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Anti-ulcer and gastric anti-secretory activities of seed extract of *Buchholzia coriacea* in Wistar Albino rats

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Ethanol extract of *Buchholzia coriacea* seed was evaluated for anti-ulcer as well as anti-secretory activity in rats because of its use in Nigerian folk medicine as an anti-ulcer agent. Standard pharmacological methods were used to carry out phytochemical analysis of the plant. Quantitative phytochemical analysis of the ethanol extract of *B. coriacea* revealed the presence of alkaloids (101.88 ± 0.11 mg/100 g), flavonoids (46.88 ± 2.21 mg/100 g), tannins (0.16 ± 0.02 mg/100 g), oxalate (0.15 ± 0.01 mg/100 g) and terpenes (23.0 ± 0.30 µg/100 g). The extract at 200 and 400 mg/kg body weight, significantly (P<0.05) and dose-dependently suppressed the ulcerogenic effect induced by indomethacin in rat gastric mucosa relative to the controls. Similarly, the extract significantly (P<0.05) decreased histamine-mediated gastric acid secretion and also blocked histamine-induced contractile responses in isolated guinea-pig ileum in a similar fashion as the standard anti-histamine drug, chlorpheniramine. The extract had comparable ulcer protective potency with cimetidine, which is a standard drug used in the management of ulcer. The mechanism of the extract's efficacy to protect the animals against indomethacin-induced ulcer may be diverse in nature (due to the presence of a number of bioactive constituents) but suppression of mediator effect of histamine is likely to play a predominant role in the observed activity.

Key words: *Buchholzia coriacea*, anti-ulcer, cimetidine, anti-secretory and indomethacin.

INTRODUCTION

Gastric ulcer is an inflammed break in the lining of the stomach caused by increased acid production or damage to the mucus lining of the stomach (Goel et al., 1985). In most conditions, the event of gastric ulcer involves an imbalance between aggressive factors (gastric acid, pepsin, and *Helicobacter pylori*) and protective factors (mucin, prostaglandin, bicarbonate, nitric oxide, and growth factors) (Hoogenwerf and Passrichas, 2001). The conventional anti-ulcer drugs such as proton pump inhibitors, histamine receptor antagonists, prostaglandin analogues and drugs affecting mucosal barrier are currently in use (Dharmani and Palit, 2006). While the use of these drugs may be effective, they are usually expensive and sometimes associated with relapse and adverse effects (Dharmani and Palit, 2006). This has led to renewed interest in the search for new anti-ulcer drugs from natural sources. Many plants have been reported to possess anti-ulcer activities. Such plants include

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Hemidesmus indicus (Anoop and Jegadeesanj, 2003), *Asparagus racemosus* (Sairam et al*.,* 2003), *Desmodium gangeticum* (Dharmani et al*.,* 2005), *Combretum racemosum* (Okwuosa et al*.*, 2006), *Corindrum sativum* (Al-Mofleh et al., 2006), *Sida acuta* (Landeswari et al*.*, 2010) and *Cissus quadrangularis* (Enechi et al., 2013a).

Buchholzia coriacea is a perennial shrub which is widely distributed in the tropical regions of West Africa. The description of the plant morphology has been documented (Hutchinson and Dalziel, 1954). In Nigeria, the seed is commonly referred to as "wonderful kola". It is widely used for traditional herbal treatment of inflammation, ulcer, bronchitis, mild fever, headache, tonsillitis and catarrh (Moser et al*.*, 2007). It is also used to treat a number of bacterial infections (Moser et al*.*, 2007). For use, the seeds are cut into bits and soaked in local gin or clean water for some days. The mixture is then filtered and the filtrate is taken orally using measurable crucible, depending on the severity of the illness. The bark is dried and ground to form powder which is snuffed through the nostril for the relief of headache, migraine and nasal congestion.

The bark sap is also applied for chest pain, bronchitis and kidney pains. In Gabon, the leaves are used for the treatment of boils. The fruits are used for the treatment of fever and as antihelminthic (Walker, 1953).

