Journal for Research in Applied Sciences and Biotechnology

www.jrasb.com

ISSN: 2583-4053

Volume-1 Issue-5 || December 2022 || PP. 16-23

https://doi.org/10.55544/jrasb.1.5.2

In-vitro Anti-Ulcer Activities of Mallotus Japonicus

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www.jrasb.com || Vol. 1 No. 5 (2022): December Issue

Received: 15-10-2022	**Revised:** 05-11-2022	**Accepted:** 15-11-2022

ABSTRACT

Objectives: This study's objective was to investigate whether or not a methanolic extract of Mallotus japonicas could decrease H+-K+ ATPase activity and neutralise acid.

Materials and Methods: We assessed the total phenolic and flavonoid contents of the sample while it was exposed to varying amounts of standard esmoprazole and methanol extract.

Results: The proton pump inhibitory activity of the extract from stomach mucosal homogenate was found to be significant (*P<0.05) and on par with the standard.

Conclusions: Based on these findings, it may be concluded that the proton pump can be effectively blocked by the methanolic extract.

Keywords- *In-vitro*, Proton pump Inhibitor, Gastric Mucosal, Herbal plants.

I. INTRODUCTION

Mallotus japonicus, also known as "Akamegashiwa" in Japanese, is a plant that belongs to the family Euphorbiaceae and is used in gardens for its purported therapeutic benefits [1,2]. This is a plant that does well in East Asian temperatures that range from tropical to temperate. Extracts of the leaf, root, bark, and pericarp of the M. japonicus plant have demonstrated significant potential for treating a wide range of conditions, such as ulcers of the stomach and duodenum, hyperacidity, irritable bowel syndrome (IBS), haemorrhoids, rheumatism, diabetes, skin and liver disorders, and nerve pain [3-5]. According to the "17th edition of the Japanese Pharmacopoeia," M. japonicus bark is still recommended as a stomach tonic that can stimulate appetite. In the past, bark tissue was utilised as a stopgap treatment for a variety of conditions, including gallstones, gastrointestinal ailments, and cancer [6]. Additionally, it was utilised as a folk remedy for the treatment of ulcers of the stomach and duodenum. The medicinal effects of a plant come from its metabolic components, which may contain bioactive metabolites. These components are responsible for the plant's overall metabolism. These metabolites are accountable for the impacts that the plant has. In most cases, the buildup of such compounds will follow a pattern that is specific to the type of tissue that is being examined. To this day, over 143 compounds have been isolated from different species of Mallotus, with M. japonicus alone accounting

ISSN: 2583-4053
Volume-1 Issue-5 || December 2022 || PP. 16-23
https://doi.org/10.55544/jrasb.1.5.2

