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Gastro-protective and therapeutic effect of *Punica granatum* against stomach ulcer caused by *Helicobacter Pylori*

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ABSTRACT

Gastric ulcer is a chronic condition that occurs when the mucosa of the stomach is broken. There is a physiological equilibrium between aggressive factors and mucosal defense. The purpose of this research was to compare the prevention level and efficiency of herbal medicinal plants (*Punica granatum*) to the omeprazole drug. Many groups were prepared from Albino male rats, the first control group (inoculate with *H. pylori* and fed with standard pellet), the Second group, rats inoculated by *H. pylori* and prevented with Punica granatum aqueous extracts (PGAE) in two dosages (250mg/kg, 500mg/kg), and last group inoculated by *H. pylori* and prevented with standard drug omeprazole at the dose (20mg/kg). The results showed that the Ulcer Inhibition % of Punica granatum with a high dose of 500mg/kg and a low dose of 250mg/kg was 84.60±5.48 and 42.87±7.14, respectively. While in the omeprazole treatment group, Ulcer Inhibition % was 24.50±6.35 and this Ulcer Inhibition % in the Punica granatum treatment groups was significant compared to the omeprazole treatment group and the control group (P=0.0001). PGAE displayed a significant lessening in stomach index and infectious cell proliferation with much cell damage. Although the result of the current study improves, a high dosage of aqueous extracts of plants has more effectiveness than a low dosage of aqueous extracts plants.

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Introduction

Helicobacter Pylori is a highly motile, spiral-shaped, gram-negative bacterium that colonizes the intestines of 50-80% of people worldwide (1). Its infection is one of the main and important reasons for continuous bacterial infection among humans. It has a high currency for both genders and all ages. According to the studies, it is directly or indirectly responsible for a diversity of human diseases, the most repeated of which are duodenal ulcers, chronic gastritis, peptic ulcer diseases, pancreatic cancer, and stomach cancer that damage the gastric mucosa (2–5). The clinical and laboratory analysis exhibited that diabetic patients had more malignant infections in the digestive system than non-diabetic patients(6). Irregular diets affect the usual operation of the oesophagus, stomach and duodenum. This disease is made by the mucosa internal digestion by gastric acid and pepsin. So, damage to gastric mucosa will happen and causes ulcer. other factors are effective in making gastric ulcers (7). Gastric ulcer is a condition of the stomach lining that has common symptoms such as vomiting, burning, dull abdominal pain, headache, weight loss, low oral resistance, stenosis, perforation and stomach bleeding (8). The focus has been on natural plants, partially because certain pharmaceutical medicines are highly harmful to the patient or cause adverse effects. Moreover, in terms of cure and disease prevention, plant products are inexpensive and more affordable contributors to improving human health (9). A plant of the family Lythraceae, *Punica granatum* (pomegranate) has been reported to have several medicinal properties including chemopreventive, antioxidant, antifungal, anti-inflammatory and wound healing. A preventive role against obesity has also been identified. 7 Steroids, triterpenoids, saponins, glycosides, flavonoids, alkaloids, carbohydrate tannins and vitamin C have been found to contain the phytochemical screening of Punica granatum extract (10). Omeprazole is used for the treatment of conditions in which the stomach excretes large quantities of acid. It prevents a particular enzyme mechanism activity located within the acid-secreting stomach cells (in the stomach), thus preventing acid production (11). Omeprazole was being used to control: heartburn (heartburn), acute gastritis, duodenitis (duodenitis), esophagitis attributable to the recurrence of gastrointestinal material, peptic ulcer and other secretaries' diseases.

Materials and Methods

Extraction of peel part of *Punica granatum*

Fresh Pomegranate fruit was collected from a market

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in Erbil city. Mainly the yellow peel is separated from the pomegranate fruit collected in August 2020 and is used in the study. The samples were air-dried indoors to protect them from direct sunlight. The method described by (12,13) was used for the aqueous extract preparation of the finely powdered samples. Hot distilled water (85ºC) was added and left overnight to prepare a high dose (25g/250ml), and a low dose (25g/500ml), then the extract was filtered by filter paper Whatman No.1. Finally, the filtered extract, were stored inside the sterile cup at refrigerator until used.

