British Journal of Pharmaceutical Research
16(1): 1-8, 2017; Article no.BJPR.32924
ISSN: 2231-2919, NLM ID: 101631759

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antioxidant Activity of *Cnestis ferruginea* and *Uvaria chamae* Seed Extracts

Basil N. Ita[1*]

[1]*Organic Chemistry Unit, Department of Chemistry, University of Uyo, Akwa Ibom State, Nigeria.*

Author's contribution

Author BNI designed the study, did the experiments and wrote the manuscript.

Article Information

DOI: 10.9734/BJPR/2017/32924
Editor(s):
(1) Rafik Karaman, Bioorganic Chemistry, College of Pharmacy, Al-Quds University, USA.
Reviewers:
(1) Cheikh Sall, University of Thiès, Sénégal.
(2) Christine Ong'ayo Wangia, Jomo Kenyatta University of Agriculture and Technology, Kenya.
Complete Peer review History: http://www.sciencedomain.org/review-history/18784

Original Research Article

Received 23rd March 2017
Accepted 20th April 2017
Published 25th April 2017

ABSTRACT

Aim: The antioxidant activity of seed extracts of *Cnestis ferruginea* and *Uvaria chamae* was investigated in this study.
Methodology: Samples were collected between November and December in 2016 from a farm in Nigeria. The air dried seeds were macerated with acetone, ethanol and water using standard methods. The antioxidant activity of the seed extracts was investigated by measuring its DPPH, ABTS scavenging activities and its metal chelating and ferric reducing potentials. In addition, total phenolic and flavonoid content of the seed extracts was evaluated.
Results: The seed extracts exhibited potent antioxidant activity in the DPPH and ABTS assay, with the ethanolic seed extract of *Uvaria chamae* being more effective (IC_{50} = 34.3 and 29.6 µg/mL respectively) than *Cnestis ferruginea*. However, the aqueous seed extract of *Uvaria chamae* exhibited the highest metal chelating activity (IC_{50}= 23.5 µg/mL), while its ethanolic extract showed higher reducing power than the standard. In addition, high content of phenolics and flavonoids was found in the organic seed extracts.
Conclusion: Seeds of studied plants are rich in natural antioxidants that can be exploited in the treatment of diseases related to oxidative stress.

Keywords: Cnestis ferruginea; Uvaria chamae; antioxidant activity; ferric reducing activity; metal chelating activity; total phenolic; flavonoid content.

**Corresponding author: E-mail: basil_ita@yahoo.com;*

1. INTRODUCTION

Reactive oxygen species have been implicated in a lot of human diseases such as cancer, diabetes, ulcer, gastrointestinal disorders, inflammation, neurodegenerative and cardiovascular diseases due to their ability to damage vital biomolecules in the body. Antioxidants can protect against damage arising from these reactive oxygen species. However, some of them have been reported to be toxic or mutagenic, thus, the search for better antioxidants of phyto-origin becomes pertinent. Because medicinal plants are rich in phyto-compounds, they can therefore be harnessed in the search for newer and more effective antioxidants that are less toxic [1].

Cnestis ferruginea and *Uvaria chamae* are medicinal plants that grow abundantly in Nigeria. Traditionally, organs of *Cnestis ferruginea* are used to treat migraines, headaches, toothache, sinusitis, and as a laxative. It is also a component of a decoction used to treat arthritis, stroke, rheumatism, gonorrhoea, etc [2]. In addition, the biochemical and phytochemical composition of the plant as well as its analgesic, anti-inflammatory, hypoglycaemic, hepatoprotective, antioxidant and anti-stress potentials have been reported [3-6].

In traditional medicine, root of *Uvaria chamae* is used to treat nose bleeding, heart diseases and fever. Also, biological activities such as antidiabetic, antioxidant, hypolipidemic, hepatoprotective, antimicrobial, neutralization of *Naja Nigricollis* venom, antiulcer and its antiplasmodial potentials have been reported for this plant [7-15].

Despite their wide usage and medicinal values, there are few research outcomes on the seeds of these plants. We therefore determine in this study the antioxidant potential of the seeds of *Cnestis ferruginea* and *Uvaria chamae.*

2. MATERIALS AND METHODS

2.1 Plant Material and Extraction

Fruits of *Cnestis ferruginea and Uvaria chamae* were harvested from a farm in Akwa Ibom State, Nigeria and the seeds separated. The seeds were shade dried, and pulverized. This powder (700 g) was macerated in 2.5 L acetone, ethanol and water separately for 24 hrs at room temperature. The filtrate was concentrated *in vacuo*, while the aqueous extract was freeze-dried to obtain the crude acetone (AcE), ethanol (EtE) and aqueous (AqE) extracts.

2.2 Chemicals

1,1- diphenyl-2-picryl hydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), disodium salt of 3-(2-pyridil)-5,6-bis(4- phenylsulfonic acid)-1,2,4-triazine (ferrozine), Folin Ciocalteu reagent, gallic acid, quercetin, butylated hydroxyanisole (BHA), trichloroacetic acid (TCA) were purchased from Sigma – Aldrich. All other reagents were of analytical grade.

2.3 Determination of Total Phenolic Content

Total phenolic content was determined using the Folin- Ciocalteu reagent. Briefly, 10 µL of each extract was taken and the volume made to 2 mL with distilled water. 0.5 mL of Folin-Ciocalteu reagent was added and the sample incubated for 3 min. This was followed by the addition of 2 mL Na_2CO_3 (20%w/v), placed in boiling water

Uvaria chamae *Cnestis ferruginea*

Fig. 1. *Uvaria chamae* and *Cnestis ferruginea* plants

for 1 min and allowed to cool to room temperature. The absorbance of this mixture was then read at 765 nm, subtracting the absorbance of the control. Total phenolic content was expressed in mgGAE/g extract based on a standard calibration curve of gallic acid [16].

2.4 Determination of Flavonoid Content

Flavonoid content of the extracts was determined according to the modified method of Kumar *et al* [1]. Briefly, each plant extract (10 μL) was diluted with distilled water and the total volume made up to 2 mL and kept at room temperature for 3 min. At the end of this period, 3 mL of 5% $NaNO_2$ and 0.3mL of 10% $AlCl_3$ was added and incubated for a further 6 min. Then, 2 mL of 1M NaOH was added and the final volume adjusted to 10 mL with distilled water. The absorbance of this mixture was read 510 nm. Flavonoid content was calculated using a standard calibration curve prepared from quercetin [1].

2.5 Evaluation of DPPH Activity

Precisely, 1 mL of each extract at varying concentrations was mixed with1mL of 0.004% methanol solution of DPPH. The mixture was shaken vigorously and allowed to stand for 30 min at room temperature in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. The procedure was repeated for the blank (methanol) and control (BHA). The radical scavenging activity was calculated using the equation:

$$\text{DPPH scavenging effect (\%)} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Sample concentration providing fifty percent inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. BHA was used as standard [17].

2.6 Evaluation of ABTS Activity

$ABTS^{\cdot+}$ was produced by reacting 7 mM ABTS solution (absorbance = 0.7$\pm$ 0.02 at 734 nm) with 2.45 mM potassium persulfate and the mixture allowed to stand at room temperature for 12 hrs in the dark. 2.94 mL of $ABTS^{\cdot+}$ solution was mixed with 60 µL of each extract and incubated at 37℃ for 20 min in the dark. After incubation, the absorption was read at 734 nm. The percentage inhibition was calculated using the equation:

$$\text{\% inhibition} = [A_{blank} - A_{sample})/A_{blank}] \times 100$$

Sample concentration providing fifty percent inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. BHA was used as standard [18].

2.7 Evaluation of Reducing Power

The reducing power of the seed extracts was determined according to the method of Oyiazu (1986) [19]. Each extract (10 -100 µg/mL) in ethanol (2.5 mL) was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide and the mixture incubated at 50℃ for 20 min. Thereafter, 2.5 mL of 10% trichloroacetic acid (w/v) was added and the mixture centrifuged at 200g for 19 min. The upper layer (5 mL) was mixed with 5 mL of deionised water and 1mL of 0.1% ferric chloride and the absorbance measured at 700 nm against a blank. A higher absorbance indicated a higher reducing power. IC_{50} value (µg/mL) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation. Ascorbic acid was used as positive control.

2.8 Evaluation of Metal Chelating Activity

Metal chelating activity was determined according to the modified method of Decker and Welch [20]. Briefly, 0.5 mL of each extract at varying concentrations was mixed with 0.05 mL of 2 mM$FeCl_2$ and 0.1 mL of 5 mM ferrozine and the total volume made to 2 mL with methanol. This mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage inhibition rate of ferrozine – Fe^{2+} complex formation was calculated using the formula:

$$\text{Scavenging activity (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where,

$A_{control}$ = absorbance of ferrozine – Fe^{2+} complex, and A_{sample} = absorbance of sample. EDTA was used as positive control.

2.9 Statistical Analysis

All experiments were performed in triplicate. Microsoft Excel was used for all statistical analysis.

3. RESULTS

Contents of total phenolics and flavonoids in seed extract of *Uvaria chamae* and *Cnestis ferruginea* are given Table 1. The ethanol and aqueous extracts of *Uvaria chamae* was richer in total phenolics (43.8 and 33.6 mgGAE/g) than *Cnestis ferruginea* (31.7 and 25.5 mgGAE/g). Generally, the ethanol extract had higher contents than the aqueous and acetone extracts. Flavonoids were lower than total phenolics, and ranged from 5.7 to 10.8 mgQE/g for *Uvaria chamae* and 3.3 to 9.6 mgQE/g for *Cnestis ferruginea*, with the aqueous and ethanol extracts of *Uvaria chamae* and *Cnestis ferruginea* respectively having the highest concentration.

To access the antioxidant activity of the seed extracts, their DPPH. ABTS, ferric reducing and metal chelating potential was accessed. In the DPPH assay, the extracts exhibited significant DPPH scavenging ability in a dose dependent manner (Fig. 2). The ethanolic seed extract of *Cnestis ferruginea* (EtE CF) and *Uvaria chamae* (EtE UC) showed the highest scavenging activity (77.4 and 82.4% respectively) at 100 µg/mL in comparison with other seed extract. Also, the highest IC_{50} value was obtained from the exthanolic seed extract of *Uvaria chamae* (IC_{50} = 34.3 µg/mL), which did not differ significantly from the ethanolic seed extract of *Cnestis ferruginea* (IC_{50} = 39.9 µg/mL) ($p \leq 0.01$). The other seed extracts had IC_{50} values greater than 40 µg/mL (Table 1).

The ability of the extracts to quench the ABTS radical was evaluated, as given in Fig. 3. Like the DPPH assay, the scavenging ability of the extracts increased in a dose-dependent manner with the ethanol and aqueous extracts being the most effective. At 10 µg/mL, the ethanol and aqueous seed extracts of *Cnestis ferruginea* and *Uvaria chamae* showed similar ABTS radical cation scavenging activity (40.6% - 45.8%); this increased significantly to $\geq$ 70% at 100 µg/mL. Generally, the ethanol extract of *Uvaria chamae* showed the highest ABTS scavenging activity (IC_{50}=29.6 µg/mL); however this was inferior to the control.

Table 1. Total phenolics, flavonoids, and antioxidant activity of *Cnestis ferruginea* and *Uvaria chamae* seed extracts

	Cnestis ferruginea			*Uvaria chamae*			Control		
	AcE	EtE	AqE	AcE	EtE	AqE	BHA	EDTA	Vit C
Total Phenolics (mgGAE/g)	14.4	31.7	25.5	22.3	43.8	33.6	-	-	-
Flavonoids (mgQE/g)	3.3	6.7	9.6	5.7	10.8	8.2	-	-	-
DPPH activity IC_{50} (µg/mL)	66.1	39.9	44.6	55.3	34.3	54.4	0.86	-	-
ABTS activity IC_{50} (µg/mL)	57.0	33.1	36.8	49.8	29.6	33.9	1.0	-	-
Metal chelating activity IC_{50} (µg/mL)	59.7	40.5	32.1	50.1	35.8	23.5	-	< 0.1	-
Reducing power IC_{50} (µg/mL)	61.1	33.7	32.9	41.1	1.0	14.9	-	-	3.1

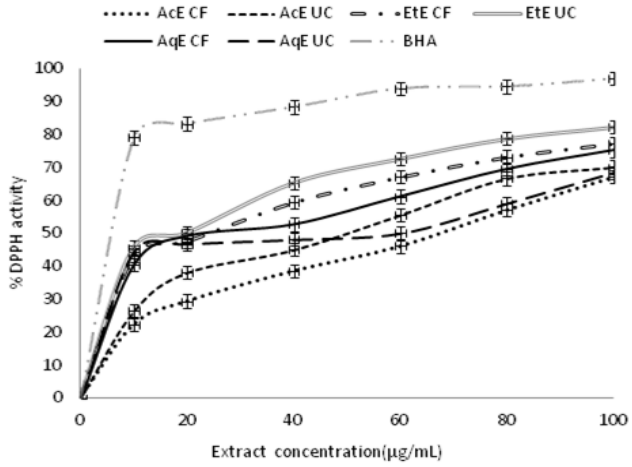

Fig. 2. DPPH radical scavenging activity of extracts

AcE CF- acetone extract of Cnestis ferruginea seeds; AcE UC - acetone extract of Uvaria chamae seeds; EtE CF- ethanol extract of Cnestis ferruginea seed; EtE UC - ethanol extract of Uvaria chamae seeds; AqE CF- aqueous extract of Cnestis ferruginea seed; AqE UC - aqueous extract of Uvaria chamae seed.

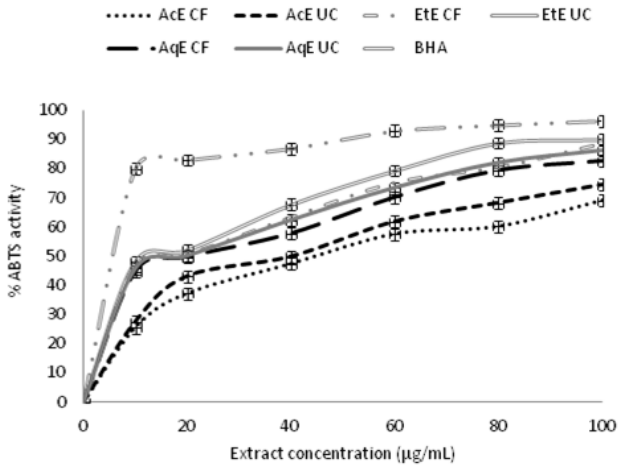

Fig. 3. ABTS radical scavenging activity of extracts

AcE CF- acetone extract of Cnestis ferruginlea seeds; AcE UC - acetone extract of Uvaria chamae seeds; EtE CF- ethanol extract of Cnestis ferruglinea seed; EtE UC - ethanol extract of Uvalria chamale seeds; AqE CF- aqueous extract of Cnestis ferruginlea seed; AqE UC - aqueous extract of Uvaria chamae seed

The reducing power of the extracts was also determined. Our findings indicate promising reducing activities (Fig. 4), and this increased with increasing concentration of the extracts. At 20 µg/mL, the ethanol and aqueous seed extracts exhibited reducing powers > 0.5, and this increased to 0.82 – 0.89 at 100 µg/mL. Generally, observed trend was: ethanol extract > aqueous extract > acetone extract. With regards to IC_{50} values (Table 1), the ethanol seed extract of *Uvaria chamae* exhibited the best reducing power (IC_{50} =1.0 µg/mL) and his was superior to vitamin C used as control.

The metal (Fe^{2+}) chelating ability of the seed extracts was also evaluated. Similar to other results, the ethanol and aqueous seed extracts showed the most promising ability to chelate Fe^{2+} in a dose-dependent pattern. The aqueous seed extract of *Uvaria chamae* exhibited the highest chelating ability at 100 µg/mL (94.7%), and this was close to that of EDTA. However, based on IC_{50} values, the chelating ability of the seed extracts was inferior to EDTA.

4. DISCUSSION

Medicinal plants will continue to play vital role in health care systems particularly amongst low income populace because they are easily accessible and are rich in secondary metabolites with varying biological properties. Results obtained from seeds of *Cnestis ferruginea* and *Uvaria chamae* indicated a rich source of total phenolics and flavonoids, however, this varied with the extracting solvent. Phenolic compounds are good free radical scavengers and metal chelators, their high content in these extracts suggest that they could play vital role in the remediation of free radical induced damage in the body. In comparison with other works, our result are higher than reports from seeds of *Hibiscus cannabinus* [21] and *Capsicum frutescens* [22] and roots of *Uvaria chamae* [7].

The antioxidant activity of the extracts in the DPPH and ABTS assay indicated that the extracts were potent free radical scavengers, with the ethanolic seed extracts of *Uvaria chamae* and *Cnestis ferruginea* being the most effective. These results indicate that the seed sextracts are capable of donating electrons or hydrogen which could deactivate organic radicals. Nwaechujor [23] reported lower DPPH scavenging activity of 73.34% at 100 µg/mL for *Uvaria chamae* root bark extract. Our result also indicate that the seed extract could scavenge DPPH radical better than the root bark at the same concentration.

The reducing power of the extracts serves as an important indicator of antioxidant activity by measuring its electron donating potentials. Our extracts exhibited promising reducing powers, with the aqueous and ethanolic seed extracts being the most potent. Lower reducing power has been reported for *Tylosema esculentum seed extracts and Uvaria chamae* roots [7,24]. Based on our result, it could be posited that the ethanol and aqueous seed extracts of *Cnestis ferruginea* and *Uvaria chamae* are rich in

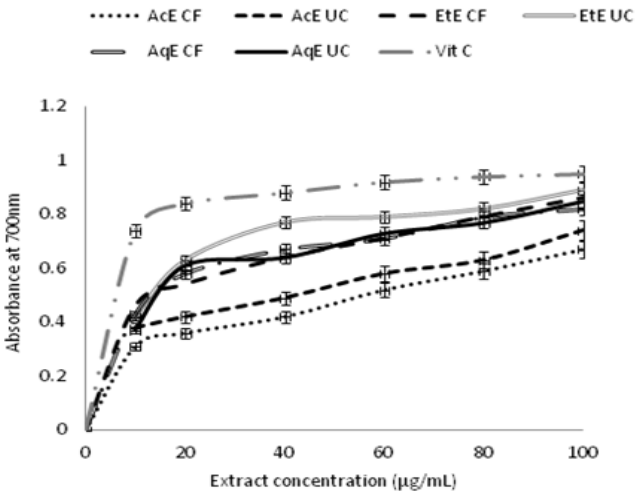

Fig. 4. Reducing power of extracts

AcE CF- acetone extract of Cnestis ferruginlea seeds; AcE UC - acetone extract of Uvaria chamae seeds; EtE CF- ethanol extract of Cnestis ferruglinea seed; EtE UC - ethanol extract of Uvalria chamale seeds; AqE CF- aqueous extract of Cnestis ferruginlea seed; AqE UC - aqueous extract of Uvaria chamae seed

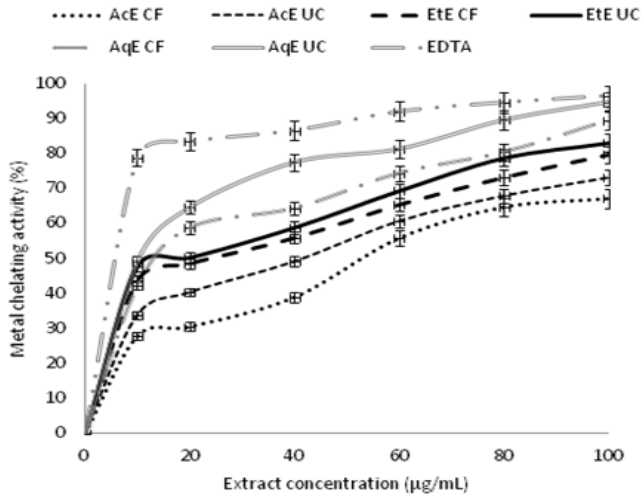

Fig. 5. Metal chelating activity of extracts

AcE CF- acetone extract of Cnestis ferruginlea seeds; AcE UC - acetone extract of Uvaria chamae seeds; EtE CF- ethanol extract of Cnestis ferruglinea seed; EtE UC - ethanol extract of Uvalria chamale seeds; AqE CF- aqueous extract of Cnestis ferruginlea seed; AqE UC - aqueous extract of Uvaria chamae seed

reductones, which are capable of donating electrons.

Iron plays many vital role in the body such as oxygen transport, cellular respiration, as a co - factor in many enzymes as well as for proper cell functioning. However, when present in excess, it can catalyse hydroperoxide decomposition and Fenton – type reactions which are capable of exaberating oxidative stress. Thus, the ability of an extract to chelate Fe^{2+} is considered as an important property of antioxidant activity. Our result showed that the extracts were potent Fe^{2+} chelators, with the aqueous seed extract of *Uvaria chamae* being the most active. These suggest that our seed extracts can prevent oxidative stress induced by transition metals in biological systems and supports its use in traditional medicine. However, our values were lower than reports for *Uvaria chamae* roots [7].

5. CONCLUSION

In conclusion, the ethanol and aqueous seed extracts of *Cnestis ferruginea* and *Uvaria chamae* can be considered as new sources of antioxidant compounds as they demonstrated notable antioxidant, metal chelating and ferric reducing potentials, with the ethanolic extract of *Uvaria chamae* being the most potent. The isolation and characterization of these phyto-compounds could play an interesting role in the fight against oxidative stress.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Kumar V, Lemos M, Sharma M, Shiram V. Antioxidant and DNA damage protecting activities of *Eulophia nuda* Lindl. Free radicals and Antioxidants. 2013;3:55-60.
2. Okwu D, Iroabuchi F. Phytochemical composition and biological activity of *Uvaria chamae* and *Clerodendoron splendiens*. E-Journal of Chemistry. 2009; 6(7):553-560.
3. Akharaiyi FC, Boboye B, Adetuyi FC. Antibacterial, phytochemical and antioxidant properties of *Cnestis ferruginea* DC (Connaraceae) extracts. Journal of Microbiology, Biotechnology and Food Sciences. 2012;2(2):592-609.
4. Ishola IO, Akindele AJ, Adeyemi OO. Analgesic and anti-inflammatory activities of *Cnestis ferrruginea* Vahl ex DC (Connaraceae) methanolic root extract. Journal of Ethnopharmacology. 2011; 135(1):55-62.
5. Ishola IO, Ashorobi RB. Anti-stress potential of aqueous root extract of *Cnestis ferruginea*. International Journal of Pharmacoology. 2007;3:295-298.
6. Ibironke GF, Odewole GA. Analgesic and anti-inflammatory properties of methanol extract of *Cnestis ferruginea* in rodents. African Journal of Medicine and Medical Sciences. 2013;41(2):205-210.
7. Monon K, Abdoulaye T, Karamoko O, Adama C. Phytochemical composition, antioxidant and antibacterial activities of root of *Uvaria chamae* P. Beauv. (Annonaceae) used in treatment of dysentery in North of Cote d'Ivoire. International Journal of Pharmacognosy and Phytochemical Research. 2015; 7(6):1047-1953.
8. Emordi JE, Agbaye EO, Oreagba JA, Iribhogbe OI. Antidiabettic and hypolipidemic activities of hydroethanolic root extract of *Uvaria chamae* in streptozotocin induced diabetic albino rats. BMC Complimentary and Alternative Medicine. 2016;16:468-473.
9. Madubunyi I. Hepatoprotective activity of *Uvaria chamae* root bark methanol extract against acetaminophen- induced liver lesions in rats. Comparative Clinical Pathology. 2016;21(3):44-49.
10. Osuagwu GGE, Ihenwosu AO. Phytochemical composition and antimicrobial activity of the leaves of *Alchornea cordifolia* (Schum and Thonn), *Sanseviera liberca* (Gerand labr) and *Uvaria chamae* (*P. Beauv*). American Journal of Phytomedicine and Clinical Therapeutics. 2014;2(1):1-12.
11. Okwuosa OM, Chukwura EI, Chukwuma GO, Okwuosa CN, Enweani IB, Agbakoba NR, et al. Phytochemical and antifungal activities of *Uvaria chamae* leaves and roots, *Spondias mombin* leaves and bark and *Combretum racemosum* leaves. African Journal of Medicine and Medical Science. 2012;9(3): 224-234.
12. Omale J, Ebiloma UG, Idoko GO. *In vivo* neutralization of *Naja nigricollis* venom by *Uvaria chamae*. American Journal of Biochemistry and Biotechnology. 2016; 9(3):224-234.
13. Chilaka PC, Unekwe PC, Ckilaka JU, Nweke IN. Studies on antiulcer properties of ethanolic leaf extracts of *Uvaria chamae*. Tropical Journal of Medical Research. 2010;14(2):23-30.
14. Okokon JE, Ita BN, Udokpoh AE. The *in-vivo* antimalarial activities of *Uvaria chamae* and *Hippocratea africana*. Annals of Tropical Medicine and Parasitology. 2006;100(7):585-590.
15. Ebi GC, Ifeanacho CJ, Kamalu TN. Antimicrobial properties of *Uvaria chamae*

stem bark. Fitoterapia. 1999;70(1):621-624.
16. Gul H, Bhakshu I, Ahmed F, Qureshi I, Ghazi I. Evaluation of *Abelmoschus moschatus* extracts for antioxidant, free radical scavenging, antimicrobial and antiproliferative activities using in vitro assays. BMC Complimentary and Alternative Medicine. 2011;11:64.
17. Subhasree B, Baskar R, Keerthana RL, Susan RL, Rajasekran P. Evaluation of antioxidant potential in selected green leafy vegetables. Food Chemistry. 2009; 115:1213-1220.
18. Obiang CS, Ondo J, Atome GN, Engonga LO, Siawaya JD, Emvo EN. Phytochemical screening, antioxidant and antimicrobial potential of stem barks of *Coula edulis* Baill, *Psedospondias longifolia* Engl. and *Carapa klaineana* Pierre. from Gabon. Asian Pacific Journal of Tropical Disease. 2016;6(7):557-563.
19. Oyiazu M. Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition. 1986;44:307-315.
20. Decker EA, Welch B. Role of ferritin as lipid oxidation catalyst in muscle food. Journal of Agricultural and Food Chemistry. 1990;38: 674-678.
21. Yusri NM, Chan KW, Iqbal S, Ismail M. Phenolic content and antioxidant activity of *Hibiscus cannabinus L* seed extracts after sequential solvent extraction. Molecules. 2012;17:12612-12621.
22. Gurnani N, Gupta M, Mehta D, Mehta BK. Chemical composition, total phenolic and flavonoid contents, and in vitro antimicrobial and antioxidant activity of crude extracts from red chilli seeds *Capsicum frutescens* L. Journal of the Taibah University for Science. 2016; 10:462-470.
23. Nwaehujor CO, Ode JO, Akande MG. *In vitro* antioxidant potentials of some herbal plants from Southern Nigeria. Journal of Medical Sciences. 2013;13(1): 56-61.
24. Kuvare A, Kandawa-Schulz M, Kapewangolo P. Comparative study of antioxidant properties, polyphenols and flavonoid contents of the tuber and seed extracts of Marama bean (*Tylosema esculentum*). International Science and Technology Journal Namibia. 2015;6:21-31.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18784