GASTROPROTECTIVE ACTIVITY OF ETHYL ACETATE FRACTION OBTAINED FROM THE STEM BARK EXTRACT OF DESERT DATE (*BALANITES AEGYPTIACA*)

*[1]Ugwah-Oguejiofor, C. J., [2]Ugwah, O. M.

[1]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria.
[2]Department of Pharmacy, Usmanu Danfodiyo University Teaching Hospital, P.M.B. 2370, Sokoto, Nigeria

*Corresponding authors' email: nenye789@yahoo.com Phone: +2348036098241

ABSTRACT

The study aimed to investigate the gastroprotective activity of the ethyl acetate fraction derived from the stem bark extract of the Desert date tree (EFDD) (Balanites aegyptiaca L. Delile; family Zygophyllaceae) using ethanol and indomethacin-induced ulcer models in Wistar rats. The study was conducted using Wistar rats and divided them into five groups (n=5). Group 1 received 10% Tween 20 (1 ml/kg), which served as the control group. Group 2 was administered the standard drug, omeprazole (20 mg/kg). Groups 3-5 were the extract groups and received doses of 125, 250, and 500 mg/kg, respectively, of EFDD. Two ulcer models were used in the study namely ethanol-induced ulcers and indomethacin-induced ulcers. After administration of the respective treatments, evaluation of the mean ulcer indices in each group and calculation of the percentage ulcer inhibition compared to the control group were carried out. The results indicated that the EFDD, at doses of 250 and 500 mg/kg, significantly reduced the mean ulcer indices in both the ethanol and indomethacin-induced ulcer models compared to the control group ($p<0.05$). This suggests that the EFDD possesses gastroprotective properties. In conclusion, the EFDD exhibits gastroprotective activity. This finding supports the traditional use of Desert date in folkloric medicine for the treatment of ulcers. However, further research is necessary to explore the underlying mechanisms responsible for the observed gastroprotective effects.

Keywords: Desert date, *Balanites aegyptiaca,* Gastroprotection, Ulcer, Ethyl acetate fraction, Phytochemistry

INTRODUCTION

Peptic ulcers are characterized by the presence of sores or lesions that extend throughout the gastrointestinal mucosa, including the *muscularis mucosae*. This condition involves various stages of increased oxidative stress, inflammation, necrosis, neutrophil infiltration, and reduction in blood flow (da Silva *et al.,* 2013). The development of peptic ulcer disease occurs when there is an imbalance between defensive and destructive factors in the stomach, with mucosal destructive factors such as pepsin, hydrochloric acid, and non-steroidal anti-inflammatory drugs (NSAIDs), and defensive factors like mucin secretion, bicarbonate barriers, prostaglandins, and mucosal blood flow (Tytgat, 2011; Proctor and Deans, 2014). When the destructive factors outweigh the defensive factors, ulcers may occur. This condition affects a significant portion of the global population, leading to increased morbidity and healthcare costs (Sharifi-Rad *et al*., 2018).

While various drug treatments have been employed to combat peptic ulcer disease, including acid-reducing pharmacological agents and H. pylori eradication regimens, these therapeutic measures have drawbacks such as high incidence of relapse, side effects, and tolerance (Sharifi-Rad *et al.,* 2018). Traditional medicines offer an alternative approach to overcome these limitations (Zewdu & Aragaw, 2020). Traditional medicines, including medicinal plants, have been used since ancient times for the treatment of diseases, and approximately 80% of the world's population relies on these traditional remedies for their healthcare needs (Hu *et al*., 2020). Numerous medicinal plants have been validated for their effectiveness in alleviating ulcer-related symptoms, including *Crepis sancta, Papaya carica, Cordia africana, Ficus thonningii, Lactuca sativa*, and *Balanites aegyptiaca* (Ebada *et al.,* 2020; Oloyede *et al.,* 2015; Yismaw *et al.,* 2020; Adane *et al*., 2021; Maheswari *et al.*, 2020; Ugwah *et al.,* 2019).

