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Potentials of *Persea americana* Mill (Avocado) (Lauraceae) Seeds as Ulcer Protective Agent

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Abstract: The seed extract of *Persea americana* has been used to treat peptic ulcer disease in folkloric medicine but its efficacy has not been validated. The present study was carried out to evaluate the ulcer-protective effects of the seed aqueous extract and its n-hexane and water fractions using rats ulcer models: dried and milled seeds of *P. americana* were soaked in distilled water for 24 h, filtered and freeze dried. The Aqueous Extract (ASEPA) was fractionated with n-hexane and residue was the water fraction. Acute toxicity study (LD_{50}) and phytochemical screening were carried out on the extract and fractions. The ulcer-protective effects of ASEPA and fractions were tested on ulcer models induced by ethanol, aspirin, stress and pyloric ligation. Cimetidine and distilled water served as the standard drug and negative control, respectively. Outcome measures were ulcer index, percentage ulcer-protection, gastric acid volume, pH, free acidity and total acidity. The effect of ASEPA and fractions on isolated tissue preparation was studied using segments of guinea pig the ileum. The oral median Lethal Dose (LD_{50}) of the extract was >5000 mg kg^{-1}. Phytochemical tests revealed the presence of secondary metabolites such as flavonoids, tannins, reducing sugars and saponins. ASEPA and fractions conferred dose-related ulcer-protection on the ulcer models used. ASEPA and fractions displayed a non-dose dependent and non specific spasmolytic effect. *P. americana* seed extract displays excellent properties that are beneficial in the treatment of peptic ulcer.

INTRODUCTION

Peptic ulcers are sores in the lining of the stomach or small intestine. They occur when the protective factors of the gastro-intestinal tract are overwhelmed by the aggressive factors (MacGill, 2018). The aggressive factors are usually *Helicobacter pylori*, HCl, pepsins, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) bile acids, ischemia, hypoxia, smoking and alcohol while the defensive factors are bicarbonate, mucus layer, mucosal blood flow, prostaglandins and growth factors (Harold *et al*., 2007). Peptic Ulcer Disease (PUD) affects a considerable number of people worldwide. The incidence of peptic ulcer has been shown to be prevalent in Africa and South Asia. When these ulcers occur in the stomach they are called gastric ulcers but when they occur

in the first portion of the intestine they are called duodenal ulcers. Peptic ulcer is the term used to describe either or both of these 2 types of ulcers. Peptic ulcer disease and its complications remain the cause of significant morbidity worldwide, representing a major burden for health care resources (Tanih *et al*., 2010). The available anti-ulcer drugs though potent are limited by several toxicities, thus, the need for alternatives (Lavanya *et al*., 2012).

Many drugs used in medicine today are directly or indirectly derived from plants reinforcing the believe that plants are good sources of drugs (Thomford *et al*., 2018) and a high percentage of the world population depends on plant-derived medicines as the first line of primary health care (Panda and Sonkamble, 2012).

Persea americana Mill (Lauraceae) is an utility medicinal plant in many parts of Africa. The fruit is commonly referred to as avocado pear, alligator pear and butter fruit. It is known as ebenmbakara (Ibibio) ube bekee (Igbo) and Ado (Yoruba) tribes of Nigeria. It is a widely distributed in the lowlands and rain forest areas of Nigeria. The leaves are thick, glossy, dark green above and paler below and are briefly shed around the time of flowering while the trees are partially self-pollinating. The fruit is a large berry containing a single large seed known as "pit" or "stone" (Morton and Dowling, 1987). The root, bark, fruits, seeds and leaves are used extensively in traditional medicine for the treatment of various ailments like diarrhea, dysentery, toothache, stomach ache, peptic ulcer and intestinal parasites (Pamploma-Roger, 1999; Owolabi *et al*., 2005). The anti-oxidant (Segovia *et al*., 2018) anti-motility (Odo *et al*., 2013) anti-bacterial (Ilozue *et al*., 2014) and anti-inflammatory (Kumar *et al*., 2017) activities of the seed have been documented. To the best of our knowledge the ulcer-protective efficacy of the seed has not been scientifically evaluated and this study was designed to fill this gap.

MATERIALS AND METHODS

Collection and identification of plant material: The fruits of *P. americana* were purchased from Oye-Nimo, Nimo, Njikoka local government area Anambra state, Nigeria in the fruiting season of April, 2016. The collection was identified and authenticated by Dr J.E. Amadi, a Taxonomist at the Department of Botany, Nnamdi Azikiwe University Awka, Nigeria and a herbarium specimen, NAUH.13 is kept in the hebarium.

