Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 3648-3653
Received: 25-03-2019
Accepted: 27-04-2019

Gabriel Ogunma Benjamin
Phytomedicine Unit, Department of Botany, University of Benin, Nigeria

Adedokun Oluwasegun Adekanmi
Department of Pharmacognosy, Igbinedion University, Nigeria

Ume Ogochukwu
Department o0f Pharmaceutical Chemistry, Igbinedion University, Nigeria

Adulkadir Nasiru
Department of Science Laboratory Technology, Auchi Polytechnic Auchi, Edo State, Benin

Correspondence
Gabriel Ogunma Benjamin
Phytomedicine Unit, Department of Botany, University of Benin, Nigeria

Antiulcer and toxicological evaluation of bi-herbal mixture of *Emilia coccinea* and *Occimum gratissimum* on Wistar rats

Gabriel Ogunma Benjamin, Adedokun Oluwasegun Adekanmi, Ume Ogochukwu and Adulkadir Nasiru

Abstract
Emilia coccinea and *Ocimum gratisimum* are employed in folk medicine in the management of gastro-intestinal tract disorders such as peptic ulcers. The bi-herbal methanol extracts of *E. coccinea and O. gratisimum* was evaluated for its anti-ulcerogenic effect and toxicity profile in rats. Graded doses of bi-herbal extracts (25, 50 and 100 mg/kg) were orally administered to the treated groups. Cimetidine at doses 100 mg/kg were used as positive controls, respectively. Stomach was opened along the greater curvature then ulceration index was determined examining the inner lining of stomach. Oral administration of the extract did not produce toxic effects to the haematological parameters and selected tissues in rats. The bi-herbal extracts (25, 50 and 100 mg/kg) showed a significant reduction in ulcer index and percentage in HCl/Ethanol-induced ulcer. Bi-herbal extracts (25, 50 and 100 mg/kg) showed antiulcer activity against ethanol-induced gastric lesions dose dependently. The extract efficiently inhibited thedevelopment of gastric lesions induced by ethanol. The results indicated that bi-herbal extract exerted an anti-ulcergenic effect associated with an increase in gastric mucosal defensive factors.

Keywords: Bi-herbal, cimetidine, *Emilia coccinea*, *Occimum gratissimum*, toxicology, ulcer

Introduction
Several plant materials with medicinal benefits are being employed in the management of diverse disease (Ncube et al., 2008) [1]. Phytochemicals are the constituentliable for most plants efficacy. *Emilia coccinea* known as scarlet tassel flower, belong to the family *Compositae*. In herbal medicine, the leaves and the root are employed in the management of several diseases (Odugbemi and Akinsulire, 2008) [2]. Ethnomedicinal report exhibited *E. coccinea* leaves being used to manage sores, treat vertigo (Burkill, 1985; Oliver, 1960) [3, 4]. More so, it is proven effective in treating ringworm, gonorrhea, ulcer, measles and convulsion in children (Odugbemi and Akinsulire, 2008; Edeoga, 2005) [2, 5]. Some of the bioactivities in this plant have been reportedsuch as, anti-diarrhoeal, anti-microbial and anti-fungal activity (Ogbebor and Adekunle, 2005; Teke, 2007; Okiei, 2009) [6 - 8]. *Ocimum gratissimum* are differently known in their local names. It is called scent leaf in English, Nchuanwu by the Igbos, Effrin-nla by the Yorubas and Daidoya by the Hausas (Odugbemi and Akinsulire, 2008; Saliu *et al.*, 2011) [2, 9]. In the southern part of Nigeria, crude aqueous extract of *O. gratissimum* is commonly used in the treatment of high fever, epilepsy, diarrhea (Effraim *et al.*, 2003) [10]. In Kenya, it is an important herbal medicine; the leaves are rubbed between the palms and sniffed as a treatment for blocked nostrils (Kokwaro, 1993) [11]. They are also used for abdominal pains, sore eyes, and ear infections, for coughs, fever, convulsions, tooth gargle, regulation of menstruation and as a cure for prolapsed of the rectum (Lexa, 2007) [12]. In India, the whole plant has been used for the treatment of sunstroke, headache, and influenza, as a diaphoretic, antipyretic and for its anti-inflammatory activity (Tania, 2006) [13].
Traditionally, peptic ulcers have been described as an imbalance between the luminal acid peptic attacks versus the mucosal defenses. Acid and pepsin components form the aggressive factors, and the mucus layer of mucin-bicarbonate secretion, phospholipid layer, tight junctions' cell proliferations, prostaglandins, and the urogastrone epidermal healing factors form the defensive factors (Sanyal, 1983) [14]. Enhancement of gastric mucus has been proposed to explain antiulcer activity (Alarcon de la Lastra *et al.*, `992) [15].

