Chemical Composition Profiling of *Epiphyllum oxypetalum* (DC.) Haw. (Queen of the Night) Grown in Akure Metropoly, Ondo State, Nigeria.

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Abstract

Phytopharmaca possesses phytochemical ingredients which usually allow them to act as remedy to several ailments. The present study determines the biochemical constituents of *Epiphyllum oxypetalum* methanol root extract. The roots were harvested, washed, shade dried at room temperature, and pulverized to powder. The extract was prepared, concentrated and analysed using standard laboratory techniques for determination of phytochemical, proximate and mineral elemental components present in the root of *E. oxypetalum*, while phytocompounds were determined using GC-MS analysis. Results of the phytochemical components of the extract of *E. oxypetalum* showed presence of appreciable qualitative and quantitative components, and a wide range of phytocompounds. Proximate analysis revealed valuable concentrations of moisture (35.77±0.08), protein (6.92±0.02), fat (1.37±0.01), ash (8.86±0.06), crude fibre (14.93±0.52) and carbohydrates (57.15±1.57). The mineral content of the roots were very outstanding which include- calcium, magnesium, phosphorus, iron, copper and zinc. The level of minerals resident in the roots of the studied plant may account for its high nutritious and medicinal values. Conclusively, it is evident that the root would not only provide adequate nutritional supplementation but may also provide the users with adequate therapeutic arsenal for the management of various health problems.

Keywords: Chemical, Composition, *Epiphyllum oxypetalum*, nutritious, phytoconstituents.

Introduction

Nowadays, much attention is focused on the vast botanical resources of African and their use as alternatives and complementary sources of medicine. This is likely to be as a result of the putative salutary effect of most Africa plants that is attributed to the presence of secondary metabolites. Medicinal plants have constituted the source of health care all over the world since time immemorial and has thus remain the mainstay of drug discovery (Beyene and Deribe, 2016); this is believed to be as a result of the presence of certain plant chemicals (phytochemicals). Phytochemicals which may also be referred to as phytonutrients/phytoconstituents are present in diverse kinds of plants which are consumed as essential components of both human and animal diet where they have marked physiological effects (Awotedu *et al*., 2019). The bioactive chemicals of note in plants are alkaloid, flavonoid, tannins, saponins and phenolic compounds; this active substances, alongside the vitamins and minerals have been implicated to contribute to their various physiologic, metabolic and protective effects (Awotedu *et al*., 2019). Other important classes of phytonutrients/phytoconstituents present in plants are the proximate and minerals. Proximate and minerals are considered as compounds and elements that possess specific metabolic functions, some of which are present in diverse range of medicinal plants (Beyene and Deribe, 2016).

The use of plants for medicinal purposes dates back to earlier records of human activity. Traditional medicines chiefly containing medicinal plants have always played a vital role as important alternative to conventional medicines in developing countries. The use of medicinal plant's products is more popular among the poor communities that inhabit rural areas and lack access to health. The healing power of medicinal plants is more felt with less or no toxic effect; probably because of the diverse phytoconstituents occurring in the natural state compared to the synthetically produced ones. More importantly, there has been enormous increase in the demand of medicinal plants across the globe for their chemical diversity and for the production of newer therapeutic moieties to control various diseases (Upendra and Khanelwa, 2012).

Nutritional compounds and elements are natural components of the earth crust; that are needed in no small measure for normal metabolic and physiological functioning of humans. To a small extent they enter our bodies via food, drinking water and air. Heavy metals include zinc, calcium, copper etc. As trace elements, some heavy metals such as Cu, Zn, and Fe and are essential for maintaining the human body metabolism, agriculture and environment (Nweze and Nwafor, 2014). However, it has been reported that climatic factors and stages of maturity could cause variation in distribution

of biomolecules and minerals present in any plant material (Bamishaye *et al*., 2011), as well as the choice of solvents, as different solvents have different extraction capabilities and spectrum of solubility for phytoconstituents (Handa, 2008). Also, soil variability and some environmental properties may alter equilibrium found in the soil and cause leaching of mineral elements tightly bound to soil particles (APHA, 1971).

