

INVESTIGATION OF IN-VITRO ANTI-OXIDANT & ANTI-ULCER ACTIVITY OF POLYHERBAL MEDICINAL PLANTS

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Abstract

Some of the plants that were investigated in this study have a long history of usage in medical practises, and scientists believe that the high levels of antioxidants found in these plants may be the reason for their effectiveness as treatments. Because flavonoids, terpenoids, and phenolic compounds all include antioxidants in their chemical structures, it is well established that they provide some protection to the body. There is a long-held belief that plants of this kind can provide some kind of protection from damage. Using in vitro testing, researchers investigated whether or not extracts of the leaves of *Rhodomyrtus tomentosa* and *Mallotus philippensis* have potential anti-ulcer and antioxidant capabilities. Both of these plants may trace their origins back to the Philippines, where they were first domesticated. This study was conducted with the intention of determining whether or not extracts of the leaves of *Rhodomyrtus tomentosa* and *Mallotus philippensis* have an inhibiting effect on H⁺-K⁺ ATPase when tested in vitro.

Keywords: Polyherbal, In-vitro, Anti-ulcer, Antioxidant.

INTRODUCTION

Countries that are considered to be first-world and countries that are considered to be third-world regularly use herbal remedies for health care. According to research that was carried out by the World Health Organization (WHO), herbal drugs are combinations of chemical compounds that are synthesised in plants. Despite this, the effectiveness of herbal drugs is reduced due to poor oral absorption[4]. Herbal remedies have been demonstrated to be effective not only in the treatment of illness but also in the prevention of illness. Medicinal products can also be sold in the form of tablets, capsules, paste, tea extracts, or even

fresh or dried herbs, depending on the market in which they are being sold. Herbs have long been admired for their ability to treat a variety of ailments; in fact, many consumers purchase herbs without first obtaining a prescription from a medical professional. When combined with other medications, certain ones can be dangerous, while others become ineffective or even counterproductive [5]. An ancient Sumerian clay slab from Nagpur that dates back approximately 5,000 years contains the earliest known written reference to the processing of medicinal plants into pharmaceuticals. The twelve drug synthesis formulas in the book cover a total of more than 250 different plants, including alkaloids such as cannabis, henbane, and mandrake [6]. The Vedas, which are considered to be India's most sacred texts, include instructions for how to use a variety of herbs, many of which are free-growing in India. Many of the plants that are used to make spices like cinnamon, cloves, nutmeg, and a whole host of others have their roots in the soil of India [7]. Some of these spices include: Documentation of the use of medicinal plants can be found in a number of national medical systems, including Ayurveda, Siddha, and Unani, as well as in the British and American Pharmacopoeias. Himachal Pradesh, a state located in the Himalayas, is home to a diverse collection of plants that can be used for medicinal purposes. This Himalayan area has conserved a significant number of medicinal plants as well as centuries' worth of medical knowledge and practise. Illegal wild cultivation and the loss of natural resources are diminishing the range of medicinal plants that are now accessible.

This puts the continued existence of some plant species in jeopardy and may even lead to their extinction in their natural habitat [9]. Even though there have been a lot of regulatory shifts, there is still a significant amount of illicit harvesting of medicinal plants going on because of how profitable the market is. Because these animals no longer exist, it is against the law to acquire them because they are considered extinct. Real-world activities are held during the workshop on Himachal Pradesh medicinal plant species that are in risk of extinction (point 10). Ulcers are a dangerous disorder that affect the digestive system and can have many different causes. Ten percent of individuals throughout the world suffer from ulcers. Chronic use of alcohol, smoking, extended stress, chronic use of nonsteroidal anti-inflammatory medicines (NSAIDs), and bacterial infection by *H. pylori* are major causes of inflammatory peptic ulcer, mucosal bleeding, and stomach discomfort [15]. Peptic ulcers, which include both intestinal and stomach varieties, afflict about 4 million individuals in the United States; each year, 350,000 new instances are diagnosed; each year, 180,000 people are hospitalised and treated with drugs; and each year, 5,000 people pass away from ulcer disease. Within the population of the United States, it is projected that 10% of men and 4% of females will acquire a peptic ulcer throughout the course of their lifespan [16]. People that consume a lot of curries, have a continual state of worry, and are always in a rush have a greater risk of developing this condition. Peptic ulcer disease is characterised by the development of inflammatory mucosal and tissue lesions or excavations that protect the gastrointestinal system. These lesions and excavations can be seen anywhere throughout the gastrointestinal tract. A connection has been made between peptic ulcers and the mucus membranes that typically protect the oesophagus, stomach, and duodenum from the damaging effects of pepsin and gastric acid. Natural plant extracts have been used as a therapy for a wide variety of disorders by humans for a number of decades now [17].