Extraction: The seeds were removed from the fruits and cut into small pieces, shade-dried for 5 days and grounded into fine powder with Binatone blender (Model BLG-401). The powder (1 kg) was soaked in 2 L of distilled water for 24 h. This was first filtered by passing through a cotton plug and further filtered with filter paper (Whatman filter paper, No. 1). The Aqueous Seed Extract of *Persea Americana* (ASEPA) was freeze-dried to a constant weight.

Fractionation: A portion (100 g) of the freeze-dried ASEPA was thoroughly mixed with 200 mL of distilled water and poured into a separating funnel. Then portions of 250 mL of n-hexane were used to wash the extract exhaustively until the n-hexane layer became clear. The residue (water fraction) was then removed from the separating funnel and the 2 fractions were dried to a constant weight using rotary evaporator at 40°C to afford the N-hexane (NF) and the Water (WF) fractions.

Animals: The study was carried out using adult albino rats (200-220 g) and guinea pigs (300-450 g) of both sexes bred in the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, Nigeria. The animals were allowed to acclimatize at Nnamdi Azikiwe University, Awka animal house for 2 weeks. The rats were fed with feed pellets, (Top Feed, Premier Feed Mills Sapele, Delta state, Nigeria) while the guinea pigs were fed on green grasses predominantly *Panicum maxima* Jacq (Poaceae). The animals were given food and water *ad libitum* throughout the experiments. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals (Pub. No. 85-23 Revised 1985) as approved by the Nnamdi Azikiwe University, Awka Ethical Committee for the use of laboratory animals.

Acute toxicity (LD_{50}) study and phytochemical analysis: The median Lethal Dose (LD_{50}) of the extract (ASEPA) was determined using the method described by Miller and Tainter (1944). Qualitative and quantitative phytochemical screening of ASEPA and fractions were carried out according to the procedures outlined by Harborne (1998) to determine the presence and concentration of the secondary metabolites.

Anti-ulcer activity: The ulcer-protective activity of the extract and fractions were evaluated in rats using ethanol, aspirin, pyloric-ligation and stress-induced ulcer models. For each model, the animals were grouped into 8 (n = 5) and treated orally as follows:

- Group 1: distilled water (10 mL kg^{-1}, nagative control)
- Group 2: cimetidine (150 mg kg^{-1}, positive control)
- Group 3: ASEPA 250 mg kg^{-1}
- Group 4: ASEPA 500 mg kg^{-1}
- Group 5: NF 250 mg kg^{-1}
- Group 6: NF 500 mg kg^{-1}
- Group 7: WF 250 mg kg^{-1}
- Group 8: WF 500 mg kg^{-1}

The animals were treated for 14 days after which ulcers were induced with the appropriate ulcerogen.

Effect of ASEPA and fractions against ethanol-induced ulcer: The animals were starved for 24 h and absolute ethanol (96% v/v) was administered (0.5 mL/100 gp.o) to each rat as a single dose. The animals were sacrificed 1 h later for ulcer determination.

Effect of ASEPA and fractions against aspirin-induced ulcer: Ulcers were induced with aspirin (150 mg kg^{-1} p.o) and the animals sacrificed 4 h later and the ulcer grading carried out as described below:

Effect of ASEPA and fractions against stress-induced ulcer: After 24 h fasting, stress ulcers were induced by forced-swimming the rats for 3 h in a glass cylinder (height 45 cm, diameter 25 cm) containing water (25°C) to the height of 35 cm (Alpine and Ward, 1999).

For each model, the animals were humanely sacrificed with excess anesthetic ether. Macroscopic evaluation of the glandular portions of the stomach was made by opening the stomach along the greater curvature, rinsed under a stream of water, pinned flat on a corkboard and viewed macroscopically with a hand lens (magnification X10). Each stomach was given a severity rating from which the ulcer index was calculated (Ganguly, 1969):

- Normal stomach..... 0
- Red coloration..... 0.5
- Spot ulcer.....…….1.0
- Hemorrhagic streak.... 1.5
- Ulcers.....…..2.0
- Perforation.....…. 3.0

$$\text{Ulcer Index (UI)} = \frac{\text{US} \times 10^{-1}}{\text{UN}}$$

Where:
UI = Ulcer Index
UN = Total Number of Ulcers per animal
US = Total number of severity score for each animal

$$\text{Protection (\%)} = \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index} \times 100}{\text{Control mean ulcer index}}$$

Effect of ASEPA and fractions against pyloric ligature-induced ulcer: This study was performed as described by Shay *et al*. (1945). The rats were grouped and treated as stated previously. Under light ether anesthesia the abdomen was opened by a small midline incision below the xiphoid process and the pyloric portion of the stomach was slightly lifted and ligated, avoiding traction to the pylorus or damage to its blood supply. The stomach was then replaced carefully and the abdominal wall closed by interrupted sutures. After 4 h of pyloric ligation the animals were humanely sacrificed with excess anesthetic ether and the stomach dissected out. The gastric content was drained into a graduated tube to determine the volume while the pH of the gastric juice was recorded using pH meter. The free acidity and total acidity were determined as stated below. The ulcer index and percentage protection were determined using the method previously stated.

