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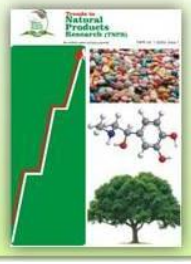

Comparison of the Antiulcer Effects of the Fractions of Ethanol Extracts of *Musa sapientum* Linn and *Brassica oleracea* Linn in Albino Rats

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Key words: *Musa sapientum*, *Brassica oleracea*, ulceration, cytoprotection

Abstract: The development of new anti-ulcer drugs from medicinal plants is an attractive field. Diverse chemical compounds with anti-ulcer activities have been isolated from plants. This study compared the antiulcer potentials of the fractions of ethanol extracts *Musa sapientum* unripe fruits and *Brassica oleracea* leaves in albino rats. Extraction was by cold maceration in ethanol to obtain the ethanol extracts of both plants. The ethanol extracts were fractionated by liquid-liquid partitioning method using n-hexane, chloroform, n-butanol and water to obtain the various fractions; HFm, CFm, BFm, and WFm of *M.sapientum* and HFb, CFb, BFb, and WFb of *B.oleracea* respectively. The oral acute toxicity (LD_{50}) of the extracts was determined in mice. Pilot study was carried out to determine the fraction with highest anti-ulcer effects which was then used for the comparative study. Doses of 100 mg/kg of WFm and WFb and standard dose of misoprostol were administered to three different groups of albino rats for 21 days after which ulcer was induced with 1ml of 98 % ethanol. One hour after ulcer induction, the animals were sacrificed under diethyl ether anaesthesia and, their stomachs were harvested and opened for ulcer ratings. The LD_{50} of the extracts were more than 5000 mg/kg. The ulcer index was decreased from 6.68 to 1.87, 2.97 and 3.85 by WFm, WFb and misoprostol respectively. The percentage ulcer protection was 72 %, 55.5 % and 12.4 % for WFm, WFb and misoprostol respectively. The results indicate that the unripe fruit of *M. sapientum* exhibited better ulcer protection than the leaves of *B. oleracea*

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INTRODUCTION

Medicinal plants are reservoirs of raw materials for drug development. The bioactive molecules isolated from crude extracts have been used as therapeutic agents or as starting materials for the synthesis of useful drugs (Onasanwo *et al*., 2013; Boetto, 2015). There is an avalanche of scientific reports on the efficacy of medicinal plant**s** in the management of ulcers of different aetiologies (Akah *et al*., 2007, Gopinathan and Naveenraj, 2013).

Ulcer is erosion in the lining of the stomach or duodenum caused by the disruption of the gastric mucosal defense systems (Gopinathan and Naveenraj, 2013).The formation of ulcer depends on decreased gastric juice PH and decreased mucosal defences (Isah *et al.*, 2019). Peptic ulcer occurs as open craters or sores in the inner lining (mucosa) of the stomach or the duodenum. The coating of mucus normally shield the stomach and duodenum from being digested. When these protective mechanisms are disturbed, digestive acids can erode the lining of the guts and cause ulcers (Gopinathan and Naveenraj, 2013). The prevalence of Peptic Ulcer Disease (PUD) is about 10 % (Akah *et al*., 2007). The pathophysiology of PUD involves an imbalance between acid, pepsin, bile and *Helicobacter pylori*, and gastric secretions, nitric oxide, prostaglandins, innate resistance of mucosal cells and growth factors (Mota *et al*., 2009; Prabha *et al*., 2011; Tyler and Premila, 2012; Agbaje and Okpara, 2013; Gopinathan and Naveenraj, 2013; Batista *et al*., 2015; Rao *et al*., 2015; Malik *et al.*, 2020). Increased use of non-steroidal anti-inflammatory drugs (NSAIDs) and low levels of antioxidants are also major factors in peptic ulcer formation (Chandrasenam *et al*., 2016; Isah *et al*., 2019; Anand and Katz, 2020; Malik *et al*., 2020).The pathogenesis of disease includes the presence of highly activated inflammatory cells such as neutrophils, dendritic cells, macrophages, and excessive production of ROS (Chandrasenam *et al*., 2016).

The prevention or cure of peptic ulcers is one of the most challenging problem in medicine because gastric ulcer therapy faces drawbacks and most of the currently available antiulcer drugs show limited efficacy and are often associated with severe side-effects (Prabha *et al*., 2011).. Reports on clinical evaluation show that there are incidences of relapses and adverse effects and danger of drug interactions during ulcer therapy (Goel and Sairam, 2002). Hence, the search for an ideal anti-ulcer drug continues and has also been extended to herbal drugs.