MATERIAL & METHODS

The Collection And Evaluation Of Various Plant Materials In the course of the study titled "The Collection And Evaluation Of Various Plant Materials," the leaves of *Rhodomyrtus tomentosa* (Myrtaceae) and *Mallotus philippensis* (Euphorbiaceae) were collected from the Central Nursery at the Forest Research Institute in Dehradun, India. The Department of Agriculture at DDGU, Gorakhpur, conducted a survey and authentication of medicinal plants in the region.

EXTRACT PROCESS

After being exposed to air and allowed to dry in the shade, the leaves of the *Mallotus philippensis* plant were processed through machinery to produce a coarse powder. The most refined version of the powder had a weight of around 600 grammes when it was measured. The powder went through an extraction process called hot continuous successive extraction in a Soxhlet apparatus, with the solvents being used in ascending sequence of polarity. Methanol, ethyl acetate, chloroform, and petroleum ether were some of the solvents that were utilised. Throughout the procedure, the temperature was maintained at a range of between 50 and 60 degrees Celsius. Extractives were dried under circumstances of reduced pressure after being heated to temperatures lower than 40 degrees Celsius. The temperature of the extractives was kept below 40 degrees Celsius during the heating process. Until further examination could be carried out, the four dry extractives were stored in a desiccator for their own protection. Before the leaves of the *Rhodomyrtus tomentosa* plant were mechanically crushed into a coarse powder, they were allowed to air-dry in the shade under shady conditions first. The weight of the coarse powder was somewhere about 800 grammes. It was measured in grammes. Using a Soxhlet apparatus and solvents in sequence of increasing polarity, the powder was processed via a hot continuous consecutive extraction in order to obtain the desired result. The following substances were listed among these solvents: petroleum ether, chloroform, ethyl acetate, aqueous alcohol (70 percent), and water. Throughout the procedure, the temperature was maintained at a range of between 50 and 60 degrees Celsius. The extractives were concentrated at a temperature that was lower than 50 degrees Celsius, and the drying process was conducted at a lower pressure than before. A desiccator was utilised so that the five dried extractives could be stored safely until more testing could be carried out on them

QUALITATIVE PHYTOCHEMICAL SCREENING

The following analyses were performed on the herbal extracts in order to determine the various phytoconstituents contained within them.

Test for Carbohydrates

After dissolving a small amount of extract in distiller's water and filtering the resulting solution, the carbohydrate content was determined.

a.) **Molisch's test:** Reddish-violet rings appeared on the treated filter after pouring a few drops of Molisch's reagent and one millilitre of concentrated sulfuric acid gently down the slides of test tubes.

b.) **Fehling's Test:** A brick-like colour is obtained by mixing equal parts of each Fehling reagent.

Test of Glycosides

a. **Borntranger's test:** A very little amount of extract was hydrolyzed with diluted hydrochloric acid on a water bath for a couple of hours. A reddish brown colour at the interface between the two layers indicates the presence of glycosides.

b. **Keller – Killiani test:** After the mixture cooled, a few drops of ferric chloride were added, and then a millilitre of glacial acetic acid was combined with a little amount of the extract. A 1 ml syringe of concentrated H₂SO₄ was then affixed to the side of a test tube and the contents were transferred there. Glycosides can be detected by looking for a reddish-brown coating that turns blue-green when left out in the light.

c. **Test for Saponins:**

a. **Foam test:** 20 millilitres of distilled water was added to the extract, and the mixture was agitated for 15 minutes in a graded cylinder. Indicating the presence of saponins, foams around a centimetre in thickness were produced.