Measurement of gastric acidity (free and total acid): The gastric juice was centrifuged at 1000 rpm for 10 min. Then, 1 mL of the gastric juice was transferred into a porcelain dish and 2 drops of Topfer's reagent was added. Colour change appeared indicating the presence of free acid. To this was added 2 drops of phenolphthalein and titrated against 0.01 N NaOH in a burette. When the trace of the red colour disappeared and is replaced by a canary yellow colour the reading was taken from the burette and used for the calculation of the free acidity (the "active" acid). The titration continued until the color of phenolphthalein appeared. The titration continued up to the point that further addition of the alkali does not deepen the pink color. The quantity of 0.01 N NaOH from the beginning was noted and used for the calculation of the total acidity. This experiment was done in triplicate and the average taken:

$$\text{Acidity (x)} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \, 100 \, \text{mEq/L}}{0.1}$$

X = The acidity (free or total)

Studies on the isolated guinea pig ileum: The spasmolytic effect of ASEPA and fractions was studied using segments of the ileum (2-3 cm long) suspended in 20 mL. Tyrode solution in a tissue bath. The composition of the Tyrode solution (mmol L^{-1}) was NaCl-136.7, KCl-2.7, CaCl2-1.8, NaHCO3-11.9, MgCl2-1.0, Na2HPO4-0.4 and glucose-5.5. The solution was maintained at 37±1°C and aerated with air. The preparation was set up under a resting tension of 0.5 g and allowed to equilibrate for 30 min. At the end of the equilibration period the extract and fractions (2.5-10 mg mL^{-1}) were added to the bath non-cumulatively, maintaining 30 sec contact time and 3 min time cycle. Responses were recorded in triplicate using 4-Channel Recorder-17304 (Ugobasile Italy). The effects of ASEPA and fractions on submaximal responses to acetylcholine (0, 2 μg) and histamine (2 μg) were also determined.

Statistical analysis: The data were expressed as mean±Standard Error of Mean (SEM). The data were analyzed by Statistical Package for Social Sciences (SPSS

Table 1: Phytochemical constituents of ASEPA and fractions

Samples	Alkaloid	Saponin	Tannin	Flavonoid	Steroid	Terpenoid	Cadiac glycosides	Reducing sugar
Constituents (%)	4.7	12.25	3.8	0.49	-	4.7	11.6	14.2
ASEPA	+	+++	+	+	_	+	++	+++
NF	+	++	+	+	_	+	+	+
WF	+	+	+	+	_	+	+	++

Key: + = trace or mildly present, ++ = moderately present, +++ = abundantly present - = absent

Table 2: Effects of ASEPA and fractions on the Ulcer Models

Treatment/dose (mg kg^{-1})	Ethanol 0.5mL/100 (mg kg^{-1})	Asprin 150 (mg kg^{-1})	Stress	Pyloric ligature
Distilled water (10 mL kg^{-1})	3.38±0.17 (0 %)	2.65±0.19 (0%)	3.63±0.16 (0%)	4.56±0.16 (0%)
Cimetidine 150	2.30±0.35 (32 %)*	0.94±0.22 (64 %)*	2.06±0.05* (43%)	2.76 ±0.16 (39 %)*
ASEPA 250	2.80±0.36 (15 %)	2.45±0.21 (8 %)	2.13±0.51 (41 %)*	4.21±0.20 (7 %)
ASEPA 500	1.45±0.1 (57 %)*	0.95±0.19 (64 %)*	1.66±0.1 (54 %)*	2.91± 0.12 (36 %)*
NF 250	2.41±0.36 (29 %)*	2.35±0.25 (11 %)	2.24±0.5 (38 %)*	3.12 ± 0.17 (32 %)*
NF 500	1.17±0.15 (65 %)*a	0.94±0.32 (65 %)*	2.01±0. (44 %)*	2.28 ± 0.14 (50 %)*
WF 250	2.68±0.37 (21 %)	2.05±0.20 (22 %)	2.95±0.48 (19 %)	4.38±0.15 (3 %)
WF 500	1.60±0.20 (53 %)*	1.55±0.17 (42 %)*	2.47±0.1 (32 %)*	2.98 ±0.10 (35 %)*

*p<0.05. Percentage ulcer-protection are in parenthesis (ASEPA = Aqueous Seed Extract of *P. Americana*, NF = N-hexane Fraction, WF = Water Fraction)

Version 20) using one way ANOVA, followed by post-hoc Turkey's test for multiple comparisons. The difference between mean were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Acute toxicity study (LD_{50}): Following oral administration of ASEPA up to 5000 mg kg^{-1}, no lethality or any obvious sign of acute toxicity was observed within 24 h. The LD_{50} was therefore, >5000 mg kg^{-1}.

