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Research Article

Evaluation of Anti-ulcer Activity of Citrus maxima (Brum.) Leaves Extract in Experimental Animals

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ABSTRACT

Citrus maxima (Brum.), Rutaceae is common in Indian folk medicine for treating ulcers but, its efficacy has not been validated. This study is to evaluate the anti-ulcer activity of ethanolic and the aqueous leaves extract of Citrus maxima in adult Wistar albino rats of both sexes. Initially, ethanolic and aqueous leaf extracts were evaluated for its acute oral toxicity study conferring to the OECD guideline, 420 based on which, 200 mg/kg p.o and 400 mg/kg p.o doses extract were selected for the study. The anti-ulcer activity of leaves extracts was studied against ethanol-induced ulcer and water immersion stress-induced ulcer models. Sucralfate (250 mg/kg p.o) and Ranitidine (100 mg/kg p.o) were used as the standard drugs. Outcome measures were ulcer score, the percent inhibition of ulcer score, ulcer index, and percent inhibition of ulcer index. Data were analyzed using one-way ANOVA followed by Dunnett's test, and $P<0.05$ was considered statistically significant. A significant ($P<0.001$) anti-ulcer activity was observed in all the models. Extracts treatment animals significantly ($P<0.001$) reduced the ulcer index as compared to the control group. Aqueous extracts (400 mg/kg p.o) showed prominent ulcer protection in water immersion stress-induced ulcers (68.29%) and in ethanol-induced ulcer models (66.76%) than in the ethanolic extracts. Phytochemical study prevails the existence of phenols, flavonoids, alkaloids, tannins, and terpenoids, which may attribute to the anti-ulcer activity of extracts. Consequently, this study confirms its anti-ulcer use in Indian folk medicine. Additional study on the isolation of specific phytochemicals and studying mechanisms of action are needed.

Keywords: Citrus maxima; Antiulcer activity; Ethanol induced ulcer; Water immersion; Stress induced ulcer

INTRODUCTION

Many people rely on the traditional medicine for primary medical problems in developing countries [1]. The demand of the herbal medicine has increased significantly over the clinical therapy as the alternative treatment system currently [2].

Citrus maxima (Brum.) is one of the medicinal plants which has been extensively used by people for treatments of various ailments [3]. It fits to family Rutaceae, found extensively in tropical and subtropical climates of Southeast Asia, Taiwan, China, India, Philippines and Nepal [4]. It has been recommended in traditional herbal medicines as antidiabetic, antimicrobial, anti-inflammatory, larvicidal, hepatoprotective, anticancer, antiulcer and antiplatelet. The decoction of leaves was used in ulcer, while it can also cure fever, gout, arthritis and kidney disorders [5-11]. It has been used as sedative in nervous affections, convulsive cough, haemorrhagic diseases and in epilepsy also [12]. Contain limonin, nerolol, nerolyl acetate and geraniol [14]. Fruits contain various compounds like hesperidin. The bark and root of C. maxima contain β-sitosterol, acridone alkaloid [13], essential oil, naringin, caffeic acid, P-coumaric acid, ferulic acid and vanillic acid [15-17].

The pain that progresses inside the stomach facing or on the small intestine is collectively called as peptic ulcers. The inequality between the aggressive factors; gastric acid, pepsin and Helicobacter pylori and the defensive factors; gastric mucus, bicarbonate secretion, prostaglandins and resistance at mucosal cells causes' ulcer [18]. Among these factors, the main cause behind peptic ulcer is the bacterial infection caused by H. pylori. H. pylori can infect the stomach and the small intestine lining which can leads to ulcer. While the use of NSAIDs like aspirin and ibuprofen if used for long time can irritate the stomach and small intestine which is the second most common cause of ulcers [19,20]. Symptoms of a peptic ulcer include burning sensation in stomach, nausea, vomiting, weight loss, bloating, felling easily full etc [21].

