academicJournals

Vol. 11(1), pp. 1-10, 3 January, 2017
DOI: 10.5897/JMPR2015.6025
Article Number: 6DEC5E762290
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Comparative physicochemical, phytochemical and acute toxicity studies of two *Ocimum* species in Western Uganda

Abayomi M. Ajayi[1,2]*, Annet Naluwuge[1], Peter Buyinza[1] and Ivan Luswata[1]

[1]Department of Pharmacology and Toxicology, Kampala International University-Western Campus, Ishaka, Bushenyi, Uganda.
[2]Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Nigeria.

Received 6 December, 2015; Accepted 28 March, 2016

***Ocimum gratissimum* and *Ocimum suave* are species belonging to the Lamiaceae family; they have been domesticated and are widely grown in gardens in Western Uganda for its claimed beneficial effect in ethnomedical practice. This study was aimed at comparative evaluation of the physicochemical, phytochemical and acute toxicity potentials of their leaves. Dried leaves of *O. gratissimum* and *O. suave* were subjected to physicochemical analysis, crude extraction in methanol and sequential extraction in n-hexane, chloroform, ethylacetate and methanol. Phytochemical constituents of the crude extracts were determined by both qualitative and quantitative methods, while acute toxicity potentials were studied in female rats. Total caffeic acid derivatives contents, total flavonoid contents and reducing power assay were evaluated in the sequential extracts. The physicochemical parameters of leaves of *O. gratissimum* and *O. suave* showed similar profiles. The extraction yield, phytochemical constituents and acute toxicity effects of the crude extracts were not significantly different. Hexane, chloroform and ethylacetate extract of *O. suave* showed significant ($p < 0.05$) higher total flavonoid contents than corresponding extracts of *O. gratissimum*. Similarly, *O. suave* extracts showed a stronger positive correlation of phenolic content to antiradical power than the corresponding extracts of *O. gratissimum.* Conclusively, the two species showed comparative physicochemical, phytochemical and acute toxicity profiles, and a positive correlation of phenolic contents to antiradical power.**

Key words: Lamiaceae, Uganda, phenolics, *Ocimum gratissimum*, *Ocimum suave*.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The therapeutic value of these medicinal plants lies in some active chemical substances that produce a definite physiological action on the human body (Edeoga et al., 2005). Nature has served as a rich repository of medicinal plants for

*Corresponding author. E-mail: yomexj@yahoo.com. Tel: +2348103861380.

thousands of years and an impressive number of modern drugs have been isolated from natural sources, notably of plant origin (Cowan, 1999). Medicinal plants were the main source for primary health care in the past centuries before the advent of conventional orthodox medicine. Herbal medicine, based on their traditional uses in the form of powders, liquids or mixtures, have been the basis of treatment for various ailments in African countries since ancient times. They provide diverse indications for which reason they are employed in the management of a wide range of conditions. Importantly, it is the most easily accessible and affordable health resource available to the local community (Mahomoodally, 2013).

The global surge in the use of medicinal plants as a result of the failures of synthetic drugs and the beliefs that herbal drugs provides safer, more reliable and cheaper phytomedicine, brings to the fore the need to improve on the quality of medicinal plants. One major problem associated with the traditional ethno-medical systems is that even though the medicinal plants seems to work very well and are readily available to the community, there are no sufficient data for quality assessment and standardization. Some of the problems associated with lack of quality include the multiple constituents of herbal drugs, absence of identified marker or active compounds in commercial quantity, and misidentification of chemo cultivars (Kunle et al., 2012). A key obstacle which has hindered the development of medicinal plant product in developing countries is the lack of documentation and stringent quality control. With this backdrop, it becomes extremely important to make an effort towards standardization of plant materials to be used as phytomedicine.

The genus *Ocimum* is ranked high among some of the astonishing herbs for having enormous medicinal potentials; they are widespread over Asia, Africa, Central and Southern America (Vani et al., 2009). All basils are member of the Lamiaceae family. Characterizations of each species in this genus (family Lamiaceae) are based on the leaves and habitat (Paton, 1992; Grayer et al., 2002). *Ocimum gratissimum* and *Ocimum suave* belong to the family Lamiaceae. Folkore medicine claims their use in many condition, they are widely grown in gardens in Uganda (Orwa et al., 2009). The leaves and flowers of *O. gratissimum* and *O. suave* are rich in essential oils, so it is used in preparation of teas and infusion. The plant materials are used throughout Uganda as home remedies (Tabuti et al., 2010). This research was conducted to compare the physicochemical, phytochemical and acute toxicity profile of two *Ocimum* species that are known locally to bear the same name (Omujaja) in Bushenyi district. This study provides scientific evidence to back the traditional use of these plants as a consequence of their chemical constituents. It will also provide documentation of phytochemical constituents and physicochemical parameters for monograph information about these two plants.

MATERIALS AND METHODS

Plants collections

The two plants were obtained from medicinal garden in Bushenyi District, Uganda, and taxonomically identified by Dr. Eunice Olet (Botanist) of the Biology Department, Faculty of Science, Mbarara University of Science and Technology and voucher specimens were retained with the voucher number LI 001 and 002 for *O. gratissimum* and *O. suave,* respectively. The plants collection was done in the early hours of the day between 9:00 a.m to 11:00 a.m. The leaves of *O. gratissimum* and *O. suave* were collected and dried under shade, then ground into powder using an electronic blender.

