PHYTOCHEMICAL COMPOSITION OF PALMWINE FERMENTED *Citrullus colocynthis*

BY

OMOLEWA, TOYOSI MOYOSORE

MCB/14/2334

A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF SCIENCE, FEDERAL UNIVERSITY OYE-EKITI.

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN MICROBIOLOGY

MARCH, 2019

CERTIFICATION

This is to certify that this project work was carried out by Omolewa, Toyosi Moyosore with Matriculation number MCB/14/2334 of the Department of Microbiology, Faculty of Science, Federal University, Oye-Ekiti.

Dr (MRS) R.A.O Gabriel-Ajobiewe .....

Supervisor Signature/ date

Prof. Bryan Ogeneh 4/4/15

Head of department Signature /date

DEDICATION

This project is dedicated to God Almighty, the giver of life. Also to my parent (pastor and Deaconess Omolewa) and to my siblings (Mr Ifedayo, Mrs Balogun, Mercy and Success).

ACKNOWLEDGEMENT

I thank my heavenly father for his loving kindness, tender mercy, for given me the privilege to complete this project work. My humble thanks goes to my project supervisor in person of Dr. (Mrs.) R.A.O. Gabriel-Ajobiewe, may God in his mercy bless you for taking out time to guide and direct me towards the success of this project work. May God increase his love upon you and your household. My gratitude goes to my Head Of Department in person of Professor Bryan Ogeneh, for his fatherly words of advice. My profound gratitude also goes to the entire lecturer in the faculty of science most especially the Department of microbiology for willingly using their own personal experience at their own disposal to sow academic seed into my life. Finally, I want to use this platform to say a big thank you to all my colleagues and friends who have contributed in one way to the project.

TABLE OF CONTENTS

CONTENTS PAGE

List of tables

List of figures

List of plates

ABSTRACT

Phytochemical compounds are known as natural remedies, which have been used since earliest times of mankind. The availability of specific phytochemicals in plant gives it specific medicinal properties. This research focused on discovering the phytochemical components of liquid state fermented *Citrullus colocynthis* with the addition of some other component such as clove (*Syzygium aromaticum*) and bark of baobab tree(*Adansonia digitata*), using different solvent such as palm wine and water. To carry out this experiment, the fruits were divided into five different groups including the control which are; CP (*Citrullus colocynthis* with palm wine), CW (*Citrullus colocynthis* with water), CPA (*Citrullus colocynthis* with palmwine, clove (*Syzygium aromaticum*) and bark of baobab tree (*Adansonia digitata*), CWA (*Citrullus colocynthis* with water, clove (*Syzygium aromaticum*) and bark of baobab tree (*Adansonia digitata*), the fruits were cut into smaller size to increase the surface area and weighed, appropriate solvent which are palmwine and water were measured appropriately and added.. Fermentation was carried out for five days on each of the samples at ratio 1: 2.5. After fermentation the sample was divided into two portions. The first portion was blended wet while the second portion was dried and also blended. Thereafter the phytochemical analysis was carried out for both wet and dried sample. The phytochemical screening shows the presence of all the nine phytochemical compounds tested for in dried and wet sample which are alkaloids, flavonoid, Terpenoid, Saponin, tannin, cardiac glycosides, phenols, steroids and anthraquinone. Except cardiac glycoside which is present in few of the samples. In wet sample the phytochemical screening shows that flavonoid, alkaloid, tannin, phenol, steroid, were abundantly present, while in dried sample, anthraquinone, tannins, flavonoid, phenols are abundantly present in most of the samples. This study also shows the potency of CP (i.e. *Citrullus colocynthis* with palmwine) against cancerous diseases and bacterial infection due to the presence of Saponin, tannin and phenols with abundance of steroids.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of study

Citrullus colocynthis (L.) schrad) is a valuable plant from Cucurbitaceae family, widely distributed in the barren region , It is a non-hardy, herbaceous perennial vine, branched from the base (Rahimi *et al.*, 2017). Genus *Citrullus* consist of basically four species: *C. colocynthis, C. ecirrhosus, C. rehnii and C. lanatus*. The fruits are oblong, ovoid or ellipsoid in shape with elliptical, flattened seeds (Rubatzky and Yamaguchi, 1997). *Citrullus colocynthis* fruits are generally documented for its broad range of pharmaceutical uses as well as medicinal and nutraceuticals potential. It is a medicinal plant traditionally used as an abortifacient and to treat constipation, oedema, bacterial infections, cancer and diabetes (Shi *et al.*, 2014). *Citrullus colocynthis* seed crop are the commonest soup condiment enjoyed in the south-east region of Nigeria (Ejiofor, 1994).

Phytochemical compounds are non-nutritive plant chemical compound that has protective and disease preventing properties (Harborne, 1973). Many different techniques may be followed in the screening of plants for pharmacological and chemical interest. Basic phytochemical screening consist of performing simple chemical test to detect the presence of alkaloids, tannins, saponins, Flavonoids, digitalis glycosides e.t.c. in plant extract (Sofowora, 1993). They can be classify into its different bioactive and anti-diseases properties; antibacterial properties are demonstrated by Terpenoid, Alkaloids and Phenolics while anticancer properties are exhibit by caronoids and Flavonoids (Sexena *et al.*, 2013). Some of these plant materials have been known to provide alternative natural products to synthetic and imported products. This is the basis for

the application of nutrition in therapy and treatment of ailment and disease as well as provision of raw materials for local pharmaceutical industries (Martin, 2000). Plants have the ability to synthesize a wide range of chemical compounds that are used to perform important biological functions, and to defend against attack from predators, such as insect, fungi, herbivorous animals etc. many of these phytochemicals have beneficial effects on long time health when consumed by man and can be useful in treating many human diseases (Rhoades, 1979).

1.2 Botanical classification OF *Citrullus colocynthis*

Kingdom: plantae

Subkingdom: Tracheobionta

Division : Magnoliophyta

Class: Mangnoliopsida

Order: cucurbitales

Family : cucurbitaceae

Genus : Citrullus Schrad

*Specie : Citrullus colocynthis(L.)*Schrad.

(Rani *et al.*, 2017).

1.3 Common names

Bitter Apple, Colocynth, Bitter Cucumber, desert gourd, Egusi, vine of Sodom or wild gourd (Eidi *et al.*, 2015).

