JPPIPA 10(9) (2024)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Macroscopic and Microscopic Gastroprotective Activity of Mucoadhesive Granule Formulations of Clove Leaf Ethanol Extract

Dhitania Ivana Harliwong[1], Ayu Wulandari[2*], Niluh Puspita Dewi[3], Sririzqi Muthmainnah[2], Joni Tandi[3], Putu Intan[1]

[1] STIFA Pelita Mas Palu, Palu, Indonesia.
[2] Department of Technology Pharmacy, STIFA Pelita Mas Palu, Palu, Indonesia.
[3] Department of Pharmacology and Clinical Pharmacy, STIFA Pelita Mas Palu, Palu, Indonesia.

Received: May 12, 2024
Revised: July 24, 2024
Accepted: September 25, 2024
Published: September 30, 2024

Corresponding Author:
Ayu Wulandari
ayusuha8@gmail.com

DOI: 10.29303/jppipa.v10i9.8949

Abstract: Gastric ulcers can be triggered by excessive HCl secretion, and the regenerative capacity of mucosal cells can be weakened by high alcohol levels and anti-inflammatory drugs, including NSAIDs. Gastrin functions to stimulate the secretion of gastric juice, particularly HCl and pepsinogen This study aims to evaluate the macroscopic and microscopic gastroprotective activity of clove leaf ethanol extract mucoadhesive granule formulations. The study used 25 rats divided into five groups: normal control, negative control, positive control, clove leaf ethanol extract group, and mucoadhesive granule formulation of clove leaf ethanol extract group. All groups except the normal control were administered acetylsalicylic acid. Treatments were conducted over 9 days, with surgical examination and observations performed macroscopically and microscopically on day 10. Macroscopic assessments revealed ulcer damage improvement in the positive control, extract, and mucoadhesive granule groups, all scored at 1. Microscopic assessments also indicated a significant difference between the negative control and the positive control, extract, and mucoadhesive granule groups, showing better gastric repair, scored at 0.5. Therefore, it can be concluded that the administration of the mucoadhesive granule formulation of clove leaf ethanol extract exhibits gastroprotective activity comparable to that of the clove leaf ethanol extract.

Keywords: Clove leaf extract; Gastroprotective; Mucoadhesive granule

Introduction

The stomach is one of the organs in the digestive system that serves as a reservoir for food where it is intensively mixed with gastric juice (Ahï, 2017). The stomach wall consists of three layers, one of which includes G cells. G cells are located in the antral mucosa and secrete the gastric hormone gastrin (Vavallo et al., 2024). Gastrin functions to stimulate the secretion of gastric juice, particularly HCl and pepsinogen (Stanforth et al., 2022; Martinsen et al., 2019). If the gastric mucosa frequently comes into contact with the backflow of duodenal juice, which is alkaline, inflammation (gastritis) is very likely to occur and can lead to gastric ulcers (Susilawati et al., 2016).

Gastric ulcers are defined as the breakdown of the upper gastrointestinal mucosa due to peptic acid digestion, resulting in ulcers that extend beyond the muscular mucosa into the submucosa. Gastric ulcer disease remains one of the most common disorders encountered (Studiawan et al., 2023; Ahmad et al., 2019; Tarasconi et al., 2020). Gastric ulcers can also be

How to Cite:
Harliwong, D. I., Wulandari, A., Dewi, N. P., Muthmainnah, S., Tandi, J., & Intan, P. (2024). Macroscopic and Microscopic Gastroprotective Activity of Mucoadhesive Granule Formulations of Clove Leaf Ethanol Extract. *Jurnal Penelitian Pendidikan IPA*, *10*(9), 6331–6337. https://doi.org/10.29303/jppipa.v10i9.8949

triggered by a decrease in the mucosal capability, which, in a healthy state, is highly resistant to the aggressive nature of HCl-pepsin. Besides excessive HCl secretion, the regenerative capacity of mucosal cells can be weakened by high levels of alcohol and anti-inflammatory drugs, including NSAIDs.

NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) are medications that have pharmacodynamic effects such as analgesic, anti-inflammatory, and antipyretic properties (Tsoupras et al., 2024; Bindu et al., 2020). One example is acetylsalicylic acid, which works as an anti-inflammatory by inhibiting cyclooxygenase (COX) types 1 and 2, thereby inhibiting prostaglandin production. Gastroprotective therapy has been proven to prevent the adverse effects of NSAIDs. Gastroprotection refers to the ability of certain endogenous factors to protect the gastric mucosa (Shofiatun & Wulandari, 2021). Several plants have been identified as having gastroprotective activities, one of which is the clove leaf (Syzygium aromaticum L.) (Batiha et al., 2020).

Clove leaves (Syzygium aromaticum L.) contain eugenol, which has gastroprotective properties and exhibits antiulcer activity and is used to treat bacterial infections (Escherichia coli), urinary tract and bile duct infections (Aziz et al., 2023). Flavonoids in cloves can reduce the inflammation associated with ulcerative lesions, thereby diminishing the severity of the disease and enhancing gastric mucosa synthesis (Mezui et al., 2023). Based on these findings, clove leaf extract has been developed in the form of mucoadhesive granules. The mucoadhesive system allows the formulation to bind to the surface of gastric epithelial cells, resulting in prolonged drug residence time at the site of absorption (Subramanian, 2021).

Method

This study was conducted from July to August 2024 at the Pharmacology Laboratory of STIFA Pelita Mas Palu and the Biopath Laboratory in Bandung, using an experimental method. The study involved 25 rats divided into five groups: the normal control group (I) received mucoadhesive granules without extract; the Negative Control group (II) was administered acetylsalicylic acid at a dose of 500 mg/kg body weight; the Positive Control group (III) received acetylsalicylic acid at 500 mg/kg body weight and ranitidine at 150 mg/kg body weight; the Extract Treatment group (IV) was given acetylsalicylic acid at 500 mg/kg body weight and clove leaf extract at 250 mg/kg body weight; and the Granule Treatment group (V) received acetylsalicylic acid at 500 mg/kg body weight and mucoadhesive granules of clove leaf extract at 250 mg/kg body weight.

Research Procedures

Preparation of Clove Leaf Ethanol Extract

Approximately 800 grams of dried simplicia was extracted by maceration with 96% ethanol over three 24-hour periods using a jar. The extract was then filtered through filter paper to obtain a filtrate, which was subsequently concentrated using a rotary vacuum evaporator at 60°C and further evaporated using a water bath until a thick extract was obtained. The concentrated extract was then ready for qualitative analysis (phytochemical screening) to determine the secondary metabolite compounds (Herawati et al., 2021).

Preparation of Mucoadhesive Granules of Clove Leaf Ethanol Extract

The mucoadhesive granules of clove leaf ethanol extract were prepared using the wet granulation method. The clove leaf extract, used in all formulas at 250 mg, was mixed with Avicel and then homogenized with Carbopol. To this mixture, PVP dissolved in water was added to form a moist mass. The moist mass was then passed through a No. 8 sieve and dried at 40°C for 3 hours. The dry granules were subsequently sieved using a No. 12 sieve (Wulandari et al., 2024).

Animal Preparation

Twenty-five rats were grouped for the study and treatments were administered orally. The normal control group received granules without clove leaf, the negative control group was given acetylsalicylic acid at 500 mg/kg body weight, the positive control group received acetylsalicylic acid at 500 mg/kg body weight plus a ranitidine suspension at 150 mg/kg body weight, the extract group was administered acetylsalicylic acid at 500 mg/kg body weight plus clove leaf extract at 250 mg/kg body weight, and the granule group received acetylsalicylic acid at 500 mg/kg body weight plus clove leaf granule formulation at 250 mg/kg body weight. All treatments were given orally once a day at the same time for 9 days, and the rats were fasted for 24 hours. On the 10th day, all rats were anesthetized using chloroform, then dissected, and their stomachs were harvested. The stomachs were cut from the esophagus junction (above the cardia) to the lower pylorus (distal part connected to the duodenum), opened, and washed with physiological saline solution.

Macroscopic Observations

Macroscopic evaluation of gastric mucosal damage was assessed based on changes in mucosal color, ulcer stains, bleeding, deeper ulcers, and perforations with the aid of a magnifying glass. The assessment utilized a scoring system ranging from 0 to 5. Scoring criteria are detailed in Table 1.