Balanites aegyptiaca, commonly known as Desert date and scientifically identified as *Balanites aegyptiaca* L. Delile of the Zygophyllaceae family, is a perennial tree primarily found in desert areas (Kabo *et al.,* 2020). Traditional medicine practitioners have utilized decoctions of the Desert date stem bark to treat stomach ulcers (Chothani & Vaghasiya, 2011). Previous scientific research has reported the antiulcer activity of the aqueous extract of this plant (Ugwah *et al.,* 2019). Given the significant impact of peptic ulcer disease on global morbidity, it is crucial to identify affordable and effective treatments that can cure the disease with minimal side effects. Our previous study on the aqueous extract has validated the claims of native traditional medicine practitioners. To further investigate whether the ethyl acetate fraction of Desert date (EFDD) could provide superior results compared to the crude aqueous extract, we conducted this study. Therefore, the aim of this study was to investigate the gastroprotective activities of the ethyl acetate fraction derived from the Desert date tree (EFDD).

MATERIAL AND METHODS

Plant material and fractionation

The stem bark of *Balanites aegyptiaca* was collected in the month of November, from Wamakko local government area in Sokoto state, Nigeria. It was identified and authenticated in the Department of Botany, Usmanu Danfodiyo University, Sokoto (UDUS). Voucher specimen (004B) of the plant was deposited at the herbarium of the same department. The stem bark was air-dried, pulverized using pestle and mortar, and extracted with distilled water using Soxhlet apparatus at 70°C. A 100 g of aqueous extract of *Balanites aegyptiaca* was dissolved in distilled water and poured into 1 L separating funnel and was exhaustively extracted by consecutive liquid/liquid partition with hexane (500 ml), chloroform (500 ml) and ethyl acetate fraction (500 ml) using a separating funnel. The ethyl acetate fraction realised was evaporated to

dryness to obtain the ethyl acetate fraction (Gandhi *et al*., 2003). The ethyl acetate fraction was used for the study. The percentage yield was calculated using this expression;

% yield = W_2/W_1 x 100

W_2 = weight of the extract in grams

W_1 = weight of plant material in grams

Experimental Animals

Male Wistar rats weighing between 150 and 200 g were procured from the animal house of the Department of Pharmacology and Toxicology at Usmanu Danfodiyo University in Sokoto. The rats were housed in well-constructed cages and allowed to acclimate for a period of 2 weeks. During this acclimation and experiment periods, the rats had unrestricted access to standard commercial chow and drinking water. The experimental protocol followed in this study adhered to the established guidelines for the care and use of laboratory animals as outlined in the guide for care and use of laboratory animals (2011), which ensures the welfare and ethical treatment of animals in research. The study received approval from the animal ethics committee of the Department of Pharmacology and Toxicology under the reference number PTAC/Ba/OT/002-18.

Phytochemical screening of the EFDD

Standard procedures were employed to conduct phytochemical screening of the EFDD. The screening aimed to identify the presence of various phytochemical compounds, including alkaloids, tannins, flavonoids, saponins, carbohydrates, steroids, and glycosides. The methods used for the screening followed established protocols as described in the literature (El-Olemy *et al*., 1994).

Acute toxicity study

The oral acute toxicity assessment of EFDD was conducted using the 'Up-and-Down' method in accordance with the guidelines outlined by the Organization for Economic Development (OECD) 425 (OECD, 2001). A single dose of 3000 mg/kg was administered orally to five female rats in this study. The procedure involved selecting one animal at a time, determining its weight, and administering an oral dose of the EFDD dissolved in 10% Tween 20, proportional to its weight. Each animal was closely observed for any signs of behavioural toxicity immediately following the administration of the fraction. Subsequently, a monitoring period of 14 days was initiated to assess any potential long-term lethal outcomes.

