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PROXIMATE COMPOSITION, PRELIMINARY QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL SCREENINGS OF *TRECULIA AFRICANA*

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ABSTRACT: *Treculia africana uses in folklore medicine showed several biological properties on circulatory, respiratory, urinary, digestive, nervous system, sexual, skin, vision and hearing organs due to it phytoconstituents. The present study is aimed at evaluating the proximate, qualitative and quantitative phytochemicals of T. africana. The proximate analysis, qualitative and quantitative are carried out using a standardized protocol to analyzed the Moisture, Crude Protein, Ash, Fibre, Lipids and Carbohydrates content. Also, Steroids, Saponins, Oxalate, Phytate, Tannins, Flavonoids, Alkaloids, Phenols, Cardiac Glycosides and Anthraquinones were screened for in T. africana aqueous and ethanol extracts via standard methods. The results showed that the extracts of T. africana with proximate composition of 4.43 % Moisture, 1.67 % Crude Protein, 3.18 % Ash, 1.02 % Fibre, 0.19 % Lipids and 89.41 % Carbohydrates. The phytochemical tests were positive with important secondary metabolites such as 1.55 g Oxalate, 3.02 g Phytate, 9.35 g Tannins, 4.25 g Flavonoids, 3.38 g Alkaloids, 0.14 g Phenols, 3.88 g Cardiac Glycosides and 0.16 g Anthraquinones. In conclusions, active compounds with broad biological activities required being isolated for medicinal purposes.*

KEYWORDS: Proximate, Phytochemistry, Treculia africana

INTRODUCTION

Since antique time, phytotherapy role in ethno-medicine to treat different diseases. Plants medicinal are herbs with active substances used for therapeutic benefits, with precursors for synthesized drugs (Sorowora, 1984). Several research studies had been performed on selected medicinal plants and have shown definite biological action on circulatory, respiratory, urinary, digestive, nervous system, sexual, skin, vision and hearing organs (Herve *et al*., 2008; Aggarwal, and Shishodia, (2006). *Treculia africana* commonly known as African breadfruit. It belongs to the family Moraceae and categorized as a medicinal plant with varieties of natural occurring compounds. It is a native to several Tropical and West Africa countries. They grow commonly in evergreen and deciduous forests, often by streams but may sometimes be planted in Nigeria where it is very common in the Western and Eastern states (Hollist, 2004).

These components acquire attractive biological properties, fascinated to researchers for elucidation of compounds needed for the advancement and development of medicine (Izzo, 2005). Phytochemicals are found in several plants used as vital components in animal and

human diet. Thus, the traditionally word nutrients are differed from phytochemicals because they enhance normal metabolism, in which their absent result in deficiency disease (Vivekananthan, 1995; Trewavas and Stewart, 2003). The medicinal benefits of African breadfruit associated with its phytochemicals.

METHODOLOGY

Plant Materials

Fresh leaves of *Treculia africana* were obtained from Government Reserve Area (G.R.A), Benin-City, Edo State, Nigeria. The leaves were identified by Dr. H.O. Akinibosun of the Botany department of University of Benin, where an herbarium was deposited and a voucher number UBH_T0185 was obtained. The leaves were rinsed with clean water to remove dust and dirt, shade-dried for three (3) weeks and pulverized using British milling machine.

Preparation of Plant Extracts

The preparation of the extract was carried out as described by (Onoagbe *et al.*, 1999). Approximately 2300 g each of the pulverized leaves were dissolved into separate jar using ten (10) liters of distilled water and nine liters of absolute ethanol. The mixtures were macerated with continuous stirring periodically for 72hr. After the various solvent mixtures were filtered using muslin cloths. The filtrates separately were transferred into suitable containers and lyophilized (freeze-dried). The freeze-dried aqueous and ethanol extracts were stored in a desiccator for further use.