Table 1. Scores and Criteria for Macroscopic Gastric Mucosal Damage

Score	Criteria
0	Normal
1	Redness
2	Ulcer Stain
3	Bleeding
4	Ulcer accompanied by bleeding
5	Perforation

Microscopic Observations

Microscopic examination was based on the depth of ulcers in the mucosa. The assessment used a mucosal lesion depth scoring scale from 0 to 5. Scoring criteria for the depth of mucosal ulcers are detailed in Table 2.

Table 2. Scores and Criteria for Evaluating Ulcer Depth

Score	Criteria
0	No changes
0.50	Erosion on the mucosal surface epithelium
1	Ulcer reaches the lamina propria
2	Ulcer extends to 2/3 or more of the mucosal layer
3	Ulcer spans the entire mucosal layer

Result and Discussion

Macroscopic Observations

This study was conducted in compliance with ethical standards. Free radical induction and oxidative stress cause gastric mucosal damage (Vona et al., 2021). Furthermore, an imbalance between aggressive and defensive factors can lead to gastric mucosal ulcers, with aggressive factors being more dominant. Free radicals are part of these aggressive factors (Tytgat, 2011; El-Meligy et al., 2017). One example of such radicals is non-steroidal anti-inflammatory drugs (NSAIDs). Acetylsalicylic acid, or aspirin, functions by inhibiting certain natural substances in the body to reduce pain and swelling, acting as an irritant (Dionisio et al., 2018; Fijałkowski et al., 2022; Osafo et al., 2017).

Table 3. Macroscopic Gastric Mucosal Damage Scores

Group	Mode Score
Normal Control	0
Negative Control (Acetylsalicylic Acid)	2
Positive Control (Ranitidine)	1
Clove Leaf Ethanol Extract	1
Mucoadhesive Granule Formulation of Clove Leaf Ethanol Extract	1

The animal model for gastric mucosal ulcer in this study was induced with acetylsalicylic acid. Acetylsalicylic acid causes defects in the mucosal barrier and results in the back diffusion of H+ ions. Histamine is stimulated to increase gastric acid secretion, leading to dilation and increased permeability of capillary vessels, gastric mucosal damage, and either acute or chronic gastric and mucosal ulcers (Rose & Harvey, 1987). This aligns with the macroscopic findings that the group administered acetylsalicylic acid exhibited ulcer stains but not bleeding (Figure 1). This could be due to insufficient dosage or duration of acetylsalicylic acid administration. Previous studies showed ulcer bleeding at 1500 mg/kg body weight (Kontoghiorghes, 2024).

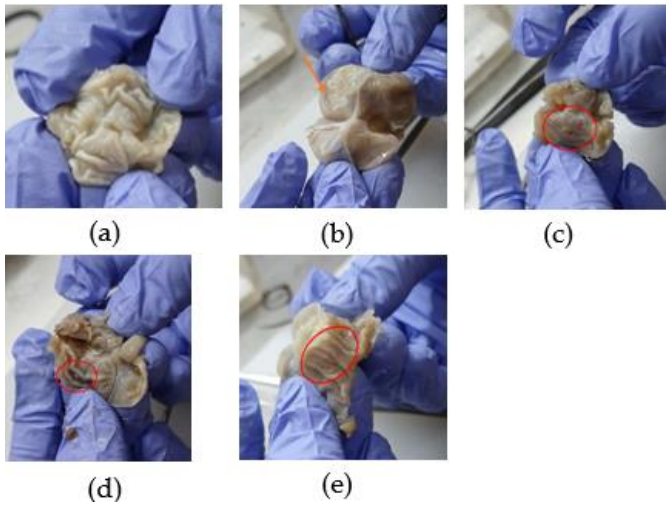

Figure 1. Macroscopic view of gastric damage. (a) Normal Control Group; (b) Negative Control Group induced with acetylsalicylic acid showing ulcer stains (arrow); (c) Positive Control Group induced with Ranitidine showing redness (circle); (d) Clove Leaf Ethanol Extract Group showing redness (circle); (e) Mucoadhesive Granule Formulation of Clove Leaf Ethanol Extract Group showing redness (circle)