Studies have shown that the seeds have antibacterial activity (Ezekiel and Onyeoziri, 2009) as well as antihelminthic activity (Ajaiyeoba et al., 2001). The anti-inflammatory, analgesic and antipyretic activities (Enechi et al., 2009) as well as the hypolipidemic potentials (Enechi et al*.,* 2013b) of the plant seed have also been reported. The pulverized seed of the plant has been demonstrated to be a good source of antioxidant vitamins (Enechi, 2011). Also, the plant has been shown to exhibit significant ($P<0.05$) free radical scavenging and anti-lipid peroxidation activities (Enechi, 2011). Although *Buchholzia* seed is used as an anti-ulcer agent in Nigerian folk medicine, there is no empirical evidence of the anti-ulcer activity. Thus, the present study was undertaken to evaluate the gastro- protective activity of the ethanol extract of *B. coriacea* against indomethacin-induced gastric ulceration.

MATERIALS AND METHODS

Pant material

Buchholzia coriacea seeds were collected from Nsukka, Enugu State, Nigeria and then identified by Mr. A. Ozioko of Bioresources Devleopment and Conservation Programme Center, Nsukka, Nigeria. Voucher specimens were retained in the Herbarium, Department of Plant Science and Biotechnology, University of Nigeria Nsukka, Nigeria.

Animals

The rats used in this study were obtained from the Animal House of the Faculty of Biological Science, University of Nigeria, Nsukka. Albino rats (Wistar strain) weighing 150 -200 g of either sex were used. The rats were housed in metal cages for at least one week in the animal room of Biochemistry Department, University of Nigeria, Nsukka, prior to testing. They were maintained under standard environmental conditions and 12 h light and dark cycles. The animals were allowed free access to standard pellets and clean water *ad libitum.* The laboratory animals were used in accordance with laboratory practice regulation and the principle of humane laboratory animal care as documented by Zimmermann (1983).

Chemicals

The chemicals used in this work were of analytical grade and included: chloroform (Riedel De Haen (RDH), Germany); ethanol (absolute) (British Drug Houses, Dorset, U.K); sodium chloride, histamine and chlorpheniramine (May and Baker, Dagenham, England); cimetidine (Sigma Chemical Company, St. Louis, U.S.A); and indomethacin (Emzor pharmaceuticals, Nigeria).

Extraction

Fresh seeds of *B. coriacea* were air dried at room temperature for two weeks and milled to a coarse powder with a Crestow milling machine. The pulverized seed was subjected to cold maceration by methods corresponding to those practiced by Nigerian traditional doctors (Safowora, 1993). One kilogram quantity of the pulverized seeds was macerated in 5 volume (w/v) 70% ethanol for 24 h with two changes of solvent. The filtrate (extract) was concentrated using a rotary evaporator. The residue was stored in a refrigerator at 4°C until used.

Phytochemical analysis

Phytochemical analysis was done using standard methods as described by Harborne (1973) and Onwuka (2004)

Indomethacin induction of ulcer

The effect of *B. coriacea* seed extract on ulcer formation was evaluated as described by Urushidiani et al*.* (1979). Prior to the test, the rats were starved of food for 24 h but they had free access to water. Twenty (20) rats were employed and grouped into four (A - D) of five rats each. Group A received 5ml/kg of b.w of normal saline p.o and served as negative control. Group B received cimetidine (100 mg/kg b.w p.o), Group C received 200 mg/kg b.w. of the extract, while Group D received 400 mg/kg b.w.of the extract (p.o). 30 min later, ulcer was induced in each rat by the oral administration of indomethacin (40 mg/kg) suspended in normal saline (5 ml/kg) to the different groups of animals. After 8 h, the animals were sacrificed and the stomachs quickly dissected out. The stomach of each animal was opened along the line of greater curvature, rinsed under a stream of water and pinned flat on a cork board. The ulcers were viewed with the aid of a magnifying lens (X10) and each given a severity rating (Main and Whittle, 1975) as follows: <1 mm=1, >1 mm<2 mm=2 and >2 mm<3 mm=3. The overall total scores divided by 10 was designated the ulcer index (UI) for that stomach. The percentage ulcer inhibition was calculated according to the method of Suzuki et al. (1976) using the following formula:

Percentage Ulcer inhibition (% UI) = (1- Ut/Uc) × 100

Where, Ut represents the ulcer index of the treated group and Uc represents the ulcer index of the control group.