Proximate Analysis

Moisture Content Determination

The moisture content of the samples was determined by weighing into moisture dish and then dish plus the sampled was weighed and then placed in an oven at 80^{o}C till constant weight of the sample was obtained (dry to a constant weight). Then brought out and allowed to cool in desiccators and then reweighed. Thus,

$$\text{Moisture (\%)} = \frac{\text{Weight of dried sample}}{\text{Weight of wet sample}} \times 100$$

Ash Content Determination

Five grams of samples were transferred into a pre-weighed porcelain crucible and weighed. The crucible was

then placed into muffle furnace for 6hr at 600°C to burn off all organic materials. The inorganic material does not volatilize at that temperature and is called ash. The furnace was allowed to cool below 200°C and maintained at this temperature for 20min. Then the crucible was placed in a dessicator with stopper lid, allowed to cool and then reweighed to measure the ash content.

$$\text{Ash (\%)} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times 100$$

Crude Protein Determination

The Kjeldahl method which is the standard method for determining protein and other nitrogen containing compounds was used. Two grams of the sample was digested with sulphuric acid to decompose it and convert nitrogen to ammonium sulphate. The digestion was speed up by adding Kjeldahl catalyst tablets to increase the boiling point. The solution was cooled and concentrated. Sodium hydroxide was added to make the solution alkaline and distilled into a weak acid (boric acid) containing methyl red indicator until solution turned from red to green. Following distillation, the ammonia was trapped as ammonium borate and quantified by titrating with a strong standard hydrochloric acid (0.01 N) until solution turned from green to wine to measure the nitrogen content. The amount of crude protein was calculated by multiplying the% nitrogen found by 6.25 (%), (CP = % Nitrogen × 6.25).

Determination of Fat Content

Beaker was placed in oven at 80°C for 10min and then removed and placed in desiccators to cool. Then the two grams of dried sample was weighed into the beaker, a glass thimble full of anhydrous diethyl either was added to the beaker and placed on the butt-type extraction apparatus. This was boiled at high temperatures for approximately 4hr by moving heat under it to volatilize the ether, then condensed and allowed to pass through the sample, extracting ether soluble materials. The extract is collected in a beaker, allowed to cool and the porous thimble removed with contents saved for crude fiber determination. Ether was distilled and collected in another container until beaker was almost dry and the remaining ether extract was then dried in oven at 80°C for 3min, cooled in the dessicator and weighed to measure the ether extract content

$$\text{Lipid (\%)} = \frac{\text{Weight of fat/oil}}{\text{Weight of sample}} \times 100$$

Crude Fibre Determination

The residue obtained from ether extracted was further treated with 1.25% sulphuric acid and 1.25% of sodium hydroxide while heating for 30min. The content was ashed in muffle furnace and reweighed.

$$\text{Crude fiber (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Qualitative Phytochemistry

Phytochemical screening for major constituent was undertaken using standard qualitative procedure as previously described by (Sofowora,1993); Trease and Evans,1989; Harbone, 1973).

Test for Tannins

About 2 g of the dried sample was added 5 ml of 45% ethanol and boiled for 5 min. The mixture was cooled and filtered. About 1ml of the filtrate was added 3 drops of lead acetate solution. A gelatinous precipitate was observed which indicates the presence of Tannins. Another 1 ml of the filtrate was added 0.5 ml of bromine water. A pale brown precipitate was observed indicating the presence of Tannins (Trease and Evans, 1989).

Test for Glycosides

About 2 g of the sample was mixed with 30 ml of distilled water and it was heated for 5min on a water bath, filtered and used as follows: About 5 ml of the filtrate was added to 0.2 ml of Fehling solution A and Fehling solution B until it turns alkaline and heated in a water bath for 2 min. A brick red precipitate indicates the presence of glycosides.

Test for Alkaloids

This was carried out by adding 0.5 g of aqueous extract in 5 ml of 1% HCL, boiled and filtered. Then Mayer's reagent was added. A creamy white color is indicative of alkaloids (Harbone, 1973).

Test for Saponins

About 0.1 g of the sample was boiled with 5 ml of distilled water for 5 min. Mixture was filtered while still hot and the filtrate was then used for the following tests (Trease and Evans, 1989). To 1 ml of the filtrates, 2 drops of olive oil was added, the mixture was shaken and observed for the formation of emulsion. About 1 ml of the filtrate was diluted with 4 ml of distilled water. The mixture was vigorously shaken and then observed on a stand for stable froth (Trease and Evans, 1989).

Test for Free Glycoside Bound Anthraquinone

According to (Wall *et al*., 1952; Sofowora, 1993), about 5 g of the extract was added to 10 ml benzene, filtered and ammonia solution added; formation of a color is an indicative of anthraquinone.