Phytochemical analysis: The qualitative phytochemical analysis of the extract and fractions revealed the presence of some bioactive substances. These phytoconstituents-alkaloids, saponins, flavonoids, tannins, terpenoids, cardiac glycosides and reducing sugars were more abundant in the extract than the fractions. The quantitative analysis of ASEPA showed that the concentration of reducing sugars was the highest (14.2%) while flavonoids was the least (0.49%) (Table 1).

Effect of the extract and fractions on ethanol-induced ulcer: The administration of 96% v/v ethanol (p.o) produced 100% ulceration in the control. ASEPA and fractions exhibited dose-related ulcer protection against ethanol-induced ulcer which manifested as the significant ($p<0.05$) reduction in ulcer index when compared to control (Table 2).

Effect of the extract and fractions on asprin-induced ulcer: The ASEPA and fractions conferred a dose-related ulcer-protective effect on aspirin-induced ulcer as shown bythe significant ($p<0.05$) reduction in ulcer indexes, especially, in the 500 mg kg^{-1} treated groups. NF 500 mg kg^{-1} caused the most potent ulcer-protective effect (Table 2).

Effect of the extract and fractions on stress-induced ulcer: Stress resulted in increase ulceration in the control. ASEPA and fraction exhibited a dose-related and significant ($p<0.05$) ulcer-protection against stress-induced ulcer (Table 2).

Effect of extract and fractions on pyloric-ligature induced ulcer: The ASEPA, NF and WF conferred a dose-related ulcer-protective effect on the pyloric-ligature induced ulcer. At 500 mg kg^{-1} there was significant ($p<0.05$) reduction in the ulcer indexes when compared to the control (Table 2).

Effect on gastric acid volume: There was a significant ($p<0.05$) reduction in gastric acid volume in all the treated groups (except WF 250 mg kg^{-1}) when compared to the negative control (Fig. 1).

Effect on gastric acid (pH): The extract and fractions caused a dose-related increase in pH. At 500 mg kg^{-1} ASEPA, NF and WF significantly ($p<0.05$) increased the pH in all the treatment groups when compared to control (Fig. 2).

Effect on free and total acidity: Significant ($p<0.05$) reductions in free and total acidity were evident in ASEPA, NF and WF (500 mg kg^{-1}) treated groups when compared to control (Fig. 3 and 4).

Effect of the extract and fractions on isolated guinea pig ileum: The ASEPA, NF and WF caused non-dose dependent spasmolytic responses on the isolated guinea pig ileum (Fig. 5). The contractile response elicited by histamine (2 μg) was reduced by NF (5 mg) and WF (5 mg) (Fig. 6). Similarly, the initial response to acetylcholine (0.2 μg) was reduced by NF (5 mg) and WF 5 mg (Fig. 6). The ASEPA also exhibited a non-dose

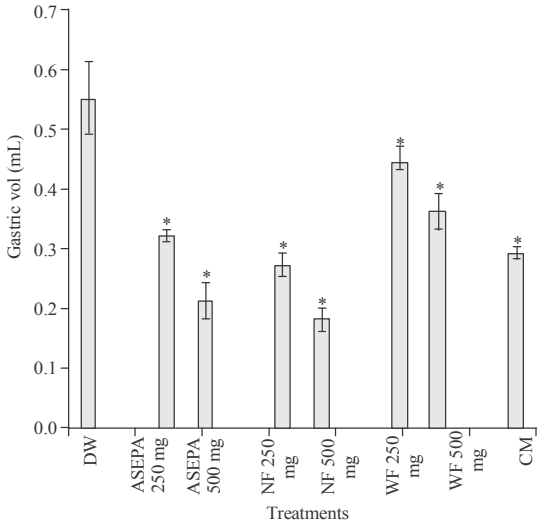

Fig. 1: Effect of ASEPA, NF and WF on gastric volume *p<0.05; DW = Distilled water (10 mL kg^{-1}), ASEPA = Aqueous Seed Extract of *P. americana*, NF = N-hexane Fraction, WF = Water Fraction, CM = Cimetidine (150 mg kg^{-1})