Determination of the effect of the extract on histamine-induced gastric acid output

The effect of *B. coriacea* seed extract on gastric acid secretion was studied on lumen perfused rat isolated stomachs by modifications of the method of Brunce and Parson (1978) as described by Nwodo et al. (2008). Five albino rats were fasted for 18 h after which they were killed by chloroform inhalation in an air-tight plastic container and the stomachs dissected out. The stomachs were quickly washed with the modified Krebs' solution (deprived of sodium bicarbonate) at room temperature. A stomach strip was placed into series of beaker labeled A-E, each containing 10 ml Krebs solution. The pH of beaker A, containing only stomach strips and Krebs' solution was determined after 5 min using a pH meter. To beaker B, a sub-maximal concentration of histamine (H) (20 µg/ml) was added and the pH of the solution was determined and recorded after 5 min. To beakers C, D and E; 0.2 mg/ml, 0.5 mg/ml and 1.0 mg/ml of the extract (E) were added respectively. After 2 min, histamine (20 µg/ml) was added to each of them and the pH values of the solutions were determined after 5 min. Acid outputs were obtained by calculating the mean pH values for 3 determinations in the respective incubates.

Effect of the extract on histamine-induced contraction of isolated guinea pig ilium

Evaluation of the effect of the extract on isolated guinea pig ileal contraction induced by histamine was carried out according to the method described by Capaso et al. (1988). Freshly isolated segments (2 cm long) of guinea pig ileum were suspended in a 10 ml organ bath containing Tyrode's solution constantly aerated and maintained at 37°C. A period of 30 min was allowed for tissue equilibration with bathing liquid. Responses of the tissue to varying doses of histamine and *B. coriacea* injection were recorded on Kymographic paper attached to a rotating drum. The contact time of each drug was 15 s with a 5 min cycle time.

Statistical analysis

The data obtained in this study were evaluated using the one-way analysis of variance (ANOVA) test between two mean groups, control and test groups, followed by Student's t-test. Significant levels were at $p<0.05$. Values were expressed as means ± standard deviation (SD)

RESULTS

Quantitative phytochemical composition of *Buchholzia coriacea* seed extract

The result of quantitative phytochemical analysis revealed the presence of high concentrations of alkaloids and flavonoids, and moderate concentrations of tannins and oxalate, while terpenes were present in low concentrations.

Effect of the extract on histamine-induced gastric acid output (pH) in rat stomach

The data presented in Table 2 shows that histamine caused an efflux of proton (H) as evidenced by the decrease in pH from 5.135 ± 0.007 to 4.705 ± 0.007. The extract at concentrations of 0.5 and 1.0 mg/ml induced significant ($p<0.05$) increase in pH from 4.705 ± 0.007 to 5.185 ± 0.049 and 5.745 ± 0.035, respectively, pointing to the ability of the extract to antagonize the acid efflux which leads to acid secretion and ulceration.

Effect of the extract on indomethacin-induced ulcer formation

Table 3 shows the effect of the extract on indomethacin-induced gastric ulceration. The ethanol extract of *B. coriacea* seed suppressed the ulceration induced by indomethacin as evidenced by the significant ($p<0.05$) dose-dependent decrease in ulcer index in the animals that were concurrently administered both the extract and indomethacin. Also cimetidine (a standard anti-ulcer drug) inhibited the ulcerogenic effect of indomethacin in a similar fashion to the inhibition by the extract.

