation of 150-1000mm per year and temperature range of 20-30°C. The leaf blades are light to dark green with nearly white veins, slightly leathery, and have a fine coat of soft hairs that rub-off. Its leaves are pubescent when young and globous on both sides on maturity. They have a waxy appearance and contain a white milky sap. Leaves are large up to 15cm long and 10cm broad with no leaf stalk. The flowers are regular, bisexual with a faint odor, they are borne in clusters of ovoid flower buds in the forks of the uppermost leaves, and each cluster contains 3-15 flowers. The fruits are follicle (8-14, 6-9cm) sub-globose to obliquely ovoid.

Its apex is rounded green, spongy, and smooth that split and invert when mature to release seed (Sharma R .2012, Thakur GS, *et al*. 2012).

1.2. MEDICINAL IMPROTANCE

Member of the family Asclepladaceae secrete milk-like latex that is known traditionally to heal wounds and stop bleeding of fresh cuts. (Boulos L, 2000).Therefore, (Shiva Prasad, *et al*) have tested the scientific validity of this knowledge on four plant species to find that the latex exhibited significant abilities of both blood coagulation and thinning, meaning the latex contains thrombin like enzymes responsible for clot formation and plasmin like action dissolving it.

Member of this family are also known for treating heart failure since they are rich in cardiac glycosides. Cardiac glycosides are cardio active compounds belonging o triterpenoids class of compounds, helps in cases of heart failure by increasing heart contraction strength. (Brian FH, *et al*., 1985). Moustafa, et al have studied the cardiac glycoside effect extracted from the aerial parts of calotropis procera on several muscles, along with one of flavonoids and saponins, they indicated a direct action of the extract on the myocardium as it increases heartbeats and heart contractions, moreover, the compound has increased the motility of smooth muscles as mentioned by (Brian *et al*., 1985) and on the other hand, it had a relaxing effect on the contracted skeletal muscles. Those effects were attributed to the glycosides actions on neutral tissues which influence the mechanical and electrical activities of the heart improving vascular resistance. Further studies on cardiac glycosides have also proven anticancer properties in, Ibrahim *et al,* (2014), have tested the methanolic extract of the root barks of calotropis procera on lung and prostrate cancer cell lines showing remarkable effect compared to the positive control cisplatin. Besides, (Nalini, *et al*., 2016)

have confirmed the anti-cancer properties of Calotropis procera, stating that it acts as ant glioblastoma against LN-18 cancer cells, they declared that it has potential application can be incorporated in developing anti-cancer drug Kazeem, *et Al.*,(2016), have evaluated the antidiabetic character of calotropis procera leaf extract, it revealed an inhibitory effect of carbohydrate hydrolyzing enzymes, alpha-amylase and alpha-glucosidase. Similarly Abd-Alrheam & Shehri (2015), have successfully used leaf extract as antidiabetic agent on male albino rats, they also recorded significant decrease in levels of serum cholesterol and triglycerides, which probably an effect of saponins content that has potential effect in the treatment of hypercholesterolemia by interfering with intestinal absorption of cholesterol. (Mallinow M R, *et al*., 1997).

On the other hand calotropis procera was found to be highly toxic when introduced to mice food, resulting in high absorption rates (Faye B (1985)).Ahmed, *et al*.,(2016) have assessed the toxic effect of latex and ethanolic leaf extract of calotropis procera, on the heart of testes of a male albino rats, proving high toxicity and recommending the use of calotropis procera as rodent control. The leaf, stem and root are also utilized in traditional medicine for treatment of sores skin disease, diarrhoea, sinus, fistula and jaundice. Extracts from plant is reported to relieve stomach pain. The sap is used for treating eye infections, and the bark of the plant is used for treatment of coughs, elephantiasis, leprosy and ulcers. (Chandra RK.,1990). The stem is utilize in native roofing of huts and also serve as source of charcoal (Ogunlesi M, *et al.*,2008). Occasionally goats and sheep eat the leaves, cattle and other livestock avoid it because of pungent smell and toxicity. (Taylor L, 2004).