Test for Flavonoids

The presence of flavonoids was determined using 1% aluminum chloride in methanol HCL, Magnesium turnings and potassium hydroxide solution (Kapoor *et al.,* 1969; Harborne, 1973). A yellow color is an indicative of flavonoids.

Quantitative Estimation of Phytochemicals

Determination of Phytic Acid

Phytic acid was determined using a standard procedure. About 2 g of each sample was placed in a 250 ml conical flask. About 100 ml of 20% concentrated hydrochloric acid (HCL) was used to soak each sample in conical flask for 3hr. This was filtered through a double layer of hardened filter papers. Approximately 50 ml of each filtrate was placed in 20 ml beaker and 107 ml of distilled water was added in each to give proper acidity. About 100 ml of 0.3% ammonium thiocyanate solution was added into each solution as indicator. This was titrated with standard iron chloride solution, which contains 0.00195 g iron per ml. The end point which was slightly brownish yellow persisted for 5 min. The percentage phytic acid was calculated using the formula.

$$\% \text{ Phytic acid} = \frac{V \times 1.198 \times 100}{\text{Wt samples}}$$

Where x = Titre value x 0.00195g.

Determination of Oxalate Ion by Permanganate Oxidation

Procedure

1. Approximately 0.20 g of unknown oxalate was weighed into a clean dry 400 ml beaker.
2. To each beaker approximately 250 ml of 7.0 M H_2SO_4 was added.
3. Into a burette, 0.1 M $KMnO_4$ solutions were added and the initial volume was noted. The volumes from the top of the meniscus each time was noted and read to 0.01 ml.
4. The oxalate mixture was warm on a ring stand and stirred frequently using a thermometer 80-90°C and keep it above 70°C at all times. The warmed mixture was titrated.
5. The end point was noted when an extremely faint pale pink color persists in the solution for 15 seconds.
6. The g/100g oxalate ion by mass in the unknown sample was calculated as follows

Calculation : Oxalate = $\frac{T \times 100 \times 0.125}{\text{Weight of sample} \times CF}$

Where cf = 1.382

Determination of Alkaloids

The alkaloids acid was determined using the procedure described by Obadoni and Ochuko, (2001). About 5 g of each sample was weighed into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added, covered and allowed to stand for 4hr. This was filtered and the extract was concentrated using a water bath to evaporate one-quarter of the original volume. Concentrated ammonium solution was added drop-wise to the extract until precipitation was completed. The entire solution was allowed to settle and the precipitate was collected by filtration, after which it was weighed.

Alkaloid g/100 g = weight of untreated sample / weight of treated sample x 100

Determination of Flavonoids

About 5 g of each plant sample was weighed in a 250 ml titration flask, and 100 ml of 80% aqueous methanol was added at room temperature and shaken for 4hr on an electric shaker. The entire solution was filtered with Whatman filter paper no. 42 and again, this process was repeated. The filtrate as a whole was later transferred into a crucible and evaporated to dryness over a water bath and weighed.

Calculation

Flavonoids (g/100 g) = weight of untreated sample / weight of treated sample x100.

Determination of Saponins

About 5 g of each sample was weighed, and dispersed in 100 ml of 20 % ethanol. The suspension was heated over a hot water bath for 4hr with continuous stirring at about 55^{o}C.

The filtrate and residue were re-extracted with another 100 ml of 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at about 90^{o}C. The concentrate was transferred into a 250ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and about 30 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight Obadoni and Ochuko, (2001).

The saponin content was calculated Saponin (g/100 g) = weight untreated sample / weight of treated sample x100

Determination of Tannins

The level of tannin in the plants was determined using the method of Van-Burden and Robinson. About 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1hr on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipette out into a test tube and then mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance of concentration was measured at 420 nm within 10min using Search tech model 721 electronic spectrophotometer.

Determination of Total Phenols

Total phenol content was determined using the method of (Makkar *et al.*, 1993). Samples (50 µl) were put in test tubes and the volume was made up to 500 µl using distilled water. Then, 250 µl of Folin-Ciocalteu reagent was added into the test tubes followed by 1.25 ml of sodium carbonate solution. The tubes were vortexes before incubated in the dark for 40 min. Absorbance of concentration was read at 725 nm using spectrophotometer.