Determination of physicochemical parameters in powdered leaves of *O. gratissimum* and *O. suave*

Standard procedures as documented in the official literature were adopted in determining the dried leaf material moisture content, ash values and water and alcohol extractive values (World Health Organization, 2003; Kokate, 2000).

Moisture content

Four grams each of powdered leaves of *O. gratissimum* and *O. suave* were weighed into pre-weighed crucibles (in triplicate), and then dried in the oven at 100°C until constant weight. The crucibles were cooled in a dessicator, loss in weight was recorded as moisture content.

Determination of ash values

Four grams (in triplicate) of powdered leaves of *O. gratissimum* and *O. suave* were weighed into silica dish previously ignited, cooled and weighed. The material was ignited to ashes in an oven at 450°C, cooled in a dessicator for up to 24 h and weighed. Acid-insoluble ash content was analyzed by boiling the total ash for 5 min with hydrochloric acid (7%, v/v); the mixture in a crucible was ignited at 450°C to a constant weight. The total ash was dissolved in 200 ml of distilled water, stirred and the mixture was filtered using pre-weighed filter paper. The weight of the dried residue was determined as insoluble ash. The insoluble ash was subtracted from the total ash to obtain the amount of water soluble ash.

Determination of cold extractive values

Four grams of the powdered sample was weighed and soaked with 100 ml distilled water (or 70% ethanol) in a conical flask with a stopper. The materials were extracted for 24 h with intermittent shaking. The extracts were filtered rapidly through a dry filter; 20 ml of the filtrate was transferred to pre-weighed evaporating dish, concentrated to almost dryness on a water bath, and then dried at 105°C for 3 h. The percentage of water-soluble or ethanol soluble extractives were calculated with reference to the dried sample. Samples were prepared in triplicate.

Determination of hot extractive values

About 4.0 g of the powdered sample was accurately weighed and soaked with 100 ml distilled water (or ethanol) in a flask. The flask was attached to a reflux condenser and boiled gently for 1 h. The extracts were filtered rapidly through a dry filter; 20 ml of the filtrate

was transferred to pre-weighed evaporating dish, concentrated to almost dryness on a water bath, and then dried at 105°C for 3 h. The percentage of water-soluble or ethanol soluble extractives were calculated with reference to the dried sample. Samples were prepared in triplicate.

Crude and sequential extraction

Crude extraction was carried out with dried powdered leaves of *O. gratissimum* and *O. suave*. 100 g of powdered leaves was macerated in 500 ml of 80% methanol and shaken for 48 h on a laboratory rotator (Nuve SL 350 Quality System, Digisystem Laboratory inc, Taiwan). Sequential extraction was performed by successive maceration of dried powdered leaves (1:5 w/v) in solvent of increasing polarity (n-hexane, chloroform, ethylacetate and methanol) for 48 h each. All extractions were performed in triplicates, and were filtered using a Whatman® filter paper. The extracts were pooled together and concentrated in a Rotary evaporator (Buchi Rotavapor R-124) under reduced pressure and dried to a constant weight in oven at 40°C. All extracts were dried in separate containers in order to determine the percentage yield of extraction. The crude methanol extract of *O. gratissimum* and *O. sauve* were labeled as ME*Og* and ME*Os*, respectively. The successive extracts were labeled as *Og*HE and *Os*HE for hexane extracts; *Og*CE and *Os*CE for chloroform extracts; *Og*EAE and *Os*EAE for ethylacetate extracts; and *Og*ME and *Os*ME for methanol extracts. All the extracts were stored in a refrigerator (4°C±2); ME*Og* and ME*Os* were dissolved in 2% Tween 80 for acute toxicity test.

Qualitative phytochemical analysis

The standard methods described by Evans (2002), Harborne (1998) and Odebiyi and Sofowora (1982) were applied in the phytochemical screening of the individual constituents of the crude extracts. The presence of the compounds were rated as positive (+) or negative (-) in the two extracts.

Quantitative phytochemical estimation in the crude methanol extracts

Quantitative assessment for crude alkaloid, tannin, saponin and flavonoid were performed in crude methanol extracts of *O. gratissimum* and *O. suave* leaves. The alkaloid content of crude methanol extract was determined by the method of Harborne (1998). Tannin content was determined by the method described by Van Buren and Robinson (1969) as modified in Edeoga et al. (2005). Saponin content was determined by the method of Obadoni and Ochuko (2001). The flavonoid contents of the extracts were estimated by the method of Bohm and Koupai-Abyazan (1994).

Determination of total caffeic acid in sequential extracts of *O. gratissimum* and *O. suave* leaves

The Arnow's reagent method of spectrophotometric estimation of caffeic acid derivatives was used for the quantification method (Benedec et al., 2012). Briefly, 0.2 ml of various extracts (1 mg/ml) was added into test tubes in triplicates and hydrochloric acid (1 ml, 0.5 N), Arnow's reagent (1 ml) and sodium hydroxide solution (1 ml, 1 N) were added to the test tubes and allowed to stand for 5 min. The absorbance was read at 500 nm in a spectrophotometer (Spectronic 21D Milton Roy, USA). Caffeic acid was used as standard for the calibration curve.

