Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(4): 927-932
Received: 19-05-2019
Accepted: 21-06-2019

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Determination of antiulcer and antioxidant activities of the ethanol leaf extract of *Uapaca staudtii* Pax (Phyllanthaceae)

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Abstract
The dried leaves of *Uapaca* staudtii Pax were extracted with 70% ethanol. The ethanol extract was subjected to phytochemical screening and partitioned to obtain n-hexane, chloroform, ethyl acetate, butanol and aqueous fractions. The extract and the various solvent fractions were evaluated for anti-ulcer and antioxidant activities using ethanol, indomethacin and reserpine- induced ulcer models for anti-ulcer evaluation while DPPH assay and determination of total flavonoid content were used to study the antioxidant effects. The result of phytochemical screening revealed the presence of flavonoids, saponins, cardiac glycosides, tannins and terpenes. Result also showed that the extracts possessed significant antiulcer and antioxidant activities compared to prototype drugs. These observed activities may be attributed to chemical compounds present in them and the study therefore justifies it use in traditional medicine.

Keywords: Evaluation, anti-ulcer, antioxidant, extract and fractions

Introduction
Uapaca staudtii Pax (Phyllanthaceae), first described as a genus in 1858 is the only genus in the subtribe Uapacinae, and is native to Africa and Madagascar [17]. It is a tree with a heavy crown and grows 7.5-30 meters tall and can specifically be found in Southern Nigeria, Cameroon, Equatorial Guinea and Gabon [17]. It is abundant along streams and coastal forests, sometimes in almost pure stands and is regarded as a fresh water mangrove found at elevations from sea level to 710 metres. The tree is sometimes harvested from the wild and used locally for its timber and edible fruit in addition to other medicinal uses [5]. Uapaca species are employed traditionally in West Africa for the treatment of skin infections, female sterility, pile, rheumatism, emesis, cough, wound, diarrhoea and as mouth wash [6]. The aim of this study was to evaluate the antiulcer and antioxidant properties of the crude ethanol extract and the partitioned fractions of the leaf of *U. staudtii* to support its numerous uses in ethnomedicinal practices.

Fig 1: *Uapaca staudtii* with fruits

Materials and method

Plant collection and authentication: The fresh leaves of *U. staudtii* were collected in September, 2015 in a swampy forest of Etinan Local Government of Akwa Ibom State, Nigeria. The plant was identified and authenticated in the Department of Botany and Ecological Studies, University of Uyo, Uyo and allotted a voucher number (UUH1815).

Preparation of plant sample and extraction: The collected leaves were cut to small pieces and air dried. The powdered leaf (500 g) was subjected to maceration using 70% ethanol for 72 hours at room temperature and filtered. The filtrate was concentrated *in-vacuo* to get the ethanol extract. The crude ethanol extract was weighed (40 g) into a 250ml beaker with the aid of an electronic weighing balance and 200 mL of distilled water was added to dissolve it. The solution was introduced into a 500 mL capacity separating funnel, then 200 mL of n-hexane was added, shaken and allowed to stand for sometimes to separate and the n-hexane layer collected. This procedure was repeated until n-hexane soluble components were exhausted. The fractionation process was repeated with chloroform, ethyl acetate and butanol thereby leading to the collection of five soluble fractions: n-hexane, chloroform, ethyl acetate, butanol and aqueous coded HF, CF, EF, BF and AF while the ethanol extract was coded EE. They were individually concentrated *in-vacuo* and stored in a refrigerator at - 4^0C from where they were used for the study.

Phytochemical screening: Phytochemical screening of the ethanol extract (EE) of *U. Staudtii* leaves was carried out using the standard method of analysis [7].

Laboratory animals: White albino rats (120-160 g) of either sex were obtained from the animal house in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo and housed in standard cages. The animals were fed with pellet feeds (vital feed of flour mill limited, Edo State, Nigeria) and water *ad libitum*. The protocols for the use of these animals were approved by the University of Uyo Institutional Animal Care and Use Committee which follows the guidelines of the internationally accepted principles for laboratory animal use and care as found in the European community guidelines.