Brassica oleracea Linn (Brassicaceae) commonly known as cabbage is the "king" of the cruciferous family of vegetables. The members of this family of vegetables have received attention for their anticancer properties (Murray and Pizzorno, 2010). Cabbage is a nutrient-dense, low-calorie food providing excellent source of many nutrients, especially vitamin C, potassium, folic acid, vitamin B6, biotin, calcium magnesium, manganese, vitamin A, and sulfur (Adam, 2009; Murray and Pizzorno, 2010). Cabbage is reported to have some phytochemical contents such as glutamine, s-methyl methionine, glucosinolate, and gefarnate (Ananya and Sandip, 2014; Sanders, 2015). It contains medicinal compounds with anti-cancer properties, as well as a natural remedy for stomach ulcers. (Chiney, 1949; Ensminger and Ensminger, 1993; Adam, 2009).*Brassica oleracea* exert marked anti-inflammatory effect (anti-ulcer) in experimental animals possibly by regulating the antioxidant and inflammatory mediators (Chandrasenam *et al*., 2016; Yang, 2018; Oancea *et al*., 2019).

Musa sapientum Linn (Musaceae), known as banana**,** is a familiar tropical fruit. Banana is not only a delicious fruit, but also has medicinal values. This fruit is composed of water (75 %), carbohydrate (23 %), proteins (1 %), fatty acids (0.3 %) and alimentary fiber (2,3 %) and their proportions depends on the level of maturation and species (Boetto, 2015). Moreover, the pulp contains vitamins A, B_2, B_1, C, B_4, B_6 and E, and minerals such as calcium, phosphorus, iron and potassium (Boetto 2015). There are reports that banana pulp protects the gastric mucosa against NSAIDs and other ulcerogens ((Prabha *et al*., 2011, Boetto 2015), and stimulates the growth of the gastric lining after damage (Prabha *et al*., 2011, Onasanwo *et al*., 2013, Boetto 2015). Studies revealed that pectin and phosphatidylcholine in green banana strengthen the mucous-phospholipid layer that protects the gastric mucosa (Reichert 1993, Boetto 2015). Other studies provided evidence on anti-ulcerogenic activity of banana pulp powder in aspirin-, indomethacin-, phenylbutazone-, prednisolone-induced gastric ulcers, and cysteamine- and histamine-induced duodenal ulcers in both rats and guinea-pigs (Boetto 2015).

The aim of this study was to compare the antiulcer effects of *Musa sapientum* and *Brassica oleracea* in albino Wistar rats using ethanol-induced ulcer model.

MATERIALS AND METHODS

Plant materials

Fresh *M. sapientum* (banana) unripe fruits and *B. oleracea* (cabbage) were purchased from Ilishan

local market, Ogun State between the months of May and June 2017. They were identified and authenticated by Nodza George of the Department of Botany, Faculty of Sciences, University of Lagos and with voucher numbers LUH: 8671 for cabbage and LUH: 8672 for banana. Voucher specimens were deposited in the Department of Botany, Faculty of Sciences, University of Lagos.

Animals

Albino Wistar rats of both sex (110 -150 g) and mice (20-25 g) were obtained from an animal breeder in Badagry, Lagos State, Nigeria. These animals were kept in cages in a well-ventilated room in the Animal House of Babcock University, Ilishan-Remo, Ogun State under room temperature (25 ± 2 ^{o}C). The animals were allowed free access to feed and water and were acclimatized for two weeks before the commencement of the study. The study was approved by Animal Ethics Committee of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State.

Drugs and Reagents

Misoprostol was obtained from a representative of Topix Pharmaceutical Company limited, Nigeria. Ethanol, diethyl ether, chloroform, acetic acid, hydrochloric acid (HCl), sulphuric acid (H_2SO_4), ammonia, n-butanol, and n- hexane were from Sigma Aldrich, St. Louis, USA.

METHODS

Extraction and fractionation of plants material

The unripe fruits of *M. sapientum* were cleaned and the greenish back carefully removed. The white flesh was cut into small pieces, dried for seven days under sunlight, and reduced to fine powder. Five hundred (500 g) of the powder and 1000 g of fresh cabbage (*B. oleracea*) were macerated separately in 70 % ethanol at room temperature for 3days with frequent agitation. The mixtures were strained, and the marc pressed using a clean white handkerchief. The exudates were filtered (Handa *et al*., 2008, Azwanida 2015) using No 1001 125 Whatman filter paper. The filtrates were concentrated in a rotary evaporator, and dried in a hot-air oven at 40 ^{o}C. Fractionation was carried out on the ethanol crude extracts by the liquid-liquid partitioning method of Adaramola *et al, (*2017) using n-hexane, chloroform, n-butanol and water to obtain hexane:(HFm), chloroform (CFm), butanol (BFm) and water (WFm) of *M. sapientum* as well as hexane (HFb), chloroform (CFb), butanol (BFb), and water (WFb) of *B. oleracea* fractions respectively. Pilot study was carried out on these fractions to determine the fractions with the best anti-ulcer activity which were then used for the comparative cytoprotective study.