1.4 Geographical distribution

Citrullus colocynthis is widely distributed across the globe from Mediterranean Europe, Cyprus, the Syrian Arab Republic, Lebanon, and Jordan to Egypt, Kuwait, Saudi Arabia, Turkey, the Islamic Republic of Iran, Pakistan, Afghanistan, India, North Africa, and Sahel (Patel *et al.*, 2012).

1.5 Cultivation

Citrullus colocynthis is a perennial plant (in wild) or an annual herb, that can be propagated both by vegetative and generative means. Its growth occurs during the season of spring summer in India, in between the period of January and October. Colocynth mostly grows in sandy soils (Patel *et al.*, 2012).

1.6 Morphological description

An annual herb with lobular tendrils, oblique branching stems and flocculent tender shoots.

1.6.1 Leaves

The leaves are acutely divided, lobes slender, thick and barren. The slanted leaves are alternatively positioned on prolonged petioles. They are almost 5-10cms in length and have approximately 3-7 lobes. The leaves of *Citrullus colocynthis* showed significant antimicrobial activity. They are very similar to water melon (Gurudeeban *et al.*, 2010).

1.6.2 Flowers

The yellow coloured flowers emerge individually at leaf axioms and are borne by yellow-greenish peduncles. Each has a subcampanulated five- lobed corolla and a five- parted calyx. They are monoecious, the male (stamens) and the female reproductive parts (pistils and ovary) are born in different flowers on the same plant. The male flowers' calyx is shorter than corolla.

They have five stamens, in which four are coupled and one is single with monadelphous anther. The female flowers have three staminoides and a three-carpel ovary. The two sexes can be distinguishable by observing the globular and hairy inferior ovary of the female flowers (Schafferman *et al.*, 1998).

1.6.3 Fruits

The fruits are Bulbous or ovoid, corpulent, indehiscent berry, 5-7.5cm in width and assorted with green and white. They are smooth and extremely bitter. Each of egusi harvest around 15-30 globoid fruit have a diameter of almost 7-10cms. Each of the three carpel bears six seeds, the outmost segment of the fruit is enclosed with an emerald coat having yellow bands. The mesocarp is made up of soft, dry, and spongy white pulp where the seeds are embedded (Gurudeeban *et al.*, 2010).

1.6.4 Seeds

Seeds are around 6mm in size, shaven, consolidate and compressed oblong-shaped. They are situated on the parietal placenta. They are grey in colour, edible and bitter but are rich in fat and protein, they can be used as oil seed. The seed contain oil content of 17-19%(w/w) consisting of 67-73% linoleic acid, 10-16% oleic acid, 5-8%stearic acid, and 9-12% palmitic acid. The seed also have large amount of arginine, tryptophan, and the sulfur containing amino (Gurudeeban *et al.*, 2010).

1.6.5 Roots and stem

Roots are large perpetual, fleshy, long and delicate and perennial, leading to a high rate of survival because of the long tap root. It has rocky vine-like stems which spreads looking for

something to climb. Shrubs and herb if they are present are preferred and climbed by means of auxiliary branching tendrils (Sen and Bhandari, 1974).

Plate 1: ***Citrullus colocynthis*** **Fruit** (Zell, 2012).

1.7 JUSTIFICATION

With the rising cost of conventional medicine and imported drugs, medicinal plants should be continually evaluated and developed from our indigenous plant for improvement and sustenance of our health care delivery system. *Citrullus colocynthis* finds extensive use in traditional medicine therefore the phytochemical evaluation is useful to determine its potency as a drug.

1.8 AIM OF STUDY

To examine the phytochemical component of the ethnomedical used i.e. *Citrullus colocynthis*

1.9 OBJECTIVE OF THE STUDY

- To determine the major phytochemical compounds in the fresh fruit of Citrullus colocynthis
- To identify the phytochemical compounds present in the palmwine fermented *Citrullus colocynthis* fruit.
- To detect the phytochemical compounds present in the fermented fruit sample using water as solvent.
- To discover the effect of the solvents used on the *Citrullus colocynthis* and their effects on phytochemical compounds.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Phytochemical compounds

Phytochemicals are naturally occurring chemicals produced by plants. These are various biologically active compounds in plant, they are chemical compounds produced by plant during their normal metabolism, generally to help them thrive or thwart competitors, predators or pathogens. They are usually produced by plant through primary or secondary metabolism. These compounds gives plants their pretty colour and aroma, protect man from cancer or possibly slow down the growth of cancer, reduce inflammation and help regulate hormones (Sofowora, 1993).

2.2 Palmwine

Palmwine the sap of palmtree (family *palmae*) obtained by local palm wine tappers as seen across west Africa. Palmwine is know by a variety of names in different countries including "EMU" in Nigeria (yoruba), Matango, Fitchuk , tumbu liquor, white stuff and Mbuh in Cameroon. It has many health benefit most especially when fresh, It carry out many important body functions, such as giving cells their structure, help in transport and the storage of nutrients, it contains antioxidant, vitamin C , it promotes lactation, reduces the risk of cancer and helps to maintain good eye health. It has high amino acid, potassium, magnesium, zinc and iron. The white liquor that is initially collected tends to be sweet and non- alcoholic before it is fermented. Palm sap begins fermenting after collection, due to natural yeast (*Saccharomyces cerevisae*) in the atmosphere. Within two hours, fermentation yields 4% alcohol content, mildly intoxicating with sweet taste. Longer fermentation yields vinegar instead of stronger wine (Ukwuru and Awah, 2013).

2.3 *Citrullus colocynthis* fruit

Citrullus colocynthis fruit is used in the treatment of ascites, biliousness, jaundice, cerebral congestion, colic, constipation dropsy, fever, worms and sciatica. It has antimicrobial, antifungal and anti-parasite properties (Thakur, 2014). *Citrullus colocynthis* has shown broad spectrum antimicrobial activity against 16 clinical microorganisms isolated from HIV positive patients (Elhadi *et al.*, 2013). Medicinal plant contains several active principles with specific therapeutic effect they represent source of chemical compounds with curative properties often not provided by synthetic chemical compounds (Fabricant and Farnsworth, 2001). In traditional medicine, several medicinal plants are used for the treatment of diabetes mellitus. Ethnobotanic inquires have recorded the plant used in such a perspective, among which *Citrullus colocynthis* represent one of the most commonly used species (Allali *et al.*, 2008).