Following ulcerogenic processes, the positive control group was administered ranitidine. Results indicated significant ulcer improvement as evidenced by the administration of ranitidine (Figure 1). Clove leaves (Syzygium aromaticum L.) contain eugenol, which has gastroprotective and antiulcer activities and is used to treat bacterial infections (Escherichia coli), urinary tract infections, and bile duct infections (Hobani et al., 2022; El-Saber Batiha et al., 2020; Pandey et al., 2024). Flavonoids in cloves can reduce the inflammatory process associated with ulcerative lesions, thereby decreasing the severity of the disease and enhancing gastric mucosal synthesis (Liñán-Atero et al., 2024). The study demonstrated that both clove leaf ethanol extract and the mucoadhesive formulation of clove leaf ethanol extract showed better ulcer improvement compared to the negative control (Table 3). However, no difference was observed between the extract and granule groups. This may be due to the granule formulation being dissolved before administration, which did not allow for prolonged contact with the gastric mucosa.

Ulcer improvement by clove leaves is attributed to their bioactive contents, particularly flavonoid

compounds, which have antioxidant activity that inhibits free radical formation and plays a role in the pathophysiology of gastritis and peptic ulcers. These compounds function as gastric cytoprotective agents by stabilizing membranes and influencing several intermediate metabolic processes. Prostaglandins help reduce gastric acid secretion, increase mucous and bicarbonate secretion, enhance mucous blood fluid, and act as antihistamines, thereby lowering histamine levels and preventing histamine release. They inhibit the gastric H+/K+ proton pump, reduce gastric acid secretion, and exhibit anti-H. pylori activity, thereby offering a more effective and less harmful therapeutic potential for treating gastrointestinal diseases, especially peptic ulcers (Kuna et al., 2019).

Microscopic Results and Discussion

Microscopic Evaluation of Gastric Mucosal Damage Microscopic evaluation of gastric mucosal damage was conducted based on the depth of ulcers, in accordance with prior research, as outlined in Table 2. Gastric mucosal damage can manifest as either erosions or ulcers. Erosion refers to the disruption of the gastric mucosa epithelial layer, while an ulcer is characterized by the disruption of mucosal continuity (epithelium, lamina propria, and muscularis mucosa) that sometimes extends into the muscular layer (Kellermann & Riis, 2021). Microscopic data on gastric mucosal damage indicated statistically significant differences among several treatment groups with a p-value of 0.000 ($p<0.05$). Microscopic views of mucosal damage are illustrated in Figure 2, and the mean values of mucosal damage are presented in Table 4.

The negative control group, in particular, exhibited white blood cell infiltration penetrating several submucosal layers and even muscle tissues (Pitot et al., 2023), as shown in Figure 2. In the normal group, glandular cells were still observed, while there was tissue disorganization forming erosions in the gastric mucosa in the positive control, extract, and mucoadhesive granule groups. This damage results from an imbalance between mucosal defense factors and causative factors such as acid and pepsin. Pepsin plays a role in protein digestion and can degrade cell protein structures, thus destroying the mucosa and causing erosion (Shafira et al., 2016).

The positive control used was ranitidine. Results indicated that the positive control significantly improved from the negative control but was not significantly different from the extract group. Various factors, including potentially inadequate ranitidine dosages making it ineffective or ranitidine being less potent in addressing acetylsalicylic acid-induced gastric ulcers, might cause this. According to research, ranitidine is less effective for gastric ulcers because it does not suppress basal gastric acid secretion, making it more effective in inhibiting acid secretion due to muscarinic drug stimulation, vagus stimulation, or gastrin (Chen et al., 2023; Arin et al., 2017). Moreover, eugenol compounds are more effective than ranitidine in preventing stress-induced gastric lesions (Elizabeth Lugo-Lugo et al., 2019).