Effect of the extract on histamine-induced contraction of isolated guinea-pig ileum

Figure 1a represents the effect of *B. coriacea* seed extract on histamine-induced contraction. When histamine was injected into the organ bath containing the isolated-guinea pig ileum preparation, there was marked contraction of the tissue. On concurrent administration of varying doses of the extract and histamine, the extract caused a reduction in the amplitude of the contractile response and this was dose-dependent. On its own the extract did not contract the tissue when administered via the bath.

Figure 1b represents the effect of a standard histamine antagonist, chlorpheniramine on histamine-induced contraction of isolated guinea- pig ileum. The figure shows that in the presence of the anti-histamine drug, the histamine-induced contractile response was blocked. The figure also shows that chlorpheniramine blocked the histamine-induced contraction of isolated guinea-pig ileum in a fashion similar to the blockade by the extract.

DISCUSSION

Extract of *B. coriacea* is used in folk medicine for the treatment of ulcers, bacterial and fungal infections, as well as malaria and inflammation (Awaad et al., 2008). In the present investigation, the ethanol extract of the plant showed potency as anti-ulcer agent against indomethacin- induced ulceration in rats. The evidence that indomethacin produced ulcers in all rats that received no further treatment (Table 3) is in consonance with the fact that indomethacin, like many anti-inflammatories, induces ulcers. Like cimetidine, the extract inhibited the ulcerogenic effect of indomethacin.

Table 1. Phytochemical composition of *Buchholzia coriacea* seed extract.

Phytochemical	Concentration (mg/100 g)
Alkaloids	101.88 ± 3.61
Flavonoids	46.88 ± 2.21
Tannins	0.16 ± 0.02
Oxalate	0.15 ± 0.01
Terpenes	$0.023 \pm 0.03 \times 10^{-3}$

Each value is the mean of 3 determinations ± S.D.

Table 2. Extract antagonism of histamine-induced gastric acid output by rat stomach strips

Treatment	pH
Krebs' solution + SS	5.135 ± 0.007
Krebs' solution + SS + HA	4.705 ± 0.007
Krebs' solution + SS + HA + Extract (0.2 mg/ml)	4.915 ± 0.092
Krebs' solution + SS + HA + Extract (0.5 mg/ml)	*5.185 ± 0.049
Krebs' solution +SS + HA + Extract (1.0 mg/ml)	*5.745 ± 0.035

SS = Stomach strips; HA = histamine (20 µg/ml). Each value represents mean ± S.D. of 3 determinations. *Significantly different at $p < 0.05$ relative to the control.

Table 3. Effect of the extract on indomethacin - induced ulcer in rat stomach

Group	Treatment	Mean ulcer index	Inhibition (%)
1	Normal saline (5 ml/kg body weight)	0.00 ± 0.00	-
2	Normal saline (5 ml/kg body weight) + extract (200 mg/kg body weight)	0.00 ± 0.00	-
3	Indomethacin (30 mg/kg body weight) + extract (200 mg/kg body weight)	*10.00 ± 0.12	32.20
4	Indomethacin (30 mg/kg body weight) + extract (400 mg/kg body weight)	*7.75 ± 0.50	47.46
5	Indomethacin (30 mg/kg body weight) + cimetidine (100 mg/kg body weight).	*5.90 ± 0.55	60.00
6	Indomethacin (30 mg/kg body weight)	14.75 ± 0.93	-

Each value represents mean ± S.D. (n=5). *Significantly different at $p < 0.05$ relative to the control.

This activity was dose-dependent and reveals that the extract has anti-ulcer effect.

Ulcer index is an established indication of ulceration in experimental animals. When rats were treated with only normal saline and also *B. coriacea* extract in normal saline, the ulcer index was zero revealing that neither the physiological saline nor the extract induced ulcers. The data presented in the Table 3 also show that indomethacin, a standard anti-inflammatory drug, induced a large scale of ulcer index. This is in consanant with well-established fact that the administration of many inflammatories on empty stomach produced ulcers.

