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Laxative Properties of Agarwood Leaves of *Aquilaria* Species: A Review

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Abstract

Agarwood leaves have long been used as herbal medicine due to their diverse pharmacological qualities, one of which being laxative abilities to relieve constipation. Constipation is interpreted as a gastrointestinal ailment in which the stools are dry and firm with difficulty and infrequent evacuation. People generally suffer in silence and choose to take over-the-counter medications rather than addressing the problem. The main issue here is that currently available laxatives induce undesirable side effects such as cramps, diarrhea, nausea, and others. Previously conducted research discovered that *Aquilaria* species agarwood leaf extract has laxative qualities and has no adverse side effects on animal testing. Genkwanin 5-O-β primeveroside and mangiferin are shown as the major bioactive compounds in agarwood leaves extract that induce laxative effects. This review focuses on the laxative characteristics of agarwood leaves, bioactive compounds, and previous studies on *in vivo* testing.

1. Introduction

Agarwood, also known as gaharu or *karas* (leaves) in Malaysia, is a dark resinous wood created by trees in response to stress [1]. The *Aquilaria* species, which are rapidly declining due to increasing demand, have been added to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II list since 2004. Major *Aquilaria* species depend on tree ability to produce agarwood, with *A. malaccensis, A. subintegra, A. sinensis,* and *A. crassna*. *A. malaccensis* is the most species found in Malaysia together with *A. subintegra, A. sinensis* [2], [3]. Even though agarwood resin is highly sought for, agarwood leaves are frequently discarded during agarwood cultivation's trimming processes [4]. To address this problem, several areas of research could potentially be conducted to create new products using agarwood leaves, primarily for the food, pharmaceutical, and cosmetics industries. Additionally, in Chinese, Ayurvedic, and Tibetan herbal medicine, agarwood leaves have long been employed [1] and previous research has also shown their powerful therapeutic advantages. Laxative qualities in treating constipation are one of the therapeutic benefits of agarwood leaves which is emphasised in this review.

Constipation is a prevalent digestive issue in Malaysia and other parts of the world. There are several symptoms, such as less than three weekly defecations, lumpy or hard stool, a need to strain, the necessity for manual defecation techniques, the feeling of incomplete bowel evacuation, and the perception of anorectal

obstructions [5]. Moreover, constipation has been connected to a higher risk of colon cancer. The quality of life is greatly impacted by the intensity of the symptoms. Medication containing magnesium oxide or sennoside (the main ingredient in senna), is commonly used to treat constipation because of its strong purgative or laxative effects. On the other hand, these drugs may have the adverse consequence of extremely severe cramping diarrhea and feeling nausea [6]. Considering this, more research has to be done on laxative alternatives that do not cause patients any side effects like diarrhea. Agarwood leaf extract has shown laxative qualities, according to a few studies which employed through in vivo analysis on constipated mice and rats [6],[7],[8].

2. Agarwood (*Aquilaria* species) Overview

Agarwood is an exceptionally valued resinous wood derived principally from the genus *Aquilaria*, which is a member of the Thymelaeceae (Malvales) family and a subfamily of the Thymelaeoideae [9]. The agarwood tree is an enormous evergreen tree with a diameter of 1.5 to 2.5 metres that may grow to heights of more than 15 to 30 metres [10]. As stated in CITES, there are 21 species of *Aquilaria* that are currently known listed [1]. Based to their capacity to generate agarwood, *A. sinensis, A. subintegra, A. crassna*, and *A. malaccensis* are four of the major species [3]. Agarwood, a fragrant resin-containing heartwood from the Aquilaria species, is produced by damaged or injured bark due to microbial infection or as a defense mechanism against pathogens [10]. In addition, it is known as *gaharu* in South East Asia, *oud* in the Middle East, *chen xiangin* in China, *jinkoh* in Japan, *agar* in India, aloeswood, eaglewood, or kalamabak in many different nations [11], [12].

