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The in-Vitro Antioxidant Properties and Phytochemical Constituents of Citrullus Colocynthis Methanolic Extract

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ABSTRACT

This study was carried out to determine the in-vitro anti-oxidant, anti-diabetic and phytochemical constituents of the methanolic leaves extract of *Citrullus colocynthis.* The results of this study show that *Citrullus colocynthis*h has strong antioxidant potentials against various radicals. The extract concentration-dependently scavenges DPPH radicals with IC_{50} of 357.14µg/ml compared to 16.32µg/ml of standard gallic acid, scavenge nitric oxide radicals with IC_{50} of 234µg/ml compared to 20.5µg/ml of standard ascorbic acid and has 20% activity of standard ascorbic acid ferric reducing power. The extract also inhibited induction of lipid peroxidation and α-Amylase activity in a concentration-dependent manner in cell free assays with highest concentration of the extract inhibiting lipid peroxidation and α-Amylase activity by 72% at 500µg/ml and 91% at 250µg/ml respectively. The phytochemical constituents of the extract were determined, qualitative assays employed revealed the presence of various phytochemicals in the extract and the amount of total phenolics, flavonoids and flavonol were found to be 58.76mg GAE/g, 2.01mg RE/g and 0.13mg RE/g respectively. Gas-chromatography analysis of the extract revealed the possible presence of albumin, ascorbic acid, beta carotene, vicilins, tocotrienols, lutin, 2-tetradcyclobutane, 2-dodecyclobutane. The presence of phenolics and various antioxidant compounds in the plants may explain the strong pharmacological potentials of the plant.

INTRODUCTION

Oxidative stress results from the imbalance between the production and neutralization of reactive oxygen species such that the antioxidant capacity of cell is overwhelmed[1]. It is estimated that every day, a human cell is targeted by the hydroxyl radical and other such species at an average of 105 times inducing oxidative stress[2]. Studies have shown the relationship between increased level of ROS and incident of diabetes mellitus[3, 4]. It has been reported in a study that increased insulin concentration is associated with increased free radical production[5], while lipid peroxidation has been implicated in the pathogenesis of many degenerative disorders including naturally occurring and chemically induced diabetes mellitus[6, 7].

Presently, the popularity of herbal medicine in primary health care is on the increase this is because, the greater population of most developing countries depends on plants for their primary health care[8]. Many bioactive compounds have been isolated and characterized in medicinal plants which are used as the active ingredients of modern medicines, or as the lead compounds for new drug discovery. These plant-derived medicines are rich in phenolic compounds, flavonoids, tannins, alkaloids etc., used in the treatment of various diseases including diabetes mellitus[9, 10]. A large number of medicinal plants have been investigated for their antioxidant properties. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive process caused by oxidative stress[10].

C. colocynthis (popularly called *Egusi Bara* in West Africa) is a perennial herb commonly found wild in the sandy lands of west Africa, Nigeria. It is also found indigenous in Arabia, West Asia, Tropical Africa and in the Mediterranean region[11]. The plant has been reported to contained many phytochemicals which includes tannins, saponins, phenolics, flavonoids, terpenoids and alkaloids. Pharmacological investigation revealed that the plant possessed antimicrobial, anticancer, anti-inflamatory, analgesic, gastrointestinal, reproductive, and many other pharmacological effects[11].
Presently, only a few studies have been conducted on the antioxidant and anti-diabetic effects of the plant Therefore, the present study was aimed to investigate the in-vitro antioxidant and antidiabetics potentials of *C. colocynthis* extract and to determine the phytochemical constituents of the leaves extract.

MATERIALS AND METHODS

Reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, thiobarbituric acid, sodium carbonate, sodium nitroprusside, naphthylethylenediamine dichloride, α-amylase and potassium hexacyanoferrate, and trichloroacetic acid were obtained from Sigma–Aldrich Chemical Co. Ltd. (England). All other reagents and chemicals used were of analytical grade.

Plant Collection and Identification

Fresh leaves of the *C. colocynthis* were plucked from the Research farm of the Faculty of Agriculture, Ladoke Akintola University of Technology, Ogbomoso. The plant was

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identified and authenticated by a Taxonomist, Dr. Afuwape Adesina, of the Herbarium unit of Pure and Applied Biology Department, Ladoke Akintola University of Technology, Ogbomoso.