Determination of Anthraquinone

For the determination of anthraquinone, 0.1 g of the sample was weighed into a conical flask and 10 ml of hot water was added. It was allowed to cool and extracted with 10 ml of benzene. About 10 ml of 5% ferric chloride and 5 ml of hydrochloric acid was added to the water fraction of the extract and refluxed for 10min. About 10 ml of benzene was used to extract again. The residue was evaporated and dissolved in 10 ml of 5% potassium hydroxide. Absorbance concentration of the solution was measured at 515 nm using anthraquinone glycoside as reference standard (Harborne, 1998).

Determination of Steroids

For the determination of steriods, one (1) gram of the sample was weighed into conical flask and 20 ml of ethanol sodium hydroxide was added followed by 1ml of tetrazolium in methanol hydroxide and 1ml of tetra-methyl ammonium hydroxide. Allowed to react for 90min, the absorbance concentration was measured at 525 nm. Using ethanol sitosterol as reference standard. (Harborne, 1998).

Determination of Cardiac Glycoside

For the determination of glycoside, 2 g of each plant sample was weighed, and was dispersed in 100 ml of warm water. The suspension was heated over a hot water bath for 30 hr with continuous stirring at about 80^{o}C. The filtrate and residue were re-extracted with another 100 ml of 10 % acetone. The extract was reduced to 10ml on heating at 80^{o}C. The concentrate was transferred into a 100 ml beaker and weighed.

The Cardiac glycoside content was calculated Cardiac glycoside (g/100 g) = weight treated sample / weight of untreated sample x 100.

Isolation and Purification of Tannins

For the isolation and purification of tannins, 50g of the ethanol extract was reconstituted with ethanol and poured into a separating funnel, run 500 ml of n-hexane six times in order to remove the chlorophyll and fat. The n-hexane fractions which contain the phytochemicals were collected and poured into a separating funnel, 500 ml of n- butanol was added and shake vigorously and allowed to separate. The butanol fraction which contain mainly tannins was collected and concentrated .The dechlorophyllized extract was then applied in a vacuum liquid chromatography packed with silica gel(60-200 mesh) and eluted using solvent system of methanol : dichloromethane in the ratio 90:10, 70:30, 50:50, 30:70, 10:90 and 0:100 (Cowan, 1999; Zainal *et al*., 2014) ,each fraction was collected. It was observed by confirmatory test that fraction A 90:10, fraction B 70:30, fraction C 50:50 and fraction F 0:100 contain tannins, the fractions were concentrated and weighed.

RESULTS

The proximate composition of the leaves of *Treculia africana* results obtained was shown in table 1.

Table1: Proximate Composition of the Leaves of *Treculia africana* Extracts

Parameters	Composition (%)
Moisture	4.43±0.74
Crude Protein	1.67±0.00
Ash	3.18±0.12
Fibre	1.02±0.02
Lipids	0.19±0.00
Carbohydrates	89.41±0.84

Sample size =3

Table 2: Semi Qualitative Phytochemical Screening of the Leaves of *Treculia africana* Extracts.

Phytochemicals	Raw	Ethanol	Aqueous
Oxalate	+++	++	+
Phytate	+++	+++	+
Tannins	+++	+++	+
Flavonoids	+++	++	+
Saponins	+++	+	++
Alkaloids	+++	++	+
Phenols	++	+	+
Cardiac glycoside	++	+++	+
Anthraquinone	++	+	++
Terpenoids	–	–	–
Steroids	–	–	–

Keys: - = absent, + = present, ++ = moderately present, +++ = highly present.

Table 3: Quantitative Phytochemical Screening of the Leaves of *Treculia africana* Extracts

Phytochemicals	Raw (g/100 g)	Ethanol (g/100 g)	Aqueous (g/100 g)
Oxalate	4.17±0.07	1.55±0.02	1.23±0.02
Phytate	2.79±0.04	3.02±0.05	0.71±0.01
Tannins	5.75±0.10	9.35±0.16	0.34 ±0. 01
Flavonoids	3.66±0.06	4.25±0.02	0.49±0.01
Saponins	5.40±0.09	0.73±0.01	3.39±0.06
Alkaloids	8.73±0.15	3.38±0.05	1.38±0.02
Phenols	1.34±0.02	0.14±0.00	0.22±0.17
Cardiac glycosides	2.68±0.04	3.88±0.06	0.29±0.01
Anthraquinones	1.67±0.02	0.61±0.61	0.17±0.01
Terpenoids	<0.005	<0.005	<0.005
Steroids	<0.005	<0.005	<0.005

Sample size =3

This was carried out using Vacuum Liquid Chromatography packed with silica gel (60-200 mesh) and eluted using solvent system of methanol: dichloromethane at varying ratios. The results obtained were shown in table 4.