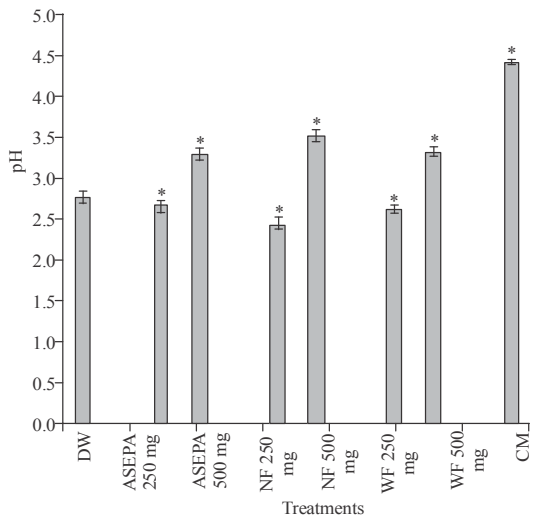

Fig. 2: Effect of ASEPA, NF and WF on gastric pH *p<0.05; DW = Distilled water (10 mL kg^{-1}), ASEPA = Aqueous Seed Extract of *P. Americana*, NF = N-hexane Fraction, WF = Water Fraction, CM = Cimetidine (150 mg kg^{-1})

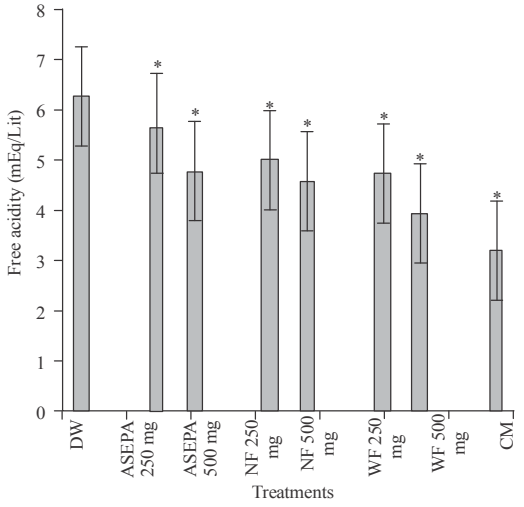

Fig. 3: Effect of ASEPA, NF and WF on free acidity *p<0.05; DW = Distilled Water (10 mL kg^{-1}), ASEPA = Aqueous Seed Extract of *P. Americana*, NF = N-hexane Fraction, WF = Water Fraction, CM = Cimetidine (150 mg kg^{-1})

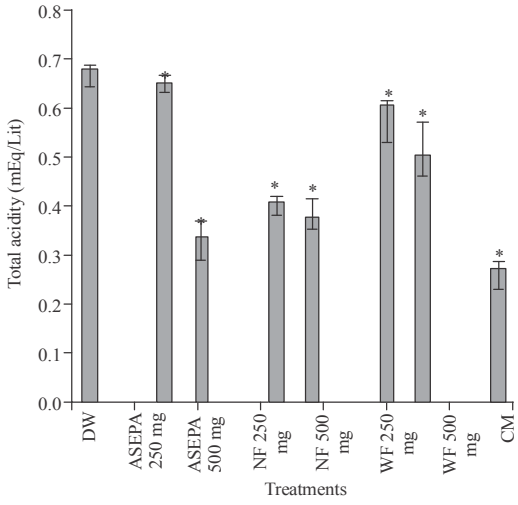

Fig. 4: Effect of ASEPA, NF and WF on total acidity *p<0.05; DW = Distilled Water (10 mL kg^{-1}), ASEPA = Aqueous Seed Extract of *P. Americana*, NF = N-hexane Fraction, WF = Water Fraction, CM = Cimetidine (150 mg kg^{-1})

dependent spasmolytic effect and significant inhibition of acetylcholine-induced responses of the isolated guinea pig ileum (Fig. 7).

The etiology of peptic ulcer is assumed in most cases, yet it is generally, accepted that it results from an imbalance between aggressive factors and the endogenous defense mechanisms (MacGill, 2018). To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucus production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis (Mohod and Bodhankar, 2011; Al-Radahe *et al*., 2013; Sharath *et al*., 2015). Plants are

