Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

ORIGINAL ARTICLE

Natural Gastroprotective Remedy from the Branches of *Spondias tuberosa* Arruda

Amanda Dias de Araújo[1,2], George Souza Feitoza[1], Fernanda Granja da Silva Oliveira[3], Bruno Oliveira de Veras[1,4], Francinete Francis Lacerda[5], Nicácio Henrique da Silva[1], Wolfgang Harand[1,2], Silvania T. Paz[6], Mário Ribeiro de Melo-Júnior[6], Jackson Roberto Guedes da Silva Almeida[3], Marcia Vanusa da Silva[1,2], Maria Tereza dos Santos Correia[1]

[1]Departments of Biochemistry, Federal University of Pernambuco, [2]National Institute of Semiarid/Ministry of Science, Technology and Innovation (INSA/MCTI), Paraiba, Brazil, [3]Center for Studies and Research of Medicinal Plants, Federal University of San Francisco Valley, Pernambuco, Brazil, [4]Post-Graduation in Tropical Medicine, Federal University of Pernambuco, Brazil, [5]Agronomic Institute of Pernambuco – IPA, Brazil, [6]Department of Pathology, Federal University of Pernambuco, Brazil

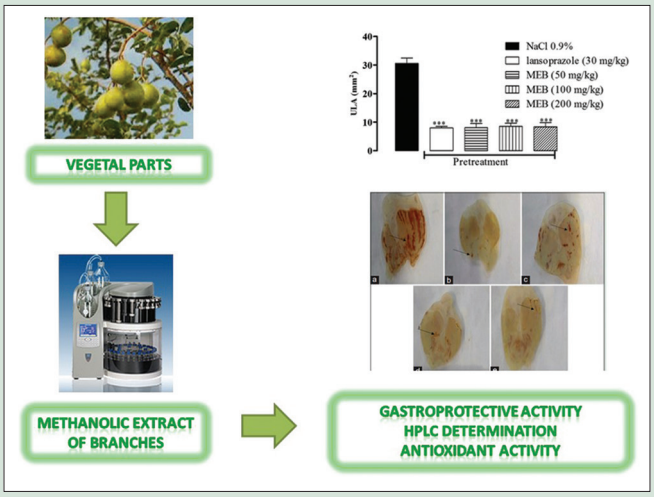
ABSTRACT

Background: Gastric ulcers are a worldwide health problem and their poor healing is one of the most important causes for their recurrence. Several species of *Spondias* are used in traditional medicine for the treatment of gastrointestinal diseases, infections, and inflammation, among other conditions. **Objectives:** The aim of this study was to investigate the antioxidant properties *in vitro* and gastroprotective effect in mice of the methanolic extract of branches of *Spondias tuberosa* (MEB). MEB was screened for antioxidant activities using different methods. **Materials and Methods:** Measurements of total phenolic compounds, flavonoids, and tannins were also evaluated. The profile of phenolic compounds of extract was performed by high-performance liquid chromatography (HPLC). MEB was investigated for acute and chronic toxicity and gastroprotective effects against ethanol-induced lesions in Swiss mice. **Results:** The extract showed antioxidant activity *in vitro* and high content of phenolic compounds, flavonoids, and tannins. The HPLC results identified the presence of gallic acid, chlorogenic acid, caffeic acid, and *t*-ferulic acid. The MEB showed no acute and chronic toxicity and in doses of 50, 100 and 200 mg/kg, v. o., reduced the area of ulcerative lesions induced by ethanol in 73.74, 72.02, and 72.40%, respectively. **Conclusion:** The oral treatment with the extract of *S. tuberosa* branches (MEB) showed gastroprotective and antioxidant activities.

Key words: Antioxidants, gastroprotective, medicinal plants, *Spondias tuberosa*, umbu

SUMMARY

- The gastroprotective activity and acute and chronic toxicity of extracts from the branches of *Spondias tuberosa* were evaluated by means of an *in vivo* study in Swiss mice, however, the MEB showed no toxicity and at doses of 50, 100 and 200 mg / kg v.o. reduced the area of ulcerative lesions. A profile of phenolic compounds was carried out and evaluated by HPLC and the extract showed high levels of phenolic compounds, flavonoids and tannins. The study indicates that the MEB of *Spondias tuberosa* showed gastroprotective activity in acute gastric lesions, with no signs of toxicity.