Omeprazole

Omeprazole is the class of medicines that have been used to treat disorders including peptic ulcers as proton pump inhibitors. Omeprazole inhibits the acid-forming enzymes in the stomach wall. The output of the stomach acid is reduced by blocking these enzymes and thus the stomach can be healed. Omeprazole was the reference anti-ulcer treatment in this sample and was extracted in Erbil city. The medication was supplied by mouth to rats at 20mg/kg body weight suspended in distilled water (5ml/kg) (14).

Animal used in the experiment

Forty-eight male albino rats were used for the trials, Rats body weight ranged from 160±20 gram 7–9-week-old and the rats were randomly allocated to four groups, each group of twelve rats were purchased from Zakho University and controlled in well-ventilated cages under sanitary conditions. The rats were provided with a regular diet for one week in the animal house of Salahaddin University's College of Education.

Induction of gastric ulcer by *H. Pylori*

Rats were given the Helicobacter pylori bacterium, which was obtained from biopsy samples of clinical patients, and this generated gastritis in the rats. The rats were held in reserve in the animal house of the Salahaddin University-College Erbil's of Education for a week and provided a basic meal. In this study, the rats were fasted in the morning and afternoon first inoculated with plant extracts then immediately inoculated with 1ml *H. pylori* suspension (5×10^8- 5×10^{10} CFU/ml) by gavage twice daily at an interval of 4h for three consecutive days, then left on normal feeding for 15 days to see the effect of the plants extracts with *H. pylori* activity (15), before inoculating animals with plants extract and *H. pylori* the animals do the antigen test via stool to ensure that did not infect with *H. pylori* before, In last period of prevention, anesthetized of the rats done by intramuscular injection of mixed xylazine-ketamine (1:9) as a single dose in the same syringe, then the Stomach was removed of each rat from each group, then dissects the stomach.

Evaluating large gastric lesions

Ulcers in the gastric mucosa appeared as extended bands of hemorrhagic lesions parallel to the long stomach axis. As a result, the damage was investigated in each stomach mucosa sample. The overall surrounding place of all lesions for each stomach was added to the size of the ulcer zone (UA) whereby the sum of tiny squares (× 4 × 1.8 = UA mm2) as described earlier by a prior with minimal modification (16). The interference proportion (I%) was computed using the following equation with minor modifications. (I%) = [(UA control - UA treated) ÷ UA control] × 100%] (17).

Acute toxicity test of pomegranate extract

Male Wistar rats were used to determine the acute toxicity in vivo. The research was initially carried out to determine the safety of the doses that were installed. Three separate dosages of 100, 1000, and 2000 mg/kg were administered orally through gastric gavage to three distinct groups of rats (n=3) in order to determine the lethal dose (LD50) that results in the death of 50% of the experimental rats in a category. This was carried out in accordance with the Organization for Economic Cooperation and Development's ethics for chemical testing (18). To track any weight changes, male Wistar rats were weighed before, during, and after the treatment. After dosing at least once in the first 30 minutes and on occasion over the following 24 hours, rats were also watched to look for any potential changes in their behavior. For 14 days, special attention was given daily throughout the first four hours. The skin, eyes, breathing, seizures, and overall health of the rats were followed for any signs of poisoning (19).

Acid content of gastric juice measurement and evaluation (pH)

The pH level of the stomach was individually recorded for evaluation of the pH level of all groups of rats to know the effect and correlation of plant extract with pH level.

Microbiological analysis

Microbiological analysis was directly carried out on the day of sample collection, Stool antigen test was done on all rat groups and was analyzed by strip *H. pylori* antigen test to see if the rats were infected with *Helicobacter pylori* or not.