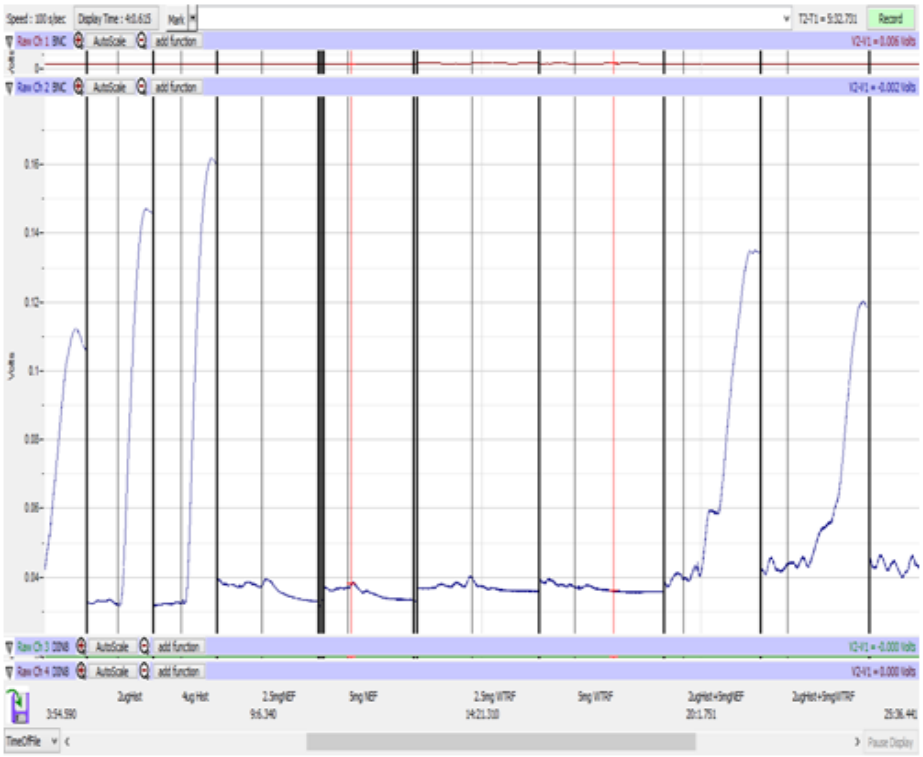

Fig. 5: Effect of NF and WF on histamine (2 µg)-induced contraction of the guinea pig ileum. NF = N-hexane Fraction, WF = Water Fraction, Ach = Acetylcholine

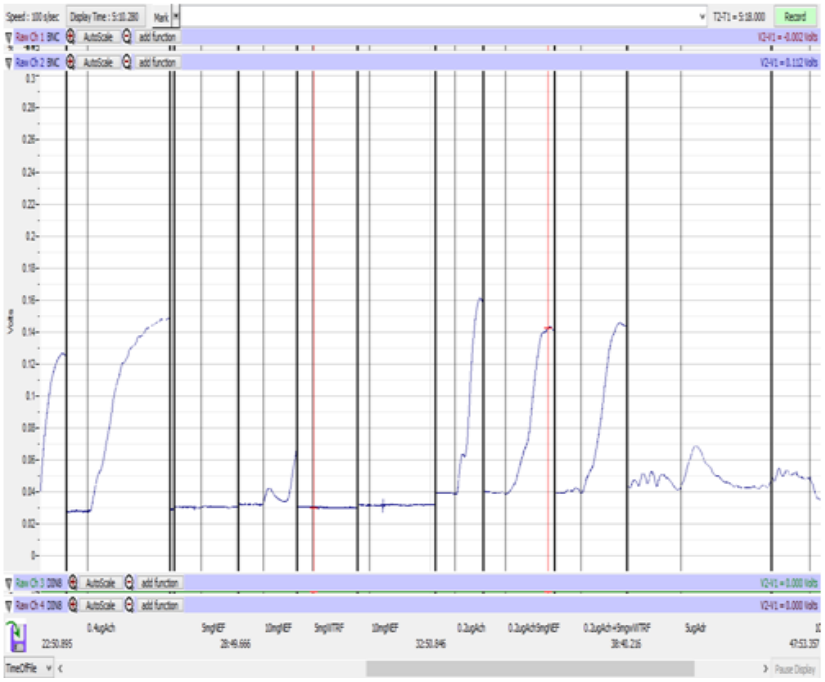

Fig. 6: Effect of NF and WF and the effect on acetylcholine (0.2 µg)-induced contraction on guinea pig ileum. NF = N-hexane Fraction, WF = Water Fraction, Ach = Acetylcholine