Abbreviations Used: HPLC: High-performance liquid chromatography, IPA: Agronomic Institute of Pernambuco, MEB: Methanolic extract of branches of *Spondias tuberosa*, NSAIDs: Nonsteroidal anti-inflammatory drugs, ROS: Reactive oxygen species, *S. tuberosa: Spondias tuberosa*, ULA: Ulcerative lesion area.

Correspondence:
Dr. Amanda Dias de Araújo,
Department of Biochemistry, Federal University of Pernambuco, 50670-901, Recife, Pernambuco, Brazil.
E-mail: amandabiologa1@gmail.com
DOI: 10.4103/pr.pr_43_20

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

Gastric ulcer is a very frequent disease in the clinical practice and a challenge in the gastroenterology research, which is considered a global health problem which affects approximately 14.5 million people worldwide.[1] Its occurrence is related largely to genetic, endogenous (pathophysiological disorders), and exogenous factors (*Helicobacter pylori* infection, stress, smoking, alcohol consumption, and use of nonsteroidal anti-inflammatory).[2,3] The progression of this condition is attributed to an imbalance between aggressive and protective factors of the mucous membranes.[4]

Moreover, the body has defense mechanisms (mucus–bicarbonate barrier, phospholipids, epithelial surface coating, lipoprotein layer of the membrane, blood flow, synthesis of prostaglandins, nitric oxide, and antioxidant system) protecting the mucosa from injury.[5] Several factors

Cite this article as: de Araújo AD, Feitoza GS, Oliveira FG, de Veras BO, Lacerda FF, da Silva NH, *et al*. Natural gastroprotective remedy from the branches of *Spondias tuberosa* arruda. Phcog Res 2020;12:455-9.

Submitted: 30-Apr-2020 **Revised:** 25-Jun-2020
Accepted: 18-Sep-2020 **Published:** 23-Jan-2021

culminated in the development of ulcers, including alcohol consumption, which leads to severe damage to the gastric mucosa directly and indirectly through mediators such as reactive oxygen species (ROS) and cytokines.[1]

Due to the growing interest in natural products and alternative therapies, extensive research is being conducted on herbs to discover and identify remedies and key compounds for the management of peptic ulcers.[6] There are numerous secondary effects associated with drugs used to treat ulcers, including arrhythmia, impotence, gynecomastia, and hematopoietic disorders. Therefore, new approaches have been sought to improve the efficiency of current drugs or to discover new potential agents that are more effective, are less expensive, and have fewer side effects associated with the health of the currently treatments used.[7] Among the numerous secondary metabolites that are associated with ulcerative process reduction are tannins, phenolic, and flavonoid compounds, which are considered compounds with high antioxidant activity, which protect cells and tissues from oxidative damage and chronic disease.[8]

Spondias tuberosa Arruda (Anacardiaceae), known as "umbuzeiro," is a tropical plant that plays an important role in the northeast of Brazil, being an important nutritional resource.[9,10] This species has a great ecological, social, and economic importance. In folk medicine, the plant is widely used for its antioxidant,[10] antibacterial,[11] and anti-inflammatory activities.[12] *S. tuberosa* is rich in phenolic compounds and flavonoids which are a group of naturally occurring secondary metabolites and have numerous pharmacological effects evaluated (anti-inflammatory, antimicrobial, and gastroprotective).[13]

In view of traditional reports on healing potential, this study aimed to evaluate the chemical composition and antioxidant activity *in vitro* and investigate the gastroprotective effects of methanolic extract from the branches of *S. tuberosa* (MEB) against gastric ulcers induced by ethanol in mice.

MATERIALS AND METHODS

Plant material and preparation of extract

The plant was collected in Catimbau National Park, Pernambuco, Northeastern Brazil (coordinates: 8°36'35"S and 37°14'40"W), being identified and authenticated in the Herbarium of the Agronomic Institute of Pernambuco (IPA), with under code number 91090.

The MEB was prepared using the branches, which were oven dried at 45°C. The material was pulverized using a mill (Tecnal/Willye mill/ET-650) to obtain the powder. The extract was obtained in an accelerated solvent extractor (ASE 350 Dionex®), equipped with a hermetically sealed stainless-steel extraction cell and 22 mm × 50 mm paper cartridge, a rinse flask, and 250 mL. The extract was concentrated under a stream of nitrogen in a heating block at 60°C. Twenty grams of powder was transferred to each cell and was extracted with methanol and then dried at 50°C using a rotary evaporator.