Dandekar *et al*. (2015a) evaluated anti-inflammatory activity of alcohol and aqueous extract of *Epiphyllum oxypetalum* leaves; and concluded that the constituents of the source material are believed to have strong ability to stifle pain and are capable of neutralizing blood clotting. Icha *et al*. (2020) reported the stem of *E. oxypetalum* to cure cardiac inffection and dropsy. He also stated in his report that the Vietnamese used the petals of the faded blooms to make soups, which are believed to have tonic and aphrodisiac properties. The aqueous and ethanol extracts of the dried leaves of *E. oxypetalum* were evaluated for antioxidant activity by Dandekar *et al*. [2015b] using hydrogen peroxide scavenging and 1,1, Dipheny-2-picrylhydrazyl radical scavenging assay (DPPH) to determine the free radical scavenging abilities of both the extracts. Results showed highest percentage of DPPH inhibition in the ethanol extract compared to aqueous extract. Upendra and Khandelwal (2012) worked on assessment of nutritive values, phytochemical constituents and biotherapeutic of *Epiphyllum oxypetalum*, and the results of the study revealed promising antimicrobial activity against all tested organisms except the tested fungi. In another study, Paralikar (2014) reported the antibacterial activities of silver nanoparticles biosynthesized from the aqueous leaf extract of *E. oxypetalum* against *Propioni bacterium acne*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* respectively. .

The aim of this study, therefore, was to evaluate the phytoconstituents of the extract obtained from the roots of *Epiphyllum oxypetalum*, as well as to study the biochemical characteristics of the phytocompounds.

Materials and Methods

Collection and Identification of Plant Material

A substantial quantity of the roots of *Epiphyllum oxypetalum* were collected from ornamental garden along Oke-Eda, Akure, Ondo State, Nigeria, and was identified and authenticated in the Phytomedicine division, Botany Unit, Department of Biological Sciences, Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria where a specimen with voucher number (no: OAUSTECH 071) was deposited.

Preparation and Extraction of Plant Material

The fresh roots of *Epiphyllum oxypetalum* were harvested, washed and dried for eight hours a day for three weeks under shed, at room temperature, to avoid loss of bioactive compounds. The dried roots were ground to powder using mechanical grinder and weighed with a Mettler balance. The powder sample was then stored in an airtight bottle for further use. Measured quantities of the powdered sample were extracted separately in 99% methanol for 72hrs followed by periodic stirring and was kept in a refrigerator to avoid any microbial growth. The extract was filtered using cheese-cloth and the filtrate re-filtered using Whatman No. 42 (125 mm) filter paper. The filtrate collected was lyophilized using a freeze-dryer (Ugo BasIle SRL-Model S174, Italy) and stored in an airtight container for further analysis.

Qualitative Phytochemical Analysis

The methanol extract of the plant material was screened for the presence of various secondary metabolites in accordance with the following procedures; Test for Alkaloids (Dragendroff's Test) using Harbone (1998) method, Tannins and Phenol using Edeoga *et al*. (2005) method, Test for Flavonoid (Alkaline reagent test) using Oluduro (2012), Steroids (Salkowski test as described by Oluduro (2012), Cardiac Glycosides by Oluduro (2012), Test for Saponins (Frothing test) using Harbone (1998) method.

Quantitative Phytochemical Assay of the Botanical

The amount of each phytochemical: saponin, alkaloids, phenols, flavonoids, tannins, steroids and cardiac glycosides present in the crude powdered sample were evaluated using standard laboratory procudures based on the methods of Obadoni and Ochuko (2001), Harborne (1973), Boham and Kocipai-Abyazan (1974), Van-Burden and Robinson (1981), and Mbaebie *et al*. (2012).

Estimation of saponin: The method of Obadoni and Ochuko (2001) was employed. Sample was ground and 20 gm of the sample put into a conical flask followed by the addition of 100 ml of 20% methanol. It was then heated over a hot water bath for 4 hours with continuous stirring at 55˚C. The mixture was filtered and the residue re-extracted with 200 ml of 20% methanol. The extract was reduced to 40 ml over water bath at 90˚C. The concentrate of the extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight. Saponin content was calculated as percentage.