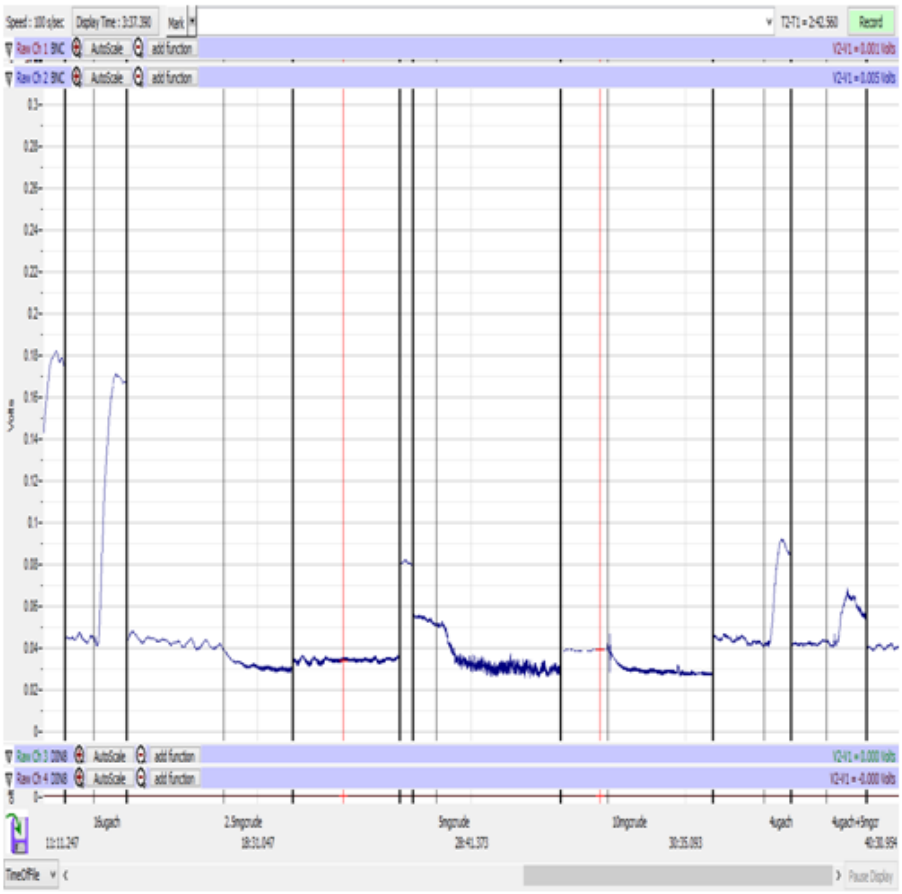

Fig. 7: Effect of ASEPA on acetylcholine (2 μ)-induced contraction of the guinea pig ileum. crude = ASEPA and Ach = Acetylcholine

one of the most attractive sources of new drugs and some have shown promise for the treatment of gastro-duodenal ulcer with minimum side effects (Welz *et al*., 2018). Oral administration of ASEPA was safe up to 5000 mg kg^{-1}. This attests to the relative safety of the extracts with only a remote chance of acute toxicity.

Phytochemical screening of ASEPA and fractions revealed the presence of bioactive compounds that have been previously associated with gastro protective activities. They include alkaloids, saponins, flavonoids, terpenoids, tannins, cardiac glycoside and reducing sugars. Idris *et al*. (2009) and Omodamiro *et al*. (2016) also reported the presence of the above secondary methabolites in *P. americana* seed extract. Anti-bacterial and anti-fungal effects of the saponins (which were in abundance in the extract) have been documented (Lanzotti *et al*., 2012). Saponins are believed to activate mucous membrane protective factors (Borrelli and Izzo, 2000). The ability of flavonoids to increase microcirculation in the gastric mucosa has also been reported (Jarial *et al*., 2018). Flavonoids act as free radical scavengers and are powerful anti-oxidants (Galleano *et al*., 2010). Anti bacterial effect of flavonoids has also been demonstrated (Jarial *et al*., 2018). Tannins on the other hand are noted for their anti-oxidant effects (Akter *et al*., 2016) and astringent property (McGee, 2004). They render the outermost layer of the mucosa less permeable to chemical irritants due to their astringent property. Tanins can also hasten the healing of wounds and inflamed mucous membrane due to their anti-inflammatory effects (Cheng *et al*., 2002) and their ability to form a protective layer over the exposed tissue, hence, keeping the wound from being infected (Quideau *et al*., 2004). Terpenoids have shown antibacterial activity and wound-healing activity (Mai *et al*., 2003). They have also been reported to possess potent activity against gastric ulcers (Mitra *et al*., 2014). Isolated pure form of alkaloids and their synthetic derivatives are used as medicinal agents for their analgesic, anti-inflammatory and anti-nociceptive properties (Bribi *et al*., 2015, 2017). There was abundance

of reducing sugars in this seed extract. Wang reported reducing sugars significantly reduced the Malondialchehyde (MDA), decreased free radical activity and enhanced the activity of Superoxide Dismutase (SOD) and Glutathione Peroxidase (GSH-Px). Therefore, the presence of these bioactive substances may be an indication of the ulcer-protective property of the extract.