Determination of total phenolic content

The total phenolic content was determined by the method of Krepsky *et al.*[14] Initially, the extract was mixed with 1 mL of Folin–Ciocalteu reagent (1:10 v/v). After 4 min, 800 µL of saturated sodium carbonate solution (75 g/L) was added. After 2 h incubation at room temperature and protected from light, the absorbance was measured at 765 nm. Gallic acid was used as standard. The results were calculated as gallic acid equivalent (GAE) per gram dry weight (DW) (mg GAE/g DW) and reported as mean value ± standard deviation (SD). All experiments were performed in triplicate.

Determination of total flavonoid content

The total flavonoid content in the extract was determined by colorimetric method, using quercetin as a standard. Five hundred microliters of the diluted sample was added to 500 µL of aluminum chloride ($AlCl_3$) solution prepared in 2% methanol. After 30 min of incubation at room temperature and protected from light, the absorbance was measured at 420 nm. The flavonoid content was estimated using a standard curve of quercetin (5–35 mg/mL), and the results were expressed in milligram equivalents of quercetin (QE) per gram DW (mg QE/g DW) and reported as mean value ± SD.[15] All experiments were performed in triplicate.

Profile of phenolic compounds by high-performance liquid chromatography

The chromatographic profile was performed with a liquid chromatograph Agilent Technologies high-efficiency brand model 1260 LC INFINITY SYSTEMS (Santa Clara, California, United States of America) with quaternary pump, photodiode array detector (DAD) with automatic injector. Data were processed in, Agilent OpenLAB CDS software (Santa Clara, CA, United States of America). (EZChrom Edition). Vers. A.04.05. Zorbax SB C_{18} column (250 × 4.6 mm, 5 µm) with a mobile phase gradient of 0.3% acetic acid (solvent A) and acetonitrile (B) gradient: 0 min: 92 (A) % and 8% (B); 15 min: 65% (A) and 35% (B); 17–20 min: 92% (A) and 8% (B). The flow was maintained constantly at 2.4 mL/min and detection was carried out at 256 to acquire ultraviolet spectra in the range of 200–400 nm. The column temperature was set at 30°C ± 0.8°C and pressure at 400 bar.

ABTS radical-scavenging assay

The methodology described by Uchôa *et al.*[10] was used in this analysis. The ABTS radical was formed by reacting 7 mM ABTS stock solution with 140 mM potassium persulfate. All experiments were performed in triplicate. The percentages of oxidative inhibition were calculated and plotted against the reference antioxidant concentration (Trolox) and expressed as trolox equivalent antioxidant capacity in the unit (µM).

2,2-diphenyl-1-picrylhydrazyl radical-scavenging assay

Free radical scavenging activity was measured by means of hydrogen donation using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH).[16] All experiments were performed in triplicate. The DPPH scavenging effect was measured using Equation 1:

$$\% = \frac{(A_c - A_s)}{A_c} \times 100$$

where A_c is the absorbance of the control and *As* the absorbance in the presence of the extract.

Phosphomolybdenum assay

The activity was performed according to Sharma methodology,[17] based on the reduction of molybdenum (VI) to molybdenum (V), in the presence of antioxidants in acid pH. All experiments were performed in triplicate. The activity (%) was calculated using Equation 2:

$$\% = \frac{(A_{aa} - A_s)}{A_{aa}} \times 100$$

where A_{aa} is the absorbance of ascorbic acid and A_s the absorbance in the presence of the extract.

Animals

Swiss male mice (*Mus musculus)* weighing 25–30 g, obtained from the Immunopathology Laboratory Keizo Asami (LIKA)-UFPE was fed with Presence® diet and free water access, under standard light-dark cycles (12/12 h), humidity (60 ± 1%), and temperature (21°C ± 2°C). Before the experiments, animals were subjected to fasting for 16 h and housed in fasting cages to prevent coprophagy. The experimental protocol was approved by the Animal Ethics Committee of UFPE (No. 23076.052951/2015-71).

Acute and chronic toxicity

The animals were subjected to fasting 12 h and divided into four groups (n = 6). The groups received vehicle (0.9% NaCl) as the negative control, or a MEB single dose of 500, 1000, and 2000 mg/kg, v.o. In order to assess the behavioral changes as well as the occurrence of death, behavioral parameters were noted during the first 24 hr and once a day until complete 72 hr.[18] During the 14 days of observation, the animals were measured daily water consumption, feed, and weight and were sacrificed at the end of the period.