For thousands of years, people have utilised and traded agarwood across the world. Due to its great economic worth, agarwood is in high demand these days. The market has spread to the many different parts of the world for a variety of uses, including religious applications in India, incense and medicinal usage in China, and perfumes in the Arab world [9]. Along with the access to its precious wood, the leaves of the Aquilaria species are also becoming more and more in demand. These leaves are mainly employed in the food, beverage, and pharmaceutical industries for things like herbal tea, coffee, and food items [13]. *A. malaccensis* is a well-known agarwood species, primarily found in Malaysia. This species has a rapid rate of development and may thrive in a variety of soil types and conditions [14]. Three species of agarwood leaf which as famous among farmers are *A. malaccensis*, *A. sinensis* and *A. subintegra* as shown in Fig. 1 with their characteristics as classified in Table 1.

(a) **(b)** **(c)**

Fig. 1 *Leaves of agarwood tree (a) Aquilaria malaccensis, (b) Aquilaria sinensis, and (c) Aquilaria subintegra*

Table 1 *Characteristics of Aquilaria leaves* [9], [15]

	A. malaccensis	*A. sinensis*	*A. subintegra*
Leaf shape	Oblong lanceolate, caudate-acuminate	Orbicular, elliptic-oblong, obovate	Eliptic-oblong
Leaf texture	Papery	Leathery	Leathery
Leaf surface	Smooth	Smooth	Smooth

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Though research on agarwood leaves is limited its medicinal value has long been recognised [13]. The discovery of bioactive compounds in *Aquilaria* leaves beneficial in seeking for chemical compounds that could potentially be effective in medicinal interventions. Numerous health benefits, such as lipid-lowering, laxative, acetylcholinesterase inhibitory, anti-inflammatory, anticancer, antitumor, antioxidant, antibacterial, antifungal, and hepatoprotective properties in the human body, are associated with this diverse range of chemical compounds in agarwood leaves [16]. This review highlights on the laxative characteristics of agarwood leaf as one of the medical advantages for treating constipation.

3. Constipation Issue

Constipation is one of the most prevalent gastrointestinal issues. It is a common chronic illness that causes symptoms to worsen but is not life-threatening. It is described by the Ministry of Health Malaysia (MOH) as a reduced in the frequency of bowel movements, stiff stools, difficulty passing waste via the mouth, and the feeling that the bowels are still not entirely cleared [17]. The common symptom of constipation includes experience hard stools, straining, difficulty urinating, ineffective attempts to pass gas, feelings of incomplete evacuation, and difficult or irregular bowel motions (Fig. 2). Constipation may arise as a side effect of another ailment, such as diabetes, hypothyroidism, or cerebrovascular disease, or it may be triggered by a medication, such as analgesics and anticholinergic drugs [18].

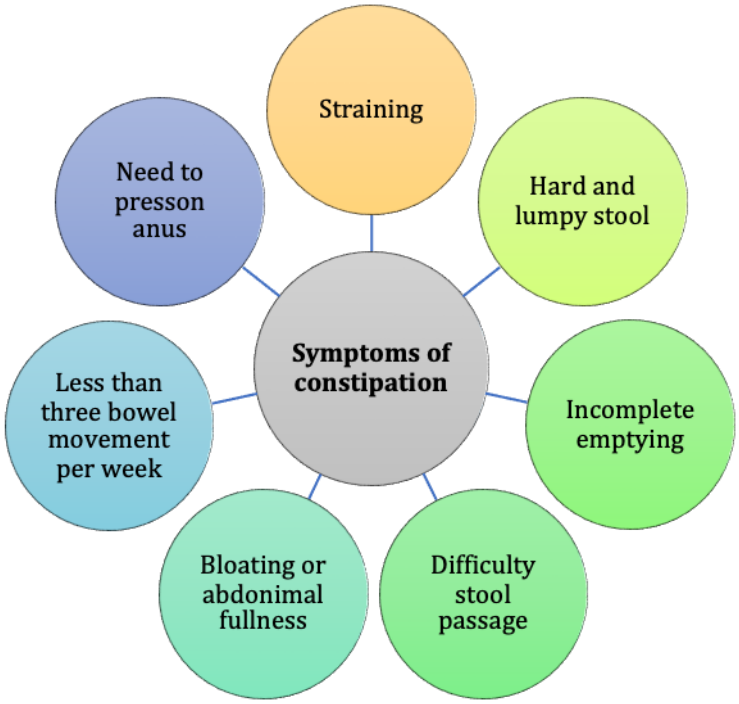


Fig. 2 *The symptoms of constipation* [19]