Preparation of Crude Methanolic extract

The leaves of *C. colocynthis* plant were air dried under shade for two weeks and then grinded into a powder. Two hundred grams (200 g) of the blended plant was macerated and extracted with methanol using soxhlet extractor for 72 hours at room temperature. The organic solvent was evaporated using rotary vacuum evaporator at 45^0C. The resulting extract (residue) was scraped into a well air-tight container and refrigerated at -20^{o}C for further use.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The assay was performed as previously described by Schelesier et al.[12]. In the DPPH assay, the radical solution is prepared by dissolving 2.4mg DPPH in 100mls of ethanol. An antioxidants reduce the free radical 2,2-Diphenyl-1-picrylhydrazyl, which has an absorption maximum at 517nm. 1.95ml DPPH was measured as blank, for the photometric assay 1.95ml DPPH solution and 50µl antioxidant solution (plant extract) were mixed. The reaction was measured after 30 minutes until $\Delta A=0.003min^{-1}$. The anti-oxidative activity was calculated by determining the decrease in absorbance at different concentrations by using the following equation.

$$\% \text{ Inhibition activity} = \frac{\text{A (DPPH)} - \text{A (Extract)}}{\text{A (DPPH)}} \times 100$$

Ferric Reducing Antioxidant property (FRAP) Assay

The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu[13]. The extract (0.75 mL) at various concentrations was mixed with 0.75 mL of phosphate buffer (0.2 M, pH 6.6) and 0.75 mL of potassium hexacyanoferrate $[K_3Fe(CN)_6]$ (1%, w/v), followed by incubating at 50^{o}C in a water bath for 20 min. The reaction was stopped by adding 0.75 mL of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 3000 r/min for 10 min. 1.5 mL of the supernatant was mixed with 1.5 mL of distilled water and 0.1 mL of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power.

Nitric oxide scavenging activity

The Nitric oxide scavenging activity was determined as previously described by Garrat[14]. Two mL of 10 mM sodium nitroprusside dissolved in 0.5 mL phosphate buffer saline (pH 7.4) is mixed with 0.5 mL of sample at various concentrations (0.2–0.8 mg/mL). The mixture is then incubated at 25^{o}C. After 150 min of incubation, 0.5 mL of the incubated solution is withdrawn and mixed with 0.5 mL of Griess reagent [(1.0 mL sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 mL of naphthylethylenediamine dichloride (0.1% w/v)]. The mixture is then incubated at room temperature for 30 min and its absorbance pouring into a cuvette is measured at 546 nm. The amount of nitric oxide radical inhibition is calculated following this equation

Inhibition of NO radical = $[A_0 - A_1]/ A_0 \times 100$

Where A_0 is the absorbance before reaction and A_1 is the absorbance after reaction has taken place with Griess reagent.

Lipid Peroxidation Inhibition Assay

For this assay, egg yolk homogenate was used as lipid source and free radicals were produced by Fenton reagent ($FeSO_4/H_2O_2$), a modified thiobarbituric acid reactive substances (TBARS) assay previously described by Jenero[15] was employed. 1 mL reaction mixture containing 0.5 mL egg yolk homogenate (10% in distilled water, v/v), 0.1 mL of extract was mixed with 0.05 mL $FeSO_4$ (0.07 M) and incubated for 30 min to induce lipid peroxidation. Free radical ruptures the lipid bilayer to form malonaldehyde as a secondary product. Two molecules of thiobarbituric acid react with one molecule of MDA to form pink colored product showing maximum absorbance at 532 nm called TBARS. When the reaction mixture was mixed with different concentrations of extract, it reduces the formation of TBARS product in concentration dependent manner in comparison to control. The percentage induction of lipid peroxidation is 100% in the control which is compared to reduction in the plant extract samples.

Inhibition of α-Amylase

The determination of α-amylase inhibition was carried out using a modified dinitrosalicylic acid (DNS) method previously described by Bernfeld[16]. 1mL of methanolic extracts of *C. colocynthis* were pre-incubated with α-amylase 1 U/mL for 30 min and thereafter 1 mL (1% w/v) starch solution was added. The mixture was further incubated at 37°C for 10 min. Then the reaction was stopped by adding 1 mL DNS reagent (12.0 g of sodium potassium tartrate tetrahydrate in 8 mL of 2 M NaOH and 96 mM 3, 5-dinitrosalicylic acid solution) and the contents were heated in a boiling water bath for 5 min. A control was prepared without plant extracts and replaced by equal quantities of buffer (20 mM Sodium phosphate buffer with 6.7 mM Sodium chloride, pH 6.9 at 20^{o}C). The absorbance was measured at 540 nm.

Calculation

% inhibition = $[A_0 - A_1]/ A_0 \times 100$

Where A_0 is the absorbance of control and A_1 is the absorbance of sample

Phytochemical screening of *C. colocynthis*

Test for flavonoids

Methodology adopted is as reported by Sofowara[17]. 50 mg of *C. colocynthis* was suspended in 100 ml of distilled water to get the filtrate. 5 ml of dilute ammonia solution was added to 10 ml of filtrate followed by few drops of concentrated H_2SO_4. Presence of flavonoids was confirmed by yellow colouration.

Test for terpenoids

The test for terpenoids employed the methodology reported by Ejikeme et al.[18]. A mixture of chloroform (2 mL) and concentrated tetraoxosulphate (VI) acid (3 mL) was added to 5 mL of *C. colocynthis* to form a layer. The presence of a reddish brown colouration at the interface shows positive results for the presence of terpenoids.

Test for tannins

The detection of tannins makes used of method reported by Ejikeme et al.[18]. 100mg of *C. colocynthis* was weighed into a test tube and boiled for 10 minutes in a water bath containing 30 mL of water. Filtration was carried out after boiling and 3 drops of 0.1% ferric chloride was added to 5 mL of the filtrate. A brownish green or a blue black colouration showed positive test.