2.4 Qualitative phytochemical screening of *Citrullus colocynthis*

Preliminary phytochemical screening of the plant showed the presence of large amount of phenolics and flavonoids. Subsequent quantification showed the presence of 0.74% (m/m) phenolics (calculated as galic acid) and 0.13%(m/m) flavonoids calculated as catechin equivalents per 100g of fresh mass. Flavonoids and other Phenolic compounds have been suggested to play preventive role against the incidence of some common disease like cancer, cardiovascular and neurodegenerative disorders (Hussain *et al.*, 2008). Chemical analysis on *Citrullus colocynthis* was carried out to predict the possible medicinal and therapeutic application (Sarkar and Tanker, 1991). The crude aqueous extract as well as the hydro-methanol extract, ethyl acetate extract, underwent phytochemical screening in order to detect the (presence or absence) of alkaloids (Dragendorff and Mayer reagent), reducing sugar (Fehling reagent),

Flavonoids (cyanidine reaction), tannin (iron chloride), and terpenoids (Liebermann Burchard reaction) (Karumi *et al.*,2004).

2.4.1 Alkaloid

Alkaloids is the terms collectively used to describe a large and diverse group of chemicals that have alkali-like properties, they are group of naturally occurring chemical compounds that contain mostly basic nitrogen atom, it contain compounds with neutral and even weakly acidc properties. Alkaloids were strongly present in *Citrullus colocynthis* (Edeoda *et al.*, 2006). The compound has a protective role in animal and it is used in medicine, especially the steroidal alkaloids which constitute most of the valuable drugs. Alkaloids are produced by a large variety of organisms including bacteria, fungi, and animals. Many alkaloids are toxic to the organisms that attack the plants that produce it.

The primary use of alkaloids however is in medicine because they act quickly on specific areas of the nervous system. They are used in the manufacture of chemicals, pharmacological, cosmetics and food products (Heinrich, 2013). Additionally, plants extracts showing active trypanocidal activity was found to contain Alkaloids, flavonoides, phnolyx and tarpanes (Le-Grand, 1989).

2.4.2 Flavonoid

Flavonoids are the largest group of phytochemicals, with more than six thousand types, they are powerful anti-oxidant with anti-inflammatory and immune system benefit with low toxicity. Flavonoids are associated with skin protection, brain function, blood sugar and blood pressure regulation. They are associated with cardiovascular disease prevention according to George mateljan foundation's. This display a remarkable array of biochemical and pharmacological

actions, viz. anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities (Middleton and kandaswami, 1993).

These compounds appear to play vital roles in defense against pathogens and predators and contribute to physiological functions such as seed maturation and dormancy. Flavonoids are important for human being due to their antioxidative and radical scavenging effect, as well as their potential estrogenic and anticancer activities. They strengthens the capillaries and prevent the small cutaneous hemorrhages so frequent in the aged, some of them relieve cramps of the smooth muscles while others improve circulation in coronary arteries, The compound is useful in disease resistance (Winkel-Shirley, 2002).

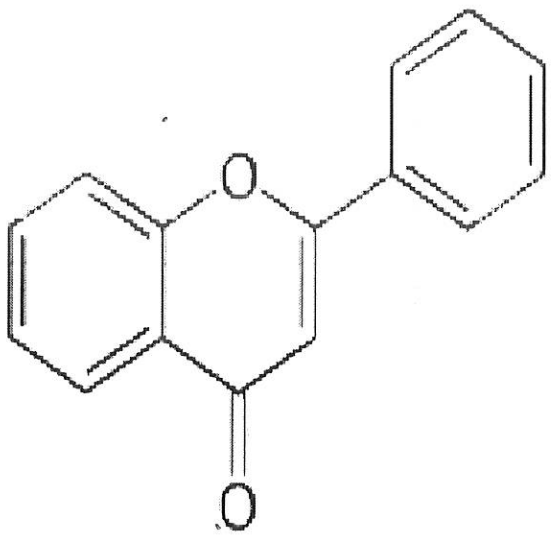

Figure 1: Structure of a Flavon (Middleton and kandaswami, 1993).

2.4.3 Saponin

Saponin is a class of chemical metabolite found in natural sources as most are found in particular abundance in various plant species. They are basically glycosides of both tripterpenes and sterol which have been detected in over 70 families of plant (Thakur *et al.*, 2011).

Their outstanding characteristics (i.e. soap-like foaming) they produce when shaken in aqueous solution explains their use as detergents and hence its name (sapo in latin meaning soap), they are therefore used as expectorant and emulsifying agent. Saponin is used in medicine and pharmaceutical industries because of its foaming ability with the production of frothy effect. It has the property of precipitating of ad coagulating red blood cell, it reduce blood cholesterol levels, reduce the risk of cancer and stimulate our immune system. Saponin when injected into the blood stream is highly toxic but less so when administered by mouth (Francis *et al.*, 2002). They are also toxic to fish (causes paralysis of gills), they are also occasionally toxic to cattle .characteristics of Saponin include hemolytic activity, cholesterol binding properties and bitterness (Okwu, 2004).

2.4.4 Phenols

These are group of aromatic chemical compounds with weakly acidic properties and are characterized by a hydroxyl (OH) group attached directly to an aromatic ring. The presence of phenol is considered to be potentially toxic to the growth and development of pathogen (Okwu., 2004). The structural classes of Phenolic compounds include the polyphenolic and monomers such as ferulic and catechol. polyphenol might interfere in several of the steps that leads to the development of malignant tumours, it play a role in inactivating carcinogens and inhibiting the expression of mutagens (Urquiaga and Leighton, 2000).

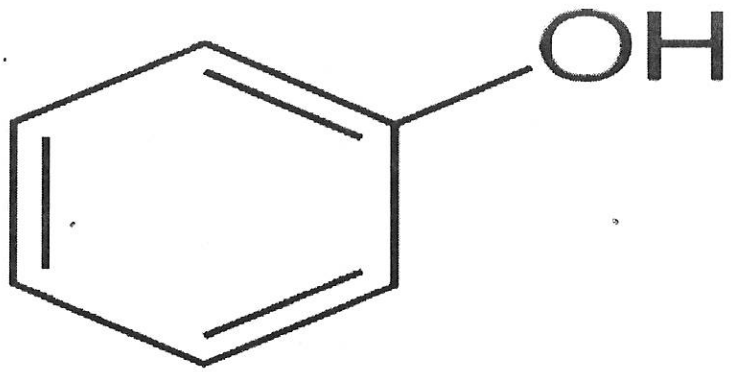

Figure 2: Structure of phenol (Okwu, 2004).