Determination of total flavonoid content in sequential extracts of *O. gratissimum* and *O. suave* leaves

The total flavonoid contents (TFC) in the extracts were determined by the spectrophotometric aluminium chloride method (Sultana et al., 2009). Briefly, 1 ml of the extract (1 mg/ml) was added into test tubes. After that, 0.3 ml of 10% (w/v) $NaNO_2$ was added to the test tubes, and left to react for 5 min. Then, 0.3 ml of 10% (w/v) $AlCl_3$ was added and left for 1 min to react. Then, 2 ml of 1M NaOH was added and the mixtures shaken. Aliquots of the mixtures were transferred to a cuvette, and the absorbance values measured at 510 nm in a spectrophotometer (Spectronic 21D Milton Roy). Rutin was used as a standard for the calibration curve. All samples were prepared in triplicates.

Determination of reducing power of sequential extracts of *O. gratissimum* and *O. suave* leaves

The reducing power was determined according to the method of Oyaizu (1986) as described in Ferreira et al. (2007). It is based on the principle that antioxidant substances react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}) which then reacts with ferric chloride to form ferrous complex that has an absorption maximum at 700 nm.

Various concentrations of the plants extracts or ascorbic acid (0.125, 0.25, 0.5, and 1.0 mg/ml) were mixed with 2.5 ml of phosphate buffer and 2.5 ml of 1% potassium ferricyanide, respectively. The mixtures were incubated in a 50°C water bath for 20 min. After cooling to room temperature (20 to 23°C), 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of freshly prepared 0.1% ferric chloride solution. The absorption was measured at 700 nm in a spectrophotometer (Spectronic 21D Milton Roy, USA). The inhibitory concentration providing 0.5 of absorbance (IC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration.

Comparative evaluation of acute toxicity effects of crude methanol extracts of *O. gratissimum* and *O. suave* leaves

Female Wistar rats were housed in the Animal Facility of the Department of Pharmacology and Toxicology, Kampala International University-Western Campus. The animals were kept in a cage lined with sawdust, at room temperature with adequate ventilation, under a naturally illuminated environment with 12 h of light and 12 h of darkness. They were fed with standard diet (Nuvita (R) Animal Feed Ltd, Jinja Uganda) and have access to clean drinking water *ad libitum*. The animal experiment was conducted according to the National Institute of Health Guide for the care and use of laboratory animals (NIH, 1996). The study was carried out after ethical consideration and approval by research committee of the Kampala International University – Western Campus.

Comparative toxicity screening was carried out in female Wistar rats (weighing 120 to 150 g) following the Organization for Economic Co-operation and Development (OECD) guidelines (423). The rats were assigned into groups (n=5) including a control group by the stratified random method according to their body weight. The route of administration was by oral gavage in accordance with the main route of intake of *O. gratissimum* and *O. suave* by humans for medicinal purposes. Single oral dose of 2000 and 5000 mg/kg per body weight of ME*Og* and ME*Os* was administered to overnight fasted animals. Animals were observed individually after dosing once during the first 30 min, periodically during the first twenty four hours, with special attention given during the first four hours and daily thereafter, for a total of 14 days. The effects of the extracts on

Table 1. Physicochemical parameters of leaves of *O. gratissimum* and *O. suave*.

Physicochemical parameter	*O. gratissimum*		*O. suave*	
	Mean ± SEM	**%**	**Mean ± SEM**	**%**
Moisture content	0.73 ± 0.07	18.3	0.50 ± 0.36	12.5
Ash values				
Total ash	0.73 ± 0.09	18.3	1.17 ± 0.09	32.5
Acid-insoluble ash	0.10 ± 0.05	2.5	0.18 ± 0.04	4.5
Water-Soluble ash	0.33 ± 0.18	8.3	0.75 ± 0.03	18.8
Extractive values				
Cold water soluble extractives	0.55 ± 0.25	13.8	0.45 ± 0.25	11.3
Cold Ethanol soluble extractives	0.37 ± 0.17	9.3	0.33 ± 0.09	8.3
Hot water soluble extractives	0.13 ± 0.03	3.3	0.27 ± 0.03	6.8
Hot ethanol soluble extractives	0.47 ± 0.07	11.8	0.33 ± 0.12	8.3

Values are Mean ± SEM (n=3). ^{ns}Values are not significantly different; $p > 0.05$ using unpaired T-test.

general behavior, feed intake and weight gain were observed in the animals for fourteen days post administration.

Statistical analysis

All data generated were presented as Mean ± Standard Error of the Mean (SEM). The reducing power activity was expressed in terms of IC_{50}, which was estimated from the non-linear regression curve using version 5 GraphPad prism®. The linear correlation coefficients between the total caffeic acid derivatives content and reducing power activity were calculated by Pearson test using the GraphPad Prism® software version 5.01 (GraphPad Software, Inc. La Jolla, CA 92037 USA). Comparative significant differences between two samples were analyzed using student's t test. All other analysis was by one-way ANOVA statistical model with Newman-Keuls Multiple Comparison post hoc test. Statistical significance was taken for $p<0.05$.

RESULTS

Physicochemical parameters of leaves of *O. gratissimum* and *O. suave*

The moisture content, ash values and extractive values of dried leaves of *O. gratissimum* and *O. sauve* were determined and are presented in Table 1. Percentage of moisture content in leaves of *O. gratissimum* (18.3%) was higher than what was obtained in *O. suave* (12.5%). The percentage total dry ash content for *O. suave* (32.5%) was greater than *O. gratissimum* (18.3%). Similarly, the acid-insoluble ash and water-soluble ash values for *O. suave* (4.5 and 18.8%) was greater than for *O. gratissimum* (2.5% & 8.3%). These differences were not significantly different ($p > 0.05$).