Histopathology investigation

After the necropsy, stomach samples were taken and preserved in 10% formalin for 48 hours. The tissue samples were cut into 0.5 cm thick slices and put in plastic cassettes to be dehydrated and cleared before being embedded in paraffin. Subsequently, the tissue samples were cut into 4 µm sections. The tissue layers used to be cautiously put in a water bath and mounted on glass slides using a hot plate. Sections of stomach tissue were deparaffinized twice with xylene for two minutes each, rehydrated three times with ethanol diluted differently (100%, 90%, and 70%) for two minutes each, and stained with hematoxylin and eosin (H&E) stain. At magnifications of 40x, 100x, 200x, and 400x, tissue sections were seen and studied under a light microscope.

Statistical method

To investigate the effect of an aqueous extract of Punica granatum and antibacterial omeprazole in gastric ulcer production and ulcer inhibition, the data were expressed as mean ± S.E.M. (number = 6 mice/group). One-way ANOVA followed by Tukey's post-mortem multiple comparison tests was performed to measure the significance of differences between groups. The significance level was determined to be less than 0.05%.

Results

Effect of PGAE and omeprazole

Animals of the second and third groups protected by PGAE showed a significant reduction in the ulcer area. The results showed that in the Punica granatum treatment group with a dose above 500 mg/kg, Ulcer Area (mm2) was 125.50±1.24 and Ulcer Inhibition % was 84.60. In the Punica granatum treatment group with a dose of 250 mg/kg Ulcer Area (mm2) was 465.60±8.54 and Ulcer Inhibition % 42.87. In the omeprazole treatment group, Ulcer Area (mm2) was 615.25±11.68 and Ulcer Inhibition was 24.50.while in the control group Ulcer Area (mm2) was 815.00 ± 24.78 and Ulcer Inhibition % 14.76And this Ulcer Inhibition % in the Punica granatum treatment groups was significant compared to the omeprazole treatment group and the control group (P=0.0001).

The ulcer area was dramatically reduced in a dose-dependent manner when compared to the control group. The largest reduction proportion of ulcer area development was (84.60%) in the rats prevented with a high dosage (500mg/kg) of PGAE. However, ulcer area was greatly decreased or suppressed from (815.00mm2 control group rats) to (125.50 mm2) as shown in Table 1.

The antiulcer activity of the drug omeprazole in a model of H. pylori-induced gastric lesion. The results demonstrated that rats' stomachs protected with omeprazole and given H. pylori solution reduced areas of gastric ulcer formation at a rate (24.50%) of ulcer area inhibition in comparison to the gastric ulcer control group.

Evaluation of pH level of gastric content between Punica granatum and antibacterial omeprazole

When PAGE was used to prevent ulcers in experimental animals, it was observed that this treatment method was associated with a decrease in gastric acidity. Examination of stomach acidity after treatment showed that the pH of stomach acid in the *P. granatum* 500 mg/kg treatment group was 6.3 and in *P.granatum* 250 mg/kg treatment group it was 5.

Stomach pH was 5.5 in the omeprazole treatment group and 3.5 in the control group, which shows the positive effects of *P. granatum* treatment in reducing stomach acidity (P=0.001) (Figure 1).

Evaluation of stomach color change in rats between *Punica granatum* and antibacterial omeprazole

The color of prevented rat stomachs with PGAE significantly changed compared with the ulcer control group, white bright color was found in the stomach of the rats with PGAE, and a white to pink color was found in each of the stomachs, otherwise, the control groups stomach was totally dark red, while the color of prevented rat stomach with omeprazole are changed comparing with the ulcer control group, red bright color was found in the stomach of the rats with omeprazole that showed in Figure 2.

Histological examinations

Figure 3 shows the histological analysis and macroscopic appearance of the stomachs of the several rat groups that were evaluated. The macroscopic inspection of the opened stomachs of normal control rats revealed normal morphological features (20), while the ulcer control group displayed hemorrhagic lesions and specks of coagulated blood. Additionally, the PGAE pre-treatment group's sto-

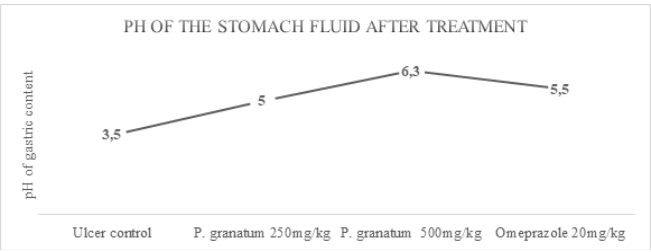

Figure 1. Evaluation of pH level of gastric content between *Punica granatum* and antibacterial omeprazole.