Constipation is a global issue that affects many individuals worldwide. Prevalence rates vary from 0.7-79% worldwide, with an overall median of 16% and a median of 33.5% among older populations. Studies show that constipation affects between 0.7 and 29.6% of children worldwide. Asian countries seem to have a lower prevalence of constipation with median of 10.8% than North America with 16%, Europe with 19.2%, and Oceania with 19.7% [20]. In Asia, the self-reported constipation issue rate ranges from 1.4-32.9% [18]. Constipation is a lifestyle problem that may exacerbate with ageing. It could be a persistent issue for which people need to use laxatives for an extended period of time. Nowadays, around 20–30% of seniors who are 60 years of age or older take laxatives several times a week. 60% of elderly persons self-report using laxatives and experiencing constipation 24–37% of the time [21].

It is critical to research therapeutic foods and plants with laxative qualities as alternatives to laxative drugs because they can have adverse consequences [22]. A laxative is a medication that stimulates or make bowel motions easier. Over-the-counter laxatives are classified into five types which are oral osmotic, oral bulk formers, oral stool softeners, oral stimulants, and rectal suppositories. The frequent possible adverse effects of the five main categories of laxatives are bloating, cramping, diarrhea, nausea and others [23]. Anthraquinoids are the most often used laxatives; they are derived from a crude extract of rhubarb or senna. Unfortunately, the powerful purgative action of anthraquinonoids renders them unsuitable for long-term use. In fact, even

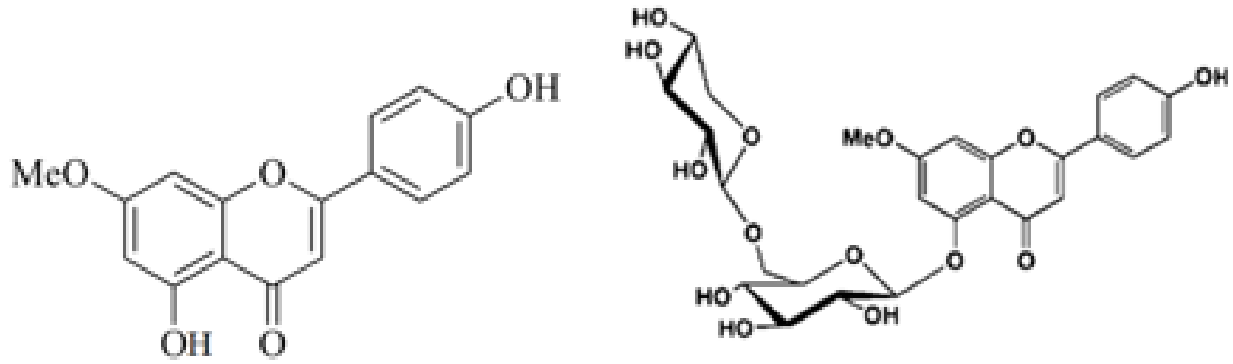
occasional intake of these laxatives can result in pseudomelanosis coli, which is a risk contributor to colon neoplasma. Agarwood has been shown in research studies to be a novel laxative source without any purgative action [6].

4. Laxative Potential's Medicinal Compound of Agarwood Leaves

Agarwood leaves have been shown through pharmacological study to have gastrointestinal regulating properties. Genkwanin 5-O-β-primeveroside and mangiferin have been shown to be the primary bioactive components that have an impact on intestinal motility [24].