The cold water extractive yield was higher for *O. gratissimum* (13.8%) compared to *O. suave* (11.3%), while hot water showed higher values for *O. suave* (6.8%) than *O. gratissimum* (3.3%). Both cold ethanol and hot ethanol gave the same soluble extractive values for *O. suave* (8.3 %). Hot ethanol gave higher values for *O. gratissimum* (11.8%) than cold ethanol extractive (9.3%). Taken together, cold extraction gave the better yield for both samples compared to hot extraction.

Crude and sequential extraction yield

Crude extraction of powdered leaves of *O. gratissimum* and *O. suave* in aqueous methanol gave a percentage yield of 1.86 and 2.23%, respectively. Sequential extraction of MEO*g* and MEO*s* gave similar yields in all solvent extracts except in the methanol extract. The yield was as follows, *Os*HE (2%) > *Og*HE (1.7%); *Og*CE (1.49%) > *Os*CE (1.48%); *Og*EAE (1.22%) < *Os*EAE (1.23%) and *Og*ME (3.73) < *Os*ME (9.78%).

Phytochemical analysis

Qualitative phytochemical screening revealed the presence of the major phytochemicals in the methanol extracts of both *O. gratissimum* and *O. suave* leaves (Table 2). Quantitative phytochemical estimations in the methanol extracts revealed higher percentages of tannins and flavonoids in the MEOg (60 and 66%) than MEO*s* (40 and 56%). Similarly, the alkaloids composition in MEO*g* (24%) was higher than in MEO*s* (20%), saponin content was however lower in MEO*g* (10%) compared to MEO*s* (22%) (Table 3). These differences were not statistically significant ($p > 0.05$).

Total caffeic acid and total flavonoid contents in sequential extracts of *O. gratissimum* and *O. suave*

The level of phenolic compounds in the different solvent

Table 2. Qualitative screening results for phytochemicals found in methanol extracts of *O. gratissimum* (ME*Og*) and *O. suave* (ME*Os*) leaves.

Substance	Test/Reagent	ME*Og*	ME*Os*	Deduction
Saponins	Frothing test	+	++	Saponins detected in both species but more in *O. suave*
Tannins	Lead acetate	+	+	Both extracts contained tannin in the two methods used
	15% ferric chloride	++	+	
Terpenoids/Steroids	Salkowski test	+	+++	More terpenoids were found in *O. suave*
Alkaloids	Mayer test	+	+	Presence of alkaloids were detected in both extracts
	Wagners test	+	+	
Flavonoids	Shinoda assay	+	+	Presence of flavonoids were detected in methanol extracts of both plants
	2% lead acetate	++	+	
	Dilute ammonia solution	+	+	
Reducing sugars	Iodine	+	+	Reducing sugars present in both extracts
Cardiac glycosides	Ferric chloride + glacial acetic acid	++	+	There were more cardiac glycosides
Lactone coumarins	Balijet test	++	+	More coumarins in *O. gratissimum*

Table 3. Quantitative phytochemical composition of methanol extracts of *O. gratissimum* (ME*Og*) and *O. suave* (ME*Os*) leaves.

Phytochemicals	ME*Og*		ME*Os*	
	Mean ± SEM	% Content	Mean ± SEM	% Content
Alkaloids	0.12 ± 0.15^{ns}	24	0.10 ± 0.03	20
Flavonoids	0.30 ± 0.10^{ns}	60	0.20 ± 0.10	40
Tannins	0.33 ± 0.05^{ns}	66	0.26 ± 0.04	52
Saponins	0.05 ± 0.03^{ns}	10	0.11 ± 0.01	22

Values are Mean ± SEM (n=3). ^{ns}Values are not significantly different; $p > 0.05$ using unpaired T-test.

extracts (hexane, chloroform, ethylacetate and methanol) of the leaves of *O. gratissimum* and *O. suave* are shown in Figure 1A and B. Total caffeic acid derivatives expressed as mgCAE/g sample was as follows, *Os*HE (47.2) > *Og*HE (33.1); *Os*CE (42.3) > *Og*CE (34.9); *Og*EAE (76.7) > *Os*EAE (53.6); and *Os*ME (85.1) > *Og*ME (74.9). The findings of this study revealed a significantly ($p < 0.05$) higher caffeic acid contents in *Og*EAE than *Os*EAE.

Similar pattern was observed in total flavonoid contents, with *O. gratissimum* showing lower contents compared to *O. suave* extracts (Figure 1B). The total flavonoid content expressed as RE/g sample was as follows: *Os*HE (32.4) > *Og*HE (24.5); *Os*CE (39.1) > *Og*CE (32.2); *Os*EAE (45.7) > *Og*EAE (41); and *Os*ME (43.8) > *Og*ME (42.2). The results showed a significant ($p < 0.05$) higher flavonoid contents in hexane, chloroform and ethylacetate extracts of *O. suave* compared to *O. gratissimum*.