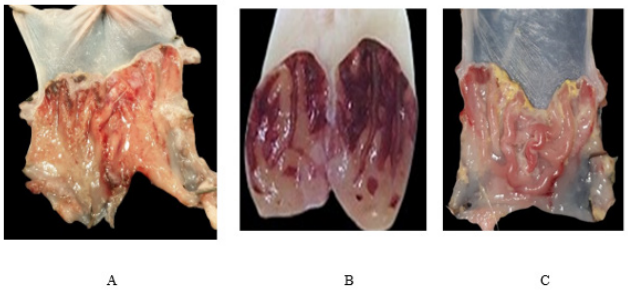

Figure 2. (a) Shown the rats stomach treated with omeprazole, (b) Stomachs affected with H.pylori, (c) rats stomachs prevented with PGAE.

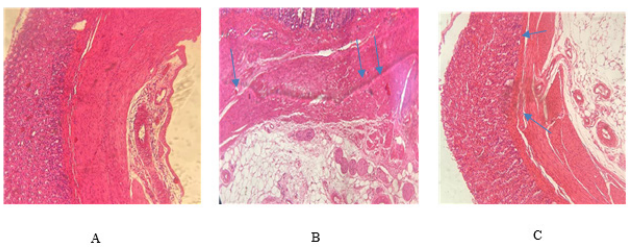

Figure 3. (a) stomach affected with H.pylori showed severe structural injuries and progress inflammations with red spots in the histological structure; (b) shown the rats stomach treated with omeprazole group with thick the lower portion (antrum) section and propagation of necrosis cells; (c) rats stomach prevented with PGAE showing insignificant treated ranges of necrosis were noticed in the antrum (400x). (p = 0.0013)

Table 1. Anti-ulcer effects of *P granatum* and omeprazole treatments in male albino rats.

Animal group	Prevention (5ml/kg) dose (*n=36*)	Ulcer Area (mm²) Mean ± SD (*n=36*)	Ulcer Inhibition %	P-value*
1	Ulcer control group	815.00±24.78	14.76	0.0001
2	High dose	125.50±1.24	84.60	
3	Low dose	465.60±8.54	42.87	
4	Omeprazole	615.25±11.68	24.50	

P-Value based on One-way ANOVA followed by Tukey's posthoc.

machs were equivalent to those of a normal control group with few mucosal lesions. The histopathology structure of the gastric layers was discovered by histologically analyzing the stomachs of healthy control rats (mucosa, submucosa, and serosa) (21). Rats without ulcers had focal gastric mucosal necrosis associated with inflammatory cell infiltration, submucosal edema, and submucosal inflammatory cell infiltration in their stomachs. The PGAE group's negligible damages demonstrated the ulcer formation's suppression (22). While other analyzed sections from the pomegranate peel treatment group exhibited no histological alterations, some of them showed minor submucosal edema (23). The ulcer scores significantly decreased after receiving PGAE treatment ($p = 0.0013$). (400x).

Discussion

The present findings agree with the results of their findings show that done (24). Gastric ulcer was prevented with PGAE, producing significant healing in an induced gastric ulcer model (25). The current investigation concluded that components of the PGAE are effective for gastric ulcer prevention, as claimed by traditional medicine practitioners (26). Numerous psychological and physical stress cause stomach ulcers in both humans and laboratory animals. PGAE acts as a protection against gastric ulcers (27).