There are multiple aetiologic factors in ulcer pathogenesis and the ability of the extract to protect against indomethacin- induced ulceration indicate its ability to inhibit one or more multiple inciting stimuli in ulcerogenesis (Awaad et al., 2008). Phytochemical analysis revealed the presence of bioactive constituents such as alkaloids, flavonoids, tannins and saponins with alkaloids and flavonoids occurring in high concentrations (Table 1). Hydrolysable tannins contain glucose moiety and have been used internally as astringent and as heavy metals antidote (John and Onabanjo, 1990).

Tannins being astringent may precipitate microproteins on the site of ulcer thereby forming an impervious protective pellicle on the lining to resist the attack of proteolytic enzymes (John and Onabanjo, 1990). This could be likened to the effect of drug such as sucralfate which act by providing a cytoprotective defense against acid peptic digestion. This is consistent with a previous report that partly attributed the anti-ulcer activity of crude extract of *Microgramma squamulosa* to the astringent action of tannins (Suffredini et al., 1999). Previous *in vitro* assessment of the antioxidant status of the ethanol extract of *Buchholzia coriacea* showed that the extract produced significant ($p<0.05$) antioxidant effect by

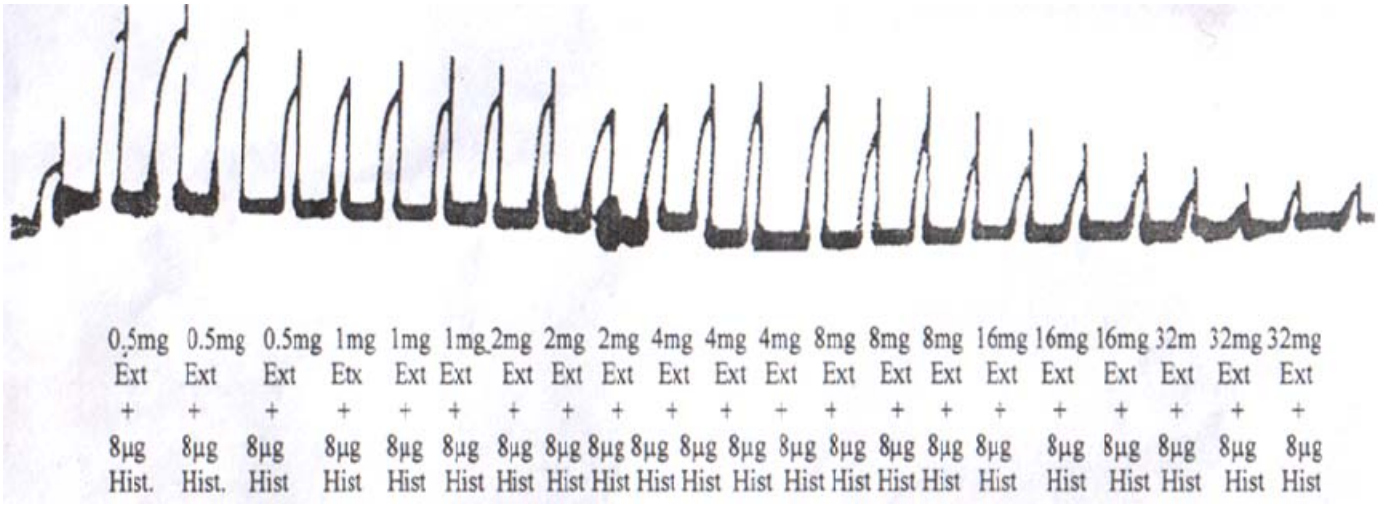

Figure 1a. Dose-dependent (0.5 to 32 mg/ml) blockade of *Buchholzia coriacea* seed extract on histamine-induced (8 µg) contraction of isolated guinea-pig ileum.