Reducing power of sequential extracts of *O. gratissimum* and *O. suave* leaves

The ability to act as antioxidant is based on the reducing potential of phenolics. To further compare the phenolic properties of the successive extracts, the reducing powers of the extracts were evaluated. A dose-dependent increase in reducing power of the extracts was observed (Figure 2). Ascorbic acid used as positive standard agent showed higher reducing power with an IC_{50} of 0.14 ± 0.08 compared to the extracts. The IC_{50} for the extracts is as presented in Table 4. All the extracts showed potent reducing potentials all within the range for the standard antioxidant (Ascorbic acid), but *Os*ME showed the best activity. Comparative anti-radical power

Table 4. Inhibitory concentration (IC50), antiradical power and Pearson correlation coefficients of successive extracts of *O. gratissimum* and *O. suave*.

Extract	Reducing power	Antiradical power	Correlation[a]
	IC_{50}	$1/IC_{50}$	TCADC[b]
*Og*HE	0.19 ± 0.07	5.26	0.9301*
*Os*HE	0.30 ± 0.08	3.33	0.9808**
*Og*CE	0.16 ± 0.05	6.25	0.8893*
*Os*CE	0.26 ± 0.05	3.84	0.9604**
*Og*EAE	0.18 ± 0.07	5.56	0.9188*
*Os*EAE	0.16 ± 0.05	6.25	0.8939*
*Og*ME	0.21 ± 0.08	4.76	0.9347*
*Os*ME	0.14 ± 0.04	7.14	0.8444^{ns}
Ascorbic acid	0.14 ± 0.08	7.14	-

[a]Pearson correlation coefficient (r). [b]Total caffeic acid derivative content. *$p < 0.05$, **$p < 0.01$ (two-tailed), ns: not significant.

Table 5. Effects of methanol extracts of *O. gratissimum* (ME*Og*) and *O. suave* (ME*Os*) leaves after single oral administration on body weight in Rats.

Treatment	No. of deaths	Body weight of experimental animals (kg) at different days[a]				
		0	4	7	10	14
ME*Og* (2 g/kg)	0/5	0.124 ± 0.005	0.131 ± 0.005	0.136 ± 0.005	0.139 ± 0.005	0.141 ± 0.005
ME*Og* (5 g/kg)	0/5	0.125 ± 0.006	0.129 ± 0.006	0.133 ± 0.006	0.136 ± 0.006	0.135 ± 0.006
ME*Os* (2 g/kg)	0/5	0.126 ± 0.006	0.131 ± 0.006	0.136 ± 0.006	0.141 ± 0.007	0.147 ± 0.005
ME*Os* (5 g/kg)	0/5	0.127 ± 0.006	0.134 ± 0.006	0.137 ± 0.006	0.140 ± 0.006	0.142 ± 0.007
Distilled water (10 ml/kg)	0/5	0.129 ± 0.007	0.136 ± 0.007	0.138 ± 0.007	0.141 ± 0.006	0.143 ± 0.006

[a]Values are Mean ± SEM (n=3). Values are not significantly different; $p > 0.05$ using one-way analysis of variance.

is as follows: *Og*HE > *Os*HE; *Og*CE > *Os*CE; *Og*EAE < *Os*EAE; and *Og*ME < *Os*ME. There were positive correlations between the reducing potential and the phenolic contents of all the extracts except *Os*ME.

Acute toxicity effects of crude methanol extracts of *O. gratissimum* and *O. suave* leaves

Comparative acute toxicity test showed no mortality in rats administered ME*Og* and ME*Os* at doses of 2 and 5 g/kg (Table 5). The two extracts did not produced significant changes in treated animals' behavioral pattern, such as locomotor activity, writhing response, fighting, convulsion, tremor, exophthalmos, ptosis, piloerection, tail elevation, traction, motor incoordination, catalepsy, pain response, pinna reflex, skin color, diarrhea, or impairment in food intake, or water consumption immediately after administration or during the 14 days observation period. There was no significant difference in body weights during the fourteen days post administration observations. However, there was a slight loss in weight of the animals administered with ME*Og* (5 g/kg) from days 10 to 14.

DISCUSSION

This study was conducted on the need to improve quality of local herbal medicines by systematic scientific studies and documentation of two *Ocimum* spp. bearing similar local names, in order to avoid the pitfall of misidentification. The physicochemical evaluation of herbal drugs is an important parameter in detecting adulteration or improper handling of drugs. These parameters are useful for identification of allied species as well as adulterants.

The low moisture content of *O. suave* (12.5%) as compared to *O. gratissimum* (18.3%) may discourage bacterial growth when the dried leaves are stored. Dried herbal materials with moisture content higher than 14% have been shown to support bacterial growth (African Pharmacopeia, 1986). The total ash is particularly important in the evaluation of purity of drugs, that is, the presence or absence of foreign inorganic matter such as metallic salts and/or silica (Musa et al., 2006). A lower acid-insoluble ash shows that a very small amount of the inorganic compounds is insoluble in acid. The implication is that adulteration of the raw materials with compounds such as silica or other metallic salts is very less likely to