Acute toxicity study: The method reported by Miller and Tainter [9] was used to study the acute toxicity of ethanol leaf extract of *U. staudtii*. Thirty six Swiss mice were randomized into groups of six mice each and starved of food 24 hours before the experiment but allowed free access to water. The ethanol extract was administered in a dose range of 400-1000 mg/kg body weight of the mice and observed for physical signs of toxicity. The number of death for each group was recorded and the median lethal dose (LD_{50}) calculated using probit versus dose graph method and the dose that corresponded to 50% probit was taken as the LD_{50}.

Ethanol-induced gastric ulceration: The rats (120-160 g) were randomized and divided into 10 groups of 5 rats per group. Food was withdrawn 24 hours and water 2 hours before the commencement of the experiment [1]. Animals in group 1 were administered with 10 mL/kg of distilled water as the negative control, groups 2-4 were administered with three doses of the ethanol extract (80 mg/kg, 160 mg/kg and 240 mg/kg), groups 5, 6, 7, 8, 9 were administered with 160 mg/kg each of HF, CF, EF, BF and AF while group 10 rats were pretreated with 40 mg/kg of propranolol as positive control group. These treatments were carried out 1hour prior to administration of 0.4 mL of ethanol for the induction of ulcer lesions [12]. The drugs, extract and fractions were administered with the aid of an orogastric cannula; animals were sacrificed 4 hours later by cervical dislocation and their stomachs removed, opened along the greater curvature and the tissues fixed with 100% formaldehyde in saline. This was followed by the examination and scoring of the lesions with the aid of a hand lens according to standard methods and ulcer index and preventive ratio determined [13]

Indomethacin induced gastric ulceration: Adult albino rats (120-160 g) were randomly divided into 10 groups of five rats each. The methodology here was similar to the one reported for ethanol-induced gastric ulceration except that after 1 hour, 600 mg/kg of indomethacin dissolved in 5% $NaHCO_3$ was administered to all the rats (p. o) for the induction of ulcer lesions. The animals were grouped, pretreated with the extract and fractions in a similar manner like in ethanol-induced model. The rats were sacrificed 6 hours later by cervical dislocation; stomachs removed and opened along the greater curvature. This was followed by macroscopic examination with the aid of a hand lens and scoring of the lesions. The tissues were fixed with 10% formaldehyde in saline. Ulcer index (UI) and preventive ratio (PR) of each of the animal groups pretreated with the extracts/fractions were calculated using standard methods [13].

Reserpine induced gastric ulceration: Adult albino rats (120-160 g) were randomized and divided into 10 groups of five rats each. The protocol here was similar to the earlier ones reported for ethanol and indomethacin models with the uniqueness of this model being the use of 8 mg/kg reserpine for the induction of ulcer lesions and 100 mg/kg cimetidine in 10% tween 80 as positive control. The ulcer index and preventive ratio of each of the animal groups pretreated with extract, fractions and standard drugs were also calculated [13].

Antioxidant evaluation

Determination of total flavonoid content: The total flavonoid content was determined using the method earlier reported [18]. 5 mL of 10% Aluminium chloride ($AlCl_3$) in methanol was mixed with the same volume of the extract (EE) solution. The absorbance of the mixture at 413 nm was measured using UV/Visible spectrophotometer and the measurement was taken after 10 minutes against a blank sample consisting of a 5 mL of EE with 5 ml methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve prepared with gallic acid (20-100 µg/mL) and was expressed as milligrams of gallic acid equivalents (GAE)/1g of extract.