Current findings suggest that the anti-ulcer effect is associated with increased secretion of adherent mucus and increased pH of gastric contents (28), which can inhibit the production of oxygen-derived free radicals and keep the contents of MDA in a normal state. our result was agreeing with the result finding in (25) PGAE prevents ulcers by increasing mucus secretion and pH level in pyloric ligation rats (29). Also shows the anti-ulcer activity of AMP in experimentally induced gastric ulcers. *H. pylori* formed widely visible black hemorrhagic injuries on the stomach mucosa (30), moreover plant extraction of pomegranate peel had an effect (84.60%) inhibition formation of stomach ulcers compared with the control group in rats gastro-protective with the high dose plant extract of PGAE (500mg/kg) and (42.87%) inhibition formation of the stomach ulcers in rats with low dose *Punica granatum* extract (250mg/kg), however, another study result (31) were agrees with our study result finding that PGAE plays a major role on inhibition formation of stomach ulcers gastro-protective in rat animal model (32).

The active chemicals found in pomegranate are assumed to be responsible for the fruit's ability to boost local gastric defense mechanisms, re-epithelialization, and regeneration of the glandular architecture of the stomach mucosa as evidenced in histological sections (33). Prostaglandins, eNOS-mediated NO generation, tissue development factors, mucosal endogenous antioxidant status, chelating oxidative agents, and anti-angiogenic factors are all enhanced by pomegranate polyphenols (34). By promoting the precipitation of microproteins at the ulcerative site, constructing a barrier against irritants across the stomach mucosa, and inhibiting gastric secretions at the damage site, tannins—another beneficial component of PGAE—also helped with ulcer healing (35). It was expected that pomegranate rind extract generated in methanol would have a better antioxidant capacity (27).

In addition to the juice, pomegranate extracts from the peels, blossoms, and seeds also exhibit a substantial anti-inflammatory effect in the gut. Some inferences can be made from all the studies included in the current evaluation (36). Also, it appears that the pure substances found in pomegranate fruits function in various ways (37).

In a study, the ability of pomegranate (*Punica granatum* Linn) flower diethyl ether extracts to cure wounds in diabetic male Wistar rats was examined (38). Healthy wounded rats were given basic ointment as treatment. Others received basic ointment base treatment (39). In groups, rats with injuries were given nitrofurazone, a common medicine, while others were given a simple base ointment containing a 1:1 combination of the two extracts (40). Histopathological examinations revealed that all-natural extract-treated rats had significantly lower wound areas than the control and nitrofurazone groups (41). Among the natural extract groups, pomegranate flower extract produced the best results, with lesions completely healed by day 18 (42). In vitro, PGAE displayed significant activity against H. pylori with a MIC of 0.156 mg/mL and significant urease inhibitory activity with an IC50 of 6 mg/mL, in contrast to the confirmed strain. PGAE and metronidazole together have a synergistic impact on *H. pylori* with a MIC of fewer than 0.5. Rats given PPEE orally for 9 days experienced a considerable decrease in H. pylori gastritis, and even at quite large dosages, PGAE exhibited no symptoms of acute in vivo toxicity. PGAE can therefore be utilized as a possible substitute or additional therapy to reduce H. pylori contamination that is accompanied by clinical symptoms.

There are many reports (43-) about the extracts of medicinal plants and their biological and therapeutic effects, and the effects of one of them on pomegranate were discussed in this research.

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Interest conflict
The authors declare that they have no conflict of interest.

Author's contribution
AJM and ZZA: Experimental design and achievement of data; AMH, RKF and ZBT: Analysis and writing; MMH and SM: Revising and reviewing

Financial interest statement
Partial financial support was received from Cihan University-Erbil.

Ethics statement
(A) This research was approved by the Ethics Committee for Animal Experimentation, Faculty of Science, Cihan University-Erbil and Department of Biology, College of Education, Salahaddin University, Erbil (No.140, D,11.4.2021) Rats recognized the principles outlined in the United States National Academy of Sciences Guide for Care and Use of Research Laboratory Animals, which was issued by the National Institutes of Health (OECD, 2002).

(B) There is no whole or partial publication of this manuscript anywhere.
(C) The manuscript is not currently being considered for publication in another journal.
(D) Manuscript read and approved by all authors.