1.3. CALOTROPIS PROCERA AS PESTICIDES

Studies have determined that some botanical compounds such as alkaloids, nicotine, anabasine and lupitin in the extract of calotropis procera latex produced high mortality against mosquito larvae, which make it very efficient in the control of several mosquito species. Additionally, the plant was discovered to be highly toxic to the white garden terrestrial snail thepapisana, by the effect of uscharin that was extracted from the latex. (Hussein HI, Kamel *A. et al*)

1.4. CALOTROPIS PROCERA AS RENEWABLE ENERGY SOURCE

Erdman MD and Erdman BA in 1981, suggested that hexane extraction of calotropis procera could be used as substitute for petroleum or petrochemical feed stocks, they claimed that calotropis procera yield high density fluid, rich in hydrocarbon. In their study the ratio of carbon and hydrogen in the extraction were similar to crude oil and the heat value content was comparable to crude oil, fuel, oil and gasoline. Besides, (Barbosa MA, *et al*; 2014) have implied the high concentration of seed oils and fatty acids from calotropis procera in different localities in Brazil, they suggested that oil contents and total biosynthesis of saturated fatty acids have been increased in temperature and drought and finally concluded that calotropis procera has potential as biodiseal feed stock.

1.5. EFFECT OF CALOTROPIS PROCERA

Calotropis is UNSAFE, especially in high doses. It contains chemicals that can interfere with heart function, particularly at high doses. It can cause serious side effects including vomiting, diarrhea, slow heartbeat, convulsions, and death. The plant is proven as toxic, and it is one of the plant not eaten by grazing animals. The latex from the plant is used by the tribal people to make

poison arrows used for hunting purpose. The latex is highly toxic to humans eyes, it causes ocular toxicity and produces loss of vision with photophobia.

Latex of calotropis procera was studied for it's inflammatory effect using pedal edema and air pouch models of inflammation in rats and could be used to evaluate anti-inflammatory drugs. Furthermore, latex also produces toxics iridocyclitis, keratoconjunctivitis, corneal endothelial cytotoxicity, and keratitis when applied accidentally on the eye.

In a study, DL and flowers of calotropis procera and its ethanolic extract were evaluated against MCF-7 and Hela cells line cultures against the MTT assay to determine the inhibitory effects of test compounds on cell growth in vitro. The standard drug tamoxifen inhibits 60.46% breast cancer (MCF-7) cells, whereas the ethanolic extract of DL and flowers showed cytotoxic properties against both MCF-7 and Hela cells in a dose dependent manner (Quazi S, Mathur K, Arora S. Calotropis procera 2013).

1.5 Statement of the Problem

Despite the traditional medicinal use of *Calotropis procera*, there is limited scientific data validating its antioxidant properties and phytochemical profile, particularly in ethanol extracts of the leaves. With the growing interest in natural antioxidants, there is a need for detailed scientific assessment of this plant's bioactivity.

1.6 AIM AND OBJECTIVES

The aim of this present study is to evaluate the antioxidant potentials of ethanol extract of Calotropis procera.

The specific objective

- To extract the oil from the plant using ethanol as solvent
- To carry out the phytochemical analysis of the extract
- To determine the anti-oxidant potential of the phytochemical constituents of the oil.

1.7 Significance of the Study

The study provides scientific validation for the ethnobotanical use of *Calotropis procera* and contributes to the discovery of potential natural antioxidants for pharmaceutical and nutraceutical applications.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1 GENERAL REVIEW

Everyday researches are searching for new drugs with better or improved therapeutic actions. Medicinal plants provide an excellent source of lead compound in discovering noble and new drugs with considerable loss side or adverse effects. (Saha S., *et al.,*2013). Traditional medicines are very important since early ancient period because of their faithfulness in the use of various ailments and human sufferings. Various types of bioactive natural compounds are derived from medicinal plants and it serves as raw materials for new drug discovery. Different types of phytochemical such as alkaloids, saponins, carbohydrate, glycosides, flavonoids, gums, steroids, tannins, phenolic compounds, volatile oils etc are synthesized from numerous types of medicinal plants that have potential therapeutic and pharmacological activities and are used in different disorders. (Akinmoladun AC. *et al.,* 2007).