Rapid radical scavenging assay: The antioxidant properties of the ethanol extract (EE), fractions (HF, CF, EF, BF and AF) and ascorbic acid were determined using the method similar to an earlier one [19] with slight modification. 20 mL of chloroform and methanol (90:10) was prepared as a solvent system and emptied into a thin layer chromatographic (TLC) tank and covered for 30 minutes. The extract, fractions and standard drug (ascorbic acid) were spotted on a pre-coated analytical TLC plates (10 cm x 10 cm) with thickness of 0.25 nm. The spotted samples were then allowed to dry at ambient temperature for about 10 minutes after which the plate was introduced into the already saturated tank for development.

After development, the chromatogram was dried and sprayed with 0.3 mm solution of the stable radical DPPH and yellow spots formed against purple background were taken as positive results. The speeds of development of the yellow colour were noted [4].

2, 2 – Diphenyl–I–Picryhydrazyl – (DPPH) radical assay: A stock concentration of 1 mg/mL of the ethanol extract, fractions and standard drug was prepared by dissolving 5 mg of extract and fractions in 5 mL of methanol. Aliquots of the stock were diluted to 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/mL, respectively using methanol. 2 mL of each concentration of the leaf extract/fractions were mixed with 4mL of 2, 2-Diphenyl-I-picrydrazyl (DPPH) radical-methanol solution. The mixtures were vortexed and incubated in a dark chamber for 30 minutes at room temperature [8]. The absorbance values of these solutions were measured and recorded on an ultraviolet/visible spectrometer against a blank at 517 nm. The experiment was repeated three times and percentage (%) inhibitions calculated using a the formula below [8].

$$\% \text{ Scavenging Activity} = \frac{\text{Absorbance of control} - \text{Absorbance of Extract}}{\text{Absorbance of control}} \times 100\%$$

Results and discussion

Acute toxicity testing produced a median lethal dose (LD_{50}) of 800 ± 63.64 mg/kg for the ethanol leaf extract of *U. staudtii*. The mice exhibited signs of toxicity ranging from convulsion, restlessness, decreased movement, writhing and death. However, these observed signs were dose related with resultant death of all animals in the group that received 1000 mg/kg. The doses for the study was determined as 0.1, 0.2 and 0.3 of the LD_{50} of the ethanol leaf extract of *U. staudtii*.

Phytochemical Screening: The result of phytochemical screening of the ethanol leaf extract (Table 1) revealed the presence of flavonoids, tannins, cardiac glycosides, terpenes and saponins. Flavonoids have been reported to protect the gastric mucosa from damage by increasing the mucosal prostaglandin content and by inhibiting histamine secretion from mast cells through the inhibition of histidine decarboxylase and free radical scavenging ability [13] while terpenes involvement in anti-ulcer activity is by forming protective mucus on the gastric mucosa [2]. Cardiac glycosides are reported to block sodium pump of cardiac cells used to treat heart diseases and also the proton pump of the stomach selectively in the treatment of gastric ulcers. Also, tannins have been shown to precipitate microproteins at the site of ulcers thereby forming an impervious protective pellicle over the lining to prevent absorption of toxic substances, hence preventing the effect of proteolytic enzyme while saponins are known to have a soothing effect on wounds [16]. Therefore, these phytoconstituents may in part be responsible for the observed biological effects.

Table 1: Result of phytochemical screening of ethanol extract of leaf of *U. staudtii*

Test	Observation
Tannins	
Ferric Chloride	++
Bromine water	++
Saponins	
Frothing	+
Flavonoids	
Shinoda Test	+
Alkaloids	
Dragendorff	-
Cardiac glycosides	
Salkowski	++
Lieberman	+++
Keller Keliani	+++
Anthraquinones	
Borntrager's	-

Key: + Present in trace, ++ Present in moderate amount, +++ Present in abundance, - Absent