4.1 Genkwanin

Genkwanin is one of the flavonoid lipid molecules found in agarwood *Aquilaria* species. It is a bitter-tasting chemical compounds present in a wide range of foods, mostly plants and vegetables. The molecular formula of Genkwanin is $C_{16}H_{12}O_5$ with a molecular weight of 284.26 g/mol [25]. Genkwanin 5-O-β-primeveroside is an O-methylated flavone, which is an instance of flavonoid. In the chemical structure, the substance is made up of a sugar moiety termed β-primeveroside that is coupled to the C5 oxygen atom [26]. Genkwanin 5-O-β-primeveroside is formed from its parent compound known as genkwanin which is a member of 7-O-methylated flavonoids with methoxy groups linked to the C7 of the flavonoid backbone. The chemical structure of genkwanin and genkwanin 5-O-β-primeveroside are as shown in Fig. 3. Regarding genkwanin 5-O-β-primeveroside, it has been shown that this primary active ingredient responsible for the laxative action is exclusively present in *Aquilaria* species [8] and in *Daphne genkwa* [27]. Genkwanin 5-O-β-primeveroside has the chemical formula $C_{27}H_{30}O_{14}$, and a molecular weight of 578.43 g/mol [26].



(a) **(b)**

Fig. 3 *Chemical structure of (a) Genkwanin* [8], *(b) Genkwanin 5-O-β-primeveroside* [6]

Genkwanin, notably genkwanin 5-O-β-primeveroside, is a bioactive molecule found in the leaves of *Aquilaria* species. That promotes laxative effects. The laxative action occurs when the intestines relax and loosen, and it is frequently used to treat constipation over a long period of time. According to research by Ito *et al.* [8], 1000mg/kg of agarwood leaves of water and ethanol extract had a potential to have a laxative impact on loperamide-induced mice. In accordance with study by Hara *et al.* [6], the minimal effective dosage of genkwanin 5-O-β-primeveroside for ethanol extract of agarwood leaves is 100mg/kg. Furthermore, agarwood leaves extract taken once daily at doses of around 150, 300, and 600 mg/kg has shown to markedly enhance stool weight, frequency, and water content in rats which has been induced with constipated due to a low-fiber diet [7].

4.2 Mangiferin

Mangiferin is an important xanthone-type chemical discovered in *Aquilaria* species. Xanthones are secondary metabolites discovered in higher plant groups, fungi, and lichens [28]. A basic xanthone structure has the chemical formula $C_{13}H_8O_2$. Mangiferin is a naturally emerging C-glucoside xanthone that is abundant in several parts of the mango tree primarily *Mangifera indica*, a member of the Anacardiaceae family. Other plant sources from which mangiferin can be extracted include *Aquilaria* species, *Bombax malabaricum*, *Gentiana lutiae* and *Swertia chirata*. Mangiferin has molecular weight of 422.34 g/mol and the chemical formula is $C_{19}H_{18}O_{11}$ [29]. Fig. 4 depicts the molecular structure of mangiferin.

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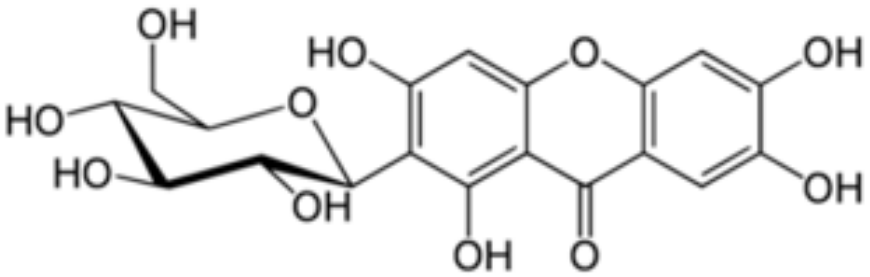

Fig. 4 *Chemical structure of mangiferin* [29]

Numerous pharmacological actions, such as those that are antidiabetic, anti-inflammatory, antioxidant, and laxative, have been linked to mangiferin. The most well-known and extensively studied bioactivity associated with mangiferin is its antidiabetic effect. Moreover, it has been shown that this substance inhibits several carbohydrates, such as sucrase, alpha-glucosidase, maltase, isomaltase, alpha-amylase, and aldose reductase [30]. According to Kakino *et al.* [7], acetylcholine receptor stimulation is responsible for mangiferin's laxative activity. Additionally, it promotes gastrointestinal content activity and increases small intestine muscle tension in mice, resulting in an increase in the number and weight of stool beads [7], [24].