Test for Phenolic Compounds and tannins

In a separate container, some of the extract was combined with aq. Learn the correct procedure for detecting phenolic chemicals, and then implement your findings. Add 10% W/v lead acetate solution: Observed for white color precipitates.

Test for Amino acid and protein:

Following the dissolution of a tiny amount of extract in a few millilitres of distilled water, the following reagents were applied to the mixture.

a. **Millon's reagents:** The colour of the colour red It can be seen from the precipitate that proteins and amino acids are present.

b. **Ninhydrin reagents:** a. The presence of protein and amino acid may be determined by the colour purple that is present.

Test for Flavonoids:

Shinoda Test:To this, a small quantity of extract was added to ethanol, and then a tiny bit of metallic magnesium or zinc was sprinkled in. After adding two drops of strong hydrochloric acid, the colour changed to pink.

Ammonia Test: A minute quantity of extract was mixed with ethanol and shaken. A strip of filter paper was dipped in an alcoholic solution, the strip was then made ammoniated, and the resulting colour was monitored as it changed from white to orange.

IN VITRO ANTI-OXIDANT STUDY

DPPH METHOD

Antioxidant activity was determined by measuring the extracts' ability to quench the stable free radical DPPH[69]. Reducing agents for the DPPH radical include hydrogen donors, yielding the equivalent hydrazine. After reacting with hydrogen donors, the purple DPPH radical turns yellow. In this decolorizing experiment, the antioxidant is added to a DPPH solution in methanol, and the resulting drop in absorbance is assessed.

Method for Inactivating H₂O₂ Radicals

One of the least reactive molecules is hydrogen peroxide, which is why it is one of the most common forms of oxidising agents. Without metal ions, it is relatively stable at normal pH and temperature. However, the enzyme superoxide dismutase can catalyse a dismutation step to produce these molecules from superoxide anion

Nitric Oxide Method

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which then reacts with oxygen to produce nitrite ions. Spectroscopic techniques can be used to measure this[71]. When this method of producing nitric oxide comes into touch with dissolved oxygen and water, it produces nitric acid and nitrous acids. The amount of nitrous acid released is calculated using Griess reagent, which turns the material under study a purple colour and acts as a free radical scavenger.

IN-VITRO ANTIULCER ACTIVITY

H⁺/K⁺ -ATPase Inhibition Activity:

Preparation of the H⁺/K⁺ ATPase Enzyme: The stomach was opened and the fundal mucosa was removed. The parietal cell lining was scraped away to get a sample of H⁺/K⁺ - ATPase enzyme. Paraetal stomach cells were homogenised in 16 mM Tris buffer (pH 7.4) with 10% Triton X-100, centrifuged at 6000 rpm for 10 minutes, and the supernatant solution was used to block H⁺/K⁺ - ATPase. Protein concentration may be calculated using Bradford's technique with bovine serum albumin (BSA) as a reference. Anti-ATPase H⁺/K⁺ Ion Balance: The sample reaction mixture was incubated at 37 degrees Celsius for 60 minutes, and it contained 0.1 ml of enzyme extract (300 g) and plant extract with varying concentrations (20 g, 40 g, 60 g, 80 g, 100 g)

Statistical Analysis

Statistically significant differences between means were indicated by the standard error of the mean. Using One-Way Analysis of Variance, we compared the means of our experimental data to those of the vehicle-control group (ANOVA). Dunnett's test and Tukey's multiple comparison test were then used to further analyse the data. Statistical significance was defined as a p-value less than 0.5, and Graph Pad Prism V.4 was used to conduct the analysis.