Test for phenols

Presence of phenols in *C. colocynthis* was carried out by adding few drops of ferric chloride solution to 10 mg of *C. colocynthis*. Formation of bluish black colour indicates the presence of phenol[19].

Test for saponins

The ability of saponins to produce emulsion with oil was used for the screening test[20]. 20 mg of *C. colocynthis* was

boiled in 20 mL of distilled water in a water bath for five min and filtered. 10 ml of the filtrate was mixed with 5 mL of distilled water and shaken vigorously for froth formation. 3 drops of olive oil were mixed with froth, shaken vigorously and observed for emulsion development.

Test for phytosterol

Presence of terpenoids in *C. colocynthis* was carried out by adding 5ml of chloroform to small quantity of *C. colocynthis*. A few drops of concentrated Sulphuric acid was later added to this mixture and allowed to stand for a minute. Formation of brown ring indicates the presence of phytosterol[20].

Test for alkaloids

0.4 g of *C. colocynthis* was stirred with 8 ml of 1% HCl and the mixture was warmed and filtered[20]. 2 ml of filtrate was treated with few drops of potassium mercuric iodide (Mayer's reagent). Turbidity or precipitation with the reagent was taken as evidence for existence of alkaloids.

Quantitative Phytochemical Analysis

Determination of total phenolic compound in methanolic extract of *C. colocynthis*

The total phenolic in *C. colocynthis* were determined by Folin-Ciocalteu method[21]. For the preparation of calibration curve, 1ml of aliquots of 0.24, 0.075, 0.0105 and 0.3mg/ml ethanol gallic acid solutions were mixed with 1ml Folin-Ciocalteu reagent (diluted ten-fold) and 4ml (75g/L) sodium carbonate. The absorbance was read after 30mins at 20^{o}C at 765nm and the calibration curve was drawn. 1ml of *C. colocynthis* was mixed with 1ml Folin-Calteus reagent and 4ml (75g/L) sodium carbonate and after 30minutes the absorbance was read for the determination of plant phenolic. All determination was performed in triplicate. Total content of phenolic compounds in *C. colocynthis* (Gallic acid equivalent) was calculated using the following formula

$C = c. v/m$

Where C is the total content of phenolic compound (mg/g plant extract, in GAE)

c is the concentration of Gallic acid established from the calibration curve in mg/ml

v is the volume of extract in ml

m is the weight of pure plant extract.

Determination of Flavonoids in *C. colocynthis*

The content of flavonoids was determined by a pharmacopeia method[22], using rutin as a reference compound. One ml of *C. colocynthis* in methanol (10g/L) was mixed with l ml aluminium trichloride in ethanol (20g/L) and diluted with ethanol to 25ml. The absorption at 415 nm was read after 40 min at 20^{0}C. The blank samples were prepared from l ml plant extract and l drop of acetic acid, and diluted to 25ml. The absorption of rutin solutions was measured under the same conditions. Standard rutin solution was prepared at a concentration of 0.1 mg/ml. All determinations were carried out in duplicate. The amount of flavonoids in plant extracts in rutin equivalents (RE) was calculated by the following formula:

$X = (A \cdot m_o \cdot 10) / A_o \cdot m)$

Where: X - flavonoid content, mg/g plant extract in RE: A-the absorption of plant extract solution: A_o- the absorption of standard rutin solution: m-the weight of plant extract, g; m_o-the weight of rutin in the solution, g.

Determination of Flavonols in *C. colocynthis*

The content of flavonols was determined by Yermakov's method[23]. The rutin calibration curve was prepared by mixing 2ml of 0.5, 0.4, 0.3, 0.2, 0.166, 0.1, 0.05, 0.025, and 0.0166 mg/ml rutin ethanol solutions with 2ml (20g/L) aluminum chloride and 6ml (50g/l) sodium acetate. The absorption at 440 nm was read after 2.5h at 20^{0}C. The same procedure was carried out with 2ml of plant extract (10g/L) instead of rutin solution. All determinations were carried out in duplicates. The content of flavonols, in rutin equivalents (RE) was calculated by the following formula:

$X=C \cdot V/m$

Where, X-flavonols content, mg/g plant extract in RE: C-the concentration of rutin solution, established from the calibration curve, mg/ml: V, m-the volume and weight of plant extract, ml, g.

Gas chromatography

Gas chromatography (GC) spectra were run on a Shimadzu GC-17A gas chromatography outfitted with autosampler and a Flame Ionization detector (FID). GC column used was, fused silica capillary column OV-1, DB-1 (30 m x 0.53 mm, 0.5 µm film thickness), at 75 °C and programmed to 75 °C at 240 °C/min and 5 min hold. Injector and detector were at 240 and 250 °C respectively. About 1 µL of each sample were injected in an splitless mode. Relative quantity of the chemical compounds present in extract of *C. colocynthis* was expressed as percentage based on peak area produced in the chromatogram. The identification of *C. colocynthis* constituents was carried out by comparison of GC retention times of *C. colocynthis* with GC retention times of desired standards compounds.