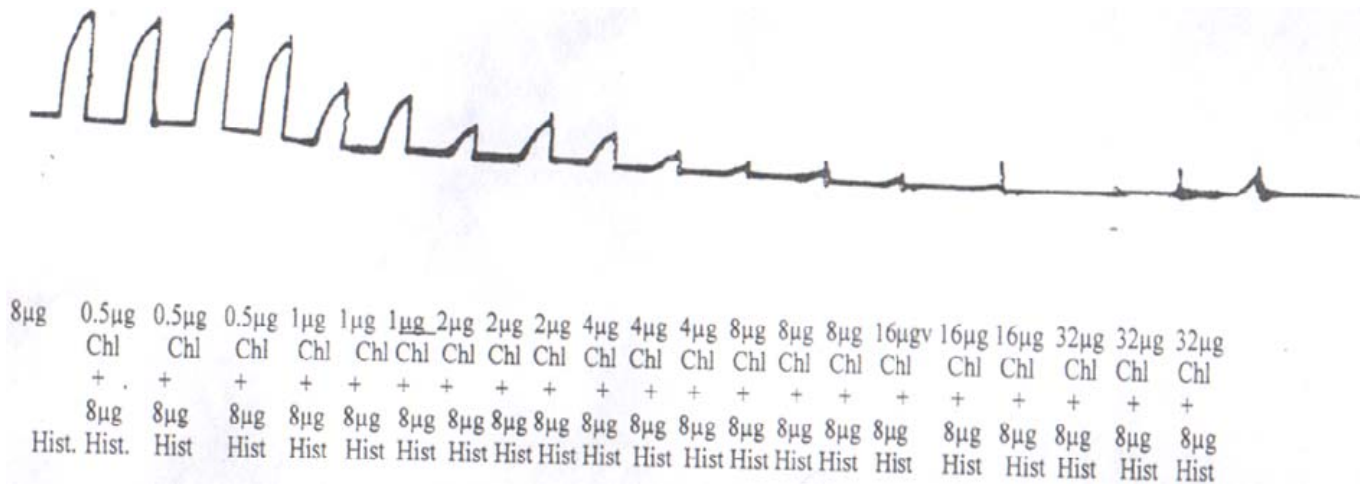

Figure 1b. Dose-dependent (0.5 to 32 mg/ml) blockade of chlorpheniramine (reference anti-histamine drug) on histamine-induced (8 µg) contraction of isolated guinea-pig ileum.

exerting significant ($p<0.05$) nitric oxide free radical scavenging activity (Enechi, 2011). The extract also significantly inhibited ferrous sulphate and CCl_4-induced lipid peroxidation in rat liver homogenate (Enechi, 2011). Anti-oxidants play a protective role against cellular damage by scavenging free radicals (Szabo, 1989). The extract may therefore exert a cytoprotective effect against ulcer formation by inhibiting free radical-mediated cell damage. The anti-ulcer activity of the extract may be due to flavonoids, which are abundant in the extract and have been shown in previous studies to possess anti-ulcerogenic and anti-ulcer activities (Tamotsu et al., 1978). Flavonoids, which are in relative abundance in this plant (46.88 ± 2.21 mg/100 g) are associated with free radical scavenging activity (Musonda and Clipman, 1998) and have been shown to protect various cell types from oxidative stress-mediated cell injury (Nakayama et al., 1993; Sasaki et al., 2002) and the flavonoids may play some roles in scavenging of free radicals in ulcerogenesis. Inhibition of prostaglandin synthesis is considered as the biochemical mechanism for the action of anti-inflammatory drugs (Vane, 1971). Ulcer induction may thus be consonant with inhibition of prostaglandin synthesis. Prostaglandins regulate many physiological processes including secretion of mucus that protect the gastric mucosa from acid and proteolytic enzymes in the stomach. Prostaglandins also inhibit gastric acid secretion. Histamine (H_2) receptor activity stimulates adenylate cyclase system and in turn causes increases in calcium ion concentrations (Nwodo et al., 2003), which ultimately