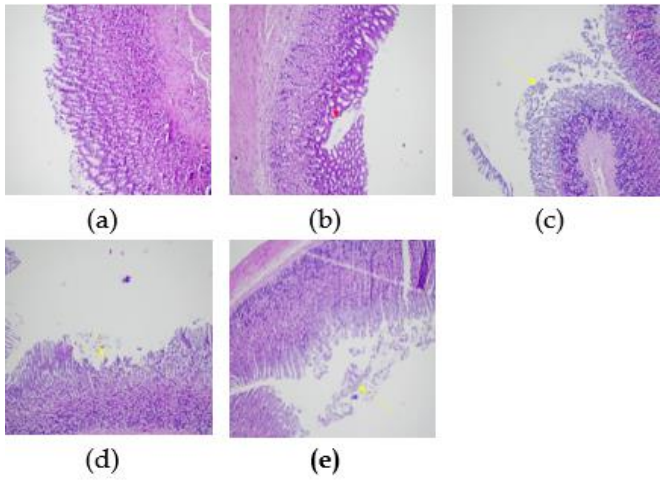

Figure 2. Microscopic view of gastric damage. (a) Normal Control Group; (b) Negative Control Group induced with acetylsalicylic acid showing ulcers extending through several mucosal layers; (c) Positive Control Group induced with Ranitidine showing epithelial surface mucosa erosion; (d) Clove Leaf Ethanol Extract Group showing epithelial surface mucosa erosion; (e) Mucoadhesive Granule Formulation of Clove Leaf Ethanol Extract Group showing epithelial surface mucosa erosion

According to Indiarto et al. (2024), the flavonoid content in clove leaves can reduce gastric acid secretion. One mechanism involved is through the inhibition of histidine decarboxylase enzyme, which is responsible for histamine formation in the gastric mucosa. By inhibiting this enzyme's activity, histamine formation in the stomach is reduced, ultimately inhibiting gastric acid production (Fitrya et al., 2022). Additionally, flavonoids possess gastroprotective activities as anti-secretory agents, antioxidants, and cytoprotective agents. Flavonoids' effects on the gastric mucosa involve blocking acid secretion, regulating prostaglandin E2-dependent secretion, and as antioxidants that bind to ROS (Jomova et al., 2023). Flavonoids also reduce intestinal motility and secretion, thereby mitigating chronic inflammatory injury (Sari & Yanuarty, 2022).

Conclusion

Based on the research conducted, it can be concluded that ranitidine induction demonstrates gastric damage both macroscopically and

microscopically. The administration of mucoadhesive granule formulations of clove leaf ethanol extract improves gastric damage, thereby exhibiting gastroprotective activity on par with that of clove leaf ethanol extract, both macroscopically and microscopically.

Acknowledgments
The authors would like to thank the Pharmacology Laboratory of STIFA Pelita Mas Palu, Biopath Laboratory Bandung, and the Pelita Mas Palu Foundation for facilitating the funding of this research.

Author Contributions
Conceptualization, D. I. H and A. W; methodology, N. P. D and S. M.; validation, D. I. H., and P. I.; data analysis, A.W., investigation, J.T.;resources, D. I. H and A. W.; data curation, J. T and N. P. D.; writing—original draft preparation, D. I. H and A. W.; writing -review and editing, D. I. H and P. I.; supervision, A. W and J. T.; all author have read and agreed to the published version of the manuscript.

Funding
This research received funding support from the Pelita Mas Foundation.

Conflicts of Interest
The authors declare there is no conflict of interest.

References

Ahï, B. (2017). Thinking about digestive system in early childhood: A comparative study about biological knowledge. *Cogent Education*, *4*(1), 1278650. https://doi.org/10.1080/2331186X.2017.1278650

Ahmad, A. A., Kasim, K. F., Ma'Radzi, A. H., & Gopinath, S. C. B. (2019). Peptic ulcer: Current prospects of diagnostic and nanobiotechnological trends on pathogenicity. *Process Biochemistry*, *85*, 51–59. https://doi.org/10.1016/j.procbio.2019.06.024

Arin, R. M., Gorostidi, A., Navarro-Imaz, H., Rueda, Y., Fresnedo, O., & Ochoa, B. (2017). Adenosine: Direct and Indirect Actions on Gastric Acid Secretion. *Frontiers in Physiology*, *8*, 737. https://doi.org/10.3389/fphys.2017.00737

Aziz, A. H. A., Rizkiyah, D. N., Qomariyah, L., Irianto, I., Che Yunus, M. A., & Putra, N. R. (2023). Unlocking the Full Potential of Clove (Syzygium aromaticum) Spice: An Overview of Extraction Techniques, Bioactivity, and Future Opportunities in the Food and Beverage Industry. *Processes*, *11*(8), 2453. https://doi.org/10.3390/pr11082453

Batiha, G. E. S., Alkazmi, L. M., Wasef, L. G., Beshbishy, A. M., Nadwa, E. H., & Rashwan, E. K. (2020). Syzygium aromaticum l. (Myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules*, *10*(2). https://doi.org/10.3390/biom10020202