References

1. Palamides P, Jolaiya T, Idowu A, Loell E, Onyekwere C, Ugiagbe R, et al. Helicobacter pylori patient isolates from South Africa and Nigeria differ in virulence factor pathogenicity profile and associated gastric disease outcome. Sci Rep 2020;10:1–13.
2. Kassid OM, Khalaf Raheem A, Shamikh Hassan A. Prevalence and Risk Factors of Helicobacter Pylori Infection in Misan, Iraq: A Cross-Sectional Screening Study Using Stool Antigen Test. J Med Chem Sci 2022;5:1177–82.
3. Geng C, Xu X, Nie X, Lu D, Li J, Chen Q, et al. Expression of helicobacier pylori antibody in patients with pancreatic cancer. Acta Medica Mediterr 2019;35:51–4.
4. Piskinpasa N, Piskinpasa ME. Helicobacter pylori prevalence in patients getting dialysis and its correlations to endoscopy findings. Acta Medica Mediterr 2019;35:155–8.
5. Yulizal OK, Lelo A, Ilyas S, Kusumawati RL. The effect of Channa striata extract and standard eradication regimen on asymmetric dimethylarginine in Helicobacter pylori gastritis rat model. Vet World 2020;13:1605.
6. Yaseen MM, Alkubaisy SA, Mohammad WT, Jalil AT, Dilfy SH. Cancer and Complications of Peptic Ulcer in Type 2 Diabetes Mellitus patients at Wasit province, Iraq. J Med Chem Sci 2023;6:335–45.
7. Wu Q, Sun Q. Preventive effect of fresh yam extract on gastric ulcers in mice. Acta Medica Mediterr 2022;38:3295–303.
8. Mashayekhi-Sardoo H, Razavi BM, Ekhtiari M, Kheradmand N, Imenshahidi M. Gastroprotective effects of both aqueous and ethanolic extracts of Lemon verbena leaves against indomethacin-induced gastric ulcer in rats. Iran J Basic Med Sci 2020;23:1639.
9. Youssef H, El-Mahmoudy AM. Evaluation of the antimicrobial potential of Punica Granatum leaves hydro-methanolic extract against selected pathogens. Am J Curr Microbiol 2019;7:23–33.
10. Nuraddin SM, Amin ZA, Sofi SH, Osman S. Antibacterial and anti-ulcerogenic effects of Punicagranatum peel extract against ethanol-induced acute gastric lesion in rats. Zanco J Med Sci (Zanco J Med Sci) 2019;23:308–14.
11. Xiao F, Mao J. Treatment of gastroesophageal reflux-related cough with proton pump inhibitors and prokinetic agents. Acta Medica Mediterr 2019;35:3131–7.
12. Taha ZB, Odabaş-Serin Z, M Hussein A. Effect of Isatis spp. Extraction on the Growth of Aspergillus niger and Candida albicans. Cihan Univ Sci J 2020;4:85–9.
13. Lakshmidevi J, Appa RM, Naidu BR, Prasad SS, Sarma LS, Venkateswarlu K. WEPA: a bio-derived medium for added base, π-acid and ligand free Ullmann coupling of aryl halides using Pd (OAc) 2. Chem Commun 2018;54:12333–6.
14. Pedernera AM, Guardia T, Calderón CG, Rotelli AE, de la Rocha NE, Di Genaro S, et al. Anti-ulcerogenic and anti-inflammatory activity of the methanolic extract of Larrea divaricata Cav. in rat. J Ethnopharmacol 2006;105:415–20.
15. Nasr NM, Khider M, Metry W, Atallah K. Antibacterial Activity of Lactic Acid Bacteria against Helicobacter pylori Evidence by in vivo and in vitro Studies. Int J Curr Microbiol App Sci 2017;6:4235–47.
16. Kauffman Jr GL, Grossman MI. Prostaglandin and cimetidine inhibit the formation of ulcers produced by parenteral salicylates. Gastroenterology 1978;75:1099–102.
17. Njar VCO, Adesanwo JK, Raji Y. Methyl angolensate: the antiulcer agent of the stem bark of Entandrophragma angolense. Planta Med 1995;61:91–2.