Ethanol-induced gastric ulcers

The animals were divided into five groups (n = 6) and were subjected to fasting for 16 h and divided into groups pretreated orally. Group I served as negative control which received only vehicle (0.9% NaCl, v. o.); Group II served as positive control and received lansoprazole (30 mg/kg, v. o.); Groups III, IV, and V received MEB in the doses 50, 100, and 200 mg/kg, v.o., respectively. After 50 min, 0.2 ml of an absolute ethanol (damaging agent solution) was administered orall and 1 h later, the animals were euthanized. The stomachs were removed, opened along the greater curvature, washed, and photographed to determine the ulcerative lesion area (ULA-mm^2)[19,20] using computerized planimetry and software *ImageJ*. The percentage of inhibition was calculated using Equation 3:

$$\% = \left[\frac{(\text{ULA negative control - ULAtreatedgroup})}{(\text{ULA negative control})}\right] \times 100$$

Table 1: Quantification of phenolic compounds, flavonoids, and tannins in methanolic extract of branches of *Spondias tuberosa*

Parameters	MEB
Total Phenolic Compounds (mg EAG/g DW)	193.95±0.10
Flavonoids (mg EQ/g DW)	21.61±0.70

Values are expressed as mean±SEM of three replicates. EAG: Equivalent of gallic acid; EQ: Equivalent to quercetin; EC: equivalent to catechin; DW: Dry weight; MEB: Methanolic extract of branches of *Spondias tuberosa*; SEM: Standard error of mean

Table 2: Phenolics compounds identified in extract of branches of *Spondias tuberosa* (methanolic extract of branches of *Spondias tuberosa*) by high-performance liquid chromatography

Polyphenolic compounds	Retention time (min)	MEB (µg/mg of extract)
Gallic acid	1.81	9.8
Chlorogenic acid	4.24	33.6
Catechin	4.45	25.2
Caffeic acid	5.44	4.8
Rutin	8.20	0.82
t- Ferulic acid	8.36	3.22

Values are expressed as mean±SEM of three replicates. SEM: Standard error of mean; MEB: Methanolic extract of branches of *Spondias tuberosa*

Statistical analysis

The results were expressed as mean ± SD, and analysis of variance was used followed by Dunnett's posttest and analyzed with GraphPad® Prism version 5.0 (San Diego, California, United States of America).

RESULTS

The yield of MEB was 26.33%. The MEB is rich in phenolic componds, with flavonoids, as shown in Table 1.

From the chromatographic analysis of the MEB, it was possible to identify the presence of gallic acid, chlorogenic acid, catechin, caffeic acid, rutin, and ferulic acid [Table 2]. All these compounds are considered to have antioxidants and health benefits.[21] In the present work, these compounds showed promising results for gastroprotection.

The results obtained in the gastric ulcer model induced by absolute ethanol showed that MEB significantly decreased gastric lesions when compared to the control group.

Figures 1 and 2 show that lansoprazole (30 mg/Kg; 73,84 %) and MEB at doses of 50, 100, and 200 mg/kg protected the gastric mucosa in 73.74%, 72.02%, and 72.40%, respectively, compared to the group pretreated with 0.9% NaCl solution.

The results obtained indicate that MEB is a source of bioactive compounds, being promising in studies of new natural antioxidant and gastroprotective agents, serving as a basis for the development of new drugs with possible application in the prevention and treatment of various diseases.

DISCUSSION

The presence of phenolic compounds and flavonoids has been positively associated with antioxidant activity with potential beneficial health effects, such as stress oxidation reduction, cancer prevention, blood lipid control and related diseases, and prevention of cardiovascular problems and complications of diabetes.[21] This activity is due to its ability to eliminate and neutralize free radicals.[22]

Studies show that the consumption of phenolic compounds found in MEB prevents diseases. Sato *et al.*[23] showed the antioxidant activity of chlorogenic acid *in vitro* and *in vivo* and its main role without protective effect against ischemia-reperfusion injury. Catechins has been described as a strong gastroprotective agent, as in the study by Sato *et al.*[23] who found that this flavonoid has a protective effect on gastric mucous cells, a protective effect on the gastric mucosal barrier, and an inhibitory effect on gastric acid secretion. Gallic acid has been described with antimicrobial activity against *H. pylori* and antiulcerative activity.[24] Rutin is one of the phenolic compounds found in abundance, which has anticancer, antioxidant, antibacterial, and antiulcerative activities.[25] Studies show the antibacterial activity of caffeic acid against *H. pylori,* and it's antiulcerative activity.[24,26] Ferulic acid has antibacterial, antioxidant, and anti-inflammatory activities.[27] All compounds found in MEB indicate its great potential in the fight against gastric ulcer formation, among other diseases.