2.4.5 Tannin

The term tannin (from tanna an old german word for oak or firtree, as in Tannenbaum) refer to the use of wood tannins from oak in tanning animals hides into leather, hence the word "taning'' for treatment of leather. Tannin is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups to form complexes with protein and other macromolecules. Tannins are water soluble Phenolic compound; they have the ability to precipitate gelatin and other proteins. Tannin compounds play a role in protection from predation and as pesticides and in plant growth. The astringency from tannin is what causes the dry and pucker feeling in the mouth following the consumption of unripe fruit. likewise the destruction of tannin with time plays an important role in the ripening of fruit and the ageing of wine. Tannin prevent the development of bacteria since the protein necessary for their nutrition are moved and their own protein content are precipitated (Hotollman, 1983).

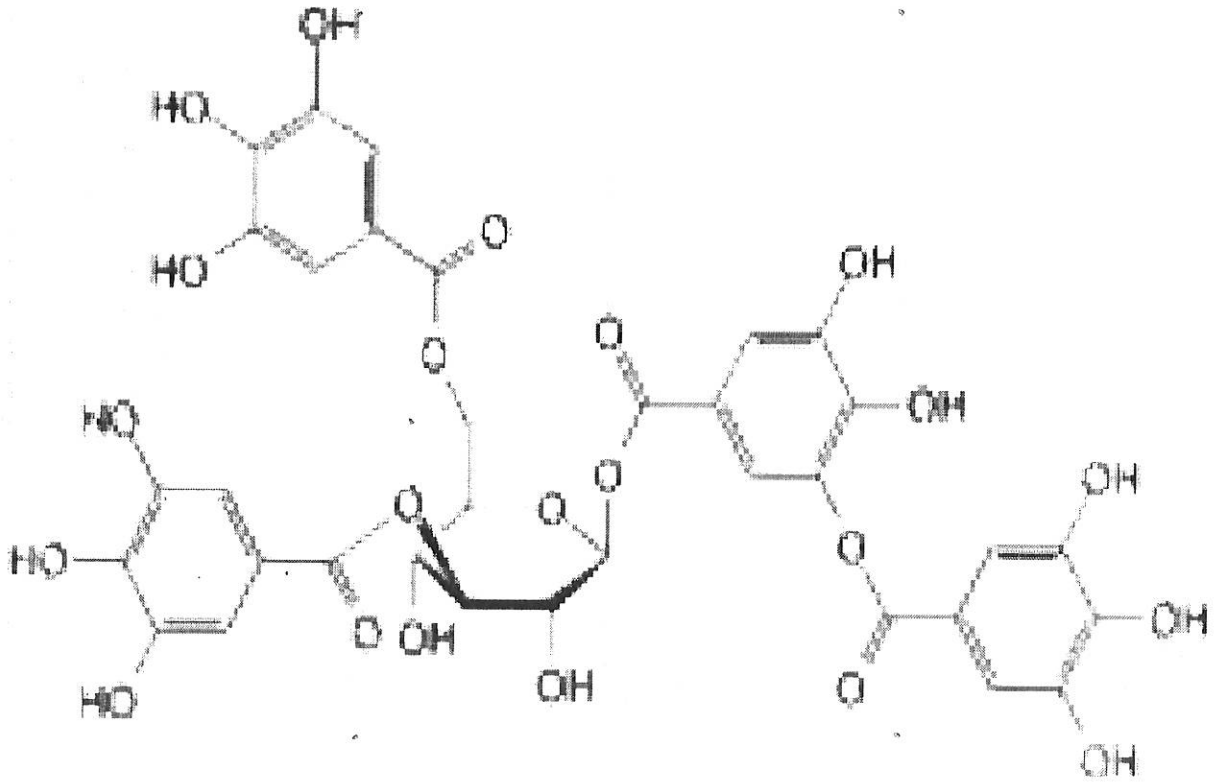

Figure3 : Structure of Tannin (Hotollman, 1983).

2.4.6 Terpenoid

They are a large and diverse class of naturally occurring organic chemicals derived from terpenes. Terpenoid essential oils are the main compounds found in the volatile steam distillation fraction responsible for the characteristic scent, odour or smell found in many plant. Some essential oils possesses medicating properties and are used in the pharmaceutical industry. The essential triterpenoids are saponins, steroids and cardiac glycosides which occur mainly as glycosides. Triterpenes occur especially in the waxy coatings of leaves and on fruit and may serve a protective function in repelling insects and microbial attack (Harborne, 1973).

2.4.7 Anthraquinone

It is a yellow, highly crystalline solid, poorly soluble in water but soluble in hot organic solvent. This compound is refer to as building block of many dye hence synthetic dye are often derived from it, They can also be used in bleaching pulp for making paper it can also be used as a digester additive in production of paper pulp by alkaline process. Derivative of 9,10-anthraquinone include many important drugs (collectively called anthracenediones). They include laxative, antimalerial, antineoplastics(used in treatment of cancer) and DNA dye (Goor *et al.*, 2007).

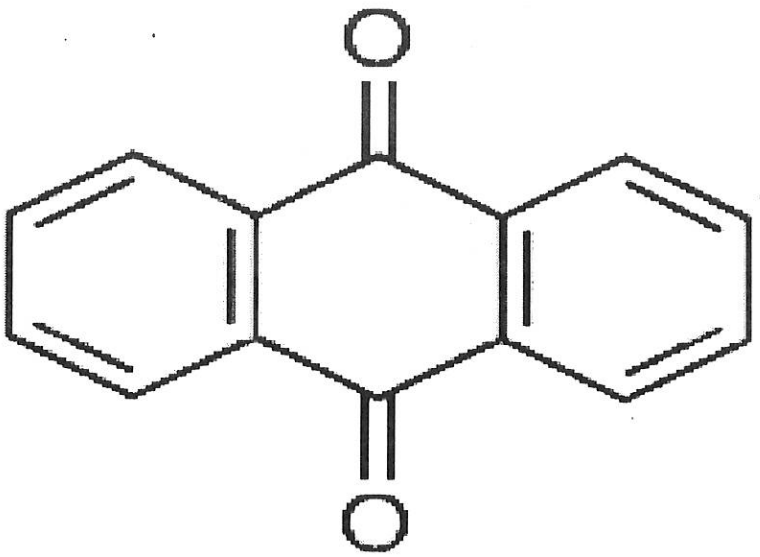

Figure 4: Structure of anthraquoinone (Goor et al., 2007).