Materials and Methods
Plant collections
The two plants were collected freshly from Plant Biology and Biotechnology botanical garden, University of Benin, Edo State. It was identified and authenticated by Dr. Akini Bosun of the

Department of Plant Biology and Biotechnology, University of Benin. Voucher specimens were deposited in the herbarium unit of Department of Botany, University of Benin, Nigeria.

Plant Preparation

The freshly collected leaves from the two plants were rinsed and air dried until properly dried, the dried samples were pulverized using British grinder. Equal weights of the two powered samples were taken and dissolved into methanol for 72hours using 1:1. The filtrate was concentrated and stored for further use.

Experimental Animals

The study was carried out using male albino rats, weighing 180 – 200 g. The rats were obtained from animal house of Biochemistry Department, University of Benin University, Benin City. The animals were housed under 12 h light-dark cycle and received pelleted food and water *ad libitum*. They were allowed to acclimatize to laboratory conditions for 2 weeks. The animals were handled according to standard protocols for the use of laboratory animals.

Ethical clearance

Animal experiments were performed in accordance with the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes, approved by the local Animal Care (ethical clearance from experimental animal ethical committee of College of Pharmacy, Igbinedion University, Nigeria) / Ethical Commission, complying with recently published principles and standards for reporting animal experiments.

***In vivo* antiulcer bioassays**

Hydrochloric acid/Ethanol-induced ulcer

Albino rats were divided into five (5) groups as reported by Gohar and Zaki, (2014) [16] and fasted for 18 hours prior to oral dosing with the distilled water (0.5 ml/kg), Cimetidine (100 mg/kg). Bi-herbal methanol extracts (25, 50 and 100 mg/kg). One hour following treatments, the whole animals were exposed to 0.5 ml of a 0.3M HCl/60% Ethanol solution orally. Animals were euthanized by cervical dislocation 1 h after administration of HCl/Ethanol solution; the stomachs were isolated and exposed along the greater curvature. Gastric contents and blood clots were removed then the stomachs were interpreted and rinsed in formaldehyde for histological study. The gastric mucosa walls were observed for lesions using binocular stereomicroscope (Nikon SMZ-10).

1 Ulcer index was estimatedusing the method of (Meyer *et al.*, 2004) [17]. The number and severity of lesions were evaluated. The following scores were used: light (I), presence of edema, hyperemia and single petechiae; moderate (II), presence of sub-mucosal hemorrhagic lesions with small erosions; severe (III), presence of hemorrhagic lesions with severe erosions.

2 Ulcer Index (UI) = (nI) + (nII) 2 + (nIII) 3\Number of animals

3 Where: n is the number of lesions.

4 The preventive effect was calculated by the method of Drury and Wallinton, (2013) [18] as follow:

5 Prevention index (%) = UI Control – UI Treated\UI Control × 100

6 UI Control = ulcer index in the negative control group

7 UI Treated = ulcer index in the group receiving HEGG

Ethanol-induced ulcer

The test was implemented by slight modification of method described by Havsteen, (1983) [19]. Animals werefasted for 18 hours prior to oral dosing with the distilled water (0.5 ml/kg), Cimetidine (100 mg/kg). Bi-herbal methanol extracts (25, 50 and 100 mg/kg). 60 minutes after treatments, the whole animals were exposed to 1 ml 75% Ethanol solution orally. Animals were euthanized by cervical dislocation 60 minutes after administration of Ethanol solution; the stomachs were isolated and exposed along the greater curvature.And the procedure of calculation was adopted from the above method

Toxicological evaluation

Hematology Assays

3 ml of blood were collected across the groups at day 14 into a 5 ml EDTA tubes by abdominal aortal using syringes and needles. The haematological analysis was done almost immediately using an automatic haemato-analyser (Sysmex, KX-21, Japan).