Free radicals are constantly generated in living beings and can cause maximum damage to biomolecules, leading to different types of disease. An alternative solution of this problem is to produce natural antioxidants from plant products. Many phytochemical compounds are known to support bioactive activities and thus responsible for the antioxidant activity.[28,29] The elimination capacity of the radical cation ABTS of MEB was evaluated, presenting a value of 2145.56 ± 10.18 µM Trolox/g DW. The extract showed antioxidant activity dependent of the concentration with IC_{50} 103.75 ± 0.50. The antioxidant activity of the MEB determined by the phosphomolybdenum method was 34.98 ± 0.06% compared to that of the ascorbic acid, which was considered to be 100%. The

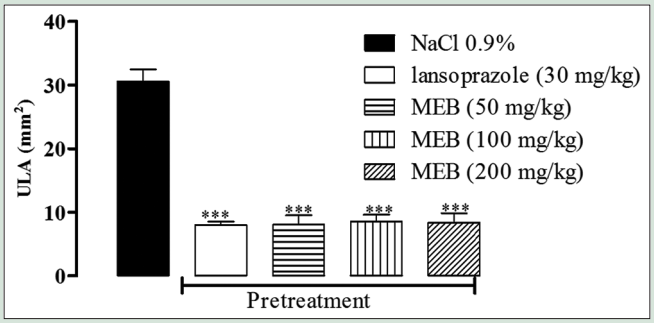


Figure 1: Effect of the oral pretreatment of methanolic extract of branches of *Spondias tuberosa* gastric lesions induced by ethanol in mice. The results are expressed as the mean ± standard deviation analysis of variance followed by Dunnett test, ***$P < 0.001$ ($n = 6$)

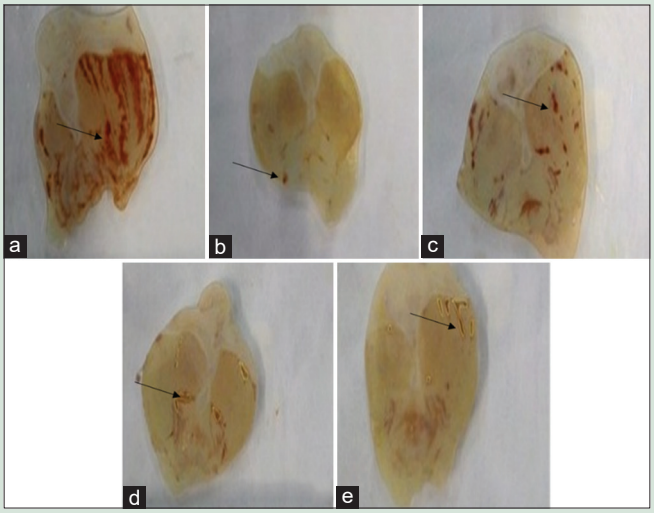


Figure 2: Macroscopic appearance of rat stomachs with ethanol-induced gastric ulcers after treatment with (a) NaCl 0.9% (v.o.), (b) lanzoprazole (30 mg/kg, v.o.), (c) methanolic extract of branches of *Spondias tuberosa* (50 mg/kg, v.o.), (d) methanolic extract of branches of *Spondias tuberosa* (100 mg/kg, v.o.) and (e) methanolic extract of branches of *Spondias tuberosa* (200 mg/kg, v.o.). Arrows indicate ulcer formation

antioxidant activity of MEB may be related to higher content of total phenols and flavonoids.

Many studies with the genus Spondias have demonstrated the presence of compounds, which were responsible for several biological activities, such as antioxidant, anti-inflammatory, antiulcerative and antimicrobial, indicating the high therapeutic potential of the species *S. tuberosa*.[24,30] Silva *et al.*[31] verified that the methanol extract of leaves of *S. tuberosa* has antibacterial activity against Gram-positive and Gram-negative bacteria. Santos *et al.*[32] verified antioxidant activity in the MEB, indicating that food intake of this fruit can bring beneficial health effects through this activity. Cabral *et al.*[33] found that the leaves of *Spondias mombin* have high anti-inflammatory activity, and this was associated with the presence of chlorogenic acid, which has antioxidant activity.