RESULT & DISCUSSION

EXTRACTION AND USE OF PLANT MATERIAL

Table 1: Extractions Prepared From and Phytochemical Studies Carried Out on *Rhodomyrtus tomentosa* & *Mallotus philippensis* Leaves

		<i>Rhodomyrtus tomentosa</i>		<i>Mallotus philippensis</i>	
EXTRACT	NATURE OF EXTRACT	YIELD VALUE %W/W	phytochemical investigation	YIELD VALUE %W/W	phytochemical investigation
P.ETHER	Solid with a greenish-brown colour	0.6	Steroid., terpenoid.	1.4	Steroid., terpenoid.
Chloroform.	A solid brown colour	1.4	Alkaloid., phenol, terpenoid	1.6	Alkaloid., phenol, terpenoid
Ethyl acetate	A brownish solid	2.8	Flavonoid., phenol., tannin	2.2	Flavonoid., phenol., tannin
AQ. Alcohol	A brownish semisolid colour	4.6	Flavonoid., phenol., saponin., terpenoid., tannin..	6	Flavonoid., phenol., saponin., terpenoid., tannin..
Distilled water	Dark brown and semisolid	2.5	Flavonoid., phenol., saponin., terpenoid., tannin.		Flavonoid., phenol., saponin., terpenoid., tannin.

Table: 2 *Rhodomyrtus tomentosa* & *Mallotus philippensis* leaf extracts phenolic content

	<i>Rhodomyrtus tomentosa</i>	<i>Mallotus philippensis</i>
Extract sample	Gallic acid milligram per g (mg/g) of phenolic content*	Gallic acid milligram per g (mg/g) of phenolic content*
Pet. Ether extract	0.48 ± 0.64	1.4 ± 0.24
Chloroform extract	1.26 ± 0.24	3.6 ± 0.40
Ethyl acetate extract	3.26 ± 0.24	3.8 ± 0.48
Aqueous alcoholic extract (70%)	4.04 ± 0.22	4.1 ± 0.18

EVALUATION OF PHENOLIC MATERIALS

Rhodomyrtus tomentosa Significant Level of Phenolic Content

When the phenolic content of each extract of *Rhodomyrtus tomentosa* leaves was measured in terms of gallic acid, the results were as follows: 0.48-0.64 mg GAE/g dry weight for the petroleum ether extract, 1.26-0.24 mg GAE/g dry weight for the chloroform extract, 3.26-0.24 mg GAE/g dry weight for the ethyl acetate extract, and 4.04-0.22 mg GAE Table 2 provides a rundown of the findings in their entirety.

Absorption of Phenolic Compounds into *Mallotus philippensis*

Gallic acid was used as the unit of measurement for the phenolic content of *Mallotus philippensis* extracts, and it was observed that the petroleum ether, chloroform, ethyl acetate, and methanolic extracts contained 1.40-2.4, 3.60-4.4, 3.80-4.8, and 4.10-1.8 mg GAE/g dry weight, respectively. Table 2 provides an overview of the results.

Experimental Studies of Antioxidants

Antioxidant Activity of *Rhodomyrtus tomentosa* Extracts

As shown in Figs. 1, 2, & 3 several *Rhodomyrtus tomentosa* extractives and standards were tested at a range of concentrations for their ability to scavenge free radicals such as nitric oxide, hydrogen peroxide, and dihydroxyphenyl phosphate (DPPH). In all cases, increasing extractive concentrations resulted in a higher percentage of inhibition.