Antiulcer Study: The result of ethanol-induced ulceration of the ethanol extract and fractions of U. *staudtii* (Table 2) showed that, the ethanol extract was able to reduce ulceration caused by ethanol, an ulcerogen in a dose-dependent manner relative to control (distilled water) but not as the prototype drug, propranolol and this reduction was statistically significant ($p \leq 0.05$) while the result of indomethacin-induced ulceration (Table 3) revealed that the extract acted in a dose-related manner in inhibiting ulcer caused by indomethacin with butanol fraction (BF) and aqueous fraction (AF) being more potent amongst the fractions in this model with percentage inhibitions of 72% and 56%, respectively. Also, the result of reserpine-induced ulcer of the ethanol extract/fractions of *U. staudtii* leaf in rat (Table 4) showed a significant ($p \leq 0.05$) dose-dependent pattern in inhibiting injury caused by reserpine with butanol fraction (BF) being the most active when considering the fractions.

Oral administration of ethanol has been reported to cause gastric mucosal damage by stimulating the formulation of leukotriene C_4 (LTC4). It also causes disturbances in gastric secretion, damage to the mucosa, alterations to the permeability of gastric mucosa and free production of free radicals. The ability of the extract/fractions in inhibiting ulceration caused by ethanol in rats is suggestive that it may have blocked these ulcer mechanisms [10].

Indomethacin is a non-selective inhibitor of cycloxoygenase (COX-1 and COX-II) enzymes that enhances prostaglandin synthesis from arachidonic acid [14]. Prostaglandins protect the stomach from injury by enhancing the secretion of bicarbonate and mucus which aid in the maintenance of mucosal blood flow resulting in mucosal turn over and repair [12]. Suppression of prostaglandin synthesis by indomethacin results in increased susceptibility of the stomach to mucosal injury and gastro duodenal ulceration. The observed ability of

the extract and fractions in reducing mucosal damage in the indomethacin-induced ulcer model in a dose-dependent way, suggests possible mobilization and involvement of endogenous prostaglandin in the gastric mucosa [11].

Reserpine as an ulcerogen, causes mucosal damage by vasoconstriction. This implies that the extract and fractions inhibited ulcer lesions by blocking this mechanism. In all the ulcer models in this study, butanol fraction demonstrated the highest preventive ratio but not as potent as the standard drugs, propranolol and cimetidine.

Table 2: Effect of ethanol leaf extract/fractions of *Uapaca staudtii* on ethanol-induced ulceration in rats

Treatments	Dose (mg/kg)	Ulcer index	Preventive ratio (%)
Distilled water	10	5.00 ± 0.02	-
US Extract	80	3.69 ± 0.02*	27
US Extract	160	2.69 ± 0.02*	46
US Extract	240	2.00 ± 0.02*	60
HF	160	2.51 ± 0.10*	50
CF	160	2.45 ± 0.10*	51
EF	160	2.33 ± 0.02*	53
BF	160	2.23 ± 0.01*	55
AF	160	4.00 ± 0.02	20
Propranolol	40	0.33 ± 0.02*	93

Data expressed as mean + SEM significant at *$p \leq 0.05$, when compared to control, n=5

Table 3: Result of ethanol leaf extract/fractions of *U. staudtii* on indomethacin-induced ulceration in rats

Treatment	Dose (mg/kg)	Ulcer index	Preventive ratio (%)
Distilled water	10	3.00 ± 0.10	-
US Extract	80	2.00 ± 0.10	33
US Extract	160	1.67 ± 0.10*	44
US Extract	240	1.33 ± 0.10*	56
HF	160	2.46 ± 0.10	18
CF	160	2.02 ± 0.14	33
EF	160	1.67 ± 0.14*	44
BF	160	0.83 ± 0.10*	72
AF	160	1.33 ± 0.10*	56
Cimetidine	100	0.33 ± 0.10*	89

Data were expressed as mean + SEM significant at *$p \leq 0.05$, when compared to control, n=5

Table 4: Effect of ethanol leaf extract/fractions of *U. staudtii* on reserpine-induced ulceration in rat

Treatments	Dose (mg/kg)	Ulcer index	Preventive ratio(%)
Control (water)	10	3.0 ± 0.00	-
US Extract	80	1.67 ± 0.10*	44
US Extract	160	1.00 ± 0.00*	67
US Extract	240	0.30 ± 0.00*	90
NF	160	2.43 ± 0.10	19
CF	160	2.20 ± 0.10	27
EF	160	2.33 ± 0.10	22
BF	160	0.16 ± 0.10*	94
AF	160	2.33 ± 0.19	22
Cimetidine	100	0.00 ± 0.00*	100

Data expressed as mean + SEM significant at *$p \leq 0.05$, when compared to control, n=5.