18. OECD. OECD Guidelines for the Testing of Chemicals. Organization for Economic; 1981.
19. Laaraj N, Bouhrim M, Kharchoufa L, Tiji S, Bendaha H, Addi M, et al. Phytochemical Analysis, α-Glucosidase and α-Amylase Inhibitory Activities and Acute Toxicity Studies of Extracts from Pomegranate (Punica granatum) Bark, a Valuable Agro-Industrial By-Product. Foods 2022;11:1353.
20. Ghazaleh M, Mohammad S, Gholamreza H, Mahnaz K, Mannan H. Anti-ulcerogenic activity of the pomegranate peel (Punica granatum) methanol extract. Food Nutr Sci 2013;2013.
21. Alam MS, Alam MA, Ahmad S, Najmi AK, Asif M, Jahangir T. Protective effects of Punica granatum in experimentally-induced gastric ulcers. Toxicol Mech Methods 2010;20:572–8.
22. Gharzouli K, Khennouf S, Amira S, Gharzouli A. Effects of aqueous extracts from Quercus ilex l. root bark, Punica granatum l. fruit peel and Artemisia herba-alba Asso leaves on ethanol-induced gastric damage in rats. Phyther Res An Int J Devoted to Pharmacol Toxicol Eval Nat Prod Deriv 1999;13:42–5.
23. Stefanou V, Timbis D, Kanellou A, Margari D, Trianti M, Tsaknis I, et al. Wound Healing Properties of Pomegranate. Arch Microbiol Immunol 2021;5:263–91.
24. Abd el-Rady NM, Dahpy MA, Ahmed A, Elgamal DA, Hadiya S, Ahmed MAM, et al. Interplay of biochemical, genetic, and immunohistochemical factors in the etio-pathogenesis of gastric ulcer in rats: a comparative study of the effect of pomegranate loaded nanoparticles versus pomegranate peel extract. Front Physiol 2021;12:649462.
25. Patrick AT, Samson FP, Madusolumuo MA. Antiulcerogenic Effects of Aqueous Stem-Bark Extracts of Pterocarpus erinaceus on Indomethacin-Induced Ulcer in Albino Rats. J Biochem Cell Biol 2018;1:2.
26. Tache AM, Dinu LD, Vamanu E. Novel Insights on Plant Extracts to Prevent and Treat Recurrent Urinary Tract Infections. Appl Sci 2022;12:2635.
27. Yassin MT, Mostafa AA-F, Al Askar AA. In Vitro Evaluation of Biological Activities and Phytochemical Analysis of Different Solvent Extracts of Punica granatum L.(Pomegranate) Peels. Plants 2021;10:2742.
28. Karim S, Alkreathy HM, Ahmad A, Khan MI. Effects of methanolic extract based-gel from Saudi pomegranate peels with enhanced healing potential on excision wounds in diabetic rats. Front Pharmacol 2021;12:704503.
29. Zade A, Vyas J, Paithankar V, Wankhede A. Evaluation of antiulcer activity of herbal drugs on experimental animal: Myrica nagi (Myricaceae). GSC Biol Pharm Sci 2022;20:138–44.
30. Singh MP, Chawla V, Kaushik D, Chawla PA, Sisodia SS. Pharmacodynamic Stance of Phytoconstituents as a Gastric Ulcer Protective Mechanism: An Overview. Curr Mol Med 2022;22:431–41.
31. Abubakar US, Abdulrazak A, Fatima A, Aisha SS, Salma SG, Aisha JS, et al. Medicinal Plants Used for the Management of Ulcer in Kano State, Nigeria 2022.
32. Gupte PA, Mahajan MP, Kole MSR, Mandlecha AH, Tatke PA, Naharwar VA, et al. Efficacy and acceptability of pomegranate effervescent granules in patients suffering from acid peptic disorders. Indian J Pharmacol 2022;54:7.
33. Onoja SO, Chinyere BC, UGOJI D, UKWUEZE JI, MADUBUIKE KG, EZEJA MI. Anti-ulcer property of methanol fraction of Callichilia subsessilis leaf extract in albino rats. Not Sci Biol 2021;13:10886.
34. Hebel-Gerber S, García-Cancino A, Urbina A, Simirgiotis MJ,