In the last few years, there has been great focus on the possible health benefits of natural substances with antioxidant, antimicrobial, analgesic, anticancer, antidiabetic, and other activities. This has resulted in an enormous increase of research on different medicinal plants to find lead compounds responsible for such pharmacological activity. Calotropis procera are widely used

traditional medicinal plants to treat various ailments. In order to provide a scientific basis for traditional uses of Calotropis procera, their ethanolic, aqueous, n-Butanol and petroleum ether extracts of various parts were tested against human pathogenic microorganisms (pseudomonas aeruginosa, Escherichia coli, cogulase positive staphylococci, cogulase albicans and candida parapsilosis) (ref). The antimicrobial potentials of calotropis procera against human pathogenic microorganisms were investigated. Their isolated phyto-constituents were evaluated for their antimicrobial potential. Antimicrobial activity of various plant extracts was compared with commercially available antibiotics. The antimicrobial potential of the above plants extract was seen against the test organisms using agar gel diffusion susceptibility test by standard techniques of opara and Anasa (1993).

The phytochemical estimation was carried out according to the methods describe by Trease and Evans (1989). Ethanolic and n-Butanol extract showed considerably good antibacterial activity against all bacteria and fungi. Among all solvents used, ethanol extract gave the highest zone of inhibition. Shimmer *et al.,*(1994) reported that plants used for traditional medicine generally contain a number of compounds which may be a potential natural antimicrobial combination and which serve as an alternative, effective, cheap and safe antimicrobial agents for treatment of common microbial infections.

A study reported that X-15- mycotansgenic mice treated with DL (400mgl kg) for a period of 15 weeks, protected mice from malignant changes occurring in liver while sinusoidal architecture and cellular integrity were slightly disrupted as compared to normal and hydropic changes were observed. DL produced a significant decrease in serum vascular endothelial growth factor (VEGF) levels of X15-myc transgenic mice from 12.18± 0.64 µg/l to 9.76± 0.27 µg/l (P= 0.002) while the level of VEGF in normal mice was 7.00±0.55 µg/l. The methanolic extract of dry

latex was included cell death in cell lines Viz, Huh-7 and Cos-1 cells. It was evaluated by using tetrazolium (MTT) assay. The ME was subjected to silica gel G. Step column chromatography using combination of non polar and polar solvents and li fractions were obtained. Out of li fractions, fraction 8 exhibited potent cytotoxic effect on both the cell lines. However, a marginal effect on the killing of non-cancerous cell lines suggested a high degree is sensitivity for transformed sells. Such differential killing of cancerous cell could relate to their altered metabolic status and/or membrane properties. The cytotoxic effect of dry latex was accompanied by intracellular fragmentation of target cell DNA observed (Choedon *et al.,* 2006).

2.2. MAJOR MILESTONE OF CALOTROPIS PROCERA

Photochemistry of Calotropis procera has always attracted the attention of researchers because despite its toxicity, it employs wide applications in traditional medicinal system till date. Dating back to (1936, Hesse *et al.)* identified calotropin as the first compound from this plant. Further Hesse and his coworkers, isolated heart poisons or cardiac glycosides namely calotropin, calotoxin, calactin, uscharin, voruscharin and uscharidin. Root powder of this plant is used in tribes to induce abortion in women and as an uterotonic since ancient period. Later it was found that it was due to the compound calotropin. (Gupta *et al)* administers calotropin to gerbils and rabbits and observed reduction in spermatids count by 65% and 94% respectively.In (1955, Rajagopalan *et al*) identified chemical constituents of seed viz. coroglaucigenin, corotoxigenin and frugoside (cardenolides). Later (Bruschweiler *et* al) identified three additional cardenolides viz. uzarigenin, syriogenin and procerosid. A novel cardenolide, 200-oxovoruscharin was isolated from the root bark by (Quaquebeke *et al*) and modified into its semisynthetic derivative, i.e., UNBS1450. (Akhtar and Malik) isolated a new cardenolide named proceragenin from the hexaneinsoluble fraction of C. procera.

A fascinating feature of the plant is its potential to curb Alzheimer's disease (AD), the most predominant root cause of dementia, a neurodegenerative disease. Its dried latex showed attenuation of b-amyloid deposition in mouse brain and cerebral protective activities. Hence, it is imperative to evaluate the mechanism of metabolites, so that it can lead to promising direction to search new scaffolds for AD treatment. In 2015, (Mohamed *et* al) isolated three non-glycosidic cardenolides namely calactroprocin, procegenin A and procegenin B from the latex. A patent claimed that polar extract of calotropis procera showed anti-ulcerative colitis activity in dose dependent manner in a subject mammal and was found to be more effective than the standard drug prednisolone.

