

Phytochemical and Antiulcer Study on the Ethanol Leaf Extract and Fractions of *Spondias dulcis* L. Anacardaceae in Rodents

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Abstract

The leaf of *Spondias dulcis* (Anacardaceae) is used by the indigenes of Usaka in Ikot Ekpene Local Government Area, Akwa Ibom State for the management of abdominal pains/aches. This study was structured to screen the ethanol leaf extract of *S. dulcis* for phytochemicals, quantify total flavonoids and phenolic contents and also, study its ulcer protective effect. Fresh leaves were shade-dried, milled, macerated with aqueous ethanol (60%) for 72 hours and the filtrate concentrated *in vacuo*. Part of the crude extract (75g) was partitioned with n-hexane, dichloromethane, ethyl acetate and butanol for their partitioned fractions. Phytochemical screening and quantification were achieved by standard protocols while ulcer protective effect was studied using ethanol and indomethacin ulcer models in rats. Phytochemical screening unveiled the major metabolites present to be flavonoids, alkaloids, saponins, tannins, phlobatannins and terpenoids. The ethanol extract had a total phenolic content of 2.586 mg/g while among the fractions, n-butanol fraction (NBT) revealed the highest total phenolic content of 2.434 mg/g and also, highest flavonoid content of 1.875 mg/g. The ethanol extract of *S. dulcis* protected the intestinal lining against ulcers in the two experimental models in a dose-related manner. The result of this study supports the folkloric application of *S. dulcis* leaf in abdominal pain diseases.

Keywords: Phytochemical, Antiulcer, Leaf, Anacardaceae

Introduction

Spondias dulcis is a specie in the Anacardaceae family employed in many diseases in folk medicine including management of abdominal disorders across different cultures [1, 2]. This plant is commonly known as ambarella, june plum, golden apple and pommecythere and locally in Nigeria, *S. dulcis* is known as Ngwu (Igbo), Tsadar masar (Hausa), Iyeye (Yoruba) and Nsukakara (Ibibio) [3].

The astringent property of *S. dulcis* bark allows easy usage as a remedy for diarrhea treatment in Cambodia while in Bangladesh, it enhances eyesight and for treatment of eye infections [4]. The fruit is reported as folkloric remedy for skin inflammation that is accompanied with itching, sore throat, ulceration of internal organs and as an antidote [5]. The high phenolic and fiber contents in the fruits made it possible to be employed as an anti-anemia, regulation of blood glucose levels and digestive aids by the natives of Malaysia, Vietnam, Sri Lanka and India [6]. In Philippine, fruits and leaves are either eaten fresh or added to other salad ingredients for digestive issues and as a mild diuretic. In some Papua New Guinean cultures, the leaves and bark are also used to address digestive complaints and skin ailments [7].

Biological data has it that *S. dulcis* has strong antimicrobial activity [8] and also has protective effects against DNA damage induced by benzo[a]pyrene and cyclophosphamide using the comet assay and micronucleus test, hence proving its efficacy in protection against cell damage [9]. The extracts of fruits and leaves are reported to have antioxidant, cytotoxic and thrombolytic properties [8,10]. Other reported biological properties are anticancer, antimutagenic, antigenotoxic and immunomodulation [9, 11, 12]. This present study was designed to lend scientific credence to the ethno medicinal application of *S. dulcis* leaves by the Usaka indigenes of Ikot Ekpene Local Government Area in Akwa Ibom State, Nigeria in the alleviation of stomach ulcers and also study its phytoconstituents.

Materials and Methods

Preparation of extract and fractions

The collection of plant material (Figure 1) was carried out in a forest at Usaka, a village in Ikot Ekpene Local Government Area in Akwa Ibom State. Identification of plant sample was achieved by Dr. Imoh Imeh Johnny of Department of Pharmacognosy and Natural Medicine and Prof Margaret Bassey, of Department of Botany and Ecological Studies both in the University of Uyo and a voucher specimen (UUPH 3(d)) deposited in the herbarium. The leaves were detached, shade-dried, reduced to powder with an electrical milling machine and plant sample (1 kg) was extracted with aqueous ethanol (60%). After 72 hours, it was filtered and concentrated *in vacuo*. A portion (75 g) of the ethanol extract was partitioned successively with nhexane, dichloromethane, ethyl acetate and butanol for their respective fractions. These fractions were individually concentrated and stored in a freezer (-4°C) for use in the various experiments.