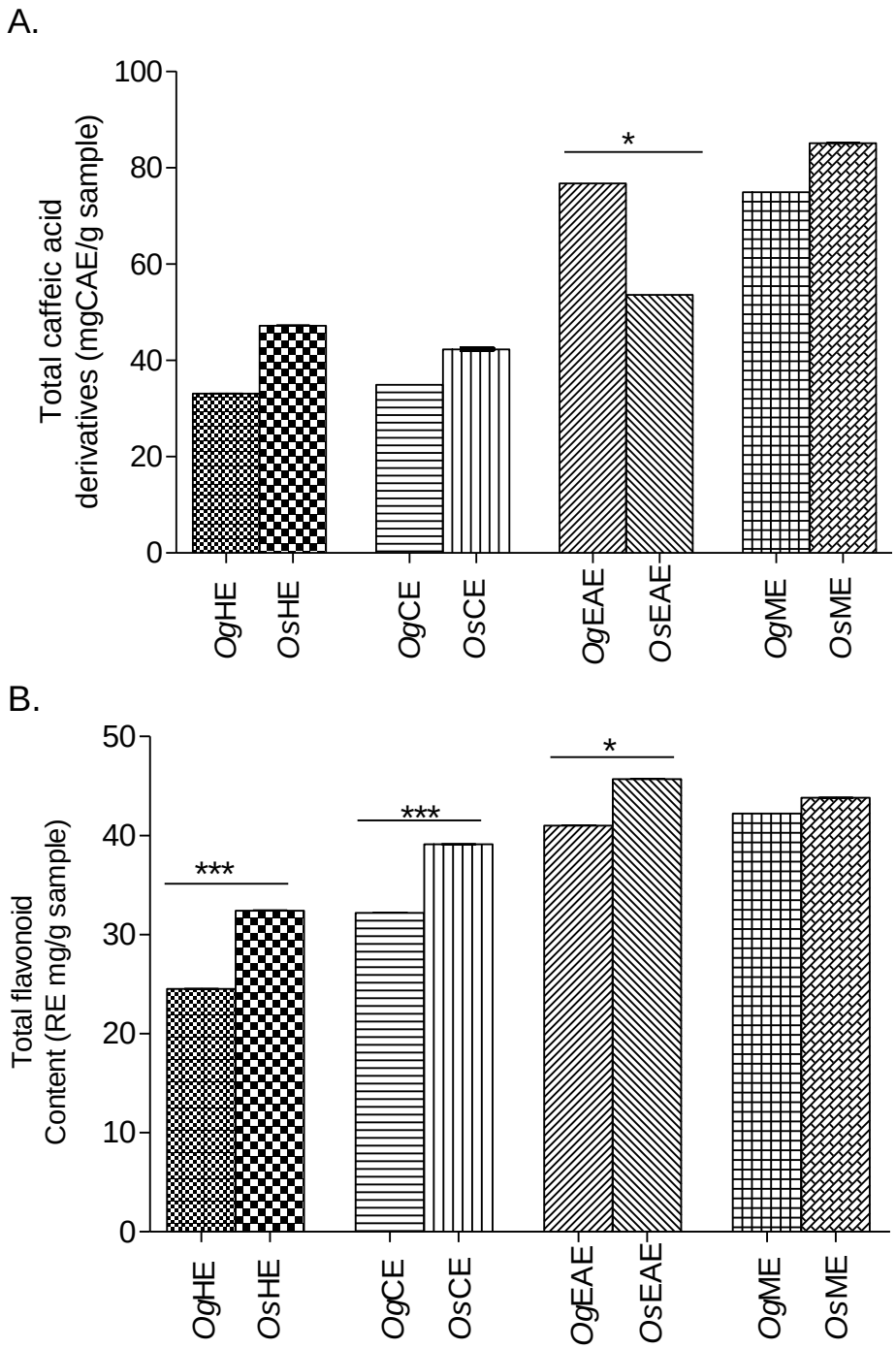

Figure 1. Phenolic contents of successive extracts of *O. gratissimum* and *O. suave* leaves. (A) Total caffeic acid derivatives content, and (B) Total Flavonoid content. *$p < 0.05$ using student t-test.

happen. The acid insoluble ash value may also mean improved oral absorption of the extracts of the leaves in the gastrointestinal tract.

The sequential extraction showed that *O. suave* phytochemicals showed relatively higher solubility than *O. gratissimum* in solvent of increasing polarity. The fact remains that plant extracts usually occur as a combination of various type of bioactive compounds or phytochemicals, which makes their separation a big challenge. Polarity based solvent extraction from medicinal plants is known to selectively extract components that are soluble in particular solvents.