Table 4. Column Chromatography Ratio Between Polar and Non-Polar of the Leaves of *Treculia africana* Extracts

Fractions	Methanol	Dichloromethane	Confirmatory test
A	90	10	+
B	70	30	+
C	50	50	+
D	30	70	-
E	10	90	-
F	0	100	+

KEYS: + = PRESENT, - = ABSENT.

DISCUSSION

There are several thousand of known and unknown phytochemicals. Plants have been known to synthesize these chemicals responsible for their protection. Current researches reveal many phytoconstituent with therapeutic benefits to against diseases (Herve *et al*., 2008; D'Incalci *et al*., 2005). They are non-essential nutrients required not for human body in life sustenance, rather with promising properties either as a prophylaxis or to suppress diseases (Herve *et al*., 2008; Barnes *et al*., 2007; Fennell *et al*., 2004). The results of the present study showed that the proximate analysis (Table 1) of the leaves of *Treculia africana* contains 4.43% Moisture, 1.67% Crude Protein, 3.18% Ash, 1.02% Fibre, 0.19% Lipids and 89.41% Carbohydrates (Irvine, 1981). Also, the qualitative phytochemical screening (Table 3) using standard procedure showed that the leave of *Treculia africana* contains various phytochemicals with varying amount of concentration (Doughari *et al*., 2009). Based on the quantitative phytochemical analysis of the leave, aside the raw extract, the ethanol extract produced higher amount when compare with aqueous extract. The quantitative phytochemical analysis of ethanol extract showed that the leave of *Treculia africana* contains 1.55 Oxalate, 3.02 Phytate, 9.35 Tannins, 4.25 Flavonoids, 3.38 Alkaloids, 0.14 Phenols, 3.88 Cardiac Glycosides and 0.16 Anthraquinones with the exception of Saponins which is higher in aqueous extract as opposed the others (0.73 Saponins in ethanol extract and 3.39 Saponins in aqueous extract), the results were expressed in g/100g (Falodun *et al*., 2006). The leave also contains trace amount of Terpenoids (< 0.005) and Steroids (< 0.005). Based on this finding, the results obtained from proximate and phytochemical analysis of the leaves of *Treculia africana* showed that the leaves are a good source of carbohydrates, tannins, cardiac glycosides, alkaloids and flavonoids (Kris-Etherton *et al*., 2002). Also, the organic solvent (ethanol) extracts showed more phytochemicals than the non-organic solvent (water) (Liu, 2004). This suggest that organic solvent should be use in order to get maximum extraction of phytochemicals from *Treculia africana* due to its non-polar nature.

CONCLUSION

Medicinal plants offer a wide range of benefit to human. The leaves of *Treculia africana* contain high percentage of carbohydrates, ash and moisture content. It is also a good source of bioactive phytochemicals, important in preventing chronic disease like cancer, diabetes

and coronary heart disease. Thus, we hope the important phytochemicals in the leaves of *Treculia afriacana* will be helpful in coping different diseases with further study.

REFERENCES

Aggarwal, B.B. and Shishodia, S. (2006). Molecular targets of dietary agents for prevention and therapy of cancer. *Biochemistry and Pharmacology*. 71:1397–1421.

Barnes, J., Anderson, L.A. and Phillipson, J.D. (2007). *Herbal Medicines. A guide for Healthcare Professionals, third ed*. Pharmaceutical Press, London. Pp 1-23.

Cowan, M.M. (1999). Plant products as antimicrobial agent.*Clin.Microbiol.Rev*.564-582.

D'Incalci, M., Steward, W.P. and Gescher, A.J. (2005). Use of cancer chemopreventive phytochemicals as antineoplastic agents. *Lancet Oncology*. 6:899–904.