Phytochemical screening of ethanol extract

Qualitative phytochemical screening was carried out on the ethanol extract of *S. dulcis* using standard methods to identify inherent secondary metabolites [13].

Determination of total phenolic content

The total phenolic content of the ethanol leaf extract and partitioned fractions of *S. dulcis* was achieved through spectrophotometric method with Folin-Ciocalteu reagent. In this method, 0.5 mL (1 mg/mL) of each fraction was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 7% Na₂CO₃. The mixture which resulted was vortexed for 15 seconds and incubated at 40°C for 30 minutes for colour development to occur. The absorbance was measured at 765 nm wavelength with a spectrophotometer. Gallic acid calibration curve was also determined and total phenolic content was calculated from the calibration curve. Results were expressed as mg of gallic acid equivalent per gram dry weight. This assay was carried out in triplicates and data presented as mean $\pm$ standard error of mean [14].

Determination of total flavonoid content

Total flavonoid content of ethanol leaf extract and fractions was determined by the aluminum chloride colorimetric assay method [15]. In this method, the reaction mixture consisting of 1 mL of extract and 4 mL of distilled water in a 10 mL volumetric flask, 0.30 mL of 5 % sodium nitrite was added and after 5 minutes, 10 % aluminum chloride (0.3 mL) was added and mixed. After another 5 minutes, 2 mL of 1 M sodium hydroxide was added and diluted to 10 mL with distilled water. A set of reference standard solutions of rutin (20, 40, 60, 80 and 100 μ g/mL) were prepared in the same manner as earlier described above. The absorbance for test and standard (reference) solutions was determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The assay was carried out in triplicates and data expressed as mean $\pm$ standard error of mean with the result expressed as milligram of rutin equivalent per gram (mgRTE/g) dry weight of extract/fractions.

Evaluation of anti-ulcer activity

Selection of animals

In this study, Swiss albino mice (25-30 g) and rats (180-220 g) of both sexes obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, housed under standard laboratory conditions and fed with recommended animal pellets were used. They were starved 24 h before the experiments but with free access to water.

Animal ethics

Animal handling and experimental procedures were performed strictly in accordance with international ethical guidelines concerning the care and use of laboratory animals, and all the experiments were carried out under the approval of the ethical committee of the Faculty of Pharmacy, University of Uyo (ethics approval number UU/FPEC/2023/034)

Determination of median lethal dose (LD₅₀)

The median lethal dose of the ethanol leaf extract of *S. dulcis* was estimated according to the OECD guideline [16]. Three groups of mice (three per group) were selected for the study. The first three mice were administered with 300 mg/kg of ethanol leaf extract of *S. dulcis*. After twenty four (24) hours, following the protocol, 2000 mg/kg was administered to the next two sets of three mice at interval of 24 hours.

Experimental design

Table 1: Experimental design for ethanol and indomethacin-induced ulceration for ethanol leaf extract of *S. dulcis*

Groups	No. of rats	Pretreatments
1	5	Dw 10 mL/kg
2	5	Sde 50 mg/kg
3	5	Sde 100 mg/kg
4	5	Sde 150 mg/kg
5	5	Propranolol 40 mg/kg for ethanol-induced ulceration
5	5	Cimetidine 100 mg/kg for indomethacin-induced ulceration

Sde = *Spondias dulcis* extract; Dw = distilled water

Table 2: Experimental design for ethanol-induced ulceration for partitioned fractions of *S. dulcis*

Groups	No. of rats	Pretreatments
1	5	Distilled water 10 mL/kg
2	5	n-hexane fraction (NH)100 mg/kg
3	5	Dichloromethane fraction (DCM) 100 mg/kg
4	5	Ethyl acetate fraction (ETH)100 mg/kg
5	5	Normal butanol fraction (NBT) 100 mg/kg
6	5	Aqueous fraction (AQ) 100 mg/kg
7	5	Propranolol 40 mg/kg

Ethanol-induced ulceration

Animals were randomized, divided and pretreated as shown in the experimental design (Table 1 and 2) [26]. After 1 h of pretreatments, the rats were administered with 95% ethanol intragastrically with orogastric cannula. Ketamine (1 mL) was administered to induce anaesthesia after 4 h and the animals sacrificed by cervical dislocation. The stomachs were removed, opened along the greater curvature and stomach contents washed off with running water. Microscopic examination of the prepared stomach samples was achieved with a hand lens and ulcer lesions scored [17].