RESULTS

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The *C. colocynthis* demonstrated a concentration and time dependent scavenging activity by quenching

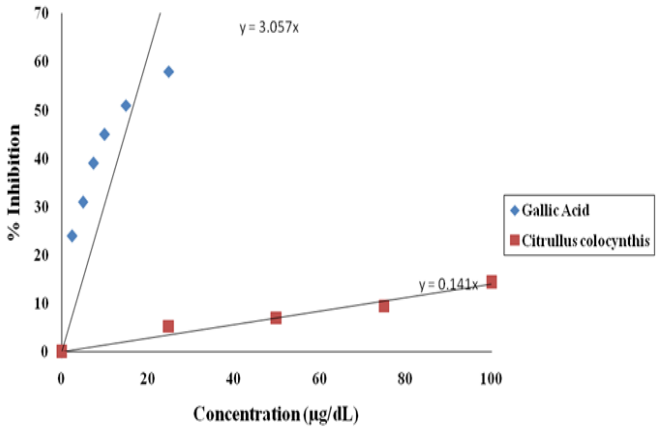

Figure 1. The effects of different concentrations of *C. colocynthis* and Gallic on the inhibition of the DPPH radical.

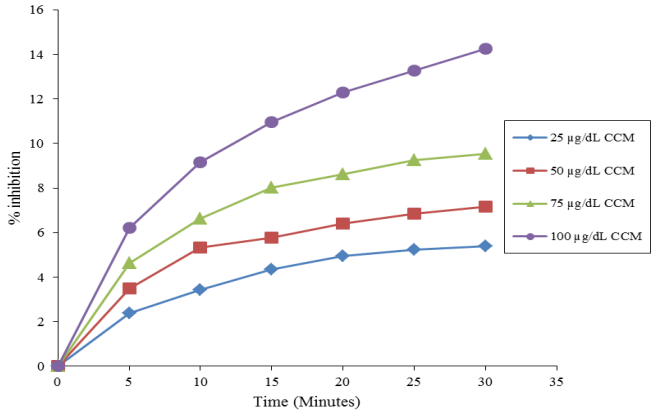

Figure 2. The effects of time on different concentration of methanolic extract of *C. colocynthis* on inhibition of DPPH radical.

DPPH radicals (Figure 1) and was compared with gallic acid, as a positive control.

The IC50 values (defined as the concentration of test compound required to produce 50% inhibition) for DPPH scavenging by *C. colocynthis* and gallic acid were 98.45 ± 3.47 µg/dL and 16.33 ± 1.50 µg/dL respectively (Figure1&2).

Ferric Reducing Antioxidant power (FRAP) Assay

The reducing abilities of *C. colocynthis* root was evaluated and compared with standard ascorbic acid. The reductive capabilities were found to increase with increasing concentration of *C. colocynthis* and standard ascorbic acid. Comparatively, the reducing abilities of *C. colocynthis* was 20% of that of standard ascorbic acid at the same concentration and experimental conditions (Figure 3).

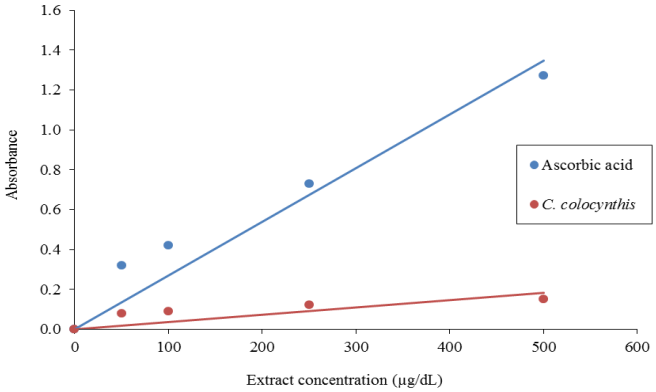

Figure 3. Ferric reducing power of *C. colocynthis* and ascorbic acid at different concentrations.

Lipid Peroxidation Inhibition Assay (TBARS)

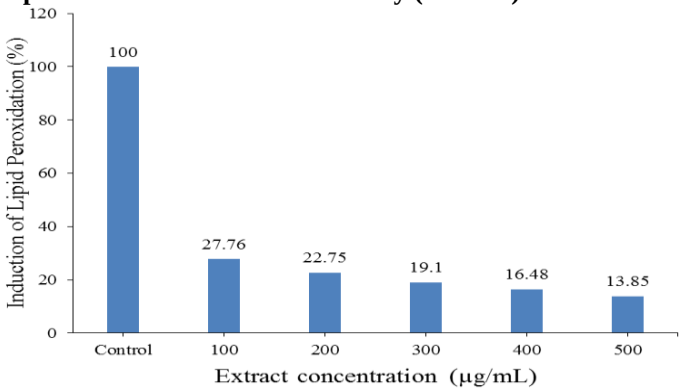

Figure 4. The effects of different concentrations of C. colocynthis on inhibition of α-amylase activity.