Histological study

The organs (Liver, Kidney) were fixed in 10 % (vol/ vol) formaldehyde, cleaned up in xylene and embedded in a paraffin wax (melting point at 56 percent). Tissue sections were prepared according to the method of (Drury and Wallinton, 2013**)** and stained with eosin/ hematoxylin. Photomicrographs were taken at ×400 using a digital camera.

Statistical analysis

Data were presented as Mean ± SEM of the respective replicates. Means of different groups were compared using ANOVA using graph pad prism 6 version computer software packages.

Results

Phytochemical screening

Table 1 shows the present of certain plant chemicals that enhances biological activities and display healing property on disease conditions.

Table 1: Phytochemical constituent of bi-herbal methanol extract

Tests	Methanol bi-herbal extract
Alkaloids	+++
Tannins	++
Saponins	+++
Flavonoids	++
Phenolics	++

Keys: presence of the constituent = +

Anti-ulcer study

Table 2 showed significance increase in the treated groups. The reference drug (100 mg/kg Ranitidine) has an ulcer inhibition of 99 % compared with the 0.0% ulcer inhibition of the negative control group. 100 mg/kg plant extract of the treated group inhibits ulcer more at 100 % likewise 25 and 50 mg/kg compared with the negative control.

Table 2: Effect of bi-herbal methanol crude extracts on ulcer Index and percentage inhibition of ulcer index (IU) % using HCL/ethanol induced ulcer model

Parameters/ Group	Dosages (mg/kg)	Ulcer Index (IU)	Percentage inhibition of Ulcer Index (IU) %
Distilled Water	0.5 ml	41.93±11.35	-
Cimetidine	100	0.42±0.23[b]	99.00
Methanol Extracts	25	9.33±2.18[a]	77.75
Methanol Extracts	50	6.22±4.35[a]	85.17
Methanol Extracts	100	0.00±0.00[c]	100

P- value(superscript [a]= 0.05, **[b]= 0.1,** *[c]=0.01)*Mean±SEM, n = 6

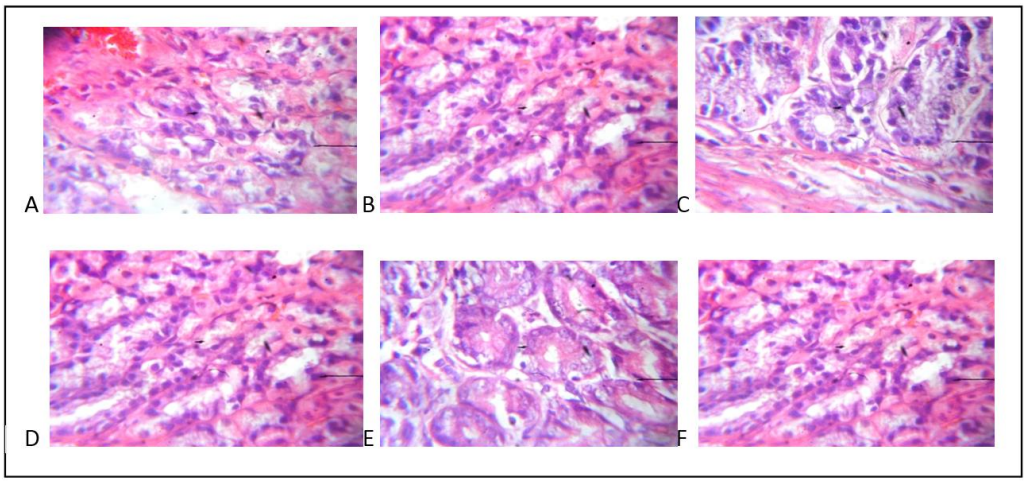

Plate I: (**B).** antiulcer activity on the stomach lining gives clearer pictures of the mucosa membrane in positive control by showing a well-defined and healthy stomach. (**C, D, E, F)** has same observation noted on the normal control and treated groups specifically at 25, 50 and 100 mg/kg compared with the (**A**) negative control which showed a devitalised stomach thereby indicating the presence of ulcer.