Indomethacin-induced ulceration

The animals were randomly divided into groups as captured in the experimental design (Table 1). After 1 h of pretreatments, indomethacin (0.2 g) was dissolved in 5% NaCO₃ in distilled water (20 mL) and administered intragastrically using orogastric cannula. Four (4) hours later, 1 mL of ketamine was administered and the animals were sacrificed by cervical dislocation. The stomachs were removed and opened along the greater curvature. Stomach contents were washed with water, microscopic examination carried out using hand lens and ulcer lesions scored [17].

Statistical analysis

Data obtained were analyzed using analysis of variance and presented as Mean $\pm$ standard error of mean. A post-hoc test was carried out using Primer statistical software and $p \leq 0.05$ was adjudged significant.

Results and Discussion

Considering the result of acute toxicity study, only one mouse from the first three mice administered with 300 mg/kg of ethanol leaf extract of *S. dulcis* died within 24 h. Following OECD guideline, a dose of 2000 mg/kg was administered to another three mice which caused 100 percent mortality. Repeated administration of another dose of 2000 mg/kg to another set of three mice also led to 100 percent death. In line with the adopted guideline, the median lethal dose (LD₅₀) was extrapolated to be 500 mg/kg.

The result of qualitative phytochemical analysis of ethanol leaf extract of *S. dulcis* (Table 3) revealed the presence of alkaloids, flavonoids, tannins and saponins. Phytochemicals in plants are usually linked to the reason why they are used as medicines and each of these classes of phytochemicals vary in their composition in the studied extract. The presence of alkaloids, flavonoids and saponins in the ethanol leaf extract of *S. dulcis* may help in the prediction of the observed anti-ulcer effects. Alkaloids as phytoconstituents are shown to have good solubility in stomach acid to exert their stomach lining protection effect [18]. The involvements of flavonoids and saponins in shielding the intestinal layer in ulcer experiments have also been reported [19-21]. The observed anti-ulcer properties of the extract of *S. dulcis* may be as a result of the presence of these plant secondary metabolites.

Table 3: Phytochemical constituents of ethanol leaf extract of *S. dulcis*

Metabolite	Test	Observation	Inference
Alkaloids	Dragendorff's reagent	Brick red precipitate	+
Flavonoids	Magnesium metals in acid	Red colouration	+
Tannins	Ferric chloride	Decolorization of bromine water	+
Saponins	Frothing	Froth that persisted after 10 min	+

+ = present

The result of total phenolic and total flavonoid contents' quantification are in Figures 2 and 3. In the quantitative study, the ethanol extract had a total phenolic content of 2.586 mg/g, while the fractions: NH (1.116 mg/g), DCM (1.186 mg/g), ETH (2.223 mg/g), NBT (2.434 mg/g) and AQ (1.405 mg/g) revealed varying quantities (Figure 2). These data further collaborate the importance of phenolic constituents of plants in mucosal protection against ulcers [20]. The total flavonoid contents of the ethanol extract and fractions as shown in Figure 3 revealed the following quantities: Extract (2.114 mg/g), NH (0.82 mg/g), DCM (0.847 mg/g), ETH (1.628 mg/g), NBT (1.875 mg/g), AQ (1.033 mg/g). These results again strengthen the involvement of flavonoids in ulcer management [22].