2.3. MISCELLANEOUS ACTIVITY

Antiapoptotic activity of latex calotropis procera was carried out by (Sayed *et al.,*(2016)) on Catfishes exposed to (100µgL^-1) 4-nonylphenol as chemical pollutant. Significant ($P<0.05$) decrease in apoptotic cells, enzymes (superoxidase dismutase, acetylcholinesterase cortisol e.t.c) and ions validified Antiapoptotic activity of the crude latex against the toxicity of 4-nonylphenol. Hence, crude latex exerted antiapoptotic activities against the toxicity of 4-nonylphenol.

Anti-hyperbilirubinemic activity of leaves was evaluated using phenyl hydrazine and paracetamol induced Wistar rats. Significant ($P<0.05$) decrease in concentrations of serum total bilirubin in hyper bilirubinemia rats proved bilirubin lowering activity of aqueous extracts of calotropis procera.

Recent studies indicated that calotropis procera has significantly broader range of beneficial effects as it contains bioactive phytochemicals with therapeutic potential. By far, only cytotoxic studies on cancer cell lines have been well established in clinical trials, whereas other activities

have been evidenced by basic studies. Most of the studies are limited to in vitro studies which lack exploration of molecular mechanism of action. Therefore, mechanism based on in vitro and in vivo studies should be carried out, which can lead to understanding of underlying mechanism related to traditional uses.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1. COLLECTION OF PLANT MATERIAL

Calotropis procera is of the family Asclepladaceae (Apocynaceae). It is a small to medium sized shrub, up to 5.5m high, occasionally branchless to a height of 2.5m. The bark is fibrous, scaly, deeply fissured when old, grey to light brown. All part of the plant exude white latex when cur or broken.

The Leaves and latex of actively growing calotropis procera were randomly and aseptically collected from different locations in Ilorin, Kwara state. Calotropis procera leaves were air dried under shed for seven (7) days and blend into powder form using electric blender and pounded with mortar and pestle. This was repeated severally to obtain finest powdered form (Fatope *et al.,* 1993) and was kept in an air tight container and kept under room temperature

3.2. PREPARATION OF SAMPLE

This was carried out in accordance with the method of (Kareem, *et al.,* 2003). Here, the leaves of calotropis procera plant were obtained as exudates by hand plucking of fresh leaves of actively growing plant and we're air dried under shed. The dried leaves were made into powder

form using mortar and pestle as described by (Fatope *et al.,* 1993), and an electric blender. The content was then stored in an air tight container until required for use.

3.3 EXTRACTION PROTOCOLS

The technique of continuous hot extraction by soxhlet extraction was carried out using solvents of different polarities and other apparatus such as weighing balance, beaker, measuring cylinder, round bottom and flat bottom flask, retort stand, separating funnel, aspirator, water bath, thimble, rotary evaporator mortar and pestle.

A quantity (50g) of the fine powder of the leaves (calotropis procera) was weighed and suspended into two conical flasks. This was percolated with 500ml of ethanol an water until the extract were colorless in the siphon tube The aqueous extract was prepared by adding 500ml of distilled water to 50g of calotropis procera leaf powder.

After 24 hours of maceration under magnetic stirring at room temperature, the mixture was centrifuged. He percolates were then filtered using a filter paper (whatman) and evaporated to dryness using a rotary evaporator at 50°C. The extract obtained served as the stock solutions, which were stored in a refrigerator at 4°C until needed for analysis.

3.4.0 QUALITATIVE ANALYSIS

To test for the phytochemical constituents present in the Bomubomu leaf (calotropis procera) .To test for the presence of

(i) Phenol

(ii) Flavonoids

(iii) Terpenoids

(iv) Alkaloid

(v) Fatty acid, in the above leaf

(vi)

3.4.1 REAGENT

Chloroform

Sulphuric acid (H_2SO_4)

Lead acetate

Diethyl ether

Mercury (II) chloride

Potassium iodide

And water (H_20)

3.4.2 PROCEDURE TO MAKE MEYER'S REAGENT

(i) Mercury (Ii) chloride (1.36g) and potassium iodide (5.00g) was mixed together with 100ml of water in a beaker, the result gotten is called MEYER'S REAGENT.

(ii) Another mixture of lead acetate with water.

3.5. DETERMINATION OF ETHANOLIC CALOTROPIS PROCERA (BOMUBOMU) EXTRACT

3.5.1 TEST FOR PPHENOL

3mls of the extract is added with 3mls of lead acetate, then mixed gently, no changes occur which shows that Phenol is observed

3.5.2 TEST FOR FLAVONOIDS

3mls of Sulphuric acid (H_2SO_4) is added with the extract. Deep Black color is observed which shows the presence of flavonoids.