leads to activation of proton pump and consequently leads to hyperacidity and ulcer (AL-Mofleh et al., 2006; Awaad et al., 2008). It is likely that protection by the extract against indomethacin-induced gastric ulceration is achieved by the suppression of acid secretion. This could be likened to the effect of anti-ulcer drugs such as proton pump inhibitors which act by providing cytoprotective defense against gastric acid secretion (Ganong, 1995). This is corroborated by the findings in the present investigation (Table 2) that the extract significantly, and in a dose-dependent manner, suppressed the histamine-induced proton efflux in the stomach, which leads to acid secretion and ulceration Thus, the mechanism of ulceration by *B. coriacea* may be by competitively binding to H_2 receptors which histamine needs to bind in order to cause H^+ efflux in the stomach. Similarly, the extract blocked histamine-induced contractile responses in a similar fashion as the standard anti-histamine drug, chlorpheniramine. The antagonism of histamine-induced contractile response by the extract seemed to suggest that the extract may possess inhibitory activity at histamine receptor sites. The anti-histamine effect of the extract could be due to one or several phytochemicals present in the plant seed. Flavonoids which occur in relative abundance in this plant have been demonstrated to antagonize the effects of histamine which is a major mediator in ulcerogenesis (Sharma et al., 1996; Macander, 1986; Capasso et al., 1988; Middleton et al., 2000).

Conclusion

The results show that the ethanol extract of *B. coriacea* seed possesses anti-ulcer activity. The ability of the extract to suppress histamine-induced gastric acid secretion as well as to antagonize histamine-induced contractile responses indicates that suppression of mediator effect of histamine might be a likely mechanism through which the extract exerts its anti-ulcer activity. The anti-ulcer effect of the extract could be due to one or several phytochemicals that have been shown to be present in the plant seed. The results also provide empirical evidence for the use of the plant seed extract for the treatment of peptic ulcer in Nigerian folk medicine. The plant can be further harnessed as a potential source of novel anti-ulcer (bioactive) compounds.

Conflicts of interest

The authors declare that they have no conflict of interest.

REFERENCES

Ajaiyeoba EO, Onocha PA, Olarenwaju OT (2001). *In vitro* anthelmintic properties of *Buchholzia coriacea* and *Gyandropsis gynandra* extract. J. Pharm. Biol. 30:217-220.

Al - Mofleh IA, Alhaider AA, Mossa JS, Al - Sohalibani MO, Raffatullah S, Qureshi S (2006). Protection of gastric mucosal damage by *Corindrum sativum* pretreatment in Wistar albino rats. Environ. Toxicol. Pharmacol. 22:64-69.

Anoop A, Jegadeean M (2003). Biochemical studies on the anti-ulcerogenic potential of *Hemidesmus indicus* R.Br. var. indicus. J Ethnopharmacol, 84: 149-56.

Capasso F, Pinto A, Mascolo N, Autore G, France MR (1988). Effects of flavonoids on PGE_2 - and LTD_4- induced contraction on the guinea-pig isolated ileum. Pharm. Res. Commun. 20:201-202.

Dharmani P, Mishra PK, Maurya R, Chauhan VS, Palit G (2005). *Desmodium gangeticum:* A plant with potent anti -ulcer effect. Indian J. Exp. Biol. 43: 517-521.

Dharmani P, Palit G (2006). Exploring Indian Medicinal plants for Anti - ulcer activity. Indian J. Pharm. 83:95-99.

Enechi OC (2011). Anti -inflammatory effect of the ethanol extract of *Buchholzia coriacea* seeds. Ph.D Thesis, University of Nigeria, Nsukka, Nigeria.

Enechi OC, Ibeafu FC, Ledee K, Nwodo OFC (2009). Anti-inflammatory, analgesic and antipyretic activities of the ethanol extract of the seeds of *Buchholzia coriacea* in experimental animals. Bio-Res. 7(1):410-413.

Enechi OC, Igbonekwu Celestina N, Ugwu Okechukwu PC (2013b). Effects of ethanol extract of *Cissus quadrangularis* on induced gastric ulcer in rats. Afr. J. Biotechnol. 12(43):6197-6202.

Enechi OC, Manyawo Lewis N, Ugwu Okechukwu PC (2013a). Effect of ethanol seed extract of *Buccholzia coriacea* (wonderful kola) on lipid profile of albino rats. Afr. J. Biotechnol. 12(32):5075-5079.