The ability of different concentration of *C. colocynthis* to reduce the induction of lipid peroxidation was compared with the control sample where lipid peroxidation induction was 100%. Decrease in the induction of lipid peroxidation was observed with increased concentration of *C. colocynthis*, 100µg/ml of the extract reduced lipid peroxidation induction by x while 400µg/ml reduced lipid peroxidation induction by y (Figure 4)

Nitric oxide scavenging activity.

The Nitric Oxide scavenging ability of *C. colocynthis* leaf extract was compared with the standard Ascorbic acid. The *C. colocynthis* demonstrated a concentration dependent scavenging ability with IC50 values (defined as the concentration of test compound required to produce 50% inhibition) for nitric oxide scavenging by *C. colocynthis* and ascorbic acid were 376.51µg/ml and 152.39µg/ml respectively (Figure 5).

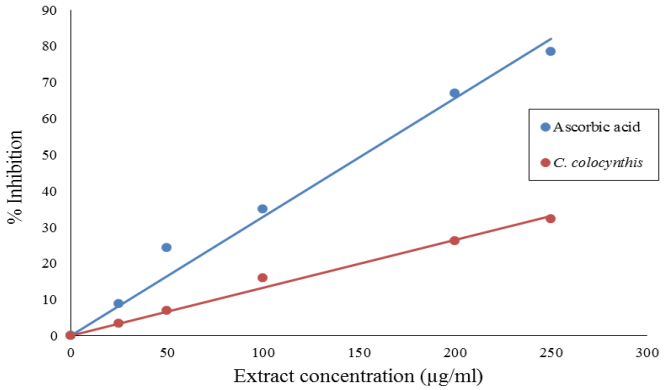

Figure 5. The effects of different concentrations of *C. colocynthis* on the inhibition of Nitric oxide radical formation.

Inhibition of α-Amylase

The methanolic extract of *C. colocynthis* significantly inhibited α–amylase activity in this study. The level of inhibition was found to be concentration dependent and maximum percentage inhibition of α–amylase activity (91.2 ± 2.61%) was obtained at 250µg of the extract (Figure 6).

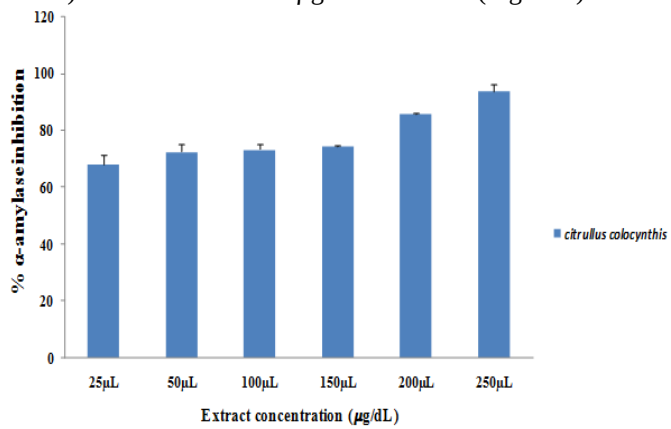

Figure 6. The effects of different concentrations of *C. colocynthis* on inhibition of α-amylase activity.

Table 1. Phytochemical composition of the methanolic leaves extract of *C. colocynthis* .

Constituents	Test	Observation	Inference
Flavonoid	Alkaline reagent	Yellow color turn Colorless	+
Terpenoids	Chloroform	Colourless	-
Tannins	Ferric chloride	Brownish green colouration	+
Phenol	Ferric chloride	Blue black coloration	+
Phytosterol	Chloroform test	No brown ring formed	-
Saponin	Foam test	Foam for some minutes	+
Flavonol	Lead Acetate	Yellow precipitate	+
Alkaloids	Mayer's test	Cream color precipitate	+

Table 2. The total phenolic content of *C. colocynthis* in mg gallic acid equivalent / g dry weight and total flavonoids and flavonols content in mg rutin equivalent / g dry weight. Values are the means of three experiments ± SEM.

Sample	Total phenolic compounds mg/g plant extract (in GAE)	Total flavonoids mg/g plant extract (in RE)	Total flavonols mg/g plant extract (in RE)	DPPH scavenging activity (IC 50)
Gallic	ND	ND	ND	16.33 ± 1.50
C. colocynthis	58.76±2.42	2.01±0.11	0.13±0.02	98.45 ± 3.47

ND: Non Detected

Phytochemical Analysis

The result of the qualitative analysis of the phytochemicals in *C. colocynthis* extract is presented in Table 1. The study revealed the presence of alkaloids, flavonoids, flavonols, tannins, phenol and saponin, in the *C. colocynthis* extract while terpenoids and phytostyrol are absent.

Quantitative determination of total phenolic, flavonoid and flavonol compound in methanolic extract of *citrullus colocynthis*.