Antioxidant Study

In antioxidant study (Table 5 and Figure 2), the result showed that the extract and fractions exhibited significant antioxidant activity in the various assays employed. For example, in the DPPH-TLC screening, ethanol extract (EE), ethyl acetate fraction (EF) and aqueous fraction (AF) were fast in bringing about the observed colour change while ascorbic acid (AA) was extremely fast in action. Also, in the DPPH radical assay, the activity of the ethyl acetate fraction (EF) was more pronounced than all other fractions but the standard drug (AA) took the lead.

DPPH is a stable organic free radical which absorbs at 517 nm and when it accepts an electron or a free radical, it looses this absorption thereby bringing about the observed colour change. Polyphenolic constituents of plants are known to scavenge free radicals and the presence of flavonoids, tannins and saponins in the ethanol leaf extract/fractions of *U. staudtii* as indicated in the phytochemical screening result (Table 1) may be linked to this observed antioxidant property of *U. staudtii* [3].

Table 5: Radical scavenging ability of the extract/fractions of *U. staudtii* leaf on DPPH-TLC screening

Treatments	Reaction speed	Intensity of spot
EE	Fast	++
HF	Slow	-
CF	Slow	-
EF	Fast	++
BF	Fast	++
AF	Fast	++
AA	Extremely Fast	+++

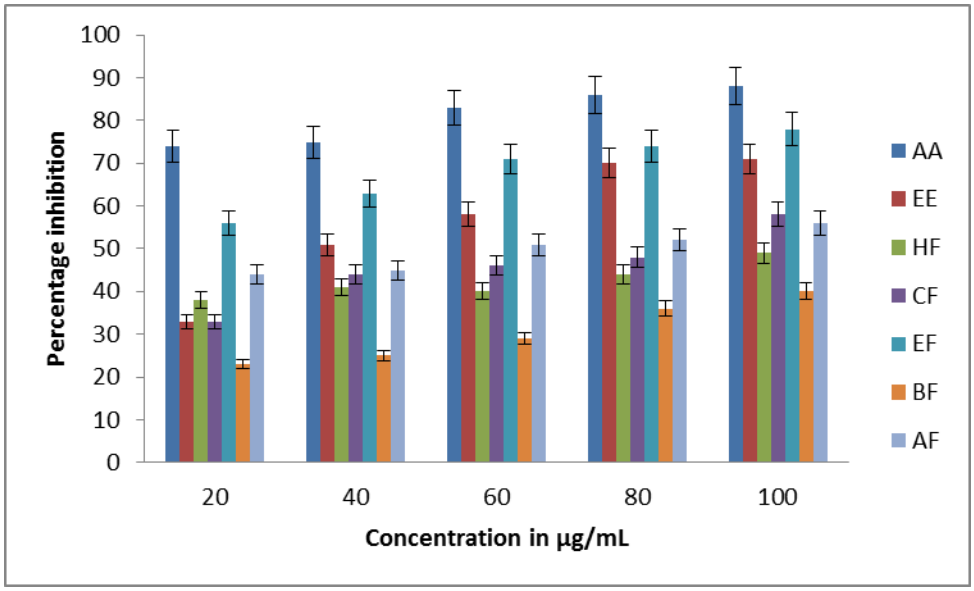

Fig 2: DPPH scavenging effect of extract/fractions of *U. staudtii*

Conclusion

The findings from this study therefore justify the ethnomedicinal uses of *U. staudtii*.