for 99 of these compounds. These chemical groups include things like alkaloids, iridoids, flavonoids, polyphenols, cardenolides, phloroglucinol, tannins, and their derivatives [7]. Other examples of these chemical groups include tannins. Tannins are considered to be part of these broader categories of chemical substances. It has been demonstrated that the leaves of M. japonicus, which are abundant in rutin and isoprenoid derivatives, are useful in the treatment of boils and swellings [8]. The antioxidant capacities of the leaves of M. japonicus, in addition to those of the plant's component components, have been investigated, and the plant itself is utilised as a dietary supplement and functional food product. It has proven to be a successful treatment for a wide range of gastrointestinal conditions [6,7,8,9,10], such as gastritis, stomach ulcers, constipation, and diarrhoea. [6,7] The bark of M. japonicus contains a significant amount of bergenin, which is a derivative of dihydroisocoumarin. For the past 45 years, drugs that treat diarrhoea have relied on M. japonicus as an active ingredient. Despite the vast commercial and medical application of M. japonicus and its potential as a source for innovative treatments, comparatively few genomes and metabolomics resources are now available for the organism [9]. This is because M. japonicus is difficult to culture. The breadth and depth of human comprehension have both grown substantially as a direct result of developments in technology that make high-throughput omics analysis possible. The omics data sets that are specific to a single species are extremely valuable. It is beneficial to look at how a biological species interacts with other related species and to develop associations with data from other omics studies [10] in order to understand more about the biological species in question. Untargeted metabolomics collects information on all metabolites, regardless of whether or not such metabolites are known to exist. In contrast, targeted metabolomics focuses on particular chemicals that are of interest. On the other hand, the information that can be obtained by focused metabolomics is restricted to just a small selection of the metabolites that are found in a specific organism. De novo transcriptome assembly based on RNA-seq has emerged as an important tool for the study of non-model plant species for whom extensive genomic data is not available [11]. This technique was initially developed for the study of model plant species. Because of the tremendous advancements that have been made in sequencing technology, this approach was developed. A better understanding of metabolic pathways and how they are distributed in plants can be gained by the analysis of the transcriptome [12]. In a similar vein, making use of the sequence similarity method in conjunction with a variety of statistical studies might be of assistance in the process of looking for genes that are perhaps involved in the production of critical metabolites. These strategies, when paired with phylogenetic approaches, can assist researchers in isolating enzymes that may be involved in the manufacture of secondary metabolites. Mallotus japonicus is a species of shrub that can be found in its natural habitat in Japan, Korea, and Taiwan, in addition to the Chinese provinces of Zhejiang and Jiangsu. This species of plant belongs to the family Euphorbiaceae. It has been hypothesised that the Mallotus japonicus plant might have some application in traditional Chinese medicine [13-15]. Because Mallotus japonicus has such significant applications in medicine, gaining an understanding of the plant's plastome and its location in the taxonomic hierarchy is a key focus of research. However, our understanding of the structure of the plastome in Mallotus japonicus is still somewhat limited. In this study, we have made the complete Mallotus japonicus plastome sequence available to the public in an effort to protect biodiversity and supply the general public with important genetic data [16]. Through the use of untargeted metabolite profiling and deep sequencing on seven different tissues, we were able to create a comprehensive metabolite library as well as genetic resources for M. japonicas (young leaf, mature leaf, young stem, mature stem, bark, central cylinder, and inflorescence). We were successful in determining the precise accumulation pattern of a number of different classes of specialised metabolites in M. japonicus by employing a method that is known as untargeted metabolomics. The expression data from the collected transcripts as well as the intensities of the MS2 verified metabolites were used in a WCNA-based network analysis that was carried out [17-20]. The goal of this research was to find modules, which are groups of genes or metabolites that have certain properties in common with one another. Utilizing a pairwise correlation between the transcript and metabolite modules allowed for the discovery of the connections that exist between genes and the products that they produce. This led to the discovery of potential genes that are responsible for the manufacture of specialised metabolites [21]. Because of these investigations, we were able to locate a transcript module in M. japonicus that is accountable for the biosynthesis of rutin. The functional importance of the transcript modules that were shown to correlate highly with the metabolic modules was investigated using gene ontology enrichment analysis as well. This demonstrated that the body possesses specialised modules that are responsible for processing the large number of metabolites that it has amassed [22]. Researchers are able to gain a better understanding of the mechanisms involved in the production of M. japonicus' specialised metabolites by making use of the data obtained from the metabolome and transcriptome as a result of this work.

Peptic ulcers are painful sores in the digestive tract that can be caused by a variety of conditions, including gastritis and acid reflux [23]. Mucosal degradation, with the defect extending into the submucosa or muscularis propria, is a defining feature of

ISSN: 2583-4053

Volume-1 Issue-5 || December 2022 || PP. 16-23

https://doi.org/10.55544/jrasb.1.5.2

peptic ulcers. Peptic ulcer disease is still considered to affect between 5 and 10 percent of the general population, despite the fact that recent epidemiological studies have demonstrated a decline in the disease's incidence, hospitalisation rates, and mortality rates [24]. The reduction in the number of illnesses caused by Helicobacter pylori (H. pylori) is almost certainly attributable to the introduction of cutting-edge treatments as well as the general adoption of improved hygiene practises.