Medicinal plants have been recognized for their healing power from various diseases such as diabetes, cancer, Alzheimer's, and gastritis. However, some have toxicity, making their use unfeasible.[34] MEB was evaluated for acute and chronic toxicity in Swiss mice. During the toxicity test, when mice were administered doses of 100 and 5000 mg/kg, the MEB did not alter the body mass of animals. During treatment, there were no clinical signs of toxicity and no death was recorded, as well as there was no change in water consumption and animal feed. The results show that oral administration of the extract did not produce toxic effects in adult Swiss mice, indicating toxicological safety for its use.

There is evidence that ethanol administration promotes oxidative stress by increasing ROS and decreasing cellular oxidative defences in a process triggered by the activation of neutrophils, causing a sequential induction of ROS mediated by lipid peroxidation and protein oxidation.[35] The generation of ROS caused by the administration of absolute ethanol induces an inflammatory response, which releases several inflammatory cytokines such as tumor necrosis factor-α and interleukin-6.[36,37] Several compounds of vegetable origin (flavonoids, saponines, tannins, gums, and mucilages) show antiulcer activity.[38] According to Potrich,[39] these compounds may have both anti-inflammatory and anti-ulcer activities, an advantage over traditional anti-inflammatory drugs that are mostly ulcerogenic.

MEB was promising for gastroprotection in animal models with ethanol-induced ulcers. This study investigated the potential of MEB of *S. tuberosa* as a protector of the gastric mucosa in gastric ulcer model induced by ethanol in mice. The gastroprotection presented in this study can be explained by the powerful antioxidant activity of the extract. There are several reports in the literature emphasizing that the administration of natural antioxidant products can prevent gastric lesions induced by ethanol action,[40] including species of *Spondias*.[41] This study proved that the MEB *S. tuberosa* acts as a natural antioxidant with gastroprotective action. This can be attributed to their secondary metabolites, such as gallic acid, chlorogenic acid, caffeic acid, and *t*-ferulic acid. In summary, the *in vitro* and *in vivo* studies confirmed the antioxidant as well as gastroprotective effect potential of extract of *S. tuberosa* branches and it was comparable with lanzoprazole, a standard gastroprotective drug.

CONCLUSION

The results indicate that the MEB of *S. tuberosa* has gastroprotective activity in acute gastric injury models induced by ethanol, which can be related to its powerful antioxidant activity, with no signals of toxicity. In addition, it contains molecules with great biotechnological potential for the development of new drugs with potential application in the prevention and treatment of pathologies triggered by the action of free radicals.

Acknowledgements

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for supporting and funding research through the Green Socioeconomic project in the Caatinga Biome in the face of Climate Change Project No. 441227/2017-1 Public Notice 19/2017 CNPq.

Financial support and sponsorship

This study was financially supported by he Green Socioeconomic project in the Caatinga Biome in the face of Climate Change Project No. 441227/2017-1 Public Notice 19/2017 CNPq.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Rozza AL, Meira de Faria F, Souza Brito AR, Pellizzon CH. The gastroprotective effect of menthol: Involvement of anti-apoptotic, antioxidant and anti-inflammatory activities. PLoS One 2014;9:e86686.
2. Kang JM, Seo PJ, Kim N, Lee BH, Kwon J, Lee DH, *et al.* Analysis of direct

medical care costs of peptic ulcer disease in a Korean tertiary medical center. Scand J Gastroenterol 2012;47:36-42.