Bindu, S., Mazumder, S., & Bandyopadhyay, U. (2020). Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. *Biochemical Pharmacology*, *180*, 114147. https://doi.org/10.1016/j.bcp.2020.114147

Chen, D., Hagen, S. J., Boyce, M., & Zhao, C. (2023). Neuroendocrine mechanism of gastric acid secretion: Historical perspectives and recent developments in physiology and pharmacology. *Journal of Neuroendocrinology*, *35*(11), e13305. https://doi.org/10.1111/jne.13305

Dionisio, K. L., Phillips, K., Price, P. S., Grulke, C. M., Williams, A., Biryol, D., Hong, T., & Isaacs, K. K. (2018). Data Descriptor: The Chemical and Products Database, a resource for exposure-relevant data on chemicals in consumer products. *Scientific Data*, *5*, 1–10. https://doi.org/10.1038/sdata.2018.125

Elizabeth Lugo-Lugo, D., De Jesús Pozos-Guillén, A., Zapata-Morales, J. R., Rodríguez-Chong, A., De Jesús Rangel-López, A., Saavedra-Leos, M. Z., & Vértiz-Hernández, A. A. (2019). Antinociceptive local activity of 4-allyl-1-hydroxy-2-methoxybenzene (Eugenol) by the formalin test: An anti-inflammatory effect. *Brazilian Journal of Pharmaceutical Sciences*, *55*, 1–7. https://doi.org/10.1590/s2175-97902019000118022

El-Meligy, R. M., Awaad, A. S., Soliman, G. A., Kenawy, S. A., & Alqasoumi, S. I. (2017). Prophylactic and curative anti-ulcerogenic activity and the possible mechanisms of action of some desert plants. *Saudi Pharmaceutical Journal*, *25*(3), 387–396. https://doi.org/10.1016/j.jsps.2016.10.008

El-Saber Batiha, G., Alkazmi, L. M., Wasef, L. G., Beshbishy, A. M., Nadwa, E. H., & Rashwan, E. K. (2020). Syzygium aromaticum L. (Myrtaceae): Traditional Uses, Bioactive Chemical Constituents, Pharmacological and Toxicological Activities. *Biomolecules*, *10*(2), 202. https://doi.org/10.3390/biom10020202

Fijałkowski, Ł., Skubiszewska, M., Grześk, G., Koech, F. K., & Nowaczyk, A. (2022). Acetylsalicylic Acid–Primus Inter Pares in Pharmacology. *Molecules*, *27*(23), 8412. https://doi.org/10.3390/molecules27238412

Fitrya, F., Amriani, A., Novita, R. P., Ahmadi, A., & Nabilah, R. (2022). Efektifitas Ekstrak Etanol Polong Petai [Parkia speciosa Hassk.] sebagai Anti Ulcer Pada Tikus Wistar yang Diinduksi Etanol Absolut. *Jurnal Sains Farmasi & Klinis*, *9*(1), 64. https://doi.org/10.25077/jsfk.9.1.64-70.2022

Herawati, E., Ramadhan, R., Ariyani, F., Marjenah, M., Kusuma, I. W., Suwinarti, W., Mardji, D., Amirta, R., & Arung, E. T. (2021). Phytochemical screening and antioxidant activity of wild mushrooms growing in tropical regions. *Biodiversitas Journal of Biological Diversity*, *22*(11). https://doi.org/10.13057/biodiv/d221102

Hobani, Y. H., Mohan, S., Shaheen, E., Abdelhaleem, A., Faruque Ahmad, M., Bhatia, S., & Abou-Elhamd, A. S. (2022). Gastroprotective effect of low dose Eugenol in experimental rats against ethanol induced toxicity: Involvement of antiinflammatory and antioxidant mechanism. *Journal of Ethnopharmacology*, *289*, 115055. https://doi.org/10.1016/j.jep.2022.115055

Indiarto, R., Herwanto, J. A., Filianty, F., Lembong, E., Subroto, E., & Muhammad, D. R. A. (2024). Total phenolic and flavonoid content, antioxidant activity and characteristics of a chocolate beverage incorporated with encapsulated clove bud extract. *CyTA-Journal of Food*, *22*(1), 2329144. https://doi.org/10.1080/19476337.2024.2329144