Previous hypotheses postulated that a hypersecretory and acidic environment, in addition to dietary factors or stress, led to the mucosal disruption that was found in individuals who suffered from acid peptic sickness. Infection with the bacteria Helicobacter pylori and the use of nonsteroidal anti-inflammatory drugs (NSAIDs) are risk factors that are shared by both stomach and duodenal ulcers [25]. In addition to drinking alcohol and smoking cigarettes, people who use nonsteroidal anti-inflammatory drugs (NSAIDs) and those who have Zollinger-Ellison syndrome are at an increased risk of developing peptic ulcers. Due to the fact that only a small fraction of people who are infected with H. pylori or who use NSAIDs develop peptic ulcer disease, an individual's vulnerability is especially significant in the early stages of mucosal injury. Peptic ulcer disease has been connected to polymorphisms in a large number of genes encoding cytokines. Polymorphisms in the gene IL1B result in an altered synthesis of interleukin 1 in the mucosa, which, in turn, contributes to H. pylori-associated gastroduodenal disorders [26].

People who use nonsteroidal anti-inflammatory drugs (NSAIDs) have a four times increased risk of developing peptic ulcers, while people who take aspirin have a two times increased risk [27]. When taken concurrently, multiple medications, including nonsteroidal anti-inflammatory drugs (NSAIDs) and aspirin, have been shown to increase the risk of bleeding in the upper gastrointestinal tract [28]. Anticoagulants, corticosteroids, and selective serotonin reuptake inhibitors are a few examples (selective serotonin reuptake inhibitors). However, it is still unknown what part non-steroidal anti-inflammatory drugs (NSAIDs) and aspirin play in the aetiology of peptic ulcer disease among the large number of patients who use these medications and who simultaneously have H. pylori infection. In spite of the fact that an infection with H. pylori is one of the primary causes of peptic ulcer disease, this is nonetheless the case. According to the findings of a meta-analysis of observational studies [29], the risk of developing peptic ulcer disease is increased if the patient takes aspirin, makes use of NSAIDs, or has the bacteria H. pylori.

According to the findings of one study, twenty percent of patients who were diagnosed with peptic ulcer disease also had idiopathic ulcers. The results of the tests for H. pylori, NSAIDs, and aspirin conducted on these individuals were all negative. Idiopathic peptic ulcer is a frequent ailment; yet, very little is understood about the pathogenic pathways that lead to the development of this disorder [30]. This condition manifests itself when the body's natural defences against jarring experiences are insufficient. According to the findings of a study conducted in Denmark, the incidence of peptic ulcers increased in line with psychiatric stress. In addition to viruses, histamine, eosinophilic infiltration, gastric bypass surgery, and metabolic disorders, medicines (steroids, chemotherapeutic agents), radiation, and ischemia are all potential triggers for this condition [31].

Since it sends more than 500,000 Americans to the hospital each year, gastrointestinal (GI) bleeding is the most prevalent GI ailment that necessitates hospitalisation in the United States. When a patient develops bleeding from the oesophagus, stomach, or duodenum, it is known as upper gastrointestinal bleeding (UGIB). Patients with UGIB who visit the emergency room eventually get admitted to the hospital in about 80% of cases[32]. Patients will be eligible for inclusion in this trial if they display clear indications of UGIB, such as bloody or coffee-grounds-like vomitus, black, tarry stools, or bloody diarrhoea (passage of red or maroon material per rectum). We will focus on the initial treatment that is given to everyone who has UGIB up until the point at which an endoscopic examination is performed[33-35]. We will focus our recommendations on individuals who have been diagnosed with ulcer bleeding because it is the most frequent cause of UGIB and the diagnosis for which the most RCTs have been performed.

II. MATERIAL & METHODS

Processing of Plant

Herbal Health Research consortium Pvt. Ltd. Vill. Khyala khurd Ram Tirath Road, Amritsar certified the authenticity of the roots and leaves of M. japonicus that were purchased in October from a local market in Dehradun, U.K. India. The market was located in Dehradun, U.K. India. After being sprayed with alcohol at a concentration of 70% and rinsed three times with running water, the plant was considered to be free of any pathogens. In order to prevent any further chemical transformations, the plant material after cleaning was air dried at room temperature. Because of the high levels of humidity, the plant material required to be inspected on a regular basis for symptoms of fungal growth. Following the drying process, the plant material was ground into a powder with the assistance of a motorised pestle. In conjunction with the Soxhlet extraction method, the use of aqueous solvents is necessary in order to accomplish the separation of the active chemicals from the inert filler.