Figure 1: *Spondias dulcis* in a natural habitat

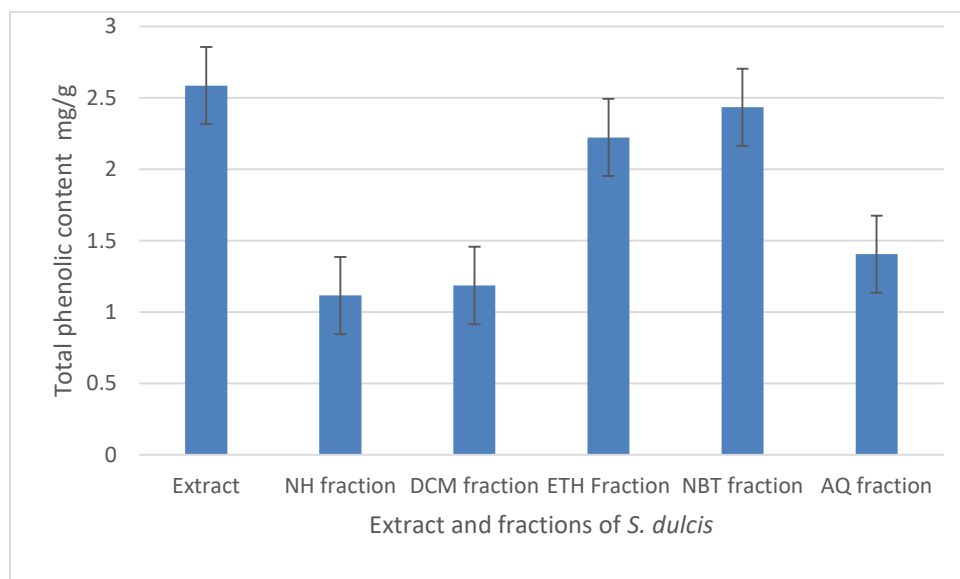


Figure 2: Quantification of total phenolic content of ethanol leaf extract and fractions of *S. dulcis*

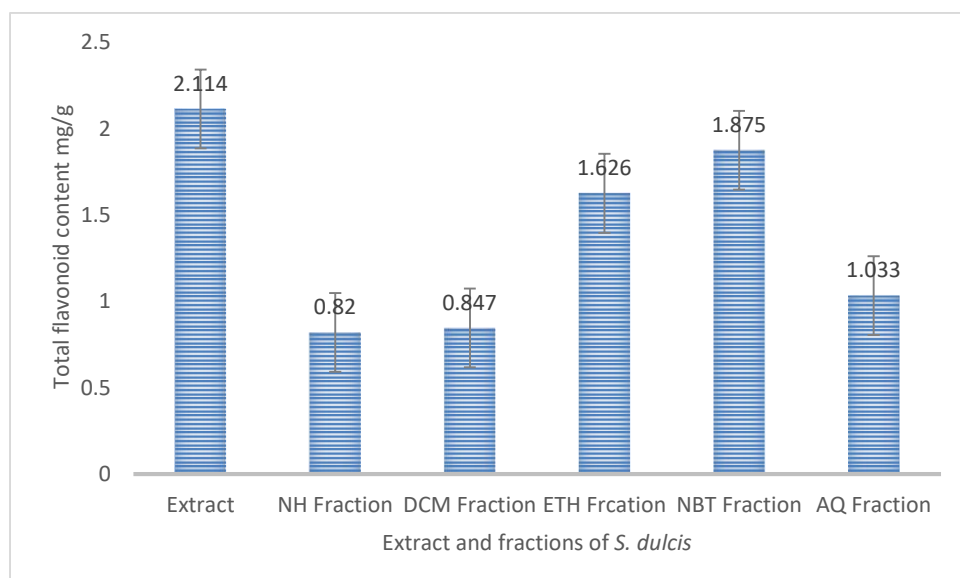


Figure 3: Quantification of total flavonoid content of ethanol leaf extract and fractions of *S. dulcis*

The data in Tables 4, 5 and 6 are the results of the effects of the ethanol extract and fractions of *S. dulcis* in the anti-ulceration experiments in rats. The result (Table 4) revealed a percentage preventive ratio of 57.0 %, 65.8 %, 82.8 % and 84.6 % for low dose, median dose, high dose and standard drug, respectively for the ethanol extract while the percentage preventive ratios for the fractions were 33 % for NH, DCM, ETH, AQ, 73 % for NBT and 93 % for propranolol (Table 6). The preventive action of *S. dulcis* was also dose-dependent and for the fractions, NBT fraction displayed a statistically significant ($p \leq 0.05$) protection when compared to control,

distilled water. The high phenolic and flavonoid contents in the NBT fraction could also account for the high percentage preventive ratio.