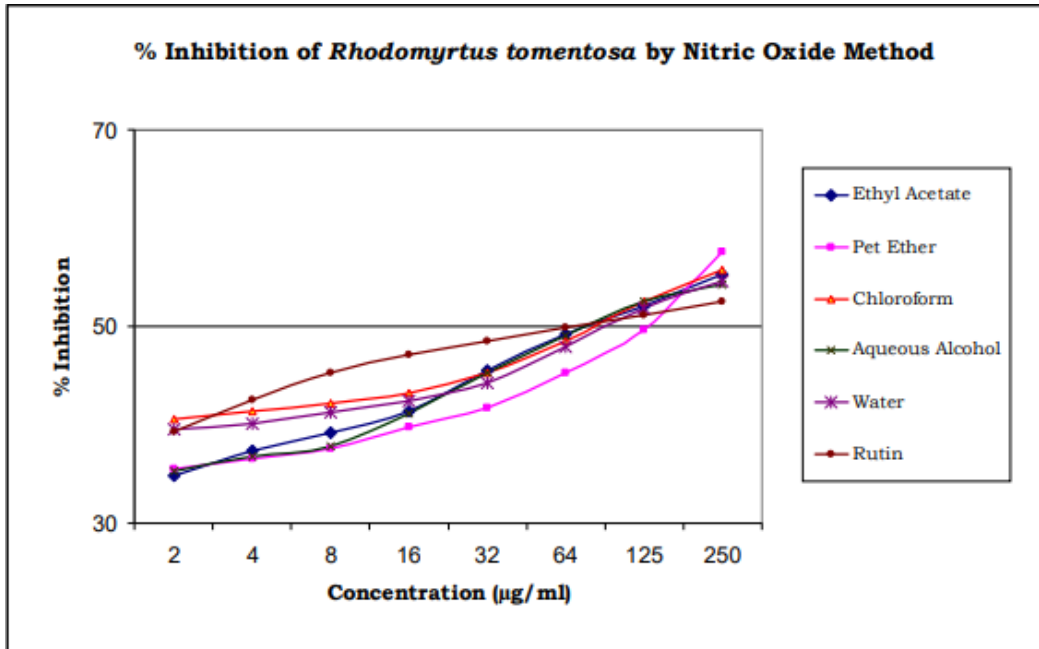


Fig:1 A comparison of the relative effectiveness of different *Rhodomyrtus tomentosa* extracts against a nitric oxide method of inhibition

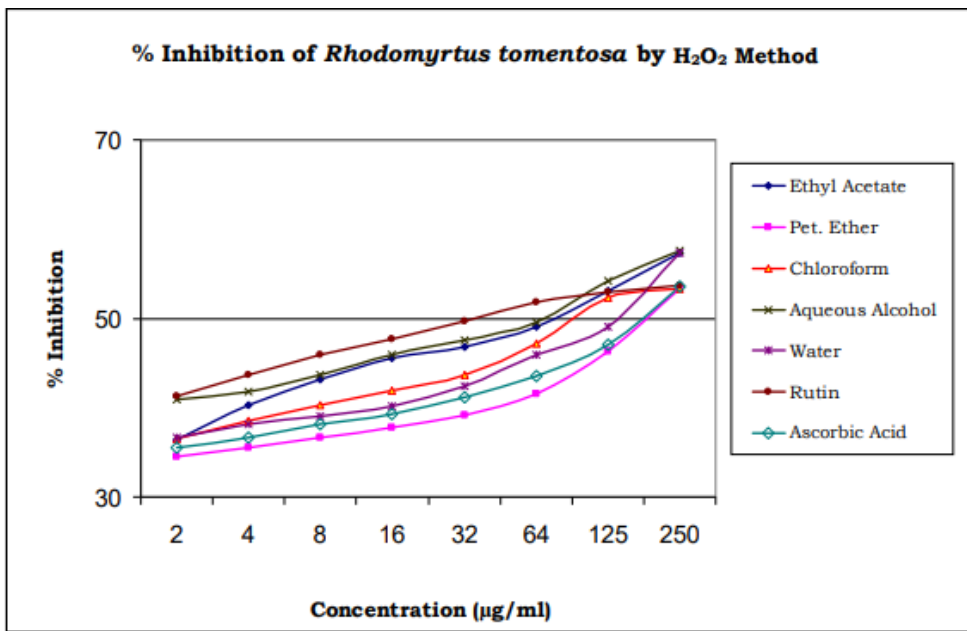


Fig: 2 A amount to which different *Rhodomyrtus tomentosa* active ingredients are inhibited by hydrogen peroxide