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Number of drugs, including Proton Pump Inhibitors (PPIs), prostaglandins analogs, histamine receptor antagonists and antacids, are accessible for healing peptic ulcer, but most of these drugs produce several side effects, toxicities, and may even modify biochemical mechanisms of the body upon chronic usage [22]. Thus, the current study was commenced to evaluate the antiulcer activity of ethanolic and aqueous extract of leaves of C. maxima against ethanol induced ulcer and water immersion stress induced ulcer in rats.

MATERIALS AND METHODS

Collection and authentication of plant materials

The leaves of C. maxima were collected from Mandaya District, South India during August 2018 and authenticated by by Dr. N.M Ganesh Babu, Head for Central for Herbal Gardens in Institute of Transdisciplinary Health Sciences and Technology, Bengaluru, South India. The specimen voucher (SACCP0125) was placed in the Department of Pharmacology, Sri Adichunchanagiri College of Pharmacy (SACCP), for future references.

Extraction of plant materials

The fresh matured leaves of C. maxima were collected, washed, air dried under shade and pulverized. Powdered plant materials were kept in airtight container until the extraction. Leaves powder 500 g was extracted through successive solvent extraction in Soxhlet apparatus using 3.5 L of ethanol and distilled water in room temperature [23,24]. The extracts were concentrated in rotary evaporator at 250-175 mbar pressure at 90 rpm and 5°C chilling temperature. Further drying was done in vacuum desiccator at pressure 60 mbar. Dried extracts were stored at 4°C in refrigerator for further experimental procedures [25,26]. The yield obtained from ethanolic and aqueous solvent was 11.35% and 32.67% respectively.

Phytochemical screening

The crude leaves extracts were assessed for the qualitative phytochemical studies, followed by quantitative evaluation of total phenol, total flavonoid, total tannin and terpenoids using the standard protocol [27,28].

Animals grouping and dosing

Wistar albino rats of either sex (150-250 g) were acquired from Sri Raghavendra Enterprises, Bangalore, India. Animals with different sexes were kept in distinct polypropylene cages at room temperature (24°C ± 2°C; relative humidity 60%-70%) in a 12 hours light dark sequence. They were nourished with typical pellet diet and water ad libitum. They were adapted to laboratory environments for seven days before beginning of the test. The study was approved by the IAEC of SACCP with registration number: 377/PO/ReBi/S/01/CPCSEA. Animals were randomly assigned to seven groups each consisting of six rats. Animals were fasted for 24 hours before the study but had free access to water. Test were conducted in accord with the guidelines of CPCSEA and doses were determined based on the acute toxicity studies as per OECD guidelines 420 [29,30].

Ethanol induced ulcer model

Standard and test drugs were administered orally 1 hour before the ethanol dose. Group I (Normal control) received normal saline (1 ml/animal); Group II (ulcer control) received absolute ethanol (5 ml/kg p.o), Group III (Standard) received sucralfate (250 mg/kg), Groups IV and V were treated with EECM (ethanolic extract of C. maxima) 200 and 400 mg/kg, respectively and Groups VI and VII were given AECM (aqueous extract of C. maxima) 200 and 400 mg/kg respectively. The animals were sacrificed after 1 hour of ulcerogen administration, and their stomachs were excised [31-33].

Water immersion stress induced ulcer model

After 30 minutes of treatment animals were kept independently in separate compartment of a stress cage and deep vertically up to the xyphoid level in a water bath for 3 hours to induce stress ulcer. Ulcers were induced by obligatory swimming in the glass cylinder (height 45 cm, diameter 25 cm) comprising water upto height of 35 cm maintained at room temperature for 3 hours [34]. The rats were divided into seven groups of six each like the absolute ethanol-induced ulcer models, but the ulcer control animals were stressed by placing in water containing cylinder and drug-treated group received ranitidine (100 mg/kg). After 3 hours animals were sacrificed, and their stomachs were excised [35].

Ulcer measurement

The stomachs were washed with normal saline and placed in 10% formalin so as to determine ulcer protection and ulcer index. The ulcer severity was scored closely with the help of a hand lens (10x). Mean ulcer score for each animal was expressed as ulcer index. The percentage protection was calculated using the formula [36] (Table 1).