2.4.8 Cardiac glycosides

These are class of organic compounds that increase the output force of the heart and increase the rate of contractions by acting on the cellular sodium-potassium ATPase pump.they have diverse range of biochemical effects regarding cardiac cell function and have been suggested for use in cancer treatment. Cardiac glycosides can be categorized based on the plant they are derived from. Its important in the treatment of heart failure and lowering blood pressure. It affect cardiovascular, neurologic, and gastrointestinal system. Due to level of toxicity, they have been replaced with synthetic drugs such as ACE inhibitor or can be used in conjunction with other treatment (Kanji and Maclean, 2012).

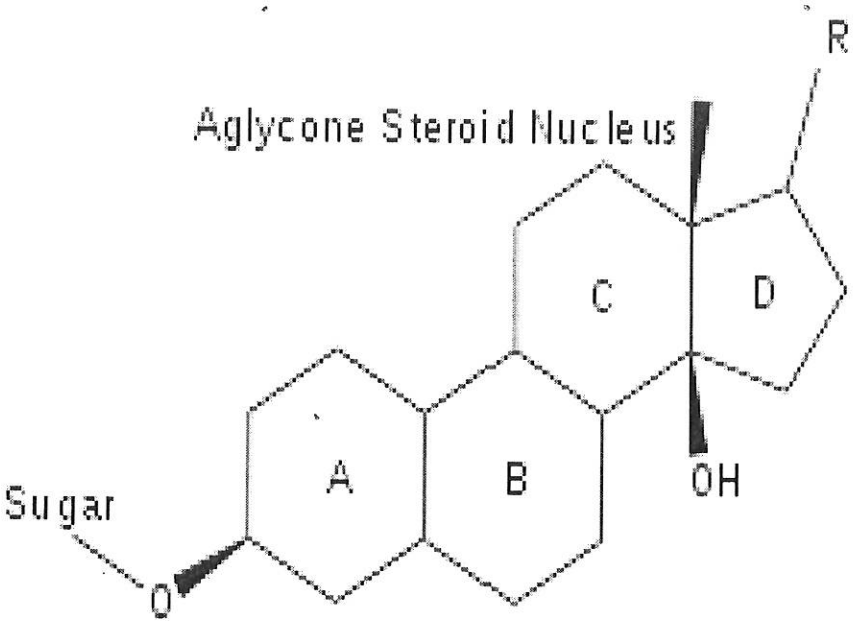

Figure 5: Structure of cardiac glycosides (Bronstein *et al.*, 2008).

2.4.9 Steroids

These are biologically active organic compound with four rings arranged in a specific molecular configuration. They are important component of cell membrane which alter membrane fluidity and also as signaling molecule. They play significant roles in a number of disorder, including malignancies like prostate cancer, where it production inside and outside the tumour promote cancer cell aggressiveness. Microbial catabolism of phytosterol side chains yields C-19 steroids, C-22 steroids, and 17- ketosteroids (Hanukoglu, 1992).

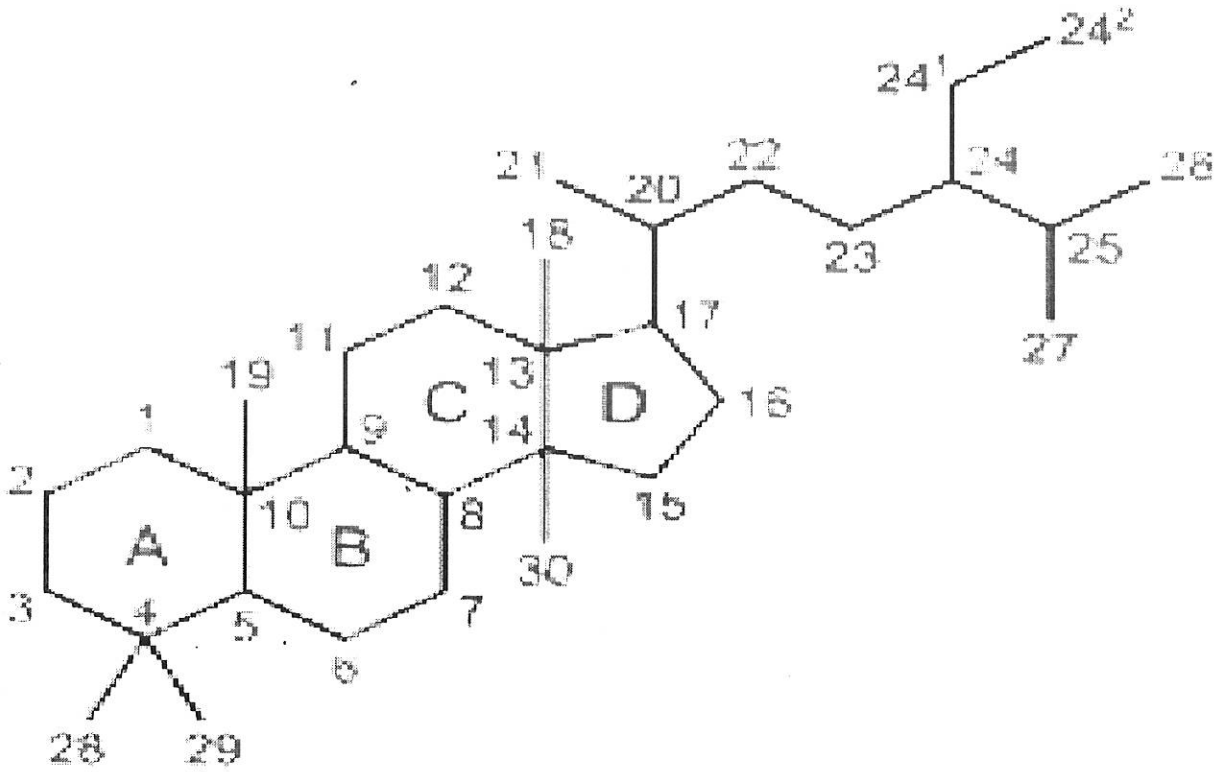

Figure 6: Structure of Trimethyl Steroids (Hanukoglu, 1992).