The phenolic content of methanolic extract of *C. colocynthis* was determined using a colometric assay (Folin-Ciocalteu), and by constructing a standard curve using Gallic acid as the standard. The total amount of phenolic compounds present in methanolic extract of *C. colocynthis* was found to be 58.76mg in Gallic acid equivalent. Also, the flavonoid and flavonols content of *C. colocynthis* extract were determined and were found to be 2.01mg/g and 0.13 mg/g of rutin equivalent respectively (Table 2).

GC ANALYSIS

Table 3. Biologically active Chemical compounds identified in methanolic extract of *C. colocynthis* leaves.

Component	Retention	Relative (%)
Albumin	1.03	23.33
Ascorbic acid	1.88	1.46
Beta carotene	2.25	15.44
Arginine	8.82	1.56
Leucine	9.68	2.96
Isoleucine	11.12	4.35
Palmitic acid	11.42	2.73
Linoleic acid	11.75	3.34
Stearic acid	12.26	6.58
Linolenic acid	12.76	5.29
Lycopene	13.48	5.32
Glutein	13.95	5.66
Globulin	14.65	1.82
Vicilin	15.35	3.55
Tocotrienols	15.68	6.94
Xanthophyls	16.55	0.87
Lutein	17.46	3.14
20teradcyclobutane	18.53	1.21
2-dodecyclobutanone	19.57	4.45

The compounds present in methanolic extract of *C. colocynthis* leaves are shown in Tables 3. Their identification and characterization were based on their elution order in a GC column.The elution time and the amount of these compounds were also presented. Based on abundance, the top two major compounds present in the methanolic extract are albumin (23.33%) and beta carotene (15.44%).The GC chromatograms of the extract presented in Figure 7 show the retention time in the column and the detected peaks which correspond to the compounds present in the extract.

DISCUSSION

Oxidative stress which results from the imbalance in the free radical generation and the scavenging ability of antioxidants in the body have been implicated in several disease states such as neurodegenerative diseases, cardiovascular diseases and most especially, type 2 diabetes mellitus[24]. Various studies have shown that high consumption of medicinal plants, fruits and vegetables containing various plant's secondary metabolite have been of great impact in ameliorating the effects of oxidative stress[25].

Among the various methods used to evaluate the total antioxidant activity of vegetables or other plants, DPPH radical scavenging assay is among the common applied method. In the present study, *C. colocynthis* showed DPPH radical scavenging activity which was found to be dependent on concentration and time. The DPPH radical scavenging activity of plants extract have been attributed to their hydrogen donating potential[26]. The ability of *C. colocynthis* extract to reduce Fe^{3+}/Fe^{2+} was determined by FRAP assay. *C. colocynthis* extract was found to have strong ferric reducing power and this ability was found to be concentration-dependent. Since FRAP assay is easily reproducible and linearly related to molar concentration of the antioxidants present, thus it can be reported that extracts of *C. colocynthis* may act as free radical scavenger, capable of transforming reactive free radical species into stable non radical products.

Nitric oxide (NO) has been implicated in chronic inflammation, cancer and other pathological conditions[27].

The NO generated at physiological pH and under aerobic conditions, reacts with oxygen to produce stable products (nitrate and nitrite). Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions[27]. *C. colocynthis* methanolic extract scavenge NO in this study in a concentration dependent manner resulting in reduced production of nitrite ions. *C. colocynthis* nitric oxide scavenging ability was compared with standard ascorbic acid and the result showed that *C. colocynthis* has strong antioxidant potential against nitrite ions formation.

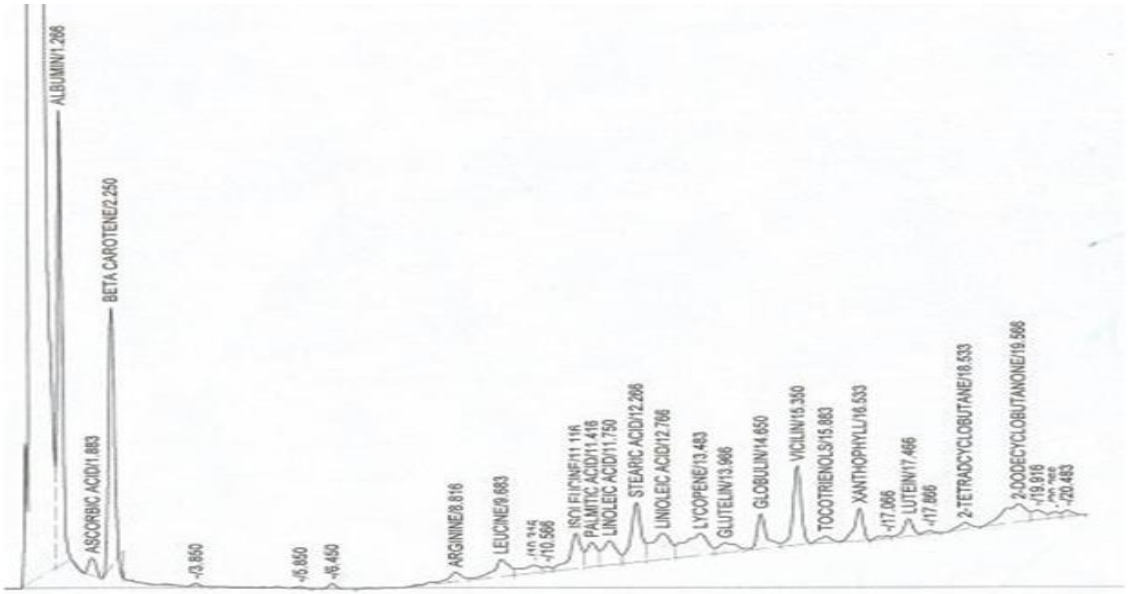

Figure 7. GC chromatogram of *Citrullus colocynthis* leaves methanol extract.