Acute toxicity study

Oral acute toxicity test was carried out on the crude ethanol extracts using Lorke (1983) method to determine the lethal dose of both plant materials.

Ulcer protective study

Twenty four albino Wistar rats were grouped into four of 6 animals per group. The animals were fasted for 24 hours with free access to water before daily oral administration of 100 mg/kg of WFm (Group 1), 100 mg/kg of WFb (Group 2), 0.0005mg/kg Misoprostol (Group 3). Group 4 received 1ml of distilled water. These treatments lasted for 21 days at the end of which the animals were fasted for 24 hours, and given 1ml of 98 % ethanol. One hour after ethanol intoxication the animals were sacrificed under diethyl ether anesthesia. Their stomachs were harvested and opened via the greater curvature. The ulcer formations were scored and rated as follows: No ulcer (no lesion) - 0, shedding of epithelium -10, petechial and frank hemorrhage- 20, one or two ulcers- 30, more than two ulcers- 40, and perforated ulcers - 50.

Ulcer index was calculated with the formula:

$UI = (US/UP) \times 10^{-1}$. (Onasanwo *et al*., 2013)

Where UI is ulcer index, US is severity of ulcer score and UP is percentage of animal with ulcer incidence. Percentage protection was also calculated using the formula:

Uc-Ut/Uc×100

(Onasanwo *et al*., 2013; Rao *et al*., 2015)

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

Histological examination

The stomach were harvested and preserved in a plain sample bottles containing 10 % normal-saline for adequate fixation (Zagrebin, 2015). The preparation was mounted on a glass slide and viewed under x100 objective of the microscope and photomicrographs were taken.

Statistical analysis

The results were expressed as mean ± S.E.M. Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test, and post hoc Newman-Keul's test using Graph-Pad PRISM 5 software. P-value < 0.05 was considered significant.

RESULTS

Yields of extraction

The percentage yield of *M. sapientum* was 4.96 % while that of *B. oleracea* was 3.6 %.

Fractionation of crude ethanol extracts

Results obtained from the fractionation of the crude extracts showed that water fractions of both plants had the highest yield, followed by n-butanol fractions, chloroform fractions with n-hexane fractions having the lowest yield (Table 1).

Acute toxicity study (LD_{50})

The acute toxicity test revealed that the LD_{50} of two extracts was greater 5000 mg/kg.

Effects of the fractions on ethanol-induced ulcer

Pretreatment with the *M. sapientum* aqueous fraction significantly ($p < 0.05$) decreased the ulcer index from 6.68 to 1.87 offering 72 % protection (Tables 2 and 3). Similarly, in the group treated with the aqueous fraction of *B. oleracea* the ulcer index was significantly ($p < 0.05$) reduced to 2.97 and percentage ulcer protection was 55.5 % (Tables 2 and 3).

Histology

Histological examination showed activation of neutrophils and their migration towards inflamed region as indicated by the arrows. The fractions remarkably reduced the area of inflamed surfaces with reduced neutrophil clustering (Figure 1).

Table 1: Yields of different fractions of *M. sapientum* and *B. oleracea*

Fractions	Yields (g)	Percentage yields (%)
WFm	18.4	92
BFm	1.2	6
CFm	0.96	4.8
HFm	0.17	0.85
WFb	22.8	76
BFb	3.0	10
CFb	1.8	6
HFb	0.16	0.53

KEY WFm – water fraction of *Musa sapientum*, BFm – n-butanol fraction of *Musa sapientum*, CFm – chloroform fraction of *Musa sapientum*, HFm – n-hexane fraction of *Musa sapientum*, WFb – water fraction of *Brassica oleracea*, BFb – n-butanol fraction of *Brassica oleracea*, CFb – Chloriform fraction of *Brassica oleracea*, HFb – n-hexane fraction of *Brassica oleracea*.

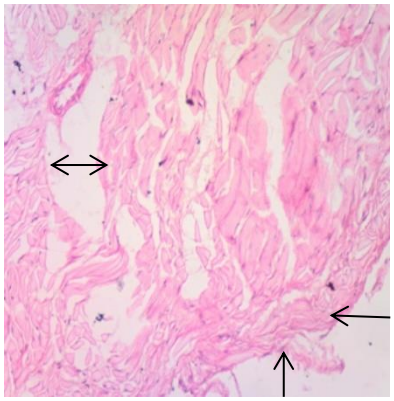
Table 2: Effect of the aqueous fractions on ethanol induced ulcer

Groups	Treatment	Ulcer index ± SEM
1	WFm (100 mg/kg)	1.87 ± 0.16*
2	WFb (100 mg/kg)	2.97 ± 0.44*
3	Misoprostol (0.0005mg/kg)	3.85 ±0. 82*
4	Distill water (1 ml)	6.68 ± 0.58