Table 1: MECHANISM OF ACTION OF SOME PHYTOCHEMICAL COMPOUNDS (Omojate *et al.*, 2014)

Phytochemicals	Activity	Mechanism of action
Flavonoids	Antimicrobial, antidiarroel	Complex with cell walls, binds to adhesions, inhibits release of autacoids and prostaglandins. inhibits contractions caused by spams gens. stimulates normalization of the deranged water transport across the mucosal cells. Inhibits GI release of acetylcholine.
Terpenoids and essential oils	Antimicrobial antidiarrheal	Membrane distruption. Inhibits release of autacoids and prostaglandin
Alkaloids	Antimicrobial, antidiarrheal,anthelmintic	Inhibits release of autacoids and prostaglandin,it suppresses transfer of sucrose from stomach to small intestine, it act on CNS, causing paralysis
Saponins	Anticancer, anthelmintic antidiarrheal	Possesses membrane permeabilizing properties, leads to vacuolization and disintegration of teguments,inhibits histamine release in vitro
Steroids	Antidiarrheal	Enhance intestinal absorption of Na+ and water.
Cardiac glycosides	Anticancer	It affect the sodium-potassium ATPase pump in cardiac muscle cells to alter their function.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The research was carried out in microbiology laboratory, Federal University Oye-Ekiti (FUOYE), Ekiti-State, and JaaGee Application and Research laboratory.

3.2 Materials used

Citrullus colocynthis fruit, palmwine (freshly tapped), bark of baobab tree (*Adansonia digitata)*, clove (*Syzygium aromaticum*), Weighing balance, filter paper, oven, hot plate, pipette, blender, stirrer, volumetric flask, cotton wool, conical flask, test tubes, measuring cylinder, masking tape, beaker, reagent bottles, spatula.

3.3 Reagents used

Mayer's reagent, Draggendoff's reagent, hydrochloric acid, ferric chloride, chloroform, sulfuric acid, aluminum solution, glacial acetic acid, acetic anhydride, benzene, ammonium.

3.4 Methodology

3.4.1 Sample collection

Citrullus colocynthis fruit was Purchased from Oja Bisi in Ado- Ekiti, Ekiti State and was transported to microbiology laboratory, Federal University Oye-Ekiti.

Freshly tapped palmwine was purchased on the day of sample preparation to ensure freshness and reduce rate of contamination using tightly capped keg.

3.4.2 Sample preparation and fermentation

The healthy fruits (*Citrullus colocynthis*) were washed thoroughly to minimize contamination and cut into small sizes aseptically to increase the surface area, and also the clove (*Syzigium aromaticum*) and the bark of baobab tree (*Adansonia digitata*) and was then weighed appropriately. Plastic buckets were washed thoroughly, cleaned and disinfected using 70% ethanol, and was labeled with different variables: CW (*Citrullus colocynthis* with water), CPA (*Citrullus colocynthis* with palmwine, clove (*Syzygium aromaticum*) and bark of baobab tree (*Adansonia digitata*), CWA (*Citrullus colocynthis* with water, clove (*Syzygium aromaticum*) and bark of baobab tree (*Adansonia digitata*), and CP (*Citrullus colocynthis* with palm wine). Appropriate solvent which can either be distilled water or palmwine were measured using measuring cylinder and poured appropriately into the bucket according to the label. The constituent of each bucket was added as labeled. Fermentation was carried out for five days (using either distilled water or palmwine) on each of the variables under aseptic condition. Weight of the fruit (284.84g), clove (*Syzygium aromaticum*) 2.62g, bark of baobab tree (*Adansonia digitata*), 93.55g, Volume of distilled water (750ml), Volume of palmwine (750ml). Solvents were added in ratio 1:2.5 while other ingredients were in a ratio of 1:10:50 with 1 belonging to the fruit, 10 to bark of baobab tree (*Adansonia digitata*) and 50 to clove(*Syzygium aromaticum*).

3.5 Qualitative analysis

phytochemical screening of the constituents in the fermented samples were carried out using standard procedures as described by (Sofowora, 1993) and (Harborne, 1973). All samples were weighed in duplicate.

3.5. 1 Determination of alkaloid

About 0.5g of extract was diluted with 10ml with acid alcohol, boiled and filtered. To 5ml of the filtrate was added 2ml of diluted ammonia. 5ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and draggendoff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggnendoff's reagent) was regarded as positive for the presence of alkaloids.

3.5.2 Determination of saponin

To 0.5g of extract was added 5ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. It was then observed for the formation of an emulsion.

3.5.3 Determination of tannin

About 0.5g of the extract was boiled in 10ml of distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for Blue-black, brownish green or green precipitate colouration.

3.5.4 Determination of terpenoids (Salkowski test)

To 0.5g each of the extract was added 2ml of chloroform in a test tube. 3ml of conc. H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish brown coloration was formed in the presence of Terpenoid as a positive result.

3.5.5 Determination of flavonoids

Three methods were used to test for flavonoid, first, dilute ammonia(5ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid(1ml) was added. A yellow colouration that dissappear on standing indicate the presence of Flavonoids

Second, a few drops of 1% Aluminium solution were added to a portion of the filtrate. A yellow colouration indicate the presence of Flavonoids. Third, a portion of the extract was heated with 10ml of ethyl acetate over a steam bath for 3min. the mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration indicate the presence of Flavonoids.

3.5.6 Determination of phenol

About 2ml of the sample was mixed with 2ml of ferric chloride. The formation of dark blue colouration indicate the presence of phenol.

3.5.7 Determination cardiac glycosides

To 0.5g of extract diluted to 5ml in water was added 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with with 1ml of concentrated sulfuric acid. A brown ring of the interface indicate a deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring. While in the acetic acid layer, a greenish ring may form just above the brown ring and gradually throughout a thin layer.