Ezekiel OO, Onyeoziri NF (2009). Preliminary Studies on the antimicrobial properties of *Buchholzia Coriacea.* Afr. J. Biotechnol. 8(3): 472-474.

Goel RK, Chakraharthy A, Sanyan AK (1985). The effect of biological Variables on the antiulcerogenic effect of vegetable plantain Banana. Planta Med. 2, 85-88

Harborne JB (1973). Phytochemical Methods a Guide to Modern Technique of Plant Analysis. 2nd ed. Chapman and Hall limited pp 90 - 191.

Hutchinson J, Dalziel JM (1954). Floral of Tropical Africa. Part 1. Academic Press London p. 86.

Landeswari S, Seuthamariai R, Valarmathi R, Shanthi S, Prema S (2010). Screening of gastric anti-ulcer activity of *Sida acuta* Burm. Int. J. Pharmtech. Res. 2(2):1644 - 1648.

Macander PJ (1986). Flavonoid affect acetylcholine prostaglandin and antigen-mediated smooth muscle contraction. Progress Clin. Biol. Res. 213:489-492.

Main IHM, Whittle NB (1975). Investigation of vasodilator and anti-secretory role of prostaglandin in the rat mucosa by use of NSAIDs. Br. J. Pharm. 53:217-224.

Middleton E, Kandaswami C, Theodarrides TC (2000). The effect of plant flavonoids on mammalian cells, implication for inflammation, heart disease and cancer. Pharm. Rev. 52:673-751.

Moser P, Danoux L, Pauly G (2007). Cosmetic and pharmaceutical uses of an Extract of a plant belonging to the genus *Buchholzia* http/www.freshpatentsonline.com.

Musonda CA, Clipman JK (1998). Quercetin inhibits hydrogen peroxide (H_2O_2)-induced NF-KappaB DNA binding activity and DNA damage in $HypG_2$ cells. Carcinogenesis, 19: 1583-1589.

Nakayama T, Yamada M, Osawa T, Kawakishi S (1993). Suppression of active oxygen-induced cytotoxicity by flavonoids. Biochem. Pharm. 45(1):265-267

Onwuka GI (2004). Food analysis Instrumentation: Theory and Practice. Naphthali Prints, Lagos. pp. 140 - 160.

Safowora A (1993). Medicinal Plants and Traditional Medicine in Africa 2nd ed. Spectrum Books limited, Ibadan, pp 1 - 50.

Sasaki N, Toda T, Kaneko T, Baba N, Matsuo M (2002). Flavonoids suppress the cytotoxicity of linoleic acid hydroperoxide toward PC12 cells. Biol. Pharm. Bull 25: 1093-1096.

Sharma ML, Singh B, Chandan BK, Khajuria A, Kaul A, Bani S, Banjerjee SK, Gambhr SS (1996). Action of some flavonoids on specific and non-specific immune mechanism. Phytomedicine, 3(2): 191-195.

Tamotsu S, Hroshi N, Shoji S (1978). A new isoflavone and the

corresponding isoflavone of ligcorice root. Chem. Pharm. Bull. 26(1):144-148.
Urushidiani T, Kasuya Y, Okabe S (1979). The mechanisms of aggravation of indomethacin-induced gastric ulcers by adrenelectomy in the rats. Japan. J. Pharmacol. 29:775.
Vane JR (1971). Inhibition of prostaglandin synthesism as mechanism of action for aspirin- like drugs. Nature New Biol. 231:22-235.
Okwuosa C, Unekwe P, Nwobodo ED, Chilaka K (2006). The anti-ulcer activities of *Combretum racemosum*. J. Biomed. Invest. 4(1):8-14.
Sairam K, Priyambada S, Aryya NC, Goel RK (2003). Gastroduodenal ulcer protective activity of *Asparagus racemosus*: an experimental, biochemical and histological study. J. Ethnopharmacol. 86:1-10.