Table 1: Scoring of ulcers.

Ulcer severity	Ulcer score
Normal stomach	0
Red coloration	0.5
Spot ulcer	1
Haemorrhagic streak	1.5
Ulcers	2
Perforation	3

Ulcer Protection=(UI of Control-UI of Test)/ UI of Control*100%

Ulcer index is calculated using this formula

UI=UN+US+UP*10-1

UN=average of number of ulcers per animal

US=average of severity score

UP=percentage of animals with ulcer

Statistical analysis

Results were expressed as a mean ± SD of six animals in each group; the treated groups were compared with the ulcer control group. The results analysed statistically using one-way analysis of variance (ANOVA) followed by Dunnett's test. P<0.05 when compared with toxicant group was considered as significant.

RESULTS

Phytochemical screening

Initial qualitative phytochemical screening of C. maxima established the occurrence of diverse secondary metabolites. The quantitative phytochemical evaluation of total phenol, total flavonoid, total tannin and terpenoids is revealed (Tables 2 and 3).

Table 1: Preliminary phytochemical screening of leaves extract of C. maxima.

Qualitative test	Procedure	Observation of extracts	
		Ethanolic	Aqueous
Alkaloids	Mayer's test	+	+
	Wagner's test	+	+
	Dragendroff test	+	+
Amino acids	Ninhydrin test	-	-
Carbohydrates	Reducing sugar	-	-
	Benedict's test	-	-
Saponin	Foam test	-	-
Glycosides	Borntrager's test	-	-
	Legal's test	-	-
Phenolics	Ferric chloride test	-	-
	Lead acetate test	+	+
Flavonoids	Aluminium hydroxide test	+	+
Protein	Biuret test	-	-
Gum and mucilage	Gum and Mucilage	-	-
Terpenoids	Salkowski test	+	+
Anthraquinone	Anthraquinone test	-	-
Tannin	Tannin test	+	+

(+) represents presence of phytochemical and (-) represents absence of phytochemicals

Table 3: Quantitative phytochemical evaluation of leaves extracts of C. maxima.

Quantitative test	Procedure	Observations of extracts	+
		Ethanolic	Aqueous
Total phenol content	Folin Ciocalteu (FC) method	123.22 ± 4.98	59.10 ± 3.35
Total flavonoid content	Aluminium chloride method	182.05 ± 0.005	68.55 ± 0.003
Total tannin content	Modified FC method	480.605 ± 0.049	119.368.55 ± 0.001
Total terpenoids content	-	32%	7.40%

Total phenol content is determined as µg gallic acid equivalent per mg of extracts, total flavonoid content is determined as µg quercetin equivalent per mg of extract, total tannin content is determined as µg gallic acid equivalent per mg of extract and the percentage yield is calculated in case of terpenoids.

Acute toxicity test

No mortality and no any behavioural and physical changes were observed during the study period. So, the LD50 of the extracts was found to be>2000 mg/kg.

Anti-ulcer effects of C. maxima

The effect of both ethanolic and aqueous leaves extracts of C. maxima on ethanol induced and water immersion stress induced ulcer was evaluated by macroscopic study (Figures 1 and 2).

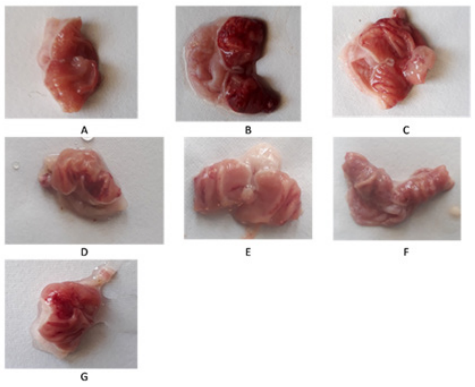

Figure 1: The gross appearance of gastric ulcers induced by ethanol (5 ml/kg) and protective effects of C. maxima (200,400 mg/kg). A:normal saline, B:ethanol, C:ethanol+sucralate (250 mg/kg), D:ethanol+ EECM (400 mg/kg), E:ethanol+sucralfate (200 mg/kg), F:ethanol+AECM (400 mg/kg), G:ethanol+AECM (200 mg/kg). Ethanol caused extensive haemorrhagic ulcers (B) and treatment with Citrus maxima at doses of 200 and 400 mg/kg attenuated gastric ulcers.

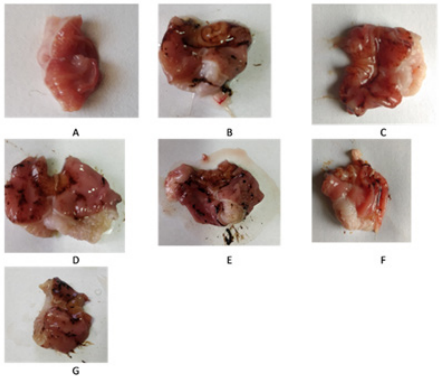

Figure 2: The gross appearance of gastric ulcers induced by stress and protective effects of Citrus maxima (200, 400 mg/kg). A:normal saline, B:stress, C:stress+ranitidine (100 mg/kg), D:stress+EECM (400 mg/kg), E:stress+EECM (200 mg/kg), F:stress+AECM (400 mg/kg), G:stress+AECM (200 mg/kg). Water immersion stress caused extensive haemorrhagic ulcers (B) and treatment with Citrus naxima at doses of 200 and 400 mg/kg attenuated gastric ulcers.

Effects of leaves extracts on ethanol induced ulcer

Both the ulcer score and ulcer index were significantly reduced by C. maxima dose of 200 mg/kg (P<0.001), 400 mg/kg (P<0.001), and the standard drug sucralfate 250 mg/kg (P<0.001). The effects of aqueous extract of C. maxima 400 mg/kg were analogous to sucralfate regarding percent reduction in ulcer scores (66.66% vs. 89.00%) and ulcer index (66.76% vs. 89.02%) (Table 4).

Table 4: Effect of Citrus maxima on ulcer index and percentage ulcer protection of absolute ethanol induced ulcer models.

Treatment	Ulcer score	Reduction in ulcer score (%)	Ulcer index	% Ulcer protection
Normal control	-	-	-	-
Ulcer control (5.0 ml/kg p.o)	3.00 ± 0.00	-	11.03±0.03	-
Sucralfate (250 mg/kg)	0.33 ± 0.09a***	89	1.21 ± 0.09 a ***	89.02
EECM (400 mg/kg)	1.16 ± 0.09 a ***	61.33	4.43 ± 0.09 a ***	59.76
EECM (200 mg/kg)	1.66 ± 0.09 a ***	44.66	6.28 ± 0.09 a ***	43.05
AECM (400 mg/kg)	1.00 ± 0.16 a ***	66.66	3.66 ± 0.16 a ***	66.76
AECM (200 mg/kg)	1.41 ± 0.14 a ***	52	5.20 ± 0.14 a ***	52.81

Values are mean ±SD, a=ulcer control group, n=6 in each group, ***P<0.001, **P<0.01 and **P<0.05 when compared with ulcer control group

Effects of leaves extracts on water immersion stress induced ulcer

Water immersion stress induced ulcer significantly (P<0.001), reduced the ulcer index and ulcer score as compared with the ulcer control group. The effects of the aqueous extract of C. maxima 400 mg/kg were analogous to the ranitidine (100 mg/kg) regarding percent reduction in ulcer scores (68.49% vs. 80.13%) and ulcer index (68.29% vs. 80.28%) (Table 5).

Table 5: Effect of Citrus maxima on ulcer index and percentage ulcer protection of water immersion stress induced ulcer models.

Treatment	Ulcer score	Reduction in ulcer score (%)	Ulcer index	% Ulcer protection
Normal control	-	-	-	-
Ulcer control (5.0 ml/kg p.o)	3.00 ± 0.00	-	11.03±0.03	-
Sucralfate (250 mg/kg)	0.33 ± 0.09a***	89	1.21 ± 0.09 a ***	89.02
EECM (400 mg/kg)	1.16 ± 0.09 a ***	61.33	4.43 ± 0.09 a ***	59.76
EECM (200 mg/kg)	1.66 ± 0.09 a ***	44.66	6.28 ± 0.09 a ***	43.05
AECM (400 mg/kg)	1.00 ± 0.16 a ***	66.66	3.66 ± 0.16 a ***	66.76
AECM (200 mg/kg)	1.41 ± 0.14 a ***	52	5.20 ± 0.14 a ***	52.81

Values are mean ±SD, a=ulcer control group, n=6 in each group, ***P<0.001, **P<0.01 and **P<0.05 when compared with ulcer control group

DISCUSSION

This research was conducted to estimate the anti-ulcer properties of ethanolic and aqueous leaves extracts of C. maxima on ethanol induced and water immersion stress induced ulcer models. The ethanolic yield 11.35% of extract is alike with 23% yield described in earlier studies in Assam, which supports that solvent holds a decent extracting capacity [37]. Ethanol and aqueous solvent issue various phytochemicals as phenols, flavonoids, tannin, terpenoids, saponin, glycosides in extracts [38]. The acute toxicity study discovered that the plant extract was non-toxic up to 2000 mg/kg and that the median lethal dose (LD50) is above 2000 mg/kg, supported by the Kundusen et al. [39,40]. Preliminary phytochemical screening shows the presence of phenols, flavonoids, tannins, terpenoids and alkaloids in both the extracts. These secondary metabolites are active as antioxidant, analgesic, anti-ulcer, anti-inflammatory, and immune stimulating agents [41,42]. Phenolics reduce the gastric acid secretion and exhibit antioxidant activity which enhances its gastroprotection activity [43]. Flavonoids act as free radical scavenger which increase the mucosal prostaglandin content, reduce histamine secretion from mast cells through inhibiting histidine decarboxylase and growth of H. pylori [44,45]. Tannins reduce the permeability of stomach irritant, while terpenoids and alkaloids play the important role against gastric ulcer [45,46].

The extract- and standard drug-treated groups showed a significant reduction (at least P<0.01) in ulcer score and ulcer index in ethanol induced ulcer model. The effect of extracts upsurges with the dose which can be linked with the standard drug. This outcome indicates that the plant extract holds a gastroprotective effect, which is as suitable as the standard drug. Pure alcohol damages the gastric mucosa, which can increase infiltration of neutrophils which are potent inflammatory mediators. While, the reduction of neutrophil infiltration during inflammation can result gastric ulcer healing. C. maxima leaves extract has also been found to show anti-inflammatory activity, so it is predicted that the gastroprotective effect of plant extract could be due to its anti-inflammatory activity [47]. Antioxidants potential of compounds are important for re-establishing gastric tissue [48]. So, ulcer treatment by extract may be related to the antioxidant activity of the plant, which is well proved in previous studies [49]. Thus, results of this study showed that the C. maxima leaves extract was capable of preventing gastric lesions formed by ethanol.

Gastric ulcerations can be the result of physical and psychological stress in animals [50]. This model is one of the finest models of stress in animals to bring ulcer [50,51]. Stress acts as the PGE2 synthesis inhibitors in gastric mucosa which finally induces the production of free radicals [52]. As a result, it reduces the mucosal blood flow and gastric mucosa which ultimately develop gastric lesion [53]. Treatment with leaves extracts at 400 and 200 mg/kg significantly reduced gastric lesions that were persuaded by stress with an inhibition rate of 68.29% which is found in this study. So, these results suggest that C. maxima leaves extract has gastro protective effects against stress-induced gastric ulcer.

CONCLUSION

This study checks the nonappearance of acute oral toxicity at given dose, and existence of anti-ulcer activity of C. maxima leaves extract. The usefulness of the extracts is equivalent with the standard drugs. Anti-ulcer effects may be linked to reduction of gastric acid secretion along with protection of the gastric mucosal barrier by phytochemicals. Thus, the current work confirms the usage of C. maxima for gastric ulcer in the Indian traditional medicine, and additional studies shall emphasis on separation of exact phytochemicals and clarifying mechanisms of action.

Data availability

Our study is the animal study and all the data is presented in the articles, if readers require more specific data then it can be provided at their mail address.

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This work was carried out for the partial fulfillment of a master's degree and college to bear all the expenses incurred.

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CONFLICT OF INTEREST

The authors declare they have no competing interest.

REFERENCES

1. Grover JK, Yadav SP. Pharmacological actions and potential uses of momordica charantia: a review. J Ethnopharmacol. 2004;93(1):123-132.
2. Lobo VC, Phatak A, Chandra N. Acute toxicity studies of some indian medicinal plants. Pharmacogn J. 2010;2(8):207-210.
3. Arias BÁ, Laca LR. Pharmacological properties of citrus and their ancient and medieval uses in the mediterranean region. J Ethnopharmacol. 2005;97(1):89-95.
4. Chaudhari SY, Ruknuddin G, Prajapati P. Ethno medicinal values of citrus genus: a review. Med J Dr D Y Patil Univ. 2016;9(5):560-565.
5. Thu DK, Thuy HT, Duyen BTT, Hang LTT, Trang NT, Nhat BS. Evaluating the acetylcholinesterase inhibitory and antioxidant activities of persea americana extracts. VNU J Sci Med Pharm Sci. 2019;35(1).
6. Buachan P, Chularojmontri L, Wattanapitayakul SK. Selected activities of Citrus maxima merr. fruits on human endothelial cells: enhancing cell migration and delaying cellular aging. Nutrients. 2014;6(4):1618-1634.
7. Diab KAE. In vitro studies on phytochemical content, antioxidant, anticancer, immunomodulatory , and antigenotoxic activities of lemon, grapefruit, and mandarin citrus peels. Asian Pacific J Cancer Prev. 2016;17(7):3559-3567.
8. Okuda T, Ito H. Tannins of constant structure in medicinal and food plants-hydrolyzable tannins and polyphenols related to tannins. Molecules. 2011;16(3):2191-2217.
9. Ou MC, Liu YH, Sun YW, Chan CF. The composition, antioxidant and antibacterial activities of cold-pressed and distilled essential oils of Citrus paradisi and Citrus grandis (L.) osbeck. Evid Based Compl Alt Med. 2015.
10. Bhandurge P, Rajarajeshwari N, Alagawadi KR, Agrawal S. Preliminary phytochemical and hypoglycemic activity of leaves of Grewia asiatica L anti-diabetic activity of holarrhena antidysenterica (Linn.) wall, bark on alloxan induced diabetic. 2018:1-3.
11. Abirami A, Nagarani G, Siddhuraju P. Antimicrobial activity of crude extract of Citrus hystrix and Citrus maxima. Int J Pharm Sci Res. 2013;4(1):1-5.
12. Singh A. Citrus maxima (burm.) merr. a traditional medicine : its antimicrobial potential and pharmacological update for commercial exploitation in herbal drugs-a review. Semantic Scholar.2017;10(5):642-651.
13. Teng WY, Huang YL, Shen CC, Huang RL, Chung RS, Chen CC. Cytotoxic acridone alkaloids from the stem bark of Citrus maxima. J Chinese Chem Soc. 2005;52(6):1253-1255.
14. Chen Y, Li T, Bai J, Nong L, Ning Z, Hu Z, et al. Chemical composition and antibacterial activity of the essential oil of Citrus maxima (burm.) merr. cv. shatian yu. J Biol Act Prod Nat. 2018;8(4):228-233.
15. Vimala G, Shoba FG. A review on antiulcer activity of few indian medicinal plants. Int J Microbiol. 2014;2014(1):519590.
16. Okwu DE, Awurum AN, Okoronkwo JI. Phytochemical composition and in vitro antifungal activity screening of extracts from citrus plants against Fusarium oxysporum of okra plant (Hibiscus esculentus). African Crop Sci Conf Proc. 2007.
17. Sapkota B, Prakash C, Jain V. Preliminary phytochemical screening and quantitative analysis of Citrus maxima (brum.) leaves extract. Int J Res Pharmacol Pharmacother. 2020;9(1):100-106.
18. Baiubon P, Kunanusorn P, Khonsung P, Chiranthanut N, Panthong A, Rujjanawate C. Gastroprotective activity of the rhizome ethanol extract of Zingiber simaoense y y qian in rats. J Ethnopharmacol. 2016;194(1):571-576.
19. Bjarnason I, Scarpignato C, Holmgren E, Olszewski M, Rainsford KD, Lanas A. Mechanisms of damage to the gastrointestinal tract from nonsteroidal anti-inflammatory drugs. Gastroenterology. 2018;154(3):500-514.
20. Ogasawara N, Sasaki M, Itoh Y, Tokudome K, Kondo Y, Ito Y. Rebamipide suppresses TLR-TBK1 signaling pathway resulting in regulating IRF3/7 and IFN-α/β reduction. J Clin Biochem Nutr. 2011;48(2):154-160.

21. Zakaria ZA, Zainol ASN, Sahmat A, Salleh NI, Hizami A, Mahmood ND,et al. Gastroprotective activity of chloroform extract of Muntingia calabura and Melastoma malabathricum leaves. Pharm Biol. 2016;54(5):812-826.

22. Iwasaki A, Aizawa T, Sugimura F. Recurrence during maintenance therapy with histamine H2-receptor antagonist in cases of gastric ulcer. Nihon Univ J Med. 1986;28(1):69-74.

23. Redfern J, Kinninmonth M, Burdass D, Verran J. Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. J Microbiol Biol Educ. 2014;15(1):45-46.

24. Gupta A, Naraniwal M, Kothari V. Modern extraction methods for preparation of bioactive plant extracts. Int J Appl Nat Sci. 2012;1(1):8-26.

25. Desai SD, Desai DG, Kaur H. Saponins and their biological activities. Pharma Times. 2009;41(3):13-16.

26. Price ML, Butler LG. Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain. J Agric Food Chem. 1977;25(6):1268-1273.

27. Senguttuvan J, Paulsamy S, Karthika K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L. for in vitro antioxidant activities. Asian Pac J Trop Biomed. 2014;4(1):S359-S367.

28. Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from ephedra intermedia indigenous to balochistan. Sci World J. 2017.

29. OECD. Test no. 420: acute oral toxicity-fixed dose procedure. Oecd Guidel Test Chem. 2001.

30. Häkkinen SH, Kärenlampi SO, Heinonen IM, Mykkänen HM, Törronen AR. Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. J Agric Food Chem. 1999;47(6):2274-2279.

31. Sathish R, Sahu A, Natarajan K. Antiulcer and antioxidant activity of ethanolic extract of Passiflora foetida L. Indian J Pharmacol. 2011;43(3):336-339.

32. Pallavi B, Sunil N, Ahamed SM, Nandheesh R. Gastroprotective effect of Sesbania grandiflora linn. seeds extract. J Biomed Pharm Res. 2020;9(2).

33. Ashoka SM, Shashidhar SC, Prakash S. Anti ulcer activity of Heliotrpium indicum leaves extract. Int J Pharm Sci Res. 2011;2(10):2490-2500.

34. Muniappan M, Sundararaj T. Antiinflammatory and antiulcer activities of Bambusa arundinacea. J Ethnopharmacol. 2003; 88(2):161-167.

35. Arumugam S, Selvaraj SV, Velayutham S, Natesan SK, Palaniswamy K. Evaluation of anti-ulcer activity of Samanea saman (Jacq) merr bark on ethanol and stress induced gastric lesions in albino rats. Indian J Pharmacol. 2011;43(5):586-590.

36. Das S, Borah M, Ahmed S. Antibacterial activity of the ethanolic extract of leaves of Citrus maxima (burm.) merr. on Escherichia coli and Pseudomonas aeruginosa. Asian J Pharm Clin Res. 2013;6(4):136-139.

37. Ngo TV, Scarlett CJ, Bowyer MC, Ngo PD, Vuong QV. Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of Salacia chinensis L. J Food Qual. 2017.

38. Sheik HS, Vedhaiyan N, Singaravel S, Material P. Acute and sub acute toxicity studies of some indigenous medicinal plants. Int J Pharm Phytopharm Res. 2013;3(2):166-169.

39. Potdar VH, Kibile SJ. Evaluation of antidepressant-like effect of Citrus maxima leaves in animal models of depression. Iran J Basic Med Sci. 2011;14(5):478-483.

40. Mohod SM, Bodhankar SL. Antiulcer activity of aqueous extract of leaves of Madhuca indica J. F. Gmel against naproxen induced gastric mucosal injury in rats. J Acute Dis. 2013;2(2):127-133.

41. Reddy VP, Sudheshna G, Afsar SK, Saran SS. Evaluation of anti-ulcer activity of Citrullus colocynthis fruit against pylorus ligation induced ulcers in male wistar rats. Int J Pharm Pharm Sci. 2012;4(2):446-451.

42. Sumbul S, Ahmad MA, Asif M, Akhtar M. Role of phenolic compounds in peptic ulcer: an overview. J Pharm Bioallied Sci. 2011;3(3):361-367.

43. Repetto MG, Llesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Braz J Med Biol Res. 2002;35(5):523-534.

44. Borrelli F, Izzo AA. The plant kingdom as a source of anti-ulcer remedies. Phyther Res. 2000;14(8):581-591.

45. Mitra P, Ghosh T, Mitra PK. Anti gastric ulcer activity of Amaranthus spinosus linn. leaves in aspirin induced gastric ulcer in rats and the underlying mechanism. SMU Med J. 2014;1(2):313-328.

46. Kundusen S, Gupta M, Mazumder UK, Haldar PK, Saha P, Bhattacharya S, et al . Exploration of anti-inflammatory potential of Citrus limetta risso and Citrus maxima (j. burm.) merr.2011;709(1):702-709.

47. Pandey BP, Thapa R, Upreti A. Total phenolic content , flavonoids content , antioxidant and antimicrobial activities of the leaves , peels and fruits of locally available citrus plants collected from kavre district of nepal. Int J Pharmacogn Chinese Med. 2019;3(3):1-6.

48. Tamboli F, More HN. Evaluation of antiulcer and antioxidant activity of Barleria gibsoni Dalz. leaves," Pharmacognosy Res. 2016;8(4):226-230.

49. Chen CY, Kuo TL, Sheu SY, Kuo TF. Preventive effects of chinese herb chai-hu-gui-zhi-tang extract on water immersion restraint stress-induced acute gastric ulceration in rats. J Vet Med Sci.2010;72(6):679-685.

50. Kim SH, Jeon BJ, Kim DH, Kim TI, Lee HK, Han DS, et al. Prickly pear cactus (Opuntia ficus indica var. saboten) protects against stress-induced acute gastric lesions in rats. J Med Food. 2012;15(11):968-973.

51. Uysal VNI, Bülbül M, Tan R, Derin N, Ustünel I, Ağar A, et al. Effect of chronic stress and L-carnitine on rat stomach. J Physiol Sci. 2007;57(3):187-192.

52. Konturek PC, Brzozowski T, Duda A, Kwiecien S, Löber S, Dembinski A, et al. Epidermal growth factor and prostaglandin E2 accelerate mucosal recovery from stress-induced gastric lesions via inhibition of apoptosis. J Physiol Paris. 2001;95(6):361-367.

53. Takezono Y, Joh T, Oshima T, Suzuki H, Seno K, Yokoyama Y, et al. Role of prostaglandins in maintaining gastric mucus-cell permeability against acid exposure. J Lab Clin Med. 2004;143(1):52-58.