3.5.8 Determination for steroids

About 2ml of acetic anhydride was added to 0.5ml of the sample with 2ml of H_2SO_4. A colour change from violet to blue or green indicates the presence of steroids.

3.5.9 Determination for anthraquinones

About 0.5g of the extract was boiled with 10ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for colour changes.

CHAPTER FOUR

RESULTS

Table 2: Phytochemical screening of mixed fermented *Citrullus colocynthis* fruit *with* clove (*Syzygium aromaticum*) plus bark of baobab tree (*Adansonis digitata*).

Test	Saponin	tannin	flavonoid	cardiac glycosides	terpenoids	steroids	anthraquinones	alkaloids	phenol
wtCWA$_1$	+ve	+ve	++ve	-ve	+ve	+ve	+ve	++ve	+ve
wtCWA$_2$	+ve	+ve	++ve	-ve	+ve	+ve	+ve	++ve	+ve
wtCP1	-ve	-ve	+ve	-ve	+ve	++ve	-ve	+ve	-ve
wtCP$_2$	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve
wtCPA$_1$	+ve	++ve	++ve	+ve	+ve	++ve	+ve	++ve	+ve
wtCPA$_2$	+ve	++ve	++ve	+ve	+ve	++ve	-ve	++ve	+ve
wtCW$_1$	+ve	+ve	+ve	-ve	+ve	++ve	+ve	+ve	+ve
wtCW$_2$	+ve	+ve	+ve	+ve	+ve	++ve	+ve	+ve	+ve
wtCON$_1$	-ve	+ve	+ve	-ve	+ve	+ve	+ve	++ve	+ve
wtCON$_2$	-ve	+ve	++ve	-ve	+ve	+ve	+ve	+ve	+ve

Key: -ve: Absent ; +ve: Present; ++ve: Abundantly present.

wtCW: *Citrullus colocynthis* + water (in wet form); wtCP: *Citrullus colocynthis*+ palmwine (in wet form) wtCWA: *Citrullus colocynthis* +water+ Clove (*Syzygium aromaticum*) +Bark of baobab tree (*Adansonis digitata*) (in wet form); wtCPA: *Citrullus colocynthis* + palmwine + Clove (*Syzygium aromaticum*) +Bark of baobab tree (*Adansonis digitata*) (in wet form); wtCON: wet Control (i .e. the fresh fruit).

Table 3: Phytochemical screening of mixed fermented *Citrullus colocynthis* fruit with clove (*Syzygium aromaticum*) plus bark of baobab tree (*Adansonis digitata*).

est	Saponins	Tannin	Flavonoids	Cardiac glycoside	Terpenoids	Steriods	Anthraquinones	Alkaloids	phen
tCWA$_1$	+ve	++ve	+ve	-ve	+ve	+ve	++ve	+ve	+v
tCWA$_2$	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+v
tCP$_1$	+ve	+ve	+ve	-ve	+ve	+ve	++ve	+ve	+v
tCP$_2$	+ve	++ve	++ve	+ve	+ve	+ve	++ve	+ve	+v
tCPA$_1$	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+v
tCPA$_2$	+ve	++ve	+ve	-ve	+ve	+ve	+ve	+ve	+v
tCW$_1$	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve
tCW$_2$	+ve	+ve	+ve	-ve	++ve	+ve	++ve	+ve	+ve
tCON$_1$	+ve	++ve	+ve	+ve	+ve	+ve	++ve	+ve	+ve
tCON$_2$	+ve	++ve	++ve	+ve	+ve	+ve	+ve	+ve	+ve

KEY: -ve: Absent; +ve: Present; ++ve: Abundantly present

dtCW: *Citrullus colocynthis* + water (in dry form); dtCP: *Citrullus colocynthis*+ palmwine (in dry form); dtCWA: *Citrullus colocynthis* +water+ Clove (*Syzygium aromaticum*) +Bark of baobab tree (*Adansonis digitata*) (in dry form); dtCPA: *Citrullus colocynthis* + palmwine + Clove (*Syzygium aromaticum*) +Bark of baobab tree (*Adansonis digitata*) (in dry form) ; dtCON: dried Control (i .e. the fresh fruit).

Table 2 shows that *Citrullus colocynthis* contain phytochemical compounds. The phytochemical compounds tested for, in both wet and dried samples are Saponin, alkaloids, flavonoid, tannin, cardiac glycosides, steroids, phenols, Anthraquinones and Terpenoid in which all were present in all tested samples except cardiac glycoside which is present in few of the samples. In this study, phytochemical screening showed that *Citrullus colocynthis* fermented using palm wine as solvent (i.e. CP) in wet form, Saponin, tannin and phenol are absent with an abundant steroids. This indicates the possibility of the potency of the CP against cancerous disease and bacterial infections. In Table 3 tannin, flavonoid, anthraquinone, and phenol are abundantly present, this shows that it can be used in treatment of wild range of disease. CPA (i.e. *Citrullus colocynthis* fermented with palmwine with addition of Clove (*Syzygium aromaticum*) and Bark of baobab tree (*Adansonis digitata*) under wet condition: tannin, flavonoids, steroid, and alkaloid are abundantly present, the mixture tends to be acidic on fermentation. This implies that the composition can be effective against skin disease, hypertension, diabetes and allergic reactions. Table 3 shows that in CPA cardiac glycoside is absence. It can be used as broad spectrum antibiotics. CWA (i.e. *Citrullus colocynthis* with water, Clove (*Syzygium aromaticum*) and Bark of baobab tree (*Adansonis digitata*) in wet form show the abundant of flavonoid and alkaloids with absence of cardiac glycoside while in dried form, cardiac glycoside is also absent, this shows that CWA can be used to regulate blood pressure, sugar level and reduce the risk of digesting toxic compound. CW (i.e. *Citrullus colocynthis* with water) in wet and dried form lack cardiac glycoside while steroid is abundant in wet form. (this can be used against the growth of cancer cells). In the control sample CON i.e. the fruit alone, Saponin and cardiac glycosides are absent in wet sample with abundance of flavonoid and alkaloid, these compounds are effective antimicrobial agent, and are capable of reducing heart disease.