ISSN: 2583-4053

Volume-1 Issue-5 || December 2022 || PP. 16-23

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Extraction with Soxhlet Apparatus

The initial step in the process of removing valuable compounds from their originating materials is known as extraction. In accordance with the extraction principle, a number of extraction processes can be carried out, including distillation, pressing, sublimation, and the use of solvents to accomplish the extraction[36]. The use of solvents for extraction is the most typical method. Following the addition of the solvent to the solid matrix comes the subsequent steps of dissolving the solute in the solvents, diffusing the solute away from the solid matrix, and finally collecting the solutes that have been extracted from the solid matrix. Anything that improves the substance's diffusivity and solubility in the stages that come before the extraction process can be used as a helping hand. The extraction efficiency will be influenced by a number of elements including the properties of the extraction solvent, the particle size of the raw materials, the ratio of solvent to solid, the temperature, and the amount of time [37].

The selection of the appropriate solvent is of the utmost significance during the process of solvent extraction. When selecting a solvent, it is essential to think about the selectivity, solubility, cost, and safety of the potential option[38]. Because of the law of similarity and intermiscibility, it stands to reason that solvents having a polarity value that is relatively near to that of the solute would be more effective, and that the opposite will also be true (like dissolves like). Alcohols (EtOH and MeOH) are the most common solvents used in the process of solvent extraction for phytochemical study.

When particles are smaller, it is simpler to separate them from their surroundings. The smaller the particle size, the easier it is for solvents and solutes to enter and diffuse throughout the material, which results in a higher extraction efficiency. On the other hand, if the particles are too small, the solute in the solid will be absorbed at an excessive pace, which will make further filtration difficult to accomplish. Both solubility and diffusion are helped forward by heating[39-42]. However, if the temperature is too high, the loss of solvents could lead to the extraction of undesired impurities as well as the breakdown of thermolabile components. When working within a constrained window of time, the extraction efficiency improves in proportion to the length of the extraction period. Adding more time to the extraction process won't make a difference once the solute has reached equilibrium both inside and outside of the solid substance. In general, a larger ratio of solvent to solids results in a higher yield from the extraction process. On the other hand, if the ratio is too high, there will be an excessive amount of extraction solvent, and the process of concentration will take an excessive amount of time. Maceration, percolation, and reflux extraction are the classic methods, and each of these processes calls for a significant amount of organic solvents in addition to a lengthy extraction time[43]. The incorporation of techniques such as super critical fluid extraction (SFC), pressurised liquid extraction (PLE), and microwave assisted extraction (MAE) into the process of extracting natural products was motivated by the benefits of using more modern or environmentally friendly extraction methods. These techniques include supercritical fluid extraction (SFC).

III. IN-VITRO EVALUATION OF ANTI-ULCER ACTIVITY

Acid- Neutralizing Capacity

We compared the acid neutralising capacity of four different concentrations of M. japonicus hydro-alcoholic extract (100 mg/ml, 200 mg/ml, 500 mg/ml, and 1000 mg/ml) to that of the gold standard antacid, AHMH (aluminium hydroxide + magnesium hydroxide - 500 mg/ml). All four concentrations of M. japonicus hydro-alcoholic extract performed better than the gold standard (ANC). After adding water to the combination that contained 5 ml of each extract, which had already been thoroughly mixed, the total volume was brought up to 70 ml so that it could be measured. Following the addition of 30 ml of 1N HCl and the subsequent 15 minutes of shaking the standard and test preparation, add two to three drops of the phenolphthalein solution. As soon as a pink tint was observed, 0.5N sodium hydroxide solution was added to the mixture in order to neutralise the excessive amount of hydrochloric acid [44].

H+K+ ATPase Inhibition

The extract was incubated at concentrations ranging from 10 to 50 g/ml in a reaction mixture containing 10 g of membrane protein, 40 mM Tris-HCl buffer (pH 7.4), 2 mM MgC12, and 1 ml. We then added 2 mM ATP Tris salt to kick off the reaction and left the mixture to sit for 20 minutes at 37 degrees Celsius. When 1 ml of ice-cold, 10% v/v trichloroacetic acid was added, the reaction immediately halted. The H+-K+ ATPase activity was measured with and without the addition of the extract and esmoprazole at a range of doses [42-44]. It was determined how much ATP was broken down into inorganic phosphate by measuring the intensity of a spectral line at 400 nm.