3. Zheng YF, Xie JH, Xu YF, Liang YZ, Mo ZZ, Jiang WW, *et al*. Gastroprotective effect and mechanism of patchouli alcohol against ethanol, indomethacin and stress-induced ulcer in rats. Chem Biol Interact 2014;222:27-36.
4. Barbosa JA, Santana MA, Leite TCC, Oliveira TB, Mota FVB, Bastos, IVGA, *et al*. Gastroprotective effect of ethyl acetate extract from Avicennia schaueriana Stapf Leechman and underlying mechanisms. Biomed Pharmacother 2019;112:108582.
5. Antonisamy P, Subash-Babu P, Alshatwi AA, Aravinthan A, Ignacimuthu S, Choi KC, *et al*. Gastroprotective effect of nymphayol isolated from *Nymphaea stellata* (Willd.) flowers: Contribution of antioxidant, anti-inflammatory and anti-apoptotic activities. Chem Biol Interact 2014;224:157-63.
6. Yeo D, Hwang SJ, Kim WJ, Youn HJ, Lee HJ. The aqueous extract from *Artemisia capillaris* inhibits acute gastric mucosal injury by inhibition of ROS and NF-kB. Biomed Pharmacother 2018;99:681-7.
7. Almasaudi SB, El-Shitany NA, Abbas AT, Abdel-dayem UA, Ali SS, Al Jaouni SK, *et al*. Antioxidant, Anti-inflammatory, and antiulcer potential of Manuka honey against gastric ulcer in rats. Oxid Med Cell Longev 2016;2016:1-11.
8. Omena CM, Valentim IB, Guedes GS, Rabelo LA, Mano CM, Bechara EJH, *et al*. Antioxidant, anti-acetylcholinesterase and cytotoxic activities of ethanol extracts of peel, pulp and seeds of exotic Brazilian Fruits. Food Research International 2012;49:334-344.
9. Sotelo T, Cartea ME, Velasco P, Soengas P. Identification of antioxidant capacity -related QTLs in Brassica oleracea. PLoS One 2014;9:e107290.
10. Uchôa AD, Oliveira EF, Pereira AP, Silva AG, Cordeiro BM, Malafaia CB, *et al*. Antioxidant activity and phytochemical profile of *Spondias tuberosa* Arruda leaves extracts. American J Plant Sci 2015;6:3038-44.
11. da Silva AR, de Morais SM, Marques MM, de Oliveira DF, Barros CC, de Almeida RR, *et al*. Chemical composition, antioxidant and antibacterial activities of two Spondias species from Northeastern Brazil. Pharm Biol 2012;50:740-6.
12. Siqueira EM, Félix-Silva J, Araújo LM, Fernandes JM, Cabral B, Gomes JAS, *et al*. *Spondias tuberosa* (Anacardiaceae) leaves: Profiling phenolic compounds by HPLC-DAD and LC-MS/MS and *in vivo* anti-inflammatory activity. Biomed Chromatogr 2016;30:1656-65.
13. Pereira AC, Lenz D, Nogueira BV, Scherer TU, Costa HB, *et al*. Gastroprotectiveactivity of theres in from *Virola oleifera*. Pharm Biol 2016;55:472-80.
14. Krepsky PB, Isidório RG, de Souza Filho JD, Côrtes SF, Braga FC. Chemical composition and vasodilatation induced by *Cuphea carthagenensis* preparations. Phytomedicine 2012;19:953-7.
15. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Analysis 2002;10:178-82.
16. de Veras BO, Dos Santos YQ, Oliveira FG, Almeida JR, da Silva AG, Correia MTDS, *et al*. Algrizea Minor Sobral, Faria Proença (Myrteae, Myrtaceae): Chemical composition, antinociceptive, antimicrobial and antioxidant activity of essential oil. Nat Prod Res 2020;34:3013-17.
17. Sharma S, Vig AP. Preliminary phytochemical screening and *in vitro* antioxidant activities of *Parkinsonia aculeata* Linn. Biomed Res Int 2014;2014:1-8.
18. Chinedu E, Arome D, Ameh FS. A new method for determining acute toxicity in animal models. Toxicol Int 2013;20:224-6.
19. Park SW, Oh TY, Kim YS, Sim H, Park SJ, Jang EJ, *et al*. *Artemisia asiatica* extracts protect against ethanol-induced injury in gastric mucosa of rats. J Gastroenterol Hepatol 2008;23:976-84.
20. Lemos AJ, Peixoto CA, Teixeira AA, Luna RL, Rocha SW, Santos HM, *et al*. Effect of the combination of metformin hydrochloride and melatonin on oxidative stress before and during pregnancy, and biochemical and histopathological analysis of the livers of rats after treatment for polycystic ovary syndrome. Toxicol Appl Pharmacol 2014;280:159-68.
21. Berlowski A, Zawada K, Wawer I, Paradowska K. Antioxidant properties of medicinal plants from peru. Food Nutrit Sci 2012;4:71-7.
22. Deepa G, Ayesha S, Muley A, Thankamani M. *In-vitro* antioxidant activity and phytochemical analysis in extracts of *Hibiscus rosa-sinensis* stem and leaves. Free Radicals Antioxidants 2012;2:41-6.