5. Animal Study on Laxative Effect of Agarwood Leaf Extract

The gastrointestinal (GI) tract of a human being is a highly complex organ that has evolved to effectively absorb nutrients from meals while also acting as a barrier to any germs that may be present. Understanding how it works in more depth is required if we are to improve nutraceutical distribution in the years to come. Due to the difficulty in directly getting information from the human gut, a range of models, including animal, cellular, and *in-vitro* models, have been employed [31]. Several factors need to be considered while selecting the most suitable animal model for pharmaceutical research [32]. Although there are still ethical considerations that need to be made, using animal models appears to have advantages over human clinical trials in terms of the range of concerns that may be addressed and the lower cost of the studies [33].

Previous research has utilised *in vivo* animal models with mice and rats to study the laxative effects of agarwood leaf extract. Crude extract of agarwood leaves were provided to loperamide induced mice [8] and low fiber-diet induced mice [7] to test their laxative capabilities. To determine the efficiency of different dosages of samples' laxative characteristics, stool parameters (frequency and weight of faeces) were examined [7], [8]. Table 2 shows a more complete summary of the preceding approaches' procedures that include agarwood leaves species, animal model utilised (including species, age and weight, feed and constipation induction), evaluation parameter and observations from each studies.

6. Conclusion

In conclusion, considering the pharmacological qualities, leaves from agarwood tree growth might be used to diversify products on the market rather than being discarded away. Agarwood leaf extract's laxative qualities may hold novel prospects for the development of nutraceutical or functional food products. Additionally, this will eventually assist farmers in earning additional revenue before harvesting the resin from agarwood trees, which can take up to ten years to develop, as the trees already produce mature leaves in the earlier years. Therefore, further research on the laxative qualities of the leaves and potential product development must be considered according to the demands of the market today.

Table 2 *Animal model and evaluation parameters of laxative effect analysis of agarwood leaf extract (in vivo analysis)*

Agarwood species	Animal model and constipation induction	Evaluation parameters	Observation/Conclusion	Ref.
***Aquilaria sinensis* and isolated compounds**	- **Species**: Male ddY (Deutschland, Denken, and Yoken) mice - **Age**: 7 to 10 weeks old - **Weight**: 20 to 35g body weight. - **Condition**: Kept in a temperature controlled environment (24.5 to 25.0°C) with a 12/12 hour light/dark cycle. - **Feed**: *Ad libitum* animal food pellets and tap water have been given. - **Control**: Distilled water (negative); Senna extract (positive)	▪ **Observe**: frequency and total stool wet weight generated ▪ **Time**: Over four successive 2 hours intervals (0-2 h, 2-4 hour, 4-6 hour, and 6-8 hour)	▪ Isolated compound (genkwanin 5-O-β primeveroside) shown significant increase in weight of stools and frequency (at dose of 100 to 1000mg/kg). ▪ Senna extract at 300mg/kg induced severe diarrhea. ▪ Agarwood leaves extract and isolated compounds did not induce diarrhea up to 1000mg/kg.	[6]
Aquilaria crassna	- **Species**: Male ddY mice - **Age**: about 6 weeks old - **Condition**: Kept in a temperature controlled environment (24.5 to 25.0°C) with a 12/12 hour light/dark cycle. - **Feed**: Food pellets (CE-2) and tap water were provided *ad libitum*. - **Constipation induced**: using loperamide hydrochloride (5mg/kg)	▪ **Observe**: frequency and total stool wet weight generated ▪ **Time**: For 8 hours at each 2 hours intervals (0-2 h, 2-4 hour, 4-6 hour, and 6-8 hour)	▪ At 1000mg/kg, water extract (at 70 and 95°C) and 60% ethanol extract significantly recovered wet weight. ▪ Water extract (at 30 and 70°C) showed a potential to restore wet weight. ▪ The frequency of stools was considerably recovered by 60% ethanol extract at 1000 mg/kg. ▪ There was a tendency for the frequency to be restored by water extract at 30, 50, 70, and 95°C.	[8]
Aquilaria sinensis	- **Species**: Male Sprague Dawley (SD) rat model - **Age** : 6 weeks old - **Condition**: Housed at a controlled room temperature (24.5-25.0°C) with a 12/12 hours light/dark cycle. - **Feed**: Food pellets (CE-2) and tap water were provided *ad libitum*. - **Constipation induced**: Fed a low-fiber diet for 5 weeks in order to induce constipation. - **Low-fiber diet content**: corn starch (41.5%), milk casein (24.5%), sucrose (10.0%), dextrin (10.0%), mineral mixture (7.0%), corn oil (6.0%), and vitamin mixture (1.0%).	▪ **Observe**: the frequency, weight, and water content of each rat's stool ▪ **Time**: 16 hours, at 2 hour intervals.	▪ Stool frequency, weight, and water content were dramatically increased after a single treatment with ethanol extract (600 mg/kg) and senna (150 and 300 mg/kg). ▪ For 14 days, ethanol extract (150 mg/kg) administered once daily resulted in a significant increase in the water content of faeces. ▪ Higher ethanol extract dosages (300 and 600 mg/kg) significantly increase stool frequency, weight, and water content. ▪ Agarwood extract does not cause diarrhea, while senna induced severe diarrhea.	[7]