Antiulcer evaluation

Ethanol-induced gastric ulcer model

The rats were assigned randomly to five groups (n=5). Prior to the experiment, the animals underwent a fasting period of 24 hours, during which they had unrestricted access to water for up to 2 hours (Ugwah-Oguejiofor *et al.,* 2017). Group 1 received 10% Tween 20 at a dosage of 1 ml/kg, while Group 2 received Omeprazole at a dosage of 20 mg/kg. Groups 3-5 were the EFDD groups and received dosages of 125, 250, and

500 mg/kg of the EFDD respectively. To induce oral gastric lesions, absolute ethanol was orally administered to the rats at a dosage of 8 ml/kg after one hour of treatment. Two hours after the administration of ethanol, the animals were euthanized by cervical dislocation. The stomachs were then excised and opened along the greater curvature. The ulcer index (UI), a measure of the severity of gastric lesions, was determined using established protocols (Arun & Asha, 2008).

Indomethacin-induced gastric model

The rats were assigned randomly to five groups (n=5). Prior to the study, the rats underwent the fasting protocol as previously described. In Group 1, the rats were orally pre-treated with 10% Tween 20 at a dosage of 1 ml/kg. Group 2 received the standard drug, omeprazole, at a dosage of 20 mg/kg. Groups 3, 4, and 5 were pre-treated with the EFDD at dosages of 125, 250, and 500 mg/kg, respectively. According to Nwafor *et al.,* (2000), after a one-hour interval, oral gastric lesions were induced by administering 100 mg/kg p.o of indomethacin to all the groups. Following a four-hour period, the animals were euthanized by cervical dislocation, and their stomachs were excised and opened according to the previously described method. The UI were then calculated.

Measurement of ulcer index (UI)

The excised stomachs were carefully cut along the greater curvature, and the mucosa was rinsed with cold saline solution to eliminate any blood contaminants. Using a transparent millimeter scale rule, the surface area of each ulcerated region in the stomach was measured (Magaji et al., 2007). The cumulative length of all the lesions present in each stomach was designated as the ulcer index (UI).

To assess the percentage inhibition of ulcer formation, the following formula was utilized:

% Inhibition = (UIC - UIT) / UIC × 100

Here, UIC represents the ulcer index in the control group, and UIT represents the ulcer index in the group of rats subjected to the test substance (Navarrete et al., 1998). The resulting percentage provides a quantification of the inhibitory effect on ulcer formation.

Statistical analysis

The results were presented as Mean ± SD (standard deviation). Statistical analysis was performed using a one-way analysis of variance (ANOVA), followed by Dunnett's t-test for multiple comparisons. The statistical calculations were conducted using GraphPad Prism 6 software (Graph Pad Software Inc., CA, USA). A significance level was set at $p < 0.05$.

RESULTS

The yield of ethyl acetate fraction of *Balanites aegyptiaca*

The percentage yield of the EFDD was 2.6% w/w.

Phytochemical studies

The EFDD showed the presence of tannins, flavonoid, saponin and steroid (Table 1).

Table 1: Phytochemical analysis of ethyl acetate fraction of Desert date

Phytochemical constituents	Ethyl acetate fraction
Alkaloids	-
Tannins	+
Flavonoids	+
Saponins	+
Carbohydrate	-
Steroids	+
Glycosides	-

+ present; - absent

Acute toxicity study

The acute toxicity study was conducted using the Up and Down method, administering the EFDD at a maximum oral dosage of 3000 mg/kg. No observable signs of toxicity were detected, and no mortality was observed within the 48-hour period following administration. Furthermore, during the 14-day long-term observation period, no adverse effects or mortality were recorded.

Antiulcer evaluation

EFDD in ethanol-induced ulcer model

The EFDD at the doses of 250 and 500 mg/kg significantly ($p<0.05$) reduced the mean UI in the rats (Table 2). The percentage inhibition was much lower than that of the standard drug group (Figure 1).

Table 2: Ethyl acetate fraction of *Balanites aegyptiaca* in ethanol-induced ulcer model

Treatment	Dose (mg/kg)	Ulcer index
10% Tween 20	1 ml/kg	10.15 1.25
EFDD	125	8.90 ± 0.55
	250	7.45 ± 0.24*
	500	6.56 ± 0.11*
Omeprazole	20	3.50 ± 0.59**

EFDD = ethyl acetate fraction of Desert date; Values are mean $\pm$ SD, n=5; *$p<0.05$, **$p < 0.01$
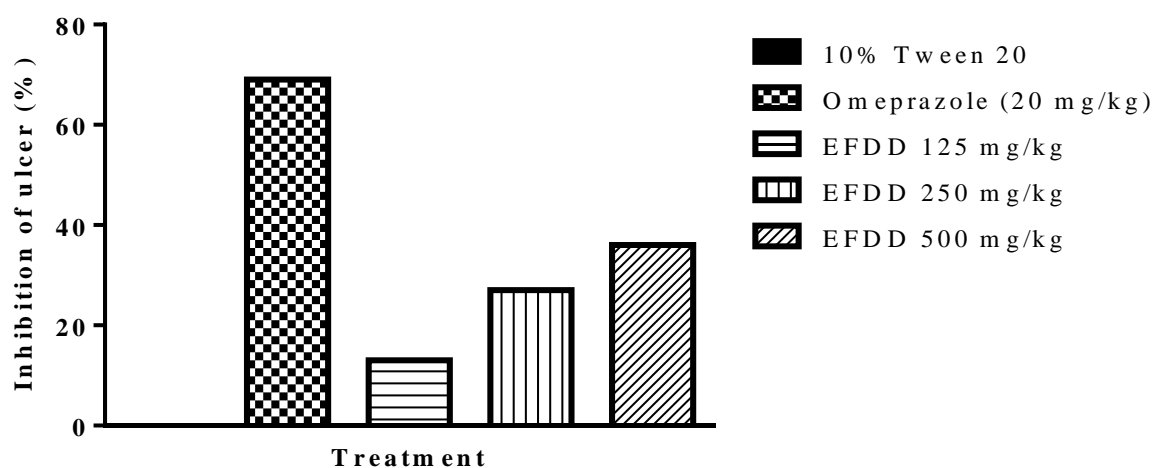

Figure 1: Ethyl acetate fraction of Desert date (EFDD) on percentage ulcer inhibition in ethanol-induced ulcers

EFDD in indomethacin-induced ulcer model

The EFDD produced a significant reduction in the mean UI ($p<0.05$) at 250 and 500 mg/kg when compared to the 10% Tween 20 group (Table 3). The percentage inhibition produced by 125 and 250 mg/kg were 10% and 6% respectively (Figure 2).

Table 3: Ethyl acetate fraction of the Desert date in indomethacin-induced ulcer model

Treatment	Dose (mg/kg)	Ulcer index
10% Tween 20	1 ml/kg	2.45 ± 0.45
EFDD	125	1.83 ± 0.36
EFDD	250	1.54 ± 0.29*
EFDD	500	1.53 ± 0.11*
Omeprazole	20	0.98 ± 0.08**

EFDD = ethyl acetate fraction of Desert date; values are mean $\pm$ SD, n=5; *$p<0.05$, **$p < 0.01$
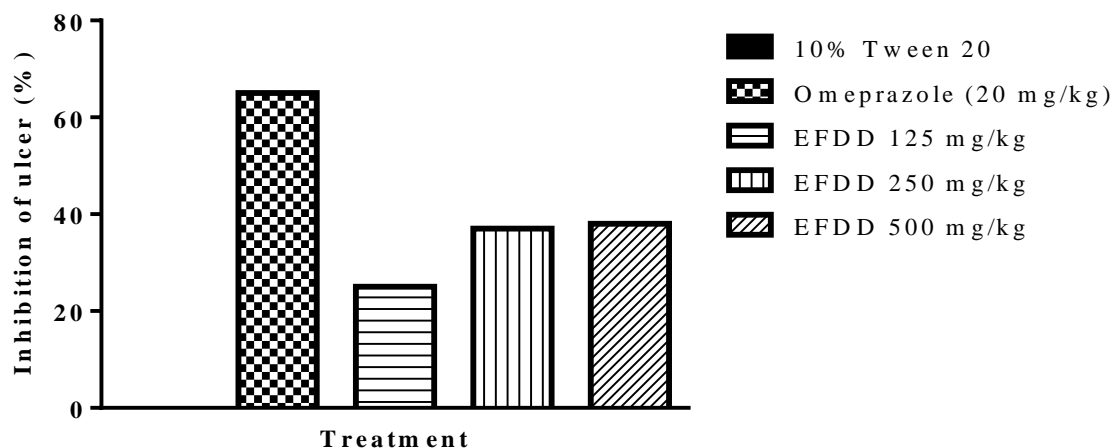
Figure 2: Ethyl acetate fraction of Desert date (EFDD) on percentage ulcer inhibition in indomethacin-induced ulcers

DISCUSSION

The study evaluated the gastroprotective activity of the EFDD using ethanol and indomethacin-induced ulcer models in Wistar rats. The data obtained showed that the fraction possessed gastroprotective property.

To study the cytoprotective activity of the EFDD, ethanol-induced gastric ulcer model was employed. Ethanol-induced ulcer formation occurs probably due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury (Guth *et al*., 1984). It induces a severe damage in gastric mucosa (impairment of defensive factor) by promoting a decrease in secretion of bicarbonate and mucus production which ultimately leads to the development of haemorrhagic and gastric mucosal injury (Hiruma-Lima *et al*.,2009). The EFDD exhibited its gastroprotective ability by inhibiting the ulcer index in the rats. This result is consistent with other gastroprotective studies using ethyl acetate fractions (Sobreira *et al*., 2017; Wang *et al*., 2018; Abubakar *et al*., 2020).

The use of indomethacin, an NSAID, is one of the principal risk factors in gastric ulcers. Through the inhibition of cyclo-oxygenase (COX-1) and (COX-2) activities, indomethacin hampers the functioning of prostaglandin synthase and directly causes cytotoxic effects on the epithelium. This process results in the suppression of prostaglandin synthesis and the induction of cellular damage within the epithelial layer (Moleiro *et al*., 2009). Prostaglandins E_2 confers Cytoprotection on the mucosal layer by inhibiting hydrochloric acid and stimulating mucus and bicarbonate secretions. In our study, pre-treatment of the rats with the fraction offered gastroprotection to the gastric mucosa against ulceration. The gastroprotective effect of the fraction may therefore be through prostaglandin-mediated pathway since non-prostanoid are known to protect gastric mucosa by mobilising endogenous prostaglandins (Ugwah *et al.,* 2019). Again this is consistent with other studies showing gastroprotective activity of agents (Ruiz-Hurtado *et al*., 2021). The phytochemical constituents of the EFDD detected were tannins, flavonoids, saponins and steroids. Tannins are astringent in nature. They form tannin-protein complex hence protect the mucosal membrane from gastric acid attack (Bigoniya *et al*., 2006). The tannin-protein complex layer apart from conferring protection to the stomach in the case of gastric ulcers by increasing chemical and mechanical resistance to injury (de Jesus *et al*., 2012), they also promote tissue repair and possess anti-*Helicobacter pylori* effects

(Vasconcelos *et al*., 2008). The presence of flavonoids in plants has been associated with their ability to effectively protect the gastrointestinal tract against ulcerative and erosive lesions, primarily due to their antioxidant properties (Harborne & Williams, 2000). Flavonoids are known to act by increasing the mucosal prostaglandin content, decreasing histamine secretion from mast cells, inhibiting histidine decarboxylase and by the inhibition of *Helicobacter pylori* growth (Yadav *et al*., 2021). Saponins is another phytochemical which was identified in the fraction. It has been shown to protect the stomach mucosa from acid by selectively inhibiting prostaglandin F_2, which causes vasoconstriction of mucosal blood vessels (Aguwa & Okunji, 1986). Plants rich in steroids have also been reported as antiulcer agent (Emmanuel *et al.,* 2020). This suggests that the antiulcer activity of the fraction may be due to the presence of these phytochemicals.

The oral acute toxicity (LD_{50}) of the EFDD was found to be >3000 mg/kg, this implies that the fraction may be considered to have a high degree of relative safety (Ani *et al*., 2021). However, additional studies are necessary to determine its toxic effects due to repeated and chronic administrations.

CONCLUSION

The EFDD demonstrated significant protection against ulcers induced by ethanol and indomethacin in the rats. This beneficial effect can be attributed to the presence of tannins, flavonoids, saponins, and steroids within the fraction. Additionally, the LD_{50} (median lethal dose) of the fraction is estimated to be greater than 3000 mg/kg, indicating a relatively high safety margin. Further investigations are warranted to isolate and identify the specific active compound(s) responsible for the observed gastroprotective effects of the fraction.

CONFLICT OF INTEREST

None declared

REFERENCES

Abubakar, I., Yankuzo, H. M., Baraya, Y. S. & Gusau, M. A. (2020). Gastroprotective effect of ethylacetate fraction of the leaves of Hannoa klaineana on aspirin and histamine-induced gastric ulcer in rats. *International Journal of Pharmacology and Toxicology*, *8*(1): 70-77.

Adane, H., Atnafie, S. A., Kifle, Z. D. & Ambikar, D. (2021). Evaluation of In Vivo Antiulcer Activity of Hydro-Methanol Extract and Solvent Fractions of the Stem Bark of *Ficus thonningii* (Moraceae) on Rodent Models. *BioMed Research International*, 2021.

Aguwa, C. N. & Okunji, C. O. (1986). Gastrointestinal studies of *Pyrenacantha staudtii* leaf extracts. *Journal of Ethnopharmacology*, 15(1): 45-55.

Ani, N., Ihim, S. A., Ikechukwu, P., Onwuka, A. & Nworu, C. S. (2021). Antiulcer activities of the methanol leaf extract and fractions of *Commelina ascendens* (Commelinaceae) using *invitro* and *invivo* Models. *Pharmacology Online*, 1: 336-348.

Arun, M. & Asha, V. V. (2008). Gastroprotective effect of *Dodonaea viscosa* on various experimental ulcer models. *Journal of Ethnopharmacology, 118*(3): 460-465.

Bigoniya, P., Rana, A. C., & Agrawal, G. P. (2006). Evaluation of the antiulcer activity of *Wrightia tinctoria* bark extract in induced acute gastric ulcers in rat. *Nigerian Journal of Natural Products and Medicine*, 10(1): 36-40.

Chothani, D. L.& Vaghasiya, H. U. (2011). A review on *Balanites aegyptiaca* Del (desert date): phytochemical constituents, traditional uses, and pharmacological activity. *Pharmacognosy reviews*, 5(9): 55.

da Silva, L. M., Allemand, A., Mendes, D. A. G., Dos Santos, A. C., André, E., de Souza, L. M., Cipriani, T.R., Dartora, N., Marques, M.C.A., Baggio, C.H & Werner, M. F. (2013). Ethanolic extract of roots from *Arctium lappa* L. accelerates the healing of acetic acid-induced gastric ulcer in rats: Involvement of the antioxidant system. *Food and chemical toxicology*, 51: 179-187.

de Jesus, N.Z.T., Falcão, H.D.S., Gomes, I.F., Leite, T.J.D.A., Lima, G.R.D.M., Barbosa-Filho, J.M., Tavares, J.F., Silva, M.S.D., Athayde-Filho, P.F.D. & Batista, L.M. (2012). Tannins, peptic ulcers and related mechanisms. *International Journal of Molecular Science*, 13(3): 3203-3228.

Ebada, S. S., Al-Jawabri, N. A., Youssef, F. S., Albohy, A., Sa'ed, M. A., Disi, A. M. & Proksch, P. (2020). In vivo antiulcer activity, phytochemical exploration, and molecular modelling of the polyphenolic-rich fraction of *Crepis sancta* extract. *Inflammopharmacology*, 28(1): 321-331.

El-Olemy, M. M., Al-Muhtadi, F. J., & Afifi, A. F. (1994). Experimental phytochemistry: A laboratory manual. King Saud University Press.

Emmanuel, A. B., Chukwuemeka, N. A., Taye, O. G., Chinonso, A. A., & Adejoh, I. P. (2020). Inhibition of gastric acid secretion is a probable mechanism underlying the anti-ulcer activity of *Alchornea cordifolia* (Schumach. & Thonn.) Mull. Arg. *International Journal of Advance Multidisciplinary Research, 7*(12): 24-30.

Gandhi, A. P., Joshi, K. C., Jha, K., Parihar, V. S., Srivastav, D. C., Raghunadh, P., Kawalkar, J., Jain, S. K. & Tripathi, R. N. (2003). Studies on alternative solvents for the extraction of oil-I soybean. *International Journal of Food Science and Technology, 38*(3): 369-375.

Guth, P. H., Paulsen, G. & Nagata, H. (1984). Histologic and microcirculatory changes in alcohol-induced gastric lesions in the rat: effect of prostaglandin cytoprotection. *Gastroenterology*, 87(5): 10831090.

Harborne, J. B. & Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry, 55*(6): 481-504.

Hiruma-Lima, C.A., Batista, L.M., de Almeida, A.B.A., de Pietro Magri, L., dos Santos, L.C., Vilegas, W. & Brito, A.R.M.S. (2009). Antiulcerogenic action of ethanolic extract of the resin from *Virola surinamensis* Warb. (Myristicaceae). *Journal of Ethnopharmacology, 122*(2): 406-409.

Hu, R., Lin, C., Xu, W., Liu, Y. & Long, C. (2020). Ethnobotanical study on medicinal plants used by Mulam people in Guangxi, China. *Journal of Ethnobiology and Ethnomedicine*, 16(1): 1-50.

Kabo, K. S., Ali, T., & Ogbesejana, A. B. (2020). Extraction And Physico-Chemical Parameter Analysis of Desert Date (Balanite aegyptiaca) Oil from Dutsin-Ma. *FUDMA Journal of Sciences*, 4(2): 409-413.

Magaji, R. A., Okasha, M. A., Abubakar, M. S. & Fatihu, M. Y. (2007). Anti-ulcerogenic and anti-secretory activity of the n-butanol portion of *Syzygium aromaticum* in rat. *Nigerian Journal of Pharmaceutical Sciences*, 6: 119-126.

Maheswari, B., Devi, P. R., Ajith, K., VedPrakash, P. & Gayatri, K. S. (2020). Evaluation of Antiulcer Activity of Ethanol Extract of Leaves of *Lactuca sativa*. *Journal of Drug Delivery and Therapeutics*, 10(4): 196-199.

Moleiro, F. C., Andreo, M. A., Dos Santos, R. D., de Mello Moraes, T., Rodrigues, C. M., de Andrade Carli, C. B., Lopes, F. C., Pellizzon, C. H., Carlos, I. Z., Bauab, T. M. & Vilegas. W. (2009). *Mouriri elliptica*: validation of gastroprotective, healing and anti-Helicobacter pylori effects. *Journal of Ethnopharmacology, 123*(3): 359-368.

National Research Council (2011). Animal care and use program. In Guide for the Care and Use of Laboratory Animals. 8th edition. National Academies Press (US).

Navarrete, A., Martínez-Uribe, L. S., & Reyes, B. (1998). Gastroprotective activity of the stem bark of *Amphipterygium adstringens* in rats. *Phytotherapy Researc, 12*(1): 1-4.

Nwafor, P.A., Okwuasaba, F. K. & Binda, L. G. (2000). Antidiarrhoeal and antiulcerogenic effects of methanolic extract of *Asparagus pubescens* root in rats. *Journal of Ethnopharmacology*, 72(3): 421-427.

Oloyede, H. O., Adaja, M. C., Ajiboye, T. O. & Salawu, M. O. (2015). Anti-ulcerogenic activity of aqueous extract of *Carica papaya* seed on indomethacin-induced peptic ulcer in male albino rats. *Journal of integrative Medicine, 13*(2): 105-114.

Organisation for Economic Development (2001). Guidelines for Testing of Chemicals No. 425.

Proctor, M. J. and Deans, C. (2014). Complications of peptic ulcers. *Surgery (Oxford)*, 32(11): 599-607.

Ruiz-Hurtado, P. A., Garduño-Siciliano, L., Dominguez-Verano, P., Martinez-Galero, E., Canales-Martinez, M. M. & Rodriguez-Monroy, M. A. (2021). Evaluation of the gastroprotective effects of *Chihuahua propolis* on indomethacin-induced gastric ulcers in mouse. *Biomedicine & Pharmacotherapy*, *137*: 111345.

Sharifi-Rad, M., Fokou, P. V. T., Sharopov, F., Martorell, M., Ademiluyi, A. O., Rajkovic, J. & Sharifi-Rad, J. (2018). Antiulcer agents: From plant extracts to phytochemicals in healing promotion. *Molecules, 23*(7): 1751.

Sobreira, F., Hernandes, L. S., Vetore-Neto, A., Díaz, I. E. C., Santana, F. C. D., Mancini-Filho, J. & Bacchi, E. M. (2017). Gastroprotective activity of the hydroethanolic extract and ethyl acetate fraction from *Kalanchoe pinnata* (Lam.) Pers. *Brazilian Journal of Pharmaceutical Sciences, 53*(1): e16027.

Tytgat, G. N. (2011). Etiopathogenetic principles and peptic ulcer disease classification. *Digestive Diseases*, *29*(5): 454-458.

Ugwah, M. O., Ugwah-Oguejiofor, C. J., Etuk, E. U., Bello, S. O. & Aliero, A. A.(2019). Evaluation of the antiulcer activity of the aqueous stem bark extract of *Balanites aegyptiaca* L Delile in Wistar rats. *Journal of Ethnopharmacology*, *239*: 111931.

Ugwah-Oguejiofor, C. J., Inuwa, F. S., Abubakar, K., Ugwah, O. M., Mshelia, E. H. & Umar, M. (2017). Evaluation of antiulcer activity of the aerial parts of *Caralluma dalzielii* N.

E. Brown in Wistar rats. *Tropical Journal of Natural Product Research*, *1*(4): 168-172.

Vasconcelos, P.C.P., Kushima, H., Andreo, M., Hiruma-Lima, C.A., Vilegas, W., Takahira, R.K. & Pellizzon, C.H. (2008). Studies of gastric mucosa regeneration and safety promoted by *Mouriri pusa* treatment in acetic acid ulcer model. *Journal of Ethnopharmacology*, *115*(2): 293-301.

Wang, Y., lou Wang, S., yi Zhang, J., ning Song, X., yong Zhang, Z., feng Li, J. & Li, S. (2018). Anti-ulcer and anti-Helicobacter pylori potentials of the ethyl acetate fraction of *Physalis alkekengi* L. var. franchetii (Solanaceae) in rodent. *Journal of Ethnopharmacology*, *211*: 197-206.

Yadav, B., Majumder, A., Sharma, H., Sharma, P. & Bind, A. (2021). Treatment and cure of ulcer by herbal plants having flavonoids as active constituents. *Journal of Pharmaceutical Sciences and Research*, *13*(5): 271-278.

Yismaw, Y. E., Abdelwuhab, M., Ambikar, D. B., Yismaw, A. E., Derebe, D. & Melkam, W. (2020). Phytochemical and antiulcer activity screening of seed extract of *Cordia africana* lam (Boraginaceae) in pyloric ligated rats. *Clinical Pharmacology: Advances and Applications*, *12*: 67.

Zewdu, W. S. & Aragaw, T. J. (2020). Evaluation of the anti-ulcer activity of hydromethanolic crude extract and solvent fractions of the root of *Rumex nepalensis* in Rats. *Journal of Experimental Pharmacology*, *12*: 325.