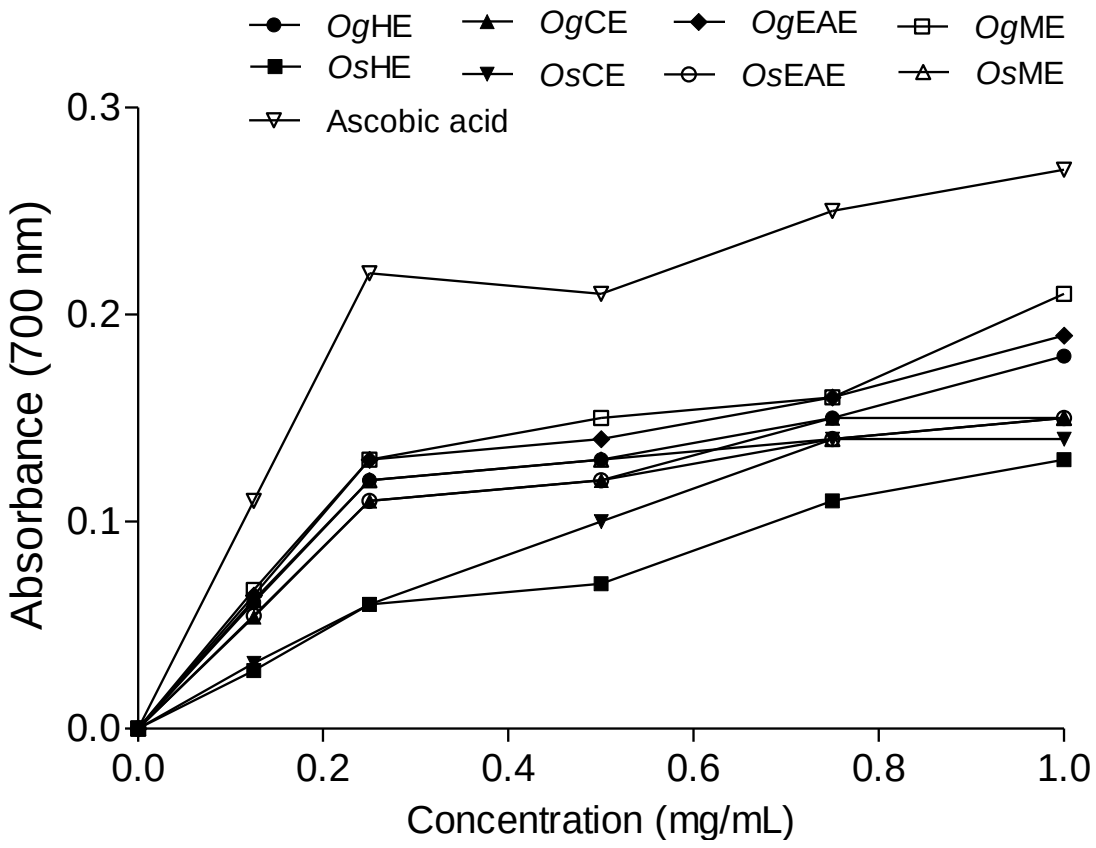

Figure 2. Reducing power assay of successive extracts of *O. gratissimum* and *O. suave* leaves.

Components vary from solvent extracts to whole extract and may be responsible for different biological effects.

Phenolic acids such as caffeic acid, gallic acid, ferulic and *p*-coumaric acid are distributed in nature in their free and bound forms. Caffeic acid and rutin were chosen, because these compounds were previously reported in the *Ocimum* spp. (Ola et al., 2009). It was observed that ethylacetate and methanol extracts have comparable amounts of the caffeic acids than hexane and chloroform. Caffeic acid (Phenolic acid) is found in many fruits, vegetables and medicinal plants existing as an esterified form of quinic acid. Caffeic acid exerts many pharmacological properties including antioxidant, anti-inflammatory and cytotoxic properties (Kelebek et al., 2015). The four major caffeic acid derivatives are caftaric acid, caffeic acid, chicoric acid and rosmarinic acid (Lee, 2010). Grayer et al. (2000) has shown the presence of rosmarinic acid in *O. gratissimum*. High performance liquid chromatographic characterization can help to differentiate and quantify the amount of the different caffeic acid derivatives in the two plants; this may serve as phytochemical markers or index for quality control and identification of the *Ocimum* spp. In a report by Hakim et al. (2004), comparative studies of phenolic content of *O. gratissimum* with seven other *Ocimum* spp., showed *O. gratissimum* to possess higher total phenolic content than the other species. It is noted that *O. suave* was absent among the *Ocimum* spp. in this report.

O. suave sequential extracts showed a more significantly positive correlation than their corresponding *O. gratissimum* extracts except in *Os*ME that was not significant. Though *Os*ME showed a higher total caffeic acid derivative contents and a comparable IC_{50} value to ascorbic acid, it however did not have a significant positive correlation; this implied that other compounds might be responsible for the reducing power other than the phenolic acids. Flavonoids and tannins are polyphenolic compounds that might be present in such extracts other than the phenolic acids. Reducing power of the extracts might be due to their hydrogen-donating ability, with a capacity to react with free radicals to stabilize and block chain reactions (Ferreira et al., 2007). Hakkim et al. (2004) demonstrated the reductive capacity of eight *Ocimum* spp., wherein *O. gratissimum* was the most effective at reducing iron (III).

Acute toxicity test provides preliminary information on the toxic nature of a material for which no other toxicological information is available. The acute toxicity study was aimed at identifying and characterizing adverse effects that can be produced in animals by a

single oral exposure to high dose of *O. gratissimum* or *O. suave* extracts. Acute toxicity results of methanol extracts of *O. gratissimum* and *O. suave* showed that none of the extracts caused death immediately after single oral administration or within fourteen days. This implied that the LD_{50} of both extracts is greater than 5 g/kg body weights of rats. There are many reasons why acute toxicity is performed, namely to serve as the basis for classification and labeling, to provide initial information on the mode of toxic action of a substance, to help arrive at a dose of a new compound and to help in dose determination in animal studies (Ukwuani et al., 2012; Balogun et al., 2014). Importantly, it also serves to validate the justification for safety and historical use of some medicinal plants. The high safety margin of the extracts of *O. gratissimum* and *O. suave* may partly explain the historical use of their leaves as infusions in the traditional management of several ailments.

Conclusion

Comparative physicochemical, phytochemical and acute toxicity analysis of two *Ocimum* spp. showed no major significant differences in the two plants. The information provided in this study will serve as an important contribution to knowledge as part of information required to prepare monographs, to promote the conservation of the two species, and in establishing quality parameters for standardization.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors acknowledge the assistance of Ronald and Pascal for the collection of the plant materials. The work was funded by the MESAU-MEPI undergraduate mentored research program, supported by Award Number 1R24TW008886 (MESAU-MEPI Programmatic Award) from the Fogarty International Center. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Fogarty International Center or the National Institutes of Health."

REFERENCES

African Pharmacopeia (1986). General Methods for Analysis 1st ed. Vol. (2) (OAU/STRC) Lagos. P 123.

Benedec D, Vlase L, Hanganu D, Oniga I (2012). Antioxidant potential and polyphenolic content of Romanian *Ocimum basilicum*. Dig. J. Nanomater. Biostruct. 7(3):1263-1270.

Bohm BA, Koupai-Abyazan MR (1994). Flavonoids and condensed tannins from leaves of *Hawaiian vaccinium vaticulatum* and *V. calycinium.* Pac. Sci. 48:458-463.

Cowan MM (1999). Plant Products as Antimicrobial Agents. Clin. Microbiol. Rev. 12(4):564-582.

Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol. 4(7):685-688.

Evans WC (2002*).* Trease and Evans Pharmacognosy. 15th ed.Habid, W.B Saunders, Edinburgh.

Ferreira IC, Baptista P, Vilas-Boas M, Lillian Barros (2007). Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. Food Chem. 100:1511-1516.

Grayer RJ, Kite GC, Abou-Zaid M, Archer LJ (2000). The Application of Atmospheric Pressure Chemical Ionization Liquid Chromatography-Mass Spectrometry in the Chemotaxonomic study of Flavonoids: Characterization of Flavonoids from *Ocimum gratissimum* var gratissimum. Phytochem. Anal. 11:257-267.

Grayer RJ, Kite GC, Veitch NC, Exkert MR, Marin PD, Senanayake P, Paton AJ (2002). Leaf flavonoid glycosides as chemosystematic characters in *Ocimum*. Biochem. Syst. Ecol. 30:327-342.

Hakkim FL, Arivazhagan G, Boopathy R (2008). Antioxidant property of selected *Ocimum* species and their secondary metabolite content. J. Med. Plants Res. 2(9):250-257.

Harborne JB (1998). Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. Chapman and Hall London 135.

Kelebek H, Selli S, Gubbuk H, Gunes E (2015). Comparative evaluation of volatiles, phenolics, sugars, organic acids and antioxidant properties of Sel-42 and Tainung papaya varieties. Food Chem. 173:912-919.

Kokate CK (2000). Practical Pharmacognocy 4th edition. Nirali Prakashan: Pune. P 149.

Kunle OF, Egharevba HO, Ahmadu PO (2012). Standardization of herbal medicines – A review. Int. J. Biodivers. Conserv. 4(3):101-112.

Lee J (2010). Caffeic acid derivatives in dried Lamiaceae and Echinacea purpurea products. J. Funct. Foods 2:158-162.

Mahomoodally MF (2013). Traditional Medicines in Africa: An Appraisal of Ten Potent African Medicinal Plants. Evid. Based Complement. Altern. Med. 2013:617459.

Musa KY, Katsayal AU, Ahmed A, Mohammed Z, Danmalam UH (2006). Pharmacognostic investigation of the leaves of Gisekia pharnacioides. Afr. J. Biotechnol. 5(10):956-957.

National Institute of Health (NIH) (1996). Guide for the Care and use of laboratory animals.

Obadoni BO, Ochuko PO (2001). Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. Glob. J. Pure Appl. Sci. 8:203-208.

Odebiyi A, Sofowora JA (1982). Phytochemical Screening of Nigerian Medicinal plants Part II. Lloydia 41(3):234-46.

Organization for Economic Co-operation and Development (OECD) (2001). Guideline for Testing of Chemicals: Acute Oral Toxicity – Acute Toxic Class Method, Organization for Economic Co-operation and Development 2001: No 423. http://www.oecd.org/chemicalsafety/risk-assessment/1948370.pdf

Ola SS, Catia G, Marzia I, Fracesco VF, Akindahunsi AA, Nadia M (2009). HPLC/DAD/MS characterisation and analysis of flavonoids and cynnamoil derivatives in four Nigerian green-leafy vegetables. Food Chem. 115:1568-1574.

Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S (2009). Agroforestree Database: A tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya. http://www.feedipedia.org/node/1650

Oyaizu M (1986). Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44:307-315.

Paton A (1992). A synopsis of *Ocimum L*. (Labiatae) in Africa. Kew Bull. 47(3):403-435.

Sultana B, Farooq A, Muhammad A (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules 14:2167-2180.

Tabuti JRS, Kukunda CB, Waako PJ (2010). Medicinal plants used by

traditional medicine practitioners in the treatment of tuberculosis and related ailments in Uganda. J. Ethnopharmacol. 127:130-136.
Ukwuani AN, Abubakar MG, Hassan SW, Agaie BM (2012). Toxicological studies of hydromethanolic leaves extract of *Grewia crenata*. Int. J. Pharm. Sci. Drug Res. 4:245-249.
Van-Buren JP, Robinson WC (1969). Formation of complexes between protein and Tannin acid. J. Agric. Food Chem. 1:77.
Vani SR, Cheng SF, Chuah CH (2009) Comparative Study of Volatile Compounds from Genus *Ocimum*. Am. J. Appl. Sci. 6(3):523-528.
World Health Organization (WHO) (2003). International Pharmacopoeia (3rd edn, Vol. 5). WHO Press: Geneva.