Generally in this study, alkaloids are abundantly present in most of the samples, Saponin is absent in wet sample of the control and it is rarely abundant in most of the sample. Tannin is present in the entire sample except in CP sample. Flavonoid is present in both wet and the dried samples. Phenol is also present in all except in wet form of CP sample. Cardiac glycoside is rarely present in most of the samples, this helps to establish the safety in the use of this plant as a medicinal plant because, this particular compound possesses a toxic nature which can be dangerous to health.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

Phytochemical compounds are chemical compounds formed during plant normal metabolism of which there are several classes including alkaloids, glycosides, Flavonoids, coumarins, gums, polysaccharides, phenol, tannins, terpenoids (Okwu *et al.*, 2004). Flavonoids, polyphenol have been identified as the active chemical constituents of some medicinal plants, and thus responsible for their pharmacological importance (Sofowora, 1993). Flavonoids and Phenolic compounds are the major group of compounds that act as primary antioxidants or free radical scavengers (Ayoola *et al.*,2008). Alkaloids, saponins, Flavonoids, polyphenol have been identified as the active chemical constituents of some medicinal plant and are therefore responsible for their pharmacological indications (Sofowora, 1993). These makes *Citrullus colocynthis* fruit a medicinal plant, effective against wild range of disease, it has long term health benefit. Furthermore, phenols and steroids are considered to be potentially toxic to the growth and development of pathogenic organisms (Okwu, 2004). The result of the phytochemical screening of *Citrullus colocynthis* fruits, leaves, and root showed the presence of Saponin, sterols, steroids, terpen, flavonoid, tannin, and alkaloids in different proportion in the tree part of plant, this result correlate with the findings of Belsem *et al.*, 2009 which proved that alkaloids were found in all extracts except roots, Flavonoids were present only in seeds; gallic tannin and coumarins only in leaves, and all of them contained steroids (Nahal, 2015).

Three major phytochemical compounds are crucial in combating microorganism such as bacteria, fungi. Such phytochemical compounds are terpenoid, alkaloid and phenol (Saxena *et al.*, 2013).

. Flavonoids are synthesized by plant in response to microbial infection that have been found in vitro to be effective against wild range of microorganism, it presence in medicinal plant helps to prevent oxidative cell damage, it also have strong anti cancer activity and protect against all stage of carcinogens (Harborne, 1973).

5.2 Conclusion

From this study, *Citrullus colocynthis* contains different phytochemical compounds which are terpenoids, phenol, Flavonoids, alkaloids, Saponin, cardiac glycosides, steroids, tannin and anthraquinone. The mixture of this fruit with certain biologically active agent such as clove and bark client enhances it performance as a medicinal plant.

It has been reported that *Citrullus colocynthis* is very potent in curing several ailment and medical conditions, the potency of this plant is due to the presence of the bioactive component.

CPA (i.e. *Citrullus colocynthis* + palmwine+ clove + bark client) is potent against wild range of disease. And cardiac glycoside which is sometimes responsible for the toxicity of different plants is rarely present in this fruit.

Nowadays, medicinal plants are showing tremendous promise for preventive intervention in the pathogenesis of many diseases, as well as their treatment.

5.3 Recommendation

As the world turns to herbal remedies in the treatment of adverse and serious medical conditions, this research will be of great importance to researchers and pharmaceutical laboratories as *Citrullus colocynthis* provides great prospect in the formulation of new drugs in the treatment of malaria, cancer, heart failure, hypertension, diabetes e.t.c.

REFERENCE

Allali H, Benmehdii H, Dib M, Tabti B, Ghalem S, and Benabadj N. (2008). Phytotherapy of Diabetes in hest Algeria. *Asian Journal Chem.* **7**: 2701-2710.

Ayoola,G., Coker, H., Adesegun, S., Adepoju-Bello, A., Obaweya, K., Ezennia E., Atangbayila, T.(2008). Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used For malaria Therapy in Southwestern Nigeria. *Tropical journal of pharmaceutical research.* 7(3): 1019-1024.

Bronstein, A., spyker D., Louis R., Green, J., Rumack, B., Griffin,s. (2008). Annual report of the American association of poison control centers' national poison data system(NPDS). *Clinical toxicology:* 47 (10): 911-1080.

Belsem,M., Ehsen .H., Nadia .F., Mahjoub A. Antibacterial activity and chemical composition of essential oil of *Pamburus missionis. Journal of ethnopharmacology.* **124(1)**: 151-153.

Edeoga H, omobona G, uche L. (2006). Chemical composition of *hyptis suaveolens* and *Ocimum gratissimum* hybrid from Nigeria. *African Journal Biotechnology* 5**(910)**: 892-895.

Eidi ,S., Azadi H., Rahbar N., Mehmannavaz H. (2015). Evaluation of antifungal activity of hydroalcoholic extracts of *Citrullus colocynthis* fruit. *Journal of Herbal Medicine.***5(1):** 36-40.

Ejiofor, M. A. N. (1994). Nutritional value of ogbono *(Irvigna gabonesis* var. excelsa). Proceedings of the ICRAF-IITA conference on *Irvigna gabonesis. Ife Journal of science Nigeria.* **14:** 177-183.

Elhadi, I. Koko,W., Dahab, M..,Imam,Y ,. and Magged, M(2013). antigiadial activity of some cucurbita species and lagenaria siceraria. *Journal of Forest Product and Industries.* **2(4):**43-47.

Fabricant D, Farnsworth N. (2001).The value of plants Used in Traditional Medicine for Drug Discovery. *Journal of Environmental Health Perspective.* **1:** 69-75.

Francis, G.,Kerem, Z., Makkar, H. and Becker, K. (2002). The biological action of saponins in animal system. *Journal of nutrition.* **88**:587-605.

Goor, G., Glenneberg, J. Jacobi, S. (2007). Hydrogen peroxide. *Ullmann's Encyclopedia of Industrial chemistry*. Weinheim: Wiley-VCH.

Gurudeeban., S., Satyavani ,K., Ramanathan, T. (2010).Bitter Apple *Citrullus colocynthis.* An overview of chemical composition and Biomedical potentials. *Asian Journal of plant sciences.* **9(7):**394-401.

Hanukoglu I (1992). Steroidogenic enzymes: structure, function, and role in regulation of steroid hormone biosynthesis. *The Journal of Steroid Biochemistry and Molecular Biology.* **43(8):**779-804.