References

1. Alphin RS, Ward JW. Action of Hexopyrronium bromide on gastric secretion in Dog and Rat. Achiever of International de Pharmacodynamic et Detherapy. 1967; 2:128-140.
2. Aguwa CN, Okunji CO. Gastrointestinal studies of *Pyrenacantha staudtii* leaf extract. Journal of Ethno pharmacology. 1986; 15(1):45-55
3. Arash KE, Rosna MT, Sadegh M, Behrooz B. Antioxidant activity and total phenolic and flavonoid content of various solvent extracts from *in vivo* and *invitro* grown *Trifolium pretense* L. (Red clover). Bio Med Res Intl. 2015; 643285:1-1
4. Blois MS. Antioxidant Determinations by the use of a stable free radical. Nature. 1958; 181:199-200.
5. Breteler FJ. Uapaca (phyllathaceae) in the Guinea Congolian forest region: A synoptic revision. Plant Ecology and Evolution. 2013; 146:75-94.
6. Dalziel JM. The useful plants of west tropical Africa. In: Flora of West Tropical, Hutchinson J. and Dalziel JM. Crown agents for the colonies, London, 1937, 406-407.
7. Evans WC. Pharmacognosy, 13th ed, Baillerel lindall, London, 1989, 638-684.
8. Guangrong H, Jiaxin J, Dai D. Antioxidative and antibacterial activity of the methanol extract of *Artemisinin anomala* S. Moore. African Journal of Biotechnology. 2008; 7(a):1335-1338.
9. Miller LC, Tainter ML. Estimation of LD_{50} and its error by means of log-probit graph paper. Proceedings of the Society for Experimental Biology and Medicine. 1944; 57:261
10. Hiruma-Lima CA, Calvo TR, Rodriguez CM, Andrade FDP, Vilegas W, Brito ARM. Antiulcerogenic activity of *Alchornea castaneaefolia* effects on somatostatin, gastrin and prostaglandin. Journal of Ethno pharmacology. 2006; 104:215-224.
11. Okokon, JE, Akpan HD, Ekaidem I, Umoh EE. Antiulcer and antidiarrheal activity of *Hippocratea africana*. Pakistan Journal of Pharmaceutical Sciences. 2011; 24:201-205.
12. Okokon JE, Umoh EE, Umoh UF, Etim EI. Antidiarrhoeal and antiulcer activities of *Mammea africana.* Iranian Journal of Pharmacology and Therapeutics. 2010; 9(2):96-101.
13. Nwafor PA, Bassey AL. Evaluation of antidiarrhoeal and antiulcerogenic potential of ethanolic extract of *Carpolobia lutea* leaves in rodents. Journal of Ethno pharmacology. 2009; 111:619-62
14. Jalilzadeh-Amin G, Najarnezhad V, Anassori E, Mostaferi M, Keshipour H. Antiulcer properties of *Glycyrrhiza glabra* L. extract on experimental models of gastric ulcer in mice. Iranian Journal of Pharmaceutical Research. 2015; 14(4):1163-1170
15. Lewis DA, Hanson D. Antiulcer drugs of plant origin. Progress in Medicinal Chemistry. 1991; 8:208-210.
16. Lorentzon P, Bayati A, Lee H, Andersen K. Selective inhibition of the gastric H^+, K^{+-}ATpase by Omeprazole and related compounds. Annals NY Acad. Sci. 1997; 834:592-599.
17. Mcpherson G. A review of Madagascan Uapaca (Euphorbiaceae S. I.) Adansonia. 2011; 33:221-231.
18. Umoh UF, Thomas PS, Etim EI, Jacobs IE, Bassey E. Antioxidant potentials of five plants used in Akwa Ibom

State ethno medicine for pain. The Pharma Innovation Journal. 2016; 5(11):31-33.
19. Koleva II, Vanbreek TA, Linssen JP, Groot AD, Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phyto chem Anal. 2002; 13:8-17.