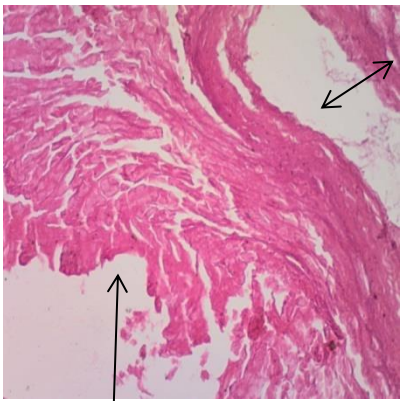
*$p < 0.05$, n=6

Table 3: Percentage ulcer protection by the fractions

Groups	Treatment	%Protection
1	WFm	72.0%
2	WFb	55.5%
3	Misoprostol	12.4%



Group 1



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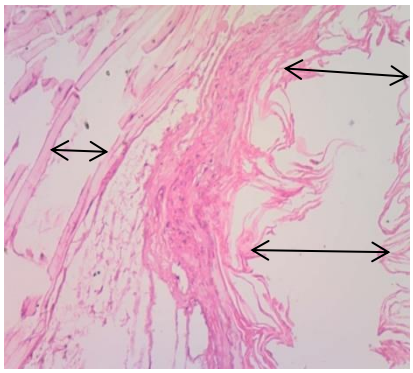
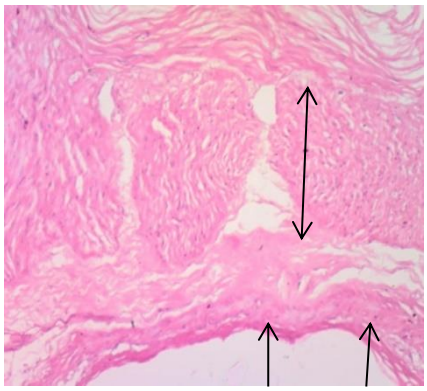


Group 3



Group 4 (+ve control)

Figure 1: The photomicrograph of histological sectioning of the stomach tissues
Arrows on the photomicrographs indicated the migration of neutrophil activated as a result of inflammation (ulcer) to the inflamed region to combat the cause of inflammation. Magnification x100

DISCUSSION

This study revealed that *M. sapietum* fruits and *B. oleracea* may possess beneficial antiulcer properties. The study by Onasanwo *et al*., (2013) reported that *M. sapientum* protected mucosal damage by 56 % whereas another study (Rao *et al*., 2015) reported 68.80 % protection. There reports do not differ significantly from the result of the present study. The variation in ulcer protection potencies might be as a result of many factors which may include, the nature of the ulcerogens used, the age of the animals used and environmental factors. However, all these studies indicate that *M sapientum* does protect gastric mucosal injury by more than 50 % irrespective of the ulcerogen used. The mechanism by which *M. sapientum* protects against ulcer was postulated to involve increase in white blood cell (WBC) production which involved neutrophil generation (Prabha *et al*., 2011), production of endogenous vasodilators like PGE_2 (Rahgozar *et al*., 2002) and by blocking of acid and pepsin secretion by the parietal cell (Rao *et al*., 2015).

Brassica oleracea extracts have been reported to offer cytoprotection by increasing the production of WBC (Oguwike *et al*., 2014) and the inhibition of gastric acid secretion (Carvalho *et al*., 2011). Many cytoprotective agents have been shown to act by enhancing the production of factors responsible for protecting gastric mucosa and preventing epithelial damage (Rao *et al*., 2015). The findings of this study therefore suggest that *M. sapientum* and *B. oleracea* may exhibit antiulcerogenic effects by producing gastric mucosal protective factors to counteract the imbalance between aggressive and protective factors altered by ethanol administration (Agbaje and Okpara, 2013). It is evident from this study that *M. sapientum* was more effective as an antiulcer agent than *B. oleracea*.

Histological showed neutrophil migrations from the no ulcer regions to the areas of ulcerations (inflammation regions). Neutrophils are component of the white blood cell (WBC), and whenever inflammation occurs, neutrophils are known to be activated and migrate to the region of inflammation from the surrounding tissues. This migration was evident in this study as the histological findings showed gradual epithelial regeneration, coupled with angiogenesis. However, regeneration and angiogenesis was moderate and near complete regeneration at the end of extract administration (Onasanwo *et al*., 2013).

CONCLUSION

The results of this study showed that both *Musa sapientum* and *Brassica oleracea* possess anti-ulcer and possibly cytoprotective properties. Comparatively, *M. sapientum* was more potent than *B, oleracea* in protecting against ethanol-induced ulceration.

Conflict of interest.

The authors declare no competing interest

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