Estimation of alkaloids: Using the method described by Harborne (1973), 5 gm of the sample was weight into a 250 ml beaker and 200 ml of 10% acetic acid in methanol was added and covered and allowed to stand for 4 hrs. The solution was then filtered and the extract was concentrated on a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid which is dried and weighed.

Estimation of phenols: This was determined using spectrophotometric method. The fat free sample was boiled with 50 ml of methanol of the phenolic component for 15 minutes, and 5 ml of the extract was pipetted into a 50 ml flask, followed by the addition of 10 ml of distilled water. Thereafter, 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The sample was made up to mark and colour developed was measured after 30 minutes at 505 nm under room temperature.

Estimation of flavonoids: Using the method of Boham and Kocipai-Abyazan (1974), 10 mg of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over water bath and weighed to a constant weight.

Estimation of tannins: Using the method of Van-Burden and Robinson (1981), 200 mg of the sample was weighed into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120 NM within 10 minutes.

Estimation of steroids and cardiac glycosides: The amount of each phytochemicals, steroids and cardiac glycosides, present in the crude powdered sample were evaluated using standard laboratory analytical procedures based on the methods described by Mbaebie *et al*. (2012).

Phytocompounds Derivatives Analysis

The phytocompounds derivatives of the extract were identified using GC-MS apparatus as cited by Bakari *et al*. (2015). A HP-5 MS capillary column was used for the GC system. Helium was used as carrier gas at a flow rate of 1 mL/min. The injection volume was 2µL. Ionization energy EI of 70 eV was used for spectroscopy detector.

The GC-MS apparatus was carried out by Chem-Station software package (Agilent Technologies). Both the injector and detector temperature were 250°C. The oven temperature was held at 100°C for 1 min, increased to 260°C at a heating rate of 4°C/min, and held for 10 min. The identification of compounds was achieved as the same method reported by Bakari *et al*. (2015), and the name, molecular weight and molecular formula of components of the test material were ascertained.

Proximate Nutritional Analysis

The moisture, crude protein, crude fat, ash, crude fibre and carbohydrates were determined in accordance with the standard laboratory analytical methods described by Akinniyi *et al*. (1986); Oluduro (2012). Representative subsamples were dried in a forced drought oven at 105-110^{0}C to a constant weight for moisture determination. Crude protein, crude fat, ash, and crude fibre were analysed by triplicate according to the aforementioned method. The total carbohydrates were determined by the difference method [100 – (protein + fats + moisture + ash in percentage)].

Mineral Elemental Analysis

The mineral analysis was done according to the standard laboratory analytical methods described by Akinniyi *et al*. (1986); Oluduro (2012). One gram of root sample was wet digested using 9 ml of freshly prepared aqua regia (a mixture of 65% nitric acid and 37% hydrochloric acid in a ratio1:3). The mixture was boiled gently over a water bath at 95^{o}C for 5h until sample is completely dissolved; filtered and made up to standard volume with deionized water. The respective minerals were analysed using Atomic Absorption Spectrophotometer (Model BC – 6500, Labmed, Incorporated, USA).

Data Analysis

Quantitative data were expressed as Mean ± SEM; analysis of variance (ANOVA) was used to detect significant difference between mean of measured parameters. SPSS version 20 was used for statistical analysis. $P < 0.05$ was considered as statistically significant difference.

Results

Phytochemical Analysis

The results of the qualitative phytochemical screening of the root of the medicinal plant, *Epiphyllum oxypetalum,* is showed in Table 1. Results revealed the presence of a variety of plant

secondary metabolites such as saponins, alkaloids, phenols, flavonoids, tannins, steroids and cardiac glycosides.

The results of the quantitative phytochemical analysis of the methanol extract of *E. oxypetalum* is presented in Table 2. It was shown that the medicinal plant had varying appreciable quantities of secondary metabolites.