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***study conception and design:*** *Khairunnisa Abdhul Muthalib, Balkis A. Talip, Hazian Saleh;* ***data collection:*** *Khairunnisa Abdhul Muthalib, Balkis A. Talip, Nadia Nabila Mohd Kodeem;* ***analysis and interpretation of results:*** *Khairunnisa Abdhul Muthalib, Balkis A. Talip, Nadia Nabila Mohd Kodeem;* ***draft manuscript preparation:*** *Khairunnisa Abdhul Muthalib, Balkis A. Talip, Nadia Nabila Mohd Kodeem. All authors reviewed the results and approved the final version of the manuscript.*

References

[1] CITES. (2022). CoP19 Doc. 62.2 (Rev. 1): Agarwood-producing taxa (*Aquilaria* spp. and Gyrinops spp.). *Nineteenth meeting of the Conference of the Parties Panama City (Panama).* Switzerland: CITES Secretariat. 1-16. https://cites.org/sites/default/files/documents/E-CoP19-62-02-R1.pdf.

[2] Adam, A. Z., Lee, S. Y., & Mohamed, R. (2017). Pharmacological properties of agarwood tea derived from *Aquilaria* (Thymelaeaceae) leaves: An emerging contemporary herbal drink. *Journal of herbal medicine*, *10*, 37-44. https://doi.org/10.1016/j.hermed.2017.06.002.

[3] Mokhtar, A.M.A., Zain, H.H.M. & Muayad, M.T.A. (2021). Overview of medicinal properties and toxicities of agarwood species. *Educatum Journal of Science, Mathematics and Technnology, 8*(2), 1-16. https://doi.org/10.37134/ejsmt.vol8.2.1.2021.

[4] Wangiyana, I. G. A. S., Nikmatullah, A., & Mulyaningsih, L. (2021). Tannin concentration of gyrinops tea taken from different agarwood plantation and different processing method. *IOP Conference Series: Earth and Environmental Science*. United Kingdom: IOP Publishing. 1-7. https://doi.org/10.1088/1755-1315/913/1/012068.

[5] Wahab, P. A., Yusoff, D. M., Kadir, A. K., Ali, S. H. & Yeh, L. Y. (2022). Prevalence, symptoms, and associated factors of chronic constipation among older adults in north-east of peninsular Malaysia. *Clinical nursing research*, *31*(2), 348-355. https://doi.org/10.1177/10547738211033934.

[6] Hara, H., Ise, Y., Morimoto, N., Shimazawa, M., Ichihashi, K., Ohyama, M. & Iinuma, M. (2008). Laxative effect of agarwood leaves and its mechanism. *Bioscience, Biotechnology, and Biochemistry, 72*(2), 335-345. https://doi.org/10.1271/bbb.70361.

[7] Kakino, M., Tazawa, S., Maruyama, H., Tsuruma, K., Araki, Y., Shimazawa, M. & Hara, H. (2010). Laxative effects of agarwood on low-fiber diet-induced constipation in rats. *BMC Complementary and Alternative Medicine, 10*(68), 1-8. https://doi.org/10.1186/1472-6882-10-68.