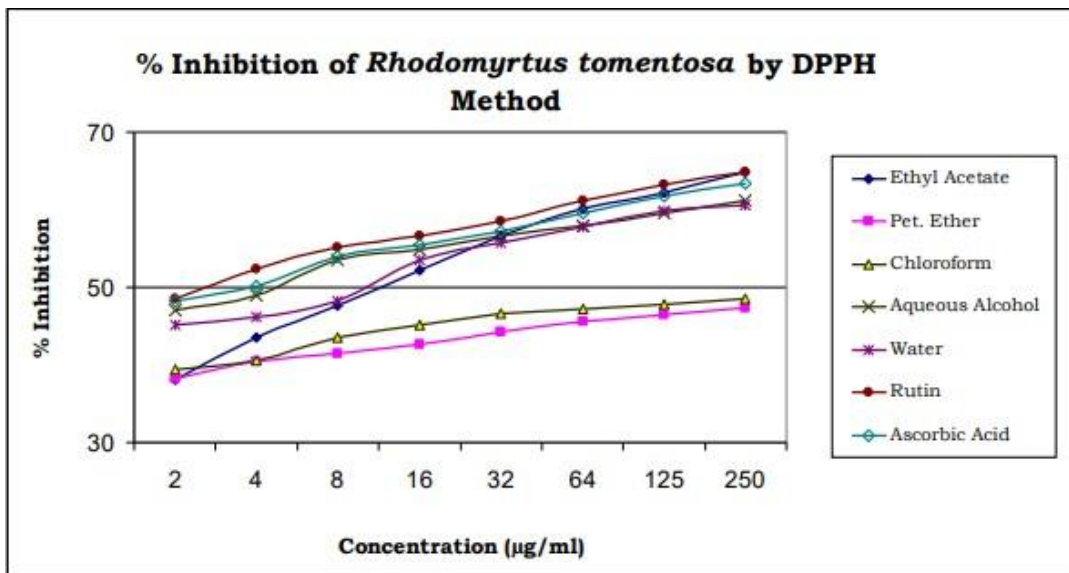


Fig: 3 *Rhodomyrtus tomentosa* Extracts Evaluated for Its Ability to Inhibit

Antioxidant Activity of *Mallotus philippensis* Extracts in vitro

Figures 4,5, 6, show the percentages of nitric oxide, hydrogen peroxide, and DPPH radicals that were scavenged by the various *Mallotus philippensis* extractives and standards utilised at varied concentrations. Concentrations of the extractives utilised in all the procedures showed an increase in the percentage of inhibition.

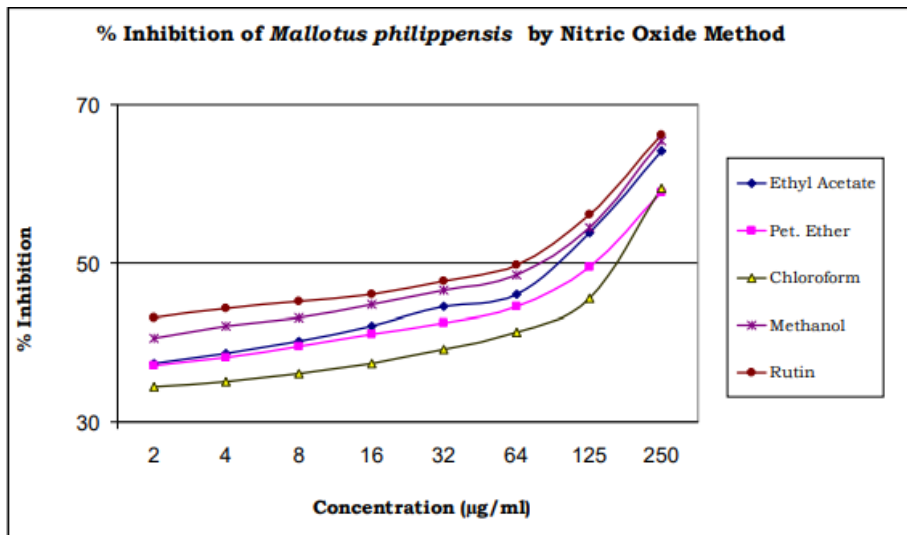


Fig: 4 Percentage Inhibition of various Extractives of *Mallotus philippensis* by Nitric Oxide Method