In the indomethacin ulcer model (Table 5), the percentage preventive ratio for the various doses of ethanol extract were 7.5 %, 81 %, 91% and also, 91% for the prototype drug, cimetidine.

Table 4: Effect of ethanol extract of *S.dulcis* on ethanol –induced ulceration in rat

Treatment	Mean Ulcer Score	Percentage Preventive Ratio
Distilled water 10 mL/kg	3.90±0.05	-
Sd extract 50 mg/kg	1.67±0.40*	57.0%
Sd extract 100 mg/kg	1.33±0.03*	65.8%
Sd extract 150 mg/kg	0.67 ±0.01*	82.8%
Propranolol 40 mg/kg	0.60 ±0.02*	84.6%

Sd = *S. dulcis*, * $p \leq 0.05$ and $n=5$

Table 5: Effect of ethanol extract of *S.dulcis* on indomethacin –induced ulceration in rat

Treatment	Mean Ulcer Score	Percentage Preventive Ratio
Distilled water 10 mL/kg	5.30±0.90	-
Sd extract 50 mg/kg	1.33±0.02*	7.49%
Sd extract 100 mg/kg	1.00±0.02*	81.1%
Sd extract 150 mg/kg	0.50 ±0.03*	90.5%
Cimetidine 100 mg/kg	0.50 ±0.02*	90.5%

Sd = *S. dulcis*, * $p \leq 0.05$ and $n=5$

Table 6: Effect of fractions of *Spondia dulcis* on ethanol-induced ulcer in rat

Treatments	Mean ulcer score	Percentage Preventive Ratio
Distilled water 10 mL/kg	5.00 ± 0.01	-
NH fraction 100 mg/kg	3.33 ± 0.02	33%
DCM fraction 100 mg/kg	3.33 ± 0.03	33%
ETH fraction 100 mg/kg	3.33± 0.02	33%
NBT fraction 100 mg/kg	1.33 ± 0.02*	73%
AQ fraction 100 mg/kg	3.33 ± 0.02	33%
Propranolol 40mg /kg	0.33 ± 0.02*	93%

NH= normal hexane, DCM= dichloromethane, ETH= ethyl acetate; NBT= normal butanol;

AQ= aqueous * $p \leq 0.05$ and $n=5$

The use of ethanol in induction of ulcer is known to cause gastric necrotic damage, infiltration of subsequent inflammatory cells with associated release of nitric oxide, bicarbonates, and gastric mucus. Also, ethanol in the induction of oxidative stress, surges malondialdehyde production while glutathione decreases [23]. Propranolol as standard drug in ethanol-induced ulcer study is known to maintain the integrity of gastric mucosa [25, 26]. The ability of the ethanol leaf extract of *S. dulcis* to prevent mucosal membrane against ethanol-induced ulceration may be connected to its ability to block these processes and also acting in a similar manner as the standard drug.

Indomethacin is thought to induce gastric damage through the inhibition of the release of protective factors like COX-1 and prostaglandin E2, bicarbonate and mucus thus causing an inflow in the amount of gastric acid and increased oxidant parameters. Thus, the ulcer ameliorating effects of the extract and fractions on indomethacin experiment may be linked to the extract/fractions blocking these mechanisms [24, 25, 28].

Conclusion

The findings of this study therefore afford scientific credence on the use of the leaf of *S. dulcis* by the indigenous people of Usaka in the management of stomach disorders and also, add to available literature on the biological actions of *S. dulcis*

Conflict of interest

None

Contribution of authors

UFU conceptualized, supervised the study and did the final vetting of the manuscript, OJE carried out animal study, MNI undertook phytochemical study, OAO drafted the original manuscript while UAS and EJ supervised collection and preparation of plant materials.

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