[8] Ito, T., Kakino, M., Tazawa, S., Watarai, T., Oyama, M., Maruyama, H., Araki, Y., Hara, H. & Iinuma, M. (2012). Quantification of polyphenols and pharmacological analysis of water and ethanol-based extracts of cultivated agarwood leaves. *Journal of Nutritional Science and Vitaminology, 58*, 136-142. https://doi.org/10.3177/jnsv.58.136.

[9] Lee, S.Y. & Mohamed, R. (2016). The origin and domestication of *Aquilaria*, an important agarwood-producing genus. In Mohamed, R. Agarwood. Tropical Forestry. Singapore: Springer. 1-20. https://doi.org/10.1007/978-981-10-0833-7_1.

[10] Zainurin, N.A.A., Samsudin, N., Hashim, Y.Z.H-Y., Al-Khatib, M.F.R., Azmin, N.F.M. and Maifiah, M.H.M. (2020). Response surface optimization of the yield of agarwood (*Aquilaria* malaccensis) leaf extract using soxhlet 70 extraction. *International Journal of Recent Technology and Engineering, 8*(6), 1926–1934. https://doi.org/10.35940/ijrte.F8013.038620.

[11] Liu, Y. Y., Wei, J. H., Gao, Z. H., Zhang, Z., and Lyu, J. C. (2017). A review of quality assessment and grading for agarwood. *Chinese Herbal Medicines, 9*(1), 22–30. https://doi.org/10.1016/S1674-6384(17)60072-8.

[12] Maharani, R., Fernandes, A., Turjaman, M., Lukmandaru, G., & Kuspradini, H. (2016). The characterization of phytochemical and GC-MS analysis on Borneo agarwood (*Aquilaria* Malaccensis Lamk) leaves and its utilization as an anti-browning in apple juice. *International Journal of Pharmacognosy and Phytochemical Research. 8*(10), 1576-1582. https://impactfactor.org/PDF/IJPPR/8/IJPPR,Vol8,Issue10,Article3.pdf.

[13] Hsiao, S. W., Wu, Y. C., Mei, H. C., Chen, Y. H., Hsiao, G., & Lee, C. K. (2021). Constituents of *Aquilaria* sinensis leaves upregulate the expression of matrix metalloproteases 2 and 9. *Molecules*, *26*(9), 2537. https://doi.org/10.3390/molecules26092537.