Table 3 showed significance increase in the treated groups. The reference drug (20 mg/kg Ranitidine) has an ulcer inhibition of 98.98% compared with the 0.0% ulcer inhibition of the negative control group. 100 mg/kg plant extract of the treated group inhibits ulcer more at 100 % likewise 25 and 50 mg/kg compared with the negative control.

Table 3: Effect of bi-herbal methanol crude extracts on ulcer Index and percentage inhibition of ulcer index (IU) %usingethanol induced ulcer model

Parameters/ Group	Dosages (mg/kg)	Ulcer Index (IU)	Percentage inhibition of Ulcer Index (IU) %
Distilled Water	0.5 ml	32.33±11.35	-
Cimetidine	100	0.33±0.33[c]	98.98
Methanol Extracts	25	11.33±3.18[a]	64.96
Methanol Extracts	50	5.83±5.33[b]	81.96
Methanol Extracts	100	0.00±0.00[c]	100

P- value (superscript [a]= 0.05, **[b]= 0.1,** *[c]=0.01)*Mean±SEM, n=6

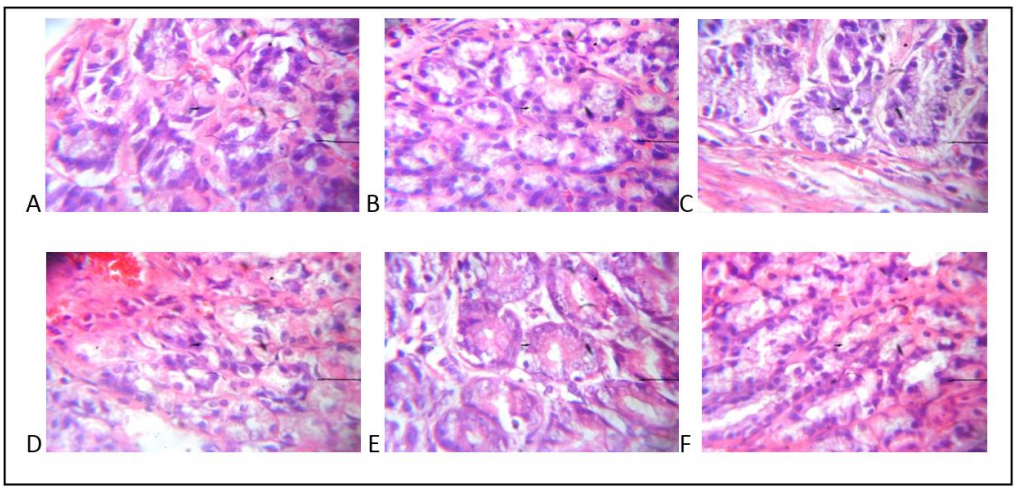

Plate 2: B. antiulcer activity on the stomach lining gives clearer pictures of the mucosa membrane in positive control by showing a well-defined and healthy stomach. **C, D, E, F.** has same observation noted on the normal control and treated groups specifically at 25, 50 and 100 mg/kg compared with the **A**. negative control which showed a devitalised stomach thereby indicating the presence of ulcer.

Toxicological evaluation

From Table 4, no toxic effect was observed on the haematological parameter, showing that its synergic action post no harm.

Table 4: Effect of bi-herbal (methanol extracts on haematological indices across the groups.

Parameters	Normal Control	25 mg/kg Methanol	50 mg/kg Methanol	100 mg/kg Methanol
BC x 10^3 / ul	17.40±1.89	12.77±3.12	14.17±0.67	14.00±4.92
LY x 10^3 / ul	12.60±1.14	6.90±0.47	6.93±0.66	8.43±2.74
MO x 10^3 / ul	2.07±0.33	1.40±0.31	1.70±0.00	2.03±0.70
GR x 10^3 / ul	2.80±0.53	4.57±2.52	5.53±1.19	4.23±1.13
LY%	72.13±1.85	58.10±8.91	49.53±6.58	57.23±3.19
MO%	11.73±0.73	11.07±0.58	11.80±0.55	13.47±0.44
GR%	16.00±1.56	30.83±9.41	38.67±7.05	29.30±3.21
RBC x 10^6 / ul	8.37±0.39	10.06±0.32	10.31±0.46	8.65±0.34
Hgb.g/d l	13.63±0.52	17.50±0.30	18.10±1.66	14.63±0.64
HCT%	42.80±1.30	50.97±1.25	52.70±5.35	43.30±1.37
MCV.f l	51.37±3.81	50.80±2.66	50.90±3.35	50.10±0.95
MCH.pg	16.23±0.52	17.37±0.89	17.43±0.98	16.87±0.33
MCHC.g/d l	31.87±1.62	34.30±0.40	34.37±0.33	33.70±0.42
RDW%	17.10±1.27	16.27±0.68	17.00±0.60	15.80±0.10
PLT x 10^3 /ul	462.0±33.25	468.3±18.77	476.7±49.71	502.3±102.1
PCT.%	0.28±0.02	0.28±0.01	0.28±0.03	0.36±0.07
MPV.f l	6.03±0.03	5.97±0.13	5.93±0.09	5.87±0.09
PDW. %	6.8j7±0.09	7.23±0.35	7.37±0.49	6.67±0.15