The ability of the gastric mucosa to resist injury by endogenous secretions (acid, pepsin and bile) and ingested irritants (e.g., alcohol, nonsteroidal anti-inflammatory drugs) can be attributed to a number of factors that have been collectively referred to as "mucosal defense" (Wallace, 2001). The concept of gastric cytoprotection against various necrotizing agents has been routinely used to assess the anti-ulcer potential of different compounds. The ethanol-induced acute gastric mucosal injury model is considered to be one of the widely used experimental models of ulcer disease (Shawon and Gautam, 2012). Ethanol easily penetrates the gastric mucosa and causes gastric ulcer 1 h after administration; producing ulceration due to a decrease in gastric mucus, prostaglandin levels, glutathione, mucosal blood flow and bicarbonate secretion (Nordin *et al*., 2014). Ethanol readily penetrates the gastric mucosa due to its ability to solubilize the protective mucous and expose the mucosa to the proteolytic and hydrolytic actions of hydrochloric acid and pepsin (Oates and Hakkinen, 1988) causing damage to the membrane. In the present study, ethanol-induced ulcer model was employed to confirm the gastric cytoprotective effect of the seed extract and fractions. The strong ulcer-prtective effect of the extract and fractions against ethanol-induced ulcer as observed by the significant ($p<0.05$) reduction in ulcer indexes might be related to the antioxidant activity of the seed which was well demonstrated in previous studies (Rodriguez-Carpena *et al*., 2011; Boadi *et al*., 2015; Ikpeme *et al*., 2014). Anti-oxidants accelerate wound healing and compounds that act as antioxidants or activate the redox system are important for restoring gastric tissue (Hussain *et al*., 2015).

Aspirin is known to induce ulcers by inhibiting prostaglandin synthetase in the cyclooxygenase pathway (Rainsford, 1987). Prostaglandins are found in many tissues including the stomach where they play a vital protective role via. stimulating the secretion of bicarbonate and mucus, maintenance of mucosal blood flow and regulating mucosal cell turnover and repair (Hayllar and Bjarnason, 1995). ASEPA and fractions conferred ulcer-protective effect against aspirin-induced ulcer as shown by the significant ($p<0.05$) reduction in ulcer indexes. The extract and fractions of *P. americana* seeds were probably exerting their activity by acting as gastro-protective agent, since, the underlying pathophysiology of NSAIDs-induced peptic ulcer is due to interference with mucosal prostaglandin synthesis.

Pyloric ligation-induced gastric ulceration may be attributed to stress-induced secretion of HCl in excess amounts from the parietal cells and autodigestion of mucosa by the accumulated gastric juice (Mohod and Bodhankar, 2011). Free radicals may also be associated since studies have shown changes in the antioxidant status following pylorus ligation-induced ulceration in rats (Sharath *et al*., 2015). The model is therefore used to determine anti-secretory effect of drugs that reduce secretion of gastric acid and pepsin. The antiulcer activity of ASEPA and fractions on this ulcer model was evidenced by the significant reduction in the ulcer indexes, volume of gastric acid, free acidity and total acidity of the gastric juice. The extract also increased the pH of the gastric acid. These results suggest ASEPA and fractions probably have anti-secretory property which may be responsible for the observed ulcer-protective effect.

Stress induced ulcers are produced due to the increased release of histamine, leading to an increase in acid secretion (histamine is a potent stimulator of gastric acid secretion). Increased histamine secretion also leads to increased gastrointestinal motility which can lead to folds in the GIT that may eventually lead to ulcer (Warzecha *et al*., 2001). The ASEPA and fractions probably conferred protection against ulcers induced by cold restraint (stress) in rats by inhibition of histamine (H_1 receptor), since, histamine mediate contraction in the gastric mucosa through H_1 receptors. This inhibition could result in decrease in gastrointestinal motility, leading to gastro-protection and ulcer healing.

The results of the in vitro study showed ASEPA and fractions exerted a non-dose dependent spasmolytic activity on the isolated guinea pig ileum. Cholinergic agents are known to increase the secretory and motor activity of the gut (Bukhari *et al*., 2013). The M_2 and M_3 receptors in the GIT play essential roles in the relaxation of the GIT (Ehlert *et al*., 1999). The possible mechanisms of GIT relaxation could be the inhibition of Acetylcholine (Ach) by blockage of the muscarinic receptors of smooth muscles of the gastro intestinal tract. Inhibition of Histamine (H_1) receptors is also a possible mechanism of the relaxant effects of these extracts, since, H_1 receptors are dominant in the gut and they mediate contraction (Ehlert *et al*., 1999).

As was observed, n-hexane fraction exhibited the highest ulcer protection and in some cases better ulcer-inhibitory effect than the standard drug cimetidine. This is in line with a report by Melese *et al*. (2011) where the aqueous leaf extract of *Plantago lanceolata* showed a better ulcer inhibition than ranitidine. Such findings strengthen the search for novel agents by tapping the rich herbal drugs used in folk medicine. The consistent better effect by n-hexane fraction could be attributed to the

ability of the solvent to extract more of the active component from the seed which was mostly in oily form. This could also explain why polar solvents like ethyl acetate, methanol, ethanol and butanol could not fractionate the extract effectively as was observed during fractionation.

CONCLUSION

The extract and fractions of *Persea americana* seeds are relatively safe and exhibited potent ulcer-protective effects. The results of this study revealed that *P. americana* seeds displayed pharmacological properties that are essential in the management of peptic ulcer disease. The use of the aqueous seed extract in the treatment of peptic ulcer disease in folklore medicine is justified.

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