23. Sato Y, Itagaki S, Kurokawa T, Ogura J, Kobayashi M, Hirano T, *et al*. *In vitro* and *in vivo* antioxidant properties of chlorogenic acid and caffeic acid. Int J Pharm 2011;403:136-8.
24. Garro MF, Salinas Ibáñez AG, Vega AE, Arismendi Sosa AC, Pelzer L, Saad JR, *et al*. Gastroprotective effects and antimicrobial activity of *Lithraea molleoides* and isolated compounds against *Helicobacter pylori*. J Ethnopharmacol 2015;176:469-74.
25. Ganeshpurkar A, Saluja AK. The Pharmacological Potential of Rutin. Saudi Pharm J 2017;25:149-64.
26. Ghosh S, Chowdhury S, Sarkar P, Sil PC. Ameliorative role of ferulic acid against diabetes associated oxidative stress induced spleen damage. Food Chem Toxicol 2018;118:272-86.
27. Ibitoye OB, Ajiboye TO. Ferulic acid potentiates the antibacterial activity of quinolone-based antibiotics against *Acinetobacter baumannii*. Microb Pathog 2019;126:393-8.
28. Moo-Huchin VM, Moo-Huchin MI, Estrada-León RJ, Cuevas-Glory L, Estrada-Mota, IA, Ortiz-Vázquez E, *et al*. Antioxidant compounds, antioxidant activity and phenolic content in peel from three tropical fruits from Yucatan, Mexico. Food Chem 2015;166:17-22.
29. Olszowy M. What is responsible for antioxidant properties of polyphenolic compounds from plants? Plant Physiol Biochem 2019;144:135-43.
30. Xie L, Lin Q, Guo K, Tong, C, Shi S, Shi FI. HPLC-DAD-QTOF-MS/MS Based Comprehensive Metabolomic Profiling of Phenolic Compounds in *Kalimerisindica* Anti-Inflammatory Fractions Industrial Crops and Products; 2019.
31. Da Silva ARA, De Morais SM, Mendes Marques MM, Oliveira DF, Barros CC, Almeida RR, *et al*. Chemical composition, antioxidant and antibacterial activities of two Spondias species from Northeastern Brazil. Pharm Biol 2012;50:740-6.
32. Santos PA, De Rezende LC, Oliveira JCS, David JM, David, JPChemical study, antioxidant and cytotoxic activities of oil seeds of *spondias tuberosa* (Anacardiaceae). Int J Fruit Sci 2018;19:1-12.
33. Cabral B, Siqueira EM, Bitencourt MA, Lima AK, Ortmann CF, Chaves VC, *et al*. Phytochemical study and and anti-inflammatory and antioxidant potential of *Spondias mombin* leaves. Rev Brasileira de Farmacognosia 2016;26:304-11.
34. Atere TG, Akinloye OA. High dose of standardised extract of Costus afer leaves potentiates cadmium reproductive toxicity in Wistar rats. Andrologia 2019;51:e13360.
35. Hoyt LR, Randall MJ, Ather JL, DePuccio DP, Landry CC, Qian X, *et al*. Mitochondrial ROS induced by chronic ethanol exposure promote hyper-activation of the NLRP3 inflammasome. Redox Biol 2017;12:883-96.
36. Du Y, Zhao W, Lu L, Zheng J, Hu X, Yu Z, Zhu L. Study on the antiulcer effects of Veronicastrumaxillare on gastric ulcer in rats induced by ethanol based on tumor necrosis factor-α (TNF-α) and endothelin-1 (ET-1). Asian Pacific J Trop Biomed 2013;3:925-30.
37. Simplicio JA, Gonzaga NA, Nakashima MA, De Martins BS, Cunha TM, Tirapelli LF, *et al*. Tumor necrosis factor-α. Tumor necrosis factor-α receptor 1 contributes to ethanol-induced vascular reactive oxygen species generation and hypertension. J Am Soc Hyperten 2017;11:684-96.
38. Oyagi A, Ogawa K, Kakino M, Hara H. Protective effects of a gastrointestinal agent containing Korean red ginseng on gastric ulcer models in mice. BMC Complement Altern Med 2010;10:45.
39. Potrich FB, Allemand A, da Silva LM, Dos Santos AC, Baggio CH, Freitas CS, *et al*. Antiulcerogenic activity of hydroalcoholic extract of *Achillea millefolium* L.: Involvement of the antioxidant system. J Ethnopharmacol 2010;130:85-92.
40. Rocha NF, Oliveira GV, Araújo FY, Rios ER, Carvalho AM, Vasconcelos LF, *et al*. (-)-α-Bisabolol-induced gastroprotection is associated with reduction in lipid peroxidation, superoxide dismutase activity and neutrophil migration. Eur J Pharm Sci 2011;44:455-61.
41. Brito SA, de Almeida CL, de Santana TI, da Silva Oliveira AR, do Nascimento Figueiredo JC, Souza IT, *et al*. Antiulcer activity and potential mechanism of action of the leaves of *Spondias mombin* L. Oxid Med Cell Longev 2018;2018:1-20.