P-valuep<0.05, Mean±SEM, n=6

From plate 3; the histological study of the hepatocyte exhibited no tissue damage to the cells, instead the bi-herbal extracts synergise to enhance the effectiveness of the liver cells and blood flow when compared with the control group.

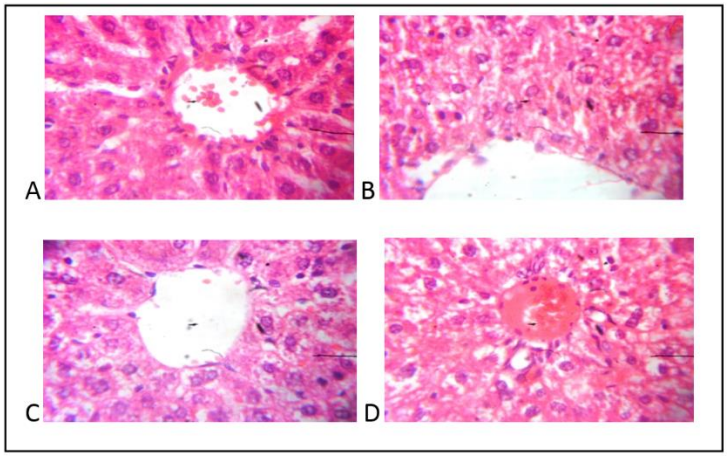

Plate 3: From the histopathological study, the picture of the hepatocytes indicated no diseases condition associated to the control (A). The same observation was noted from the graded doses across the treated groups (B, C, D).

From plate 3; the histological study of the hepatocyte exhibited no tissue damage to the cells, instead the bi-herabal extracts synergise to enhance the effectiveness of the liver cells and blood flow when compared with the control group.

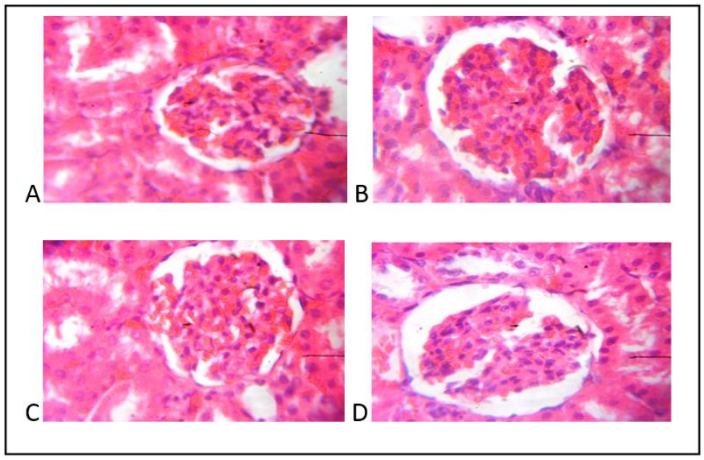

Plate 4: From the histopathological study, the picture of the kidney is an indication of no diseases condition associated to the control (A). The same observation goes to the normal control and graded doses of the treated groups (B, C, D).