3.5.3 TEST FOR TERPENOIDS

3mls of the extract is added to 1ml of chloroform, another 1ml of Sulphuric acid (H_2SO_4) is added, and there is a change in the color, which shows the presence of Tapenoids.

3.5.4 TEST FOR ALKALOIDS

3mls of the extract is added to 2ml of Meyer's reagent. It is filtered out using filter paper and allowed to evaporate; a creamy color was observed which shows the presence of Alkaloids.

3.5.5 TEST FOR FATTY ACID

1ml of the extract was added to 5mls of ether, it was filtered using a filter paper and was allowed to evaporate. If there is no transparency, it shows that Fatty acid is absent.

3.6. DETERMINATION OF ANTIOXIDANT ACTIVITIES:

The anti-oxidant potential of the methanolic extract was determined on the basis of their scavenging activity of the stable 1,1- diphenyl-2-picryl hydrazyl (DPPH) free radical. DPPH method is most widely used and easiest method to determine antioxidant activity (Uddin, .S.N., *et al,2008*). DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. The aliquots of the

different concentrations (1-500 µg/ml) of the extract were added to 3 ml of a 0.004% w/v solution of DPPH. Absorbance at 517 nm was determined after 30 min, and IC (Inhibitory concentration 50%) was determined. IC value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals (Gupta, .M. *et al,2003*)

At first 6 test tubes were taken to make aliquots of 6 conc. (1, 5, 10, 50, 100 and 500 µg/ml). Plant extract and ascorbic acid were weighed 3 times and dissolved in ethanol to make the required concentration by dilution technique. Here ascorbic acid was taken as standard. DPPH was weighed and dissolve in ethanol to make 0.004% (w/v) solution. To dissolve homogenously magnetic stirrer was used. After making the desired concentrations 3ml of 0.004% DPPH was applied on each test tube by pipette. The room temperature was recorded and kept in the test tubes for 30minutes in light to complete the reactions. DPPH was also applied on the blank test tubes at the same time where only ethanol was taken as blank. After 30minutes absorbance of each test tube were determined by UV spectrophotometer. IC50 was determined from % inhibition vs. concentration graph.

Determination of Total Phenolic Content (TPC)

TPC was measured using the Folin–Ciocalteu method. Gallic acid was used as the calibration standard, and results were expressed as mg gallic acid equivalent (GAE)/g extract.

3.5 Determination of Total Flavonoid Content (TFC)

TFC was quantified using the aluminum chloride colorimetric assay with quercetin as the standard, expressed in mg quercetin equivalent (QE)/g extract.

3.6 DPPH Radical Scavenging Assay

The free radical scavenging activity was evaluated using the DPPH method. Absorbance was measured at 517 nm, and IC_{50} values were calculated to determine antioxidant efficiency.

3.7 Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP assay was conducted based on the reduction of Fe^{3+} to Fe^{2+} in the presence of antioxidants. The absorbance was recorded at 593 nm, and results were expressed in mmol Fe^{2+} equivalents/g extract.

CHAPTER FOUR

4.0 RESULT AND DISCUSSION

4.1 Phytochemical Analysis

The phytochemical constituent of the extract are presented in the table below

Table 4.1Phytochemicals in *calotropis procera*

Parameters	Abundance
Alkaloids	+
Glycosides	+
Flavonoids	-
Steroids	-
Tannins	+
Phenols	+
Saponins	+
Terpenoids	-

Key = Interpretation; + = present; - = Absence

Table 4.1 shows the pytochemicals in *calotropis procera*. The result indicates high abundance of glycosides and tannins, moderate presence of saponins, slight presence of alkaloid and phenol while flavonoid, steroid and terpenoid were absence.