Echeverría J, Bustamante-Salazar L, et al. Chilean Rhubarb, Gunnera tinctoria (Molina) Mirb.(Gunneraceae): UHPLC-ESI-Orbitrap-MS Profiling of Aqueous Extract and its Anti-Helicobacter pylori Activity. Front Pharmacol 2021;11:583961.

35. Pandey A. A Glimpse into the Indian Traditional Medicine with Special Reference to Use of Hemidesmus indicus in Southern India: A Review n.d.
36. Sezgin GC, Ocsoy I. Anthocyanin-Rich Black Carrot (Daucus Carota Ssp. Sativus Var. Atrorubens Alef.) and Red Cabbage (Brassica Oleracea) Extracts Incorporated Biosensor for Colorimetric Detection of Helicobacter Pylori with Color Image Processing 2022.
37. Dinat S, Orchard A, Van Vuuren S. A systematic review of African natural products against gastric ulcers and Helicobacter pylori. J Ethnopharmacol 2022:115698.
38. Gul H, Geng Z, Habib G, Hayat A, Rehman MU, Khan I. Effect of ellagic acid and mesocarp extract of Punica granatum on productive and reproductive performances of laying hens. Trop Anim Health Prod 2022;54:1–10.
39. Shareef SH, Ibrahim IAA, Alzahrani AR, Al-Medhtiy MH, Abdulla MA. Hepatoprotective effects of methanolic extract of green tea against Thioacetamide-Induced liver injury in Sprague Dawley rats. Saudi J Biol Sci 2022;29:564–73.
40. Choudhary P, Roy T, Chatterjee A, Mishra VK, Pant S, Swarnakar S. Melatonin rescues swim stress induced gastric ulceration by inhibiting matrix metalloproteinase-3 via down-regulation of inflammatory signaling cascade. Life Sci 2022;297:120426.
41. Alazzouni AS, Daim MA, Gabri MS, Fathalla AS, Albrakati A, Al-Hazani T, et al. Protective Effect of Pomegranate Peels Extracts Against Stomach Peptic-Ulcer Induced By Brexin In Albino Rats 2021.
42. Nasiri E, Hosseinimehr SJ, Akbari J, Azadbakht M, Azizi S. The effects of Punica granatum flower extract on skin injuries induced by burn in rats. Adv Pharmacol Sci 2017;2017.
43. Noor A, Zebarjadi A. Introduction of Chia (Salvia hispanica L.) as an Important Oil-Medicinal Plant. Agrotech Ind Crops 2022; 2(3): 104-116. doi: 10.22126/atic.2022.8010.1060.
44. Chaghakaboodi Z, Nasiri J, Farahani S. Fumigation Toxicity of the Essential Oils of Ferula persica against Tribolium castaneum and Ephestia kuehniella. Agrotech Ind Crops 2022; 2(3): 123-130. doi: 10.22126/atic.2022.8344.1068.
45. Ganjali S, Khajeh H, Gholami Z, Jomeh-ghasemabadi Z, Fazeli-Nasab B. Evaluation of Dormancy Failure Datura stramonium Plant Seeds under the Influence of Different Treatments. Agrotech Ind Crops 2022; 2(1): 32-41. doi: 10.22126/atic.2022.7656.1049.
46. Ghamarnia H, Palash M, Dousti B. Camelina Zoning for Different Climate Conditions in Kurdistan Province. Agrotech Ind Crops 2022; 2(1): 49-56. doi: 10.22126/atic.2022.7903.1056.
47. Ghamarnia H, Mousabeygi F, Rezvani SV. Water Requirement, Crop Coefficients of Peppermint (Mentha piperita L.) and Realizing of SIMDualKc Model. Agrotech Ind Crops 2021; 1(3): 110-121. doi: 10.22126/atic.2021.6791.1019.
48. Aryafar S, Sirousmehr A, Najafi S. The Impact of Compost on Seed Yield and Essential Oil of Black Cumin under Drought Stress Conditions. Agrotech Ind Crops 2021; 1(3): 139-148. doi: 10.22126/atic.2021.7184.1026.