Table 1: Qualitative phytochemical result of the methanol root extract of *Epiphyllum oxypetalum*

Phytochemicals	Inference
Saponins	++
Alkaloids	+++
Phenols	+
Flavonoids	++
Tannins	+
Steroids	+
Cardiac glycosides	++

Legend: + = slightly present, ++ = more present, +++ = abundantly present

Table 2: Quantitative phytochemical result of the methanol root extract of *Epiphyllum oxypetalum*

Phytochemicals	Concentrations (%)
Saponins	9.83^{c}±0.35
Alkaloids	24.27^{a}±1.25
Phenols	4.33^{d}±0.22
Flavonoids	12.21^{b}±1.09
Tannins	2.94^{d}±0.02
Steroids	3.87^{d}±0.20
Cardiac glycosides	13.29^{b}±1.10

*****Values are expressed as mean and SEM of measurements; means with different superscripts differs significantly at $p < 0.05$.

Phytocompound Derivatives of the Plant Extract

Results of phytocompound derivates analysis of the methanol root extract of *E. oxypetalum*, showed that a total number of twelve (12) phytocompounds of bioactivity potentials were identified, and the names of compound, molecular formular, molecular weight, retention time and percentage composition of the identified compounds are presented in Table 3.

Tab**le 3**: Phytocompounds derivatives of methanol extract of *Epiphyllum oxypetalum* root

S/N	Name of Biochemical Compounds	Molecular Formula	Molecular Weight (g/mol)	Retention Time (mins)	Composition (%)
1	Ethanone 1-(2-hydroxyl-5-methylphenyl)-	$C_9H_{10}O_2$	150.17	9.86	2.826
2	4-Hydroxy-2-methylacetonephenone	$C_9H_{10}O_2$	150.17	9.86	2.177
3	Furazanamine,4-azido-.delta	$C_2H_3N_6O$	127.09	5.491	2.114
4	Megastigmatrienone	$C_{13}H_{18}O$	190.2814	14.79	2.442
5	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.5	15.916	0.517
6	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.11	7.485	17.664
7	2,5-Dihydroxy-4-isopropyl-2,4,6-cycloheptatrien-1-one	$C_{10}H_{12}O_3$	180.20	16.37	3.367
8	n-Hexadecanoic acid methyl ester	$C_{16}H_{32}O_2$	256.42	19.59	14.092
9	Ethanone,1-(2-hydroxy-4 methoxyphenyl)-	$C_9H_{10}O_3$	166.17	9.529	2.826
10	4-((1E)-3 Hydroxy-1-propenyl)-2-methyoxyphenol	$C_{10}H_{12}O_3$	180.20	16.37	2.023
11	Octadecanoic acid	$C_{18}H_{36}O_2$	284.48	19.59	0.510
12	Phytol	$C_{20}H_{40}O$	296.53	22.45	1.785

Nutritional Composition of the Botanical Plant

Table 4 shows the results obtained for the proximate composition analysis of the root extract of *Epiphyllum oxypetalum.* Results showed that *E. oxypetalum* had varying percentage concentrations of the tested nutrient. Meanwhile, carbohydrate had the highest concentration while fat had the least concentration.

The results of the mineral elemental screening of the botanical are presented in Table 5 and Table 6. For the qualitative analysis shown in Table 5, *Epiphyllum oxypetalum* revealed the presence of appreciable number of mineral element; equally importantly, for the quantitative analysis (Table 6), *E. oxypetalum* had varying appreciable percentage concentrations of calcium, magnesium, phosphorous, iron, copper, and zinc; with iron having the highest concentration while copper had the least.

Table 4: Proximate composition of *Epiphyllum oxypetalum* root extract

Nutrients	Concentrations (%)

Moisture	35.77^{b}±0.08
Protein	6.92^{d}±0.02
Fat	1.37^{d}±0.01
Ash	8.86^{d}±0.06
Crude fibre	14.93^{c}±0.52
Carbohydrate	57.15^{a}±1.57

*****Values are expressed as mean and SEM of measurements; means with different superscripts differs significantly at $p < 0.05$.

Table 5: Qualitative mineral elemental composition of *Epiphyllum oxypetalum* root extract

Minerals	Inference
Lead	-
Calcium	+
Magnesium	+
Phosphorus	+
Iron	+
Copper	+
Zinc	+

Legend: + = present; - = absent

Table 6: Quantitative mineral elemental composition of *Epiphyllum oxypetalum* root extract

Minerals	Concentrations (%)
Calcium	0.42^{b}±0.10
Magnesium	0.37^{b}±0.02
Phosphorus	0.29^{b}±0.01
Iron	1.92^{a}±0.31
Copper	0.068^{c}±0.002
Zinc	1.10^{a}±0.12

*****Values are expressed as mean and SEM of measurements; means with different superscripts differs significantly at $p < 0.05$.

Discussion

Plants used in the treatment of diseases contain bioactive substances with biological activity, some of which are responsible for the characteristic odour, pungencies and colour of plant, while others

give the particular plant its culinary, medicinal or poisonous virtue (Evans, 2002). Results of the qualitative phytochemical constituents of root extract of *Epiphyllum oxypetalum* showed that the tested phytochemicals (saponins, alkaloids, phenols, flavonoids, tannins, steroids and cardiac glycosides) were present in appreciable concentrations. This agrees with the report by Kasolo *et al*. (2010) who also found these phytochemicals in plants. However, Bamishaiye *et al*. (2011) and Oluduro (2012) reported absence of cardiac glycosides and steroid in the leaf extract of *Moringa oleifera*. The flavonoids, present in the methanol root extract of *E. oxypetalum* in this study have long been recognised to possess anti-allergic, anti-inflammatory, antiviral, antiproliferative and anti-carcinogenic activities as well as to affect some aspects of mammalian metabolism (Akeneme, 2008). From existing literature, *E. oxypetalum* have been implicated to possess anti-inflammatory (Dandekar *et al*., 2015a), antioxidant (Dandekar *et al*., 2015b), antimicrobial and antibacterial activities (Paralikar, 2014).

Plants produce diverse phytochemicals known as secondary metabolites. It is well known that plants produce these metabolites to protect themselves from pathogenic attacks; and invariably, used by human to contain and cure diseases. In this study, number of phytochemicals were extracted. Some of these identified biochemical compounds have been associated with several biological functions such as antioxidant, anti-inflammatory, anti-microbial, anticancer, and hypocholesterolemic activities (Meskin and Mark, 2002; Adegbola *et al*., 2019). For instance, n-hexadecanoic acid methyl ester was described to be toxic towards bacteria and fungi (Handa *et al*., 2002). Moreover, it was also reported to possess antioxidant and hypocholesterolemic activities (Chandrasekaran *et al*., 2011). The 9,12-octadecadienoic acid methyl ester identified in the root of *Epiphyllum oxypetalum* have been previously reported by Mangunwidjaja *et al.* (2006) to possess anti-inflammatory activity.

Furthermore, from the results obtained, it can be deduced that *Epiphyllum oxypetalum* root contains appreciable nutritious compounds making it to be a good source of food supplement. Appreciable level of crude nutrients in *Epiphyllum oxypetalum* root is acceptable as it could prevent the occurrence of diseases thereby promoting good health. Carbohydrate deficiency causes depletion of body tissues. Sufficiency of carbohydrate is however, necessary for optimum functioning of the brain, heart, nervous, digestive and immune system (Baker, 1996). High contents of moisture ($35.77^{b}\pm0.08$) and ash ($8.86^{d}\pm0.06$) were recorded in this study which were relatively higher than that obtained in similar researches with moisture content value of 3.21% and

ash content value of 7.13% (Anthonia, 2002). The variations in nutritional makeup of the dried *Epiphyllum oxypetalum* root analyzed in this study and that of other similar researches on *E. oxypetalum* could be attributed to the differences in genetic makeup of the plant material, and varying climatic and soil factors.

Intakes of mineral elements such as macronutrients and micronutrients are essential for physiological and metabolic processes. Results reported in this study for minerals showed that *E. oxypetalum* root contained appreciable amounts of mineral elements. Calcium ($0.42^{b}\pm0.10$) which is required for normal growth, strong muscles and skeletal development was detected in the root. Thus, *Epiphyllum oxypetalum* roots are available as a good source of calcium to farm animals or humans. The magnesium content recorded in this study was $0.37^{b}\pm0.02$. It is an essential element that the body required for structuring of skeleton and muscles (Ogbe and John, 2011). Iron is an essential trace element for normal functioning of the central nervous system and in the oxidation of carbohydrates, protein and fats. It is also a necessary component of haemoglobin and myoglobin for oxygen transport and cellular process of growth and division. The plant root contained copper, which is considered to have strong effects on the immune system. The presence of zinc in the plant material in this study is of special interest in view of important zinc in the diet of humans. Zinc is essential for the synthesis of DNA, RNA, insulin and function of several enzymes. Zinc is also required for cell reproduction and growth especially the sperm cells (Ogbe and John, 2011; Awotedu *et al*., 2019).

Conclusion

From the foregoing, it can be deduced that the botanic, *Epiphyllum oxypetalum*, is an established biomaterial that could be used as precursor in the synthesis of useful drug. The root of the plant was found to contain important phytochemicals and diverse biomolecule constituents needed to combat various kinds of infection in human; and nutritional contents which are useful for wide range of metabolic functions. The diverse biochemical constituents detected in this study has authenticated the resourcefulness of *Epiphyllum oxypetalum* in ethnomedicine. Effort should be directed towards harnessing its potentials in drug and dietary supplement formulations.

Competing Interest

The author declared no conflict of interest.

References

Adegbola, P, Adenike, AA, Fadahunsi, OS. (2019). Antioxidant property and GCMS profile of oil extracted from *Cocus nucifera* using a fermentation method. *Journal of Biotechnology, Computational Biology and Bionanotechnology*. 100 (4): 349-358.

Akaneme, FI. (2008). Identification and preliminary phytochemical analysis of herbs that can arrest threatened miscarriage in Orba and Nsukka towns of Enugu state. *African Journal of Biotechnology*. 7(1): 006-011.

Akinninyi, JA, Sultanbawa, MUA, Manawadu, D. (1986). Ethnobotany and ethnopharmacology of the Nigerian medicinal plants. In: Sofowora, A. (ed.). The State of Medicinal Plant Research in Nigeria. University Press, Ibadan, p. 164.

Anthonia, OO. (2002). Evaluation of antimicrobial property and nutritional potential of *Moringa oleifera* leaves in South-Western. *Malaysian Journal of Microbiology*. 8(2): 59- 67.

APHA (1971). Standard Methods for the Examination of Water and Waste Water, 13th Edition. Aseph Publishing, New York. Pp. 391

Awotedu, OL, Ogunbamowo, PO, Emmanuel, IB, Lawal, IO. (2019). Phytominerals and phytochemical studies of *Azadiracthta indica, Leea guineensis and Parkia biglobosa* leaves. *International Annals of Science*. 6(1): 28-34.

Bakari, S., Ncir, M., Felhi, S., Hajlaoui, H., Saoudi, M., Gharsallah, N., *et al*. (2015): Chemical composition and *in vitro* evaluation of total phenolic, flavonoid, and antioxydant properties of essential oil and solvent extract from the aerial parts of *Teucrium polium* grown in Tunisia. *Food Science and Biotechnology*, 24(6): 1943-1949.

Baker, MM. (1996). Nutrition and Dietetics for Health Care, 9th Edition. Churchill Livingstone, New York. Pp. 101.

Bamishaiye, EI, Olayemi, FF, Awagu, EF, Bamishaiye, OM. (2011). Proximate and phytochemical composition of *Moringa oleifera* leaves at three stages of maturation. *Advanced Journal of Food Science and Technology*. 3(4): 233-237.

Beyene, B. and Deribe, H. (2016). Review on application and management of medicinal plants for the livelihood of the local community. *Journal of Resources Development and Management*. 22(1): 33-39.

Boham, BA. and Kocipai-Abyazan, R. (1974). Flavonoids and condensed tannins from leaves of *Hawaiian vaccinium* and *vaticulatum calycinium*. *Pacific Science*. 48: 458-463.

Chandrasekaran, M, Senthilkumar, A, Venkatesalu, V. (2011). Antibacterial and antifungal efficacy of fatty acid methyl esters from leave of *Sesuvium portulacastrum*. *European Review and Medical Pharmacological Sciences*. 15: 775-780.

Dandekar, R, Fegade, B, Arvind, N. (2015a). Evaluation of anti-inflammatory activity of alcohol and aqueous extract of *Epiphyllum oxypetalum* leaves. *World Journal of Pharmacy and Pharmaceutical Sciences*. 4(2): 851-858.

Dandekar, R, Fegade, B, Bhaskar, VH. (2015b). Antioxidant evaluation of free radical scanvenging activities of *Epiphyllum oxypetalum*. *World Journal of Pharmacy and Pharmaceutical Sciences*. 49(70): 1301-1309.

Edeoga, HO, Okwu, DE, Mbaebe, BO. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*. 1(7): 685- 688.

Evans, WC. (2002). Trease and Evans Pharmacognosy, 15th edition. W. B Saunders Company Limited, London, Pp. 139.

Handa, SS. (2008). An Overview of Extraction Techniques for Medicine and Aromatic Plants. ***In***: Handa, SO (ed.). Extraction Technologies for Medicinal and Aromatic Plants. Trieste Publishing, Italy, p. 54.

Harada, H, Yamashita, U, Kurihara, H, Fukushi, E, Kawabata, J, Kamei, Y. (2002). Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. *Anticancer Research*. 22(2587): 2587-2590.

Harborne, JB. (1998). Phytochemical Methods. Chapman and Hall, Limited, London. Pp. 276

Kasolo, JN, Bimenya, GS, Ojok, L. (2010). Phytochemicals and uses of *Morinya oleifera* leaves in Uganda rural communities. *Journal of medicinal Plants Researches*. 4(9): 753-757.

Mangunwidjaja, DS, Kardono, SR, Iswantini, SD. (2006). Gas chromatography and gas chromatography-mass spectrometry analysis of Indonesian *Croton tiglium* seeds. *Journal of Applied Science*. 6: 1576-1580.

Mbaebie, BO, Edeoga, HO, Afolayan, AJ. (2012). Phytochemical analysis and antioxidants activities of aqueous of stem bark extract of *Schotia latifolia* Jacq. *Asian Pacific Journal of Tropical Biomedicine*. 2(2): 118-124.

Meskin, A. and Mark, S. (2002). Phytochemicals in Nutrition and Health. CRC Press, London. 123p.

Nweze, NO. and Nwafor, FI. (2014). Phytochemical, proximate and mineral composition of leaf extracts of *Moringa oleifera* from Nsukka, South – Eastern Nigeria. *Journal of Pharmacy and Biological Sciences*. 9(1): 99-103.

Obadoni, BO. and Ochuko, PO. (2001). Phytochemical studies and comparative efficacy of the crud extracts of some homeostatic plants in Edo and Delta State of Nigeria. *Global Journal of Pure and Applied Science*. 8(2): 203-208.

Ogbe, AO. and John, PA. (2011). Proximate study, mineral and anti-nutrient composition of *Moringa oleifera* leaves and potential benefits in poultry nutrition and health. *Journal of Biotechnology and Food Science.* 1(3): 296-308.

Oluduro, AO. (2012). Evaluation of antimicrobial properties and nutritional potentials of *Moringa oleifera* leaf in South-Western Nigeria. *Malaysian Journal of Microbiology*. 8(2): 59-67.

Paralikar, P. (2014). Biogenic synthesis of silver nanoparticles using leaves extract of *Epiphyllum oxypetalum* and its antibacterial activity. *Asian Journal of Biotechnology and Bioengineering*. 1(7): 1-5.

Upendra, RS. and Khandelwa, LP. (2012). Assessment of nutritive values, phytochemicals constituents and biotherapeutic of *Epiphyllum oxypetalum*. *International Journal of Pharmacy and Pharmaceutical Sciences*. 4(5): 421-425.

Van-Burden, TP. and Robinson, WC. (1981). Formation of complexes between protein and tannin acid. *Journal of Agriculture and Food Chemistry*. 1: 71-77.