Doughari, J.H., Human, I.S, Bennade, S. and Ndakidemi, P.A. (2009). Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. *Journal of Medicinal PlantsResearch*. 3: 839-848.

Falodun, A., Okunrobe, L.O. and Uzoamaka, N. (2006). Phytochemical screening and anti-inflammatory evaluation of methanolic and aqueous extracts of *Euphorbiaheterophylla* Linn. (Euphorbiaceae). *African Journal of Biotechnology*. 5:529-531.

Fennell, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M. and Van Staden, J. (2004). Assessing African medicinal plants for efficacy and safety: Pharmacological screening and toxicology. *Journal of Ethnopharmacoly*.94: 205-217.

Harborne, J.B. (1998). Phytochemical Methods. A Guide to Modern Techniques of plant Analysis. 3rd edition. Chapman and Hall, Newyork. Pp. 1-198.

Hervé, Z., Charles, K., Anoubilé, B., Janat, M.B. and Yves A.B. (2008). Phytochemical screening and determination of flavonoids in *Secamone afzelii* (Asclepiadaceae) extracts. *African Journal of Pure and Applied Chemistry* .2: 080-082.

Hollist, N.O. (2004). A Collection of Traditional Yoruba Oral and Dental Medicaments. Edited by the Book Binders and printed by Olubena Printers, Ibadan. Nigeria.

Irvine, J.I. (1981). Comparative Study of the Chemical Composition and Mineral Element Cotnent of *Treculia africana* Seeds and Seed Oils. *Journal of FoodEngineering.* 40:241-244.

Izzo, A.A. (2005). Herb-drug interactions: an overview of the clinical evidence. *Fundamental Clinical Pharmacology*. 19:1–16.

Kapoor, L.D., Singh, A., Kapoort, S.L. and Strivastava, S.N. (1969). Survey of Indian Medicinal Plantsfor Saponins, Alkaloids and Flavonoids. Lloydia. 32:297-302.

Kris-Etherton, P,M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E. and Etherton, T.D. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *American Journal of Medicine*. 113:71–88.

Liu, R.H. (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of Action. *Journal of Nutrition*. 134:3479-3485.

Makkar, H.P.S, Blummel, M., Borowy, N.K. and Becker, K. (1993).Gravimetric determination of tannins and their correlation with chemical and protein precipitation methods.*Journal of science and Food Agriculture*, 61:161-165.

Obadoni, B.O. and Ochuko, P.O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Glob.J.Pure Appl. Sci*. 86: 2003-2008.

Onoagbe, I.O., Lau, H.U., Esekheigbe, A., Dawha, I.M. and Salami, C.O. (1999). Effects of *Irvingia grandifolia* and *Spondias mombin* on blood glucose and triglyceride concentrations in streptozotocin-induced diabetic rats. Biokemistri, 9: 17-22.

Sofowora, A. (1993). Medicinal Plants and Traditional Medicines in Africa. Chichester John Wiley & Sons New York, Pp: 97-145.

Sofowora, A. (1993a). Recent Trends in Research into African Medicinal plants. *J.Ethnopharmacol*. 38: 209-214.

Sofowora, A. (1993b). Medicinal plant and Traditional Medicine in Africa. 2nd edition Spectrum Books Ibadan, Nigeria.

Trease, G.E. and W.C. Evans, (1989). Pharmacology 11thEdn., Bailliere Tindall Ltd., London, Pp: 60-75.

Trewavas, A. and Stewart, D. (2003). Paradoxical effects of chemicals in the diet on health. *Current Opinions in Plant Biology*. 6:185–190.

Vivekananthan, C. P. (1995). Phytochemicals. Drug-store in a salad. *Journal of Consumer Reports on Health*.7:133-135.

Wall, M.E., Eddy, C.R., Mc Clenna, M.L. and Klump, M.E. (1952). Detection and estimation of steroid and sapogenins in plant tissue. *Analytical Chemistry*.24:1337-1342.

Zainal, B., Abdah, M.A., Taufiq-Yap, Y.H., Roslida, A.H. Rosmin, K. (2014).Anticancer Agents from Non-Edible parts of *Theobroma cacao*.*Nat.Prod.Chem.Res*.2:134.