Jomova, K., Raptova, R., Alomar, S. Y., Alwasel, S. H., Nepovimova, E., Kuca, K., & Valko, M. (2023). Reactive oxygen species, toxicity, oxidative stress, and antioxidants: Chronic diseases and aging. *Archives of Toxicology*, *97*(10), 2499–2574. https://doi.org/10.1007/s00204-023-03562-9

Kellermann, L., & Riis, L. B. (2021). A close view on histopathological changes in inflammatory bowel disease, a narrative review. *Digestive Medicine Research*, *4*. https://doi.org/10.21037/dmr-21-1

Kontoghiorghes, G. J. (2024). The Puzzle of Aspirin and Iron Deficiency: The Vital Missing Link of the Iron-Chelating Metabolites. *International Journal of Molecular Sciences*, *25*(10), 5150. https://doi.org/10.3390/ijms25105150

Kuna, L., Jakab, J., Smolic, R., Raguz-Lucic, N., Vcev, A., & Smolic, M. (2019). Peptic ulcer disease: A brief review of conventional therapy and herbal treatment options. *Journal of Clinical Medicine*, *8*(2). https://doi.org/10.3390/jcm8020179

Liñán-Atero, R., Aghababaei, F., García, S. R., Hasiri, Z., Ziogkas, D., Moreno, A., & Hadidi, M. (2024). Clove Essential Oil: Chemical Profile, Biological Activities, Encapsulation Strategies, and Food Applications. *Antioxidants*, *13*(4), 488. https://doi.org/10.3390/antiox13040488

Martinsen, T. C., Fossmark, R., & Waldum, H. L. (2019). The Phylogeny and Biological Function of Gastric Juice—Microbiological Consequences of Removing Gastric Acid. *International Journal of Molecular Sciences*, *20*(23), 6031. https://doi.org/10.3390/ijms20236031

Mezui, C., Otto, G. L. N., Ngueke, L. N., Emakoua, J. F., Mbang, R. A. A., & Tan, P. V. (2023). Antiulcer activity of the stem bark aqueous extract of Croton oligandrum (Euphorbiaceae) against Ethanol/HCl-induced gastric mucosal injury in rats. *World Journal of Biological and Pharmaceutical Research, 5*(2), 023-030. https://doi.org/10.53346/wjbpr.2023.5.2.0073

Osafo, N., Agyare, C., Obiri, D. D., & Antwi, A. O. (2017). Mechanism of Action of Nonsteroidal Anti-Inflammatory Drugs. In A. G. A. Al-kaf (Ed.), *Nonsteroidal Anti-Inflammatory Drugs*. InTech. https://doi.org/10.5772/68090

Pandey, V. K., Srivastava, S., Ashish, Dash, K. K., Singh, R., Dar, A. H., Singh, T., Farooqui, A., Shaikh, A. M., & Kovacs, B. (2024). Bioactive properties of clove (Syzygium aromaticum) essential oil nanoemulsion: A comprehensive review. *Heliyon*, *10*(1), e22437. https://doi.org/10.1016/j.heliyon.2023.e22437

Pitot, M. A., Tahboub Amawi, A. D., Alexander, L. F., LeGout, J. D., Walkoff, L., Navin, P. J., Kawashima, A., Wood, A. J., Dispenzieri, A., & Venkatesh, S. K. (2023). Imaging of Castleman Disease. *RadioGraphics*, *43*(8), e220210. https://doi.org/10.1148/rg.220210

Rose, M. E., & Harvey, D. J. (1987). Drug metabolism, pharmacokinetics, and toxicity. In *Mass Spectrometry* (1st ed., pp. 303–372). The Royal Society of Chemistry. https://doi.org/10.1039/9781847556660-00303

Sari, D. R. A. P., & Yanuarty, R. (2022). Potensi Gastroprotektif Fraksi Etil Asetat Daun Cincau Hijau (Cyclea barbata Miers) terhadap Lambung Tikus yang Diinduksi Etanol. *PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia)*, *19*(2), 263. https://doi.org/10.30595/pharmacy.v19i2.13624

Shafira, A. N., Kairupan, C. F., & Durry, M. F. (2016). Gambaran histopatologik lambung tikus Wistar (Rattus norvegicus) yang diinduksi asam mefenamat dan diberi susu kental manis. *Jurnal E-Biomedik*, *4*(2). https://doi.org/10.35790/ebm.4.2.2016.14687