Discussion

Phytochemicals an active medicinal plants ingredient have been implicated in the management of diseases. Table 1 show the present of certain phytochemicals in the bi-herbal methanol leaves extracts. The following phytochemicals are includes; alkaloids, flavonoids, tannins, phenol and saponins were present (Harbon and Baxter, 1999) [20, 21].The antiulcer activity revealed that the ethanol extract of the bi-herbal leaves extracts showed significant reduction in the ulcer index, thus showing possibly antisecretory mechanism of the extracts. Ulcer index factor was utilized for the evaluation of antiulcer property. Since ulcer development is directly interrelated to certain factors includes gastric volume reduction and reduced liberation of total acid level in an increase pH compared with control group ($p < 0.05$) in HCL/ethanol and ethanol induced gastric ulcer model (Table 2). Moreover, the disturbance of defensive factors like mucus secretion, bicarbonate, and mucosal blood flow has been reported to cause ulcers (Goel and Bhattacharya, 1986) [22]. Oral administration of HCl-ethanol mixture at a dose of 2 ml per animal was sufficient to induce ulcer. The bi-herbal extracts at a dose of 25, 50, 100 mg/kg p.o. showed significant reduction in ulcer leision compared with the vehicle control group (Table 2 and 3). The percentage reduction of ulcer lesion was compared with standard drug cimetidine. The methanol extract of the bi-herbal leaves of *E. coccinea and O. gratisimum* exhibited significant reduction in ulcer score on Hcl/ethanol and ethanol induced stress in rats. The ulcer inhibition was found to be 77.75, 85.17 and 100% as well as264.96, 81.96 and 100 % respectively

From Table 2 and 3, hydrochloric acid -ethanol and ethanol induced gastric ulcer damage in rats possibly through leukotrienes production and also involvement of 5-lipooxygenase in the formation of ulcer lesion. Prostaglandins also play a role in ethanol–induced ulcer (Goel and Bhattacharya, 1986) [22]. Thus the protective effect of the methanol extract of the bi-herbal leaves of *E. coccinea and O. gratisimum* against the gastric damage might be due to protection against 5-lipooxygenase or leukotriene pathway (Goel and Bhattacharya, 1991) [22]. The cytoprotective action possibly stimulates the prostaglandin synthesis, which in turn is involved in cytoprotection of the gastric mucosa. It is a significant finding that methanol extract of the bi-herbal leaves of *E. coccinea and O. gratisimum* afforded a distinctively high protection against Hcl/ethanol and ethanol induced ulcer in rats. Preliminary phytochemical investigations showed the presence of alkaloids, flavonoids, tannins, phenol and saponins. Hence, the synergic antiulcer activity of bi-herbal *E. coccinea and O. gratisimum* in this experimental model may be due to the presents of phenol or flavonoids. The results demonstrated that methanol extract of the bi-herbal leaves of *E. coccinea and O. gratisimum* produced anti-ulcerogenic effects possessing anti-secretory, cytoprotective, and proton pump inhibition mechanism. This interesting observation indicates that extract of bi-herbal leaves of *E. coccinea and O. gratisimum* can be a potential source for the treatment of ulcer. However, a detailed study such as isolation of active molecules and characterization is required to confirm the phytochemicals responsible for the activity.

Similarly, there was no significant difference in the hematological indices and tissue histology of the rats in graded doses of bi-herbal extracts and control groups ($P > 0.05$). The implications of these results are that methanol extract of bi-herbal leaves of *E. coccinea and O. gratisimum* at the dosage levels engaged in this investigation exhibited no noticeable toxicity in the animals and as a result could be observed as safe doses (approximately 25, 50 and 100 mg/kg body weight) [23], the subsistence may be accountable for the less choice of the leaves as medicinal function of *E. coccinea and O. gratisimum*. Results obtained from the present study proposed that the leaves are safe in terms of toxicity. However, further studies are desirable to suitably assess the toxicity of thebi-herbal products from *E. coccinea and O. gratisimum* with long-term study procedure.

Conclusion

Since time of antiquity, herbal medicine has been employed in the management of different diseases due to the some active substance (phytochemicals). This study thereby evaluates the bi-herbal extract of *O. gratissimum* and *E. coccinea* as an anti-ulcer against ethanol-induced gastric ulcer and hydrochloric acid - ethanol induced ulcer in rats with biosafety profile.

Acknowldegement

We the authors of this work sincerely appreciate all member of staff of various laboratories involve in this research. Most especially research laboratories of Departemnt of Pharmacology and Pharmacognosy, University of Benin and Igbinedion University respectively.