Lipid peroxidation, which is widely recognized as primary toxicological event, is caused by the generation of free radicals from a variety of sources including organic hydro peroxides, redox cycling compounds and iron-containing compounds[28]. Using TBARS assay, methanolic leaves extract of *C. colocynthis* was found capable of preventing the formation of malondialdehyde (MDA) in a concentration dependent manner in this study by reducing percentage induction of MDA when compared to maximum induction of 100% observed in the control.

Management of the blood glucose level is an essential approach in the control of diabetes complications. Inhibitors of carbohydrates hydrolysing enzymes (α-amylase) have been helpful as oral hypoglycemic medicines for the control of hyperglycemia exclusively in patients with type-2 diabetes mellitus[29]. In this study, *C. colocynthis s*howed strong α-amylase inhibitory activity which could be attributed to the presence of phenolic compounds in the plant extract[30].

It has been reported that free radicals scavenging capacity of plants extracts is due to the presence of phenolic compounds in plants which could act as a hydrogen donor antioxidant[26]. The total phenolic content of methanolic leaves extract of *C. colocynthis* determined in this study was found to be 58.76 mg/g in gallic acid equivalent while total flavonoids and total flavonols were found to be 2.01 and 0.13 mg /g in rutin equivalent respectively. Therefore, it was considered that the high antioxidant potential of leaves extract of *C. colocynthis* could be attributable to its high amount of phenolic compounds content. The nature of phenolic compounds present in *C. colocynthis* extract was analyzed qualitatively. The study revealed the presence of flavonoids, tannins, phenols and saponin. Further screening of the extract on gas chromatogtraphy revealed the presence of albumin, ascorbic acid, beta carotene, vicilins, tocotrienols, lutin, 2-tetradcyclobutane, and 2-dodecyclobutane. In addition, presence of various amino acids such as arginine, leucine, isoleucine and fatty acids such as palmitic acid, linoleic acid and linolenic acid were identified in the extract.

Phenolic compounds identified to be present in *C. colocynthis* extract in this study are well known for their antioxidant properties[26]. In addition to this, several other compounds identified in *C. colocynthis* extract in this study have been shown to possess antioxidant potential in previous studies, for example beta carotene has strong antioxidant potential due to its chemical structure and interaction with biological membranes[31]. It is well-known, that beta carotene quenches singlet oxygen with a multiple higher efficiency than α-tocopherol[32]. Also, ascorbic acid identified in *C. colocynthis* extract is a standard antioxidant compound while arginine has been proved to be an important mediator of free radical scavenging by increasing the levels and activities of antioxidant enzymes such as catalase and superoxide dismutase[33].

Therefore, the pharmacological properties of *C. colocynthis* observed in this study can be traced to various antioxidants compounds and phytochemicals present in the plant extract.

CONCLUSION

The evaluation of the pharmacological properties of *C. colocynthis* methanolic extract proved that the extract has antioxidant and antidiabetic effects. There is substantial evidence that induction of oxidative stress is a key process in the onset of diabetic complications. Therefore, pharmacological properties of *C. colocynthis* observed in this study may be due to the presence various antioxidant compounds such as beta carotene and ascorbic acid and phenolic compounds which scavenge oxidants species in the plant extract.