Shofiatun, M., & Wulandari, R. L. (2021). Efek Gastroprotektif Ekstrak Etanol Buah Labu Kuning (Cucurbita moschata (Duch.) Poir) Pada Tikus Jantan Galur Wistar Yang Diinduksi Aspirin. *Jurnal Ilmu Farmasi Dan Farmasi Klinik*, *17*(2), 79. https://doi.org/10.31942/jiffk.v17i2.4071

Stanforth, K. J., Wilcox, M. D., Chater, P. I., Brownlee, I. A., Zakhour, M. I., Banecki, K. M. R. M., & Pearson, J. P. (2022). Pepsin properties, structure, and its accurate measurement: A narrative review. *Annals of Esophagus*, *5*, 31–31. https://doi.org/10.21037/aoe-20-95

Studiawan, H., Sukardiman, Lazulfa, I., & Handayani, R. (2023). Anti-Gastritis Activity of Cloves (Eugenia caryophyllata Thunberg) and Lime (Citrus aurantifolia) Leaf Extracts Combination in Absolute Alcohol Induced-Gastric Injury Mice. *Pharmacognosy Journal*, *15*(3), 378–384. https://doi.org/10.5530/pj.2023.15.88

Subramanian, P. (2021). Mucoadhesive Delivery System: A Smart Way to Improve Bioavailability of Nutraceuticals. *Foods*, *10*(6), 1362. https://doi.org/10.3390/foods10061362

Susilawati, N. M., Yuliet, Y., & Khaerati, K. (2016). Aktivitas Gastroprotektif Ekstrak Etanol Daun Gedi Hijau (Abelmoschus manihot (L.) Medik) Terhadap Tikus Putih Jantan (Rattus norvegicus L.) Yang Diinduksi Dengan Aspirin. *Natural Science: Journal of Science and Technology*, *5*(3), 296–306. https://doi.org/10.22487/25411969.2016.v5.i3.7213

Tarasconi, A., Coccolini, F., Biffl, W. L., Tomasoni, M., Ansaloni, L., Picetti, E., Molfino, S., Shelat, V., Cimbanassi, S., Weber, D. G., Abu-Zidan, F. M., Campanile, F. C., Di Saverio, S., Baiocchi, G. L., Casella, C., Kelly, M. D., Kirkpatrick, A. W., Leppaniemi, A., Moore, E. E., & Catena, F. (2020). Perforated and bleeding peptic ulcer: WSES guidelines. *World Journal of Emergency Surgery*, *15*(1), 3. https://doi.org/10.1186/s13017-019-0283-9

Tsoupras, A., Gkika, D. A., Siadimas, I., Christodoulopoulos, I., Efthymiopoulos, P., & Kyzas, G. Z. (2024). The Multifaceted Effects of Non-Steroidal and Non-Opioid Anti-Inflammatory and Analgesic Drugs on Platelets: Current Knowledge, Limitations, and Future Perspectives. *Pharmaceuticals*, *17*(5), 627. https://doi.org/10.3390/ph17050627

Tytgat, G. N. J. (2011). Etiopathogenetic principles and peptic ulcer disease classification. *Digestive Diseases*, *29*(5), 454–458. https://doi.org/10.1159/000331520

Vavallo, M., Cingolani, S., Cozza, G., Schiavone, F. P., Dottori, L., Palumbo, C., & Lahner, E. (2024). Autoimmune Gastritis and Hypochlorhydria: Known Concepts from a New Perspective. *International Journal of Molecular Sciences*, *25*(13), 6818. https://doi.org/10.3390/ijms25136818

Vona, R., Pallotta, L., Cappelletti, M., Severi, C., & Matarrese, P. (2021). The Impact of Oxidative Stress in Human Pathology: Focus on Gastrointestinal Disorders. *Antioxidants*, *10*(2), 201. https://doi.org/10.3390/antiox10020201

Wulandari, A., Chintya, G., Tuldjanah, M., & Yanuarty, R. (2024). Characterizations of Swelling Granule of Clove Leaf (Syzygium aromaticum L.) Ethanol Extract. *Sciences of Pharmacy*, *3*(1), 35–39. https://doi.org/10.58920/sciphar0301182