Table4.2 Chemical constituents of *calotropis procera*

Parameters	**Absorbance**

Saponins	1.00
Tannins	1.5
Flavonoids	0.0
Steroids	0.0
Terpenoids	0.0
Phenols	0.5
Alkaloids	0.5
Glycosides	1.5

Table4. 3 DPPH Scavenging assay **compared with standard ascorbic acid**

Concentration	**Absorbance**		
	Ascorbic µm	**Ethanol µm**	**Aqueous µm**
0	1	1	1
1	0.94	0.92	0.99
10	0.8	0.8	0.98
50	0.3	0.65	0.95
100	0.1	0.55	0.92
500	0.08	0.22	0.80

Table 4.4 DPPH Scavenging assay calotropis procera compared with standard ascorbic acid

concentration Inhibition %			
	ascorbic	Ethanol	aqueous
0	0	0	0
1	15	2	0.8
5	40	10	1.2
10	70	18	2
50	90	30	10
100	92	50	15
500	97	80	18

4.2 Antioxidant activities

DPPH is the best, easiest and widely used method for for testing preliminary free radical scavenging activity of a compound or a plant extract (Duh *et al*, 1999). In present study, ethanol extracts of the leaves of C. procera possess strong antioxidant activity. However the aqueous extract showed mild antioxidant activity. The free radical scavenging property may be one of the mechanisms by which this drug is effective as a traditional medicine. Most of the tannins and saponin are phenolic compounds and may be responsible for antioxidant properties of many plants (Lason 1988) So, this activity may be due to the presence of phenolic compounds (tannins and glycosides) present in the extract (Sadhu *et al*, 2003). Large amount of pharmaceutical raw material including medical plants are imported to manufactures drugs and medicine. A huge amount of foreign exchange can be saved if indigenous medicinal plants are used by the producer of drugs to

satisfy their need. So, further pharmacological and toxicological study is required to establish the therapeutic uses of the plant and particularly with its active principle.

The notable antioxidant potential of the ethanol extract is likely due to its rich phenolic and flavonoid contents. This corroborates findings by Edeoga et al. (2005) on the therapeutic potential of plant-derived antioxidants. The study validates the traditional use of *C. procera* and advocates for its exploration in phytomedicine development.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The plant Calotropis is one of the widely distributed along the world geographical area. The whole summation of information about the use of C. procera in the entire world is matched with available literature. There is broad categorization according to its various uses in the pharmacological as well as in traditional use. The literature showed us that it is the plant that is forgotten as the time passes. Still many scientists have worked to reveal its phytochemicals and pharmacological activity. The plants are a rich source of phyto constituents. Searching new therapeutic agents is a big challenge for the scientist of the present modern era and plants are the biggest source of these agents. Screening of plants for their pharmacological properties with the hope of finding safe and effective agents is very essential. A large number of synthetic compounds are available but due to their environmental pollution and adverse effect on the human body there use is restricted. To find the safe, effective, and environmental friendly agent from a plant source, C. procera is a plant that may present as effective one. In conclusion, the literature on C. procera suggests a huge biological potential of this plant. It is believed that the present manuscript may be useful to provide additional information with regard to its identification and in accordance to carry out further research on its use in the treatment of various diseases. In conclusion,the aqueous extract of calotropis procera is toxic and should not be recommended for medical purpose at higher doses, but can be medicinally utilized at lower concentration.

5.2 RECOMMENDATION

Further study of this feature can be beneficial in identifying which population to be targeted in medicinal industries and which in hydrocarbon synthesis. Regardless to its allelopathic effect to some crops, Calotropis procera has several ecologic roles, its ability to accumulate several elements from different soils serves as natural phyto remediation can possibly improve the quality of soil and hence rehabilitating abandoned and exhausted lands. In Egypt, there has been respective number of C. procera populations in intensive industrial regions such as Helwan south of Cairo, it's one of the most polluted areas in Egypt, consequently the presence of C. procera could act as a natural refiner for air and soil. As was mentioned by Lottermoser , C. procera had the ability to uptake high concentrations of Uranium in its tissues when grew in an abandoned mining site in Australia not to mention, vast number of heavy metals and natural elements were detected in its tissues that correlated highly to soil content. Another ecologic role, Calotropis procera is known to attract about 80 animal species, which range from casual visitors to those dependent on the plant for completing their life cycle , which provide highly productive ecosystem to abandoned lands. Furthermore, its appearance can be used as an indicator of overgrazing and poor soil and hence drive the attention to manage the stressed lands. In an overpopulating developing country such as Egypt, having C. procera is a bless, it is famous for its various medicinal and phytochemical properties along with hydrocarbon content that can be used as an alternative clean source of energy, it also can be used as a safe pesticide and rodent control without leaving harmful chemical traces. Calotropis procera presents such a rich natural resource that must be utilized industrially; it provides ecofriendly solutions to our pollution, energy limitation and habitat degradation problems.

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