Conflict of Interest

The authors declare that there is no financial or personal relationship that can be understood as representing a potential conflict of interest

References

1. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology. 2008; 7(12):1797-1806.
2. Odugbemi T, Akinsulire O. Medicinal plants by species names. In: Odugbemi .T. (Ed). Outlines and Pictures of Medicinal Plants from Nigeria, University of Lagos Press, Nigeria, 2006, 73-116.
3. Burkill HM. The Useful Plants of West Tropical Africa. The Whitefriars Press Limited, Great Britain, 1985, 960.
4. Oliver B. Medicinal Plants in Nigeria. Nigeria College of Arts, Science and Technology, 1960, 138.
5. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr. J. of Biotech. 2005; 4(7):685-688.
6. Ogbebor N, Adekunle AT. Ingibition of conidial germination and mycelia growth of *Corynesporacaiicola* (Berk and Curt) of rubber (*Heveabrasiliensismuell*. Arg.) Using extracts of some plants. Afr. J of Biotech. 2005; 4:996-1000.
7. Teke GN, Kulate JR, Ngouateu OB, Gatsing D. Antidarrhoeal and antimicrobial activities of *Emilia coccinea (Sims)* G. Don extracts. J of Ethnopharm. 2007; 112(2):278-283.
8. Okiei W, Ogunlesi M, Ademoye MA. An assessment of antimicrobial properties of extracts of various polarities *from Chasmantheradependens, Emilia coccinea and Cuscutaaustralis*, herbal medications for eye diseases. Journal of Applied Sciences. 2009; 9(22):4076-4080.
9. Saliu BK, Usman LA, Sani A, Muhammad NO, Akolade JO. Chemical composition and antibacterial (oral

isolates) activity of leaf essential oil of *Ocimum gratissimum* L. grown in north central Nigeria. International Journal of Current Research. 2011; 33(3):022-028.
10. Effraim KD, Jacks TW, Sodipo OA. Histopathological studies on the toxicity of *Ocimum gratissimum* leaves extract on some organs of rabbit. African Journal of biomedical Research. 2003; 6:21-5.
11. Kokwaro JO. Medicinal plants of East Africa, East Africa Literature Bureau, Kampala, Nairobi, and Dar-es-Salaam, 1993, 106-115.
12. Lexa GM, Josphat CM, Francis NW, Titus KM. Chemical composition and antimicrobial activity of the Essential oil of *Ocimum gratissimum* L. growing in Eastern Kenya. African Journal of Biotechnology. 2007; 6(6):760-765.
13. Tania U, Nakamura RR, Mendonca F. Antileishmanial activity of Eugenol-rich essential oil from *Ocimum gratissimum.* Parasitol International. 2006; 55:99-105.
14. Sanyal AK, Mitra PK, Goel RK. A modified methods to estimate dissolved mucosubstances in gastric juice. Indian J Exp Biol. 1983; 21:78-80.
15. Alarcon de la Lastra C, Martin MJ, Marhendra E. Gastric antiulcer activity of silymarin, a lipooxygenase inhibitor in rats. J Pharm Pharmacol. 1992; 44:929-931.
16. Gohar AA, Zaki AA. Assessment of some Herbal Drugs for Prophylaxis of Peptic Ulcer. Iran. J Pharm. Res. 2014; 13(3):6.
17. Meyer Albiero AL, AboinSertié JA, Bacchi EM. Antiulcer activity of *Sapindussaponaria* L. in the rat. J Ethnopharmacol. 2002; 82:41-44.
18. Drury RAB, Wallinton EA. Carletons Histological Technique, 16th Edn, Oxford University Press, London, 2013, 124-136.
19. Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. Biochempharmacol. 1983; 32:1141-1148.
20. Harbone HB, Baxter H. The handbook of natural flavonoids, vols 1 and 2. Chichester, UK, John Wiley and sons, 1999.
21. Goel RK, Bhattacharya SK. Gastroduodenal mucosal defense and mucosal protective agents. Indian J Exp. Biol 1991; 29:701-714.
22. Adegoke EA, Alo B. Abereamines: Water soluble seed alkaloids from *Hunteriaumbellata*. Phytochemistry, 1986; 25(6):1461-1468.