IV. RESULT & DISCUSSION

Acid Neutralizing Capacity:

In order to establish the aqueous extract's potential for neutralising an alkaline solution, it was put through a series of tests in which it was confronted with 100 mg, 500 mg, 1000 mg, and 1500 mg of the standard aluminium hydroxide $Al(OH)_3$ (500mg). According to the findings of the research, the optimal amount of the extract to use in order to neutralise acid is 1500 milligrammes table 1.

Journal for Research in Applied Sciences and Biotechnology

www.jrasb.com

ISSN: 2583-4053

Volume-1 Issue-5 || December 2022 || PP. 16-23

https://doi.org/10.55544/jrasb.1.5.2

Table 1: Acid Neutralizing Capacity

S. No.	Concentration	Volume of NAOH Used	m Eq. of Acid Consumed	ANC per gram of Antacid
1	100	37.6	13.04	110.3
2	500	29.4	17.56	36.4
3	1000	39.4	9.27	11.61
4	1500	42	12	9.36
5	500 mg Al $(OH)_3$	45.6	7.59	15.6

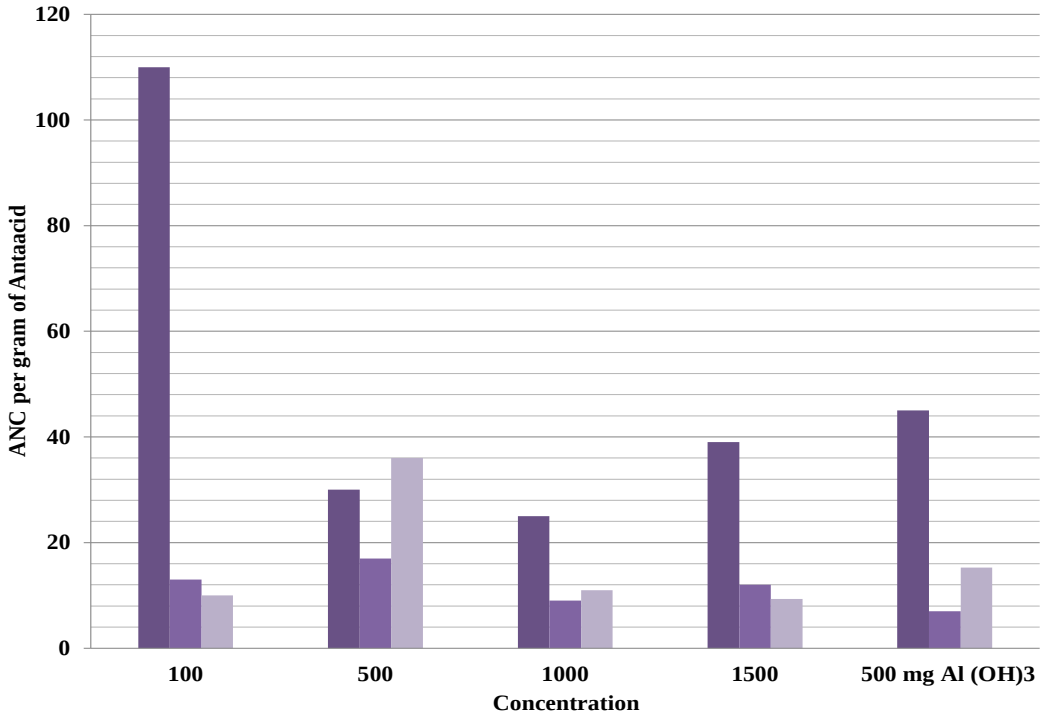

Fig. 1: Effect of aqueous extract *M. japonicus* of on acid neutralising capacity

In-vitro H+/K+ -ATPase inhibition activity

Comparing the in-vitro H+/K+ - ATPase inhibitory activities of hydro-alcoholic extract of M. japonicus roots and leaves at different concentrations (25 g, 50 g, 100 g, 200 g, and 400 g per ml) with that of the reference medicine omeprazole at similar concentrations. Inhibition of H+/K+ ATPase activity is seen to be concentration dependent, both for the test and control medications. Inhibition of H+/K+ - ATPase activity by 49.12%, 51.41%, and 54.14% was observed at doses of 100 g/ml, 200 g/ml, and 400 g/ml, respectively, using the test drug. Over 70% inhibition of H+/K+ - ATPase activity was seen at extract concentrations over 400 g/ml. These results are shown in Table 2.

Table 2: *In-vitro* H+/K+-ATPase inhibition activity

Concentration	Percentage inhibition % mean		
	Esomoprazole	Root M. *japonicus*	Leaves M. *japonicus*
25 µg	-40.52± 1.45	-20.36±1.25	-18.25±3.6
50 µg	-32.45± 1.25	-13.21±1.23	-14.23±1.45
100 µg	49.12 ± 1.56	51.41± 2.14	54.14±1.85
200 µg	62.32± 2.36	73.8± 1.45	47.63±1.74
400 µg	71.21±3.26	70.3±3.61	68±3.25

ISSN: 2583-4053

Volume-1 Issue-5 || December 2022 || PP. 16-23

https://doi.org/10.55544/jrasb.1.5.2

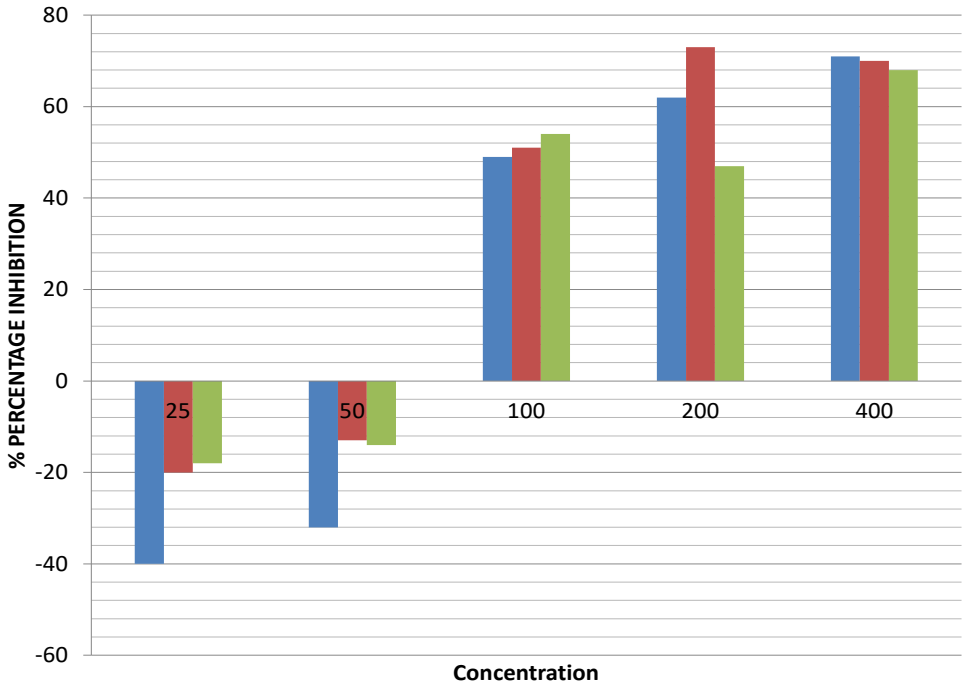

Fig:2 Percentage inhibition and concentration of *In-vitro* H+/K+-ATPase inhibition activity

V. CONCLUSION

We can conclude from these data that the aqueous extract of this species is the only candidate that has a chance of success when it comes to the development of innovative antiulcer medications. The extraction of the species' active components and the mechanisms behind its effects as an antiulcer agent will be the focus of research to be conducted in the future. This study has laid a solid platform for future work that will hopefully result in the therapeutic application of compounds that have antioxidant, antibacterial, anti-diabetic, anti-inflammatory, and analgesic actions. The findings of this study have added to the growing body of information that demonstrates the usefulness of plants that have traditionally been used to treat a wide range of human health conditions.

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