REFRENCES

1. Wiernsperger,N.F. (2003). Oxidative stress as a therapeutic target indiabetes:Revisitingthecontroversy.DiabetesMetab.29, 579-585.
2. Valko, M., Izakovic, M., Mazur, M., Rhodes, C.J., Telser, J.(2004).RoleofoxygenradicalsinDNAdamageandcancerincidence. Mol. Cell Biochem. 266, 37-56.
3. Rolo AP, Palmeira CM (2006) Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. Toxicol Appl Pharmacol 212: 167-178.
4. Ceolotto G, Bevilacqua M, PapparellaI,BaritonoE,FrancoL, *et al*. (2004) Insulin generates free radicals by an NAD(P)H, phosphatidylinositol 3'-kinase-dependent mechanism in human skin fibroblasts ex vivo. Diabetes 53: 1344-1351
5. Habib MP, Dickerson FD, Mooradian AD (2014) Effect of diabetes, insulin, and glucose load on lipid peroxidation in the rat. Metabolism 43: 1442-1445
6. Toborek M, Wasik T, Drózdz M, Klin M, Magner-Wróbel K, *et al*. (2002) Effect of hemodialysis on lipid peroxidation and antioxidant system in patients with chronic renal failure. Metabolism 41: 1229-1232.
7. Violi F, Marino R, Milite MT, Loffredo L (2009) Nitric oxide and itsroleinlipidperoxidation.
Diabetes Metab Res Rev 15: 283-288
8.Newman, D. J., Cragg, G.M. and Snader, K.M. (2000). The influence of natural products upon drug discovery, Nat. Prod. Rep. 17 (3):215-234
9.Taiz, L. & Zeiger, E. (2006). Plant physiology, 4th edn. Ch. 13. pp. 315-344. Sinauer Associates, Inc., Publishers, Massachusetts.
10.Gurib-Fakim, A. (2006). Medicinal plants: Tradition of yesterday and drugs of tomorrow. Review article. Mol. Aspects Med. 27 (1):1-93.
11.Duke JA.Handbook of energy crops. Electronic publication on the New CROPS (1983),web site. https:// www.hort.purdue.edu/newcrop/duke_ energy/ duke index. html
12.Schlesier K, Harwat M, Böhm V, Bitsch R. (2002): Assessment of antioxidant activity by using different *in vitro* methods. Free Radical Research;36(2):177-187.
13.Oyaizu M. (1986) Studies on product of browning reaction prepared from glucose amine, Japan Journal Nutrition; 44:307-315.
14.Garrat DC. (1964) The Quantitative analysis of Drugs. Chapman and Hall Ltd., Japan, 3: 456-458
15.Janero D. R, (1990) "Malondialdehyde and thiobarbituric acidreactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury," Free Radical Biology & Medicine, vol. 9, no. 6, pp. 515–540
16.Bernfeld, P. (1955) Amylases, Alpha and Beta, Vol. 1, Academic Press, NY p. 149.
17.Sofowara, A. (1993) *Medicinal Plants and Traditional Medicine in Africa*, Spectrum Books, Ibadan, Nigeria p 289
18.Ejikeme, C.M; Ezeonu, C.S and Eboatu, A.N (2014) "Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria," *EuropeanScientificJournal*,vol.10,no.18,pp.247270.
19.Santhi K. and Sengottuvel R. (2016) Qualitative and quantitative phytochemical analysis of *Moringa concanensis* Nimmo. *Int.J.Curr.Microbiol.App.Sci; 5*(1): 633-640.
20.Harborne JB (1973) Phytochemical methods. London: Chapman and Hall, Ltd; pp. 49–pp. 188.

21.Folin, O, Ciocalteu, V, (1927): Tyrosine and tryptophan dtermination in proteins. J, Biol. Chem. 73,627-650.
22.State Pharmacopeia of USSR (1989). Moscow, Medicina, 2, 324–334 (in Russian).
23.Yermakov, A. I., Arasimov, V. V., & Yarosh, N. P. (1987). Methods of biochemical analysis of plants. Leningrad: Agropromizdat (in Russian).
24.Halliwell B, Gutteridge JMC (1999). Oxidative stress: Adaptation, damage, repair and death. In: Halliwell B, Gutteridge JMC, Eds. Free Radicals in Biology and Medicine, 3rdEdition,OxfordUniversityPress,Oxford,246350.
25.Al-Snafi AE. (2015). Therapeutic properties of medicinal plants: a review of plants with antioxidant activity (part 1). InternationalJournalofPharmacologyandToxicology;6(3):159-182.
26.Owoade O A, Aborishade A B, Adetutu A, Olorunisola O S (2016). Evaluation of the Effectiveness of *Telfairia occidentalis* Leave Extracts in the Amelioration of Carbon Tetrachloride Induced Liver Injuries and Oxidative Damage in Rats. 10(2): 1-11.
27.Parul R Sukalayan; Kundu K and Pijush Saha P (2012) In vitro nitric oxide scavenging activity of methanol extracts of three Bangladeshi medicinal plants. The pharma innovation journal; 1(12): 83-88.
28.Coppen, I.N; Allen, J.C; Hamilton R.J (1983), Rancidity in foods (Applied Science Publishers, New York, USA. 67
29.Bailey, C.J (2003) New Approaches to the Pharmacotherapy of Diabetes, Vol. 2, 3rd Edition, Blackwell Science Ltd., UK (2003) p. 73.1-73.21.
30.Hanamura T, Hagiwara T, Kawagishi H (2005): Structural and functional characterization of polyphenols isolated from acerola (Malpighia emarginata DC.) fruit. Biosci Biotechnol Biochem.;69:280-6.
31.Riccioni, G. (2009) Carotenoids and cardiovascular disease. Curr. Atheroscler. Rep, 11,434-439.
32.Di Mascio, P.; Kaiser, S.; Sies, H. (1989) Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch. Biochem. Biophys., 274, 532-538.
33.Morris, K.A. Kuypers, S. Larkin, N. Sweeters, J. Simon, E.P. Vichinsky, L.A. Styles (2000): Arginine therapy: a novel strategy to induce nitric oxide production in sickle cell disease, Br. J. Haematol. 111 498–500