[14] Elias, M.F., Ibrahim, H. & Mahamod, W.R.W. (2017). A review on the Malaysian *Aquilaria* species in karas plantation and agarwood production. *International Journal of Academic Research in Business and Social Sciences, 7*(4), 1021-1029. http://dx.doi.org/10.6007/IJARBSS/v7-i4/2911.
[15] Noor, M. H. A., Mohamad, A., Othman, R., & Baharum, S. N. (2014). Morphological evaluation on leaf characteristics of common Aquilaria species in Malaysia. Retrieved on November 20, 2022, from https://inis.iaea.org/collection/NCLCollectionStore/_Public/46/116/46116554.pdf?r=1.
[16] Adam, A. Z., Tajuddin, S. N., Sudmoon, R., Chaveerach, A., Abdullah, U. H., Mahat, M. N., & Mohamed, R. (2018). Chemical constituents and toxicity effects of leaves from several agarwood tree species (*Aquilaria*). *Journal of Tropical Forest Science*, *30*(3), 342-353. https://doi.org/10.26525/jtfs2018.30.3.342353.
[17] Isa, A. (2020). Constipation. Retrieved on, October 21, 2023, from, http://www.myhealth.gov.my/en/constipation-3/.
[18] Patimah, A.W., Lee, Y.Y. & Dariah, M.Y. (2017). Frequency patterns of core constipation symptoms among the Asian adults: a systematic review. *BMC Gastroenterol, 17*, 1-12. https://doi.org/10.1186/s12876-017-0672-z.
[19] Schiller, L. R. (2019). Chronic constipation: new insights, better outcomes?. *The lancet Gastroenterology & hepatology*, *4*(11), 873-882. https://doi.org/10.1016/S2468-1253(19)30199-2.
[20] Mugie, S. M., Benninga, M. A. & Di Lorenzo, C. (2011). Epidemiology of constipation in children and adults: a systematic review. *Best practice & research. Clinical gastroenterology*, *25*(1), 3–18. https://doi.org/10.1016/j.bpg.2010.12.010.
[21] Hasim, R. (2012). Digestive Disorder. Retrieved on December 25, 2022, from http://www.myhealth.gov.my/en/digestive-disorder/.
[22] Lee, H. J., Choi, E. J., Park, S., & Lee, J. J. (2020). Laxative and antioxidant effects of ramie (*Boehmeria nivea* L.) leaf extract in experimental constipated rats. *Food Science & Nutrition*, *8*(7), 3389-3401. https://doi.org/10.1002/fsn3.1619.
[23] Frothingham, S. (2019). Common side effects of laxatives. Retrieved on October 21, 2023, from https://www.healthline.com/health/laxatives-side-effects.
[24] Kakino, M. & Hara, H. (2016). Pharmacological effects of *Aquilaria* spp. leaves and their chemical constituents. *Agarwood: Science behind the fragrance*, 125-136. https://doi.org/10.1007/978-981-10-0833-7_8.
[25] National Center for Biotechnology Information (2022). PubChem compound summary for CID 5281617, Genkwanin. Retrieved November 4, 2022 from https://pubchem.ncbi.nlm.nih.gov/compound/Genkwanin.
[26] Ngui, W.T.S. (2018). *Isolation, identification and bioactivity evaluation of mangiferin and genkwanin 5-O-β-primeveroside in gaharu plant parts and finished products for Gaharu Technologies Sdn Bhd.* University of Nottingham Malaysia Campus: Degree of Master of Philosophy's Thesis.
[27] Lin, J.H., Lin, Y.T., Huang, Y.J., Wen, K.C., Chen, R.M., Ueng, T.H. & Liao, C.H. (2001). Isolation and cytotoxicity of flavonoids from *Daphnis Genkwae Flos*. *Journal of Food and Drug Analysis, 9*(1), 6-11. https://doi.org/10.38212/2224-6614.2810.
[28] Negi, J.S., Bisht, V.K., Singh, P., Rawat, M.S.M. & Joshi, G.P. (2013). Naturally occurring xanthones: chemistry and biology. *Journal of Applied Chemistry, 2013,* 1-10. https://doi.org/10.1155/2021/8725409.
[29] Morozkina, S. N., Nhung Vu, T. H., Generalova, Y. E., Snetkov, P. P., & Uspenskaya, M. V. (2021). Mangiferin as new potential anti-cancer agent and mangiferin-integrated polymer systems—A novel research direction. *Biomolecules*, *11*(1), 1-27. https://doi.org/10.3390/biom11010079.
[30] Yoshikawa, M., Nishida, N., Shimoda, H., Takada, M., Kawahara, Y., & Matsuda, H. (2001). Polyphenol constituents from Salacia species: quantitative analysis of mangiferin with alpha-glucosidase and aldose reductase inhibitory activities. *Yakugaku zasshi: Journal of the Pharmaceutical Society of Japan*, *121*(5), 371-378. https://doi.org/10.1248/yakushi.121.371.
[31] Mackie, A. (2012). Interaction of food ingredient and nutraceutical delivery systems with the human gastrointestinal tract. In Garti, N. & McClements, D.J. *Encapsulation technologies and delivery systems for food ingredients and nutraceuticals*. United Kingdom: Woodhead Publishing. 49-70. https://doi.org/10.1533/9780857095909.1.49.
[32] Mukherjee, P., Roy, S., Ghosh, D., & Nandi, S. K. (2022). Role of animal models in biomedical research: a review. *Laboratory Animal Research*, *38*(1), 1-17. https://doi.org/10.1186/s42826-022-00128-1.
[33] Bennett, A. (2010). The use of human tissues and cells in biomedical research: the unusual suspects. *Alternatives to Laboratory Animals*, *38*(1), 5-9. https://doi.org/10.1177/026119291003801S03.