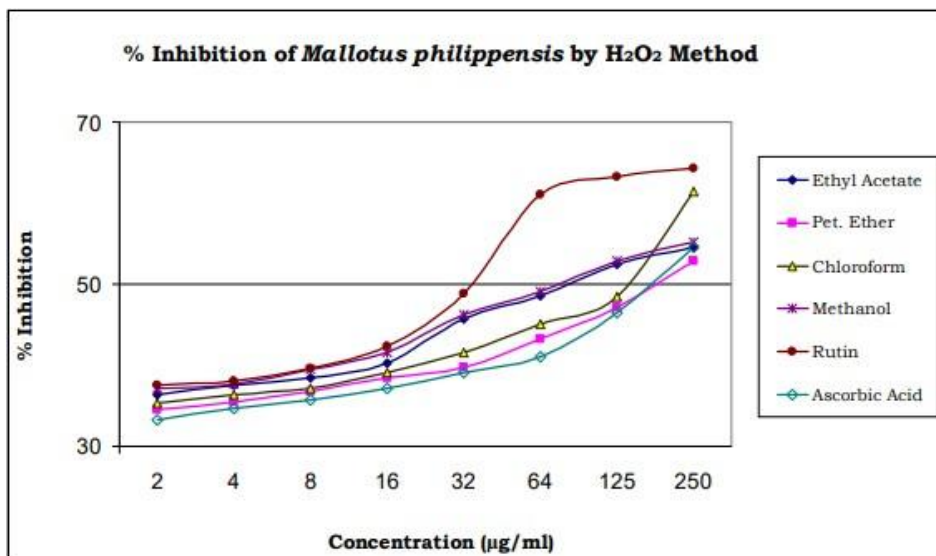


Fig: 5 Percentage Inhibition of various Extractives of *Mallotus philippensis* by H₂O₂ Method

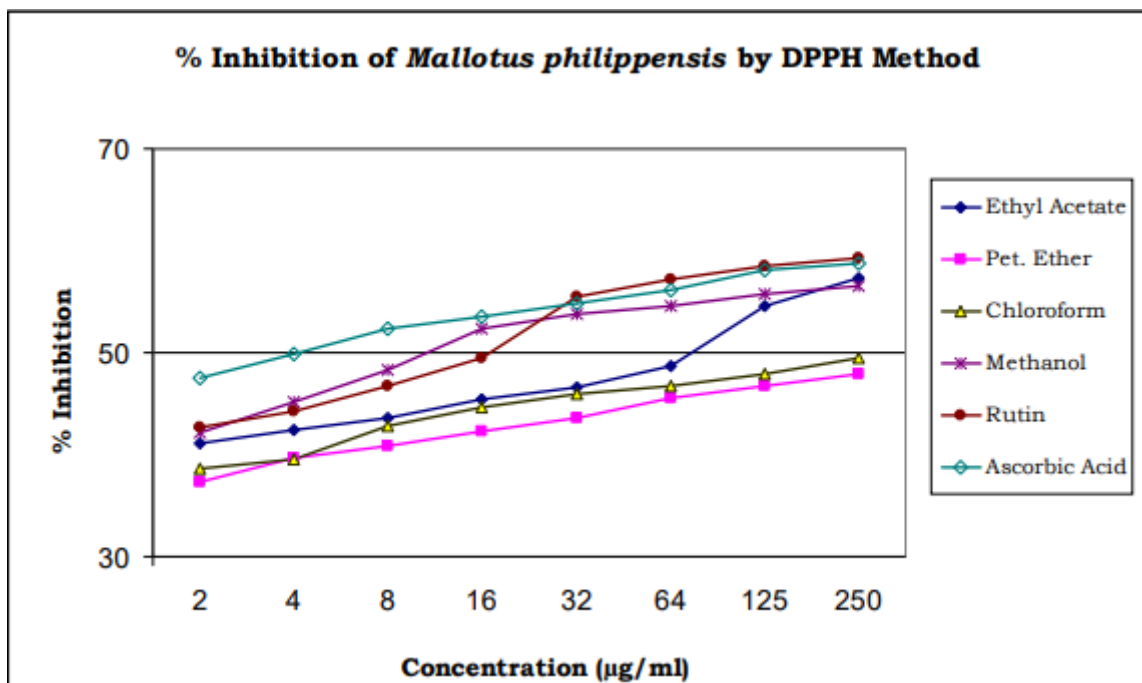


Fig: 6 Percentage Inhibition of various Extractives of *Mallotus philippensis* by DPPH Method

H⁺/K⁺ - ATPase Inhibition Activity:

The H⁺/K⁺-ATPase inhibitory activity of ethanol extract was tested at a range of concentrations (20 g, 40 g, 60 g, 80 g, and 100 g), and the results were compared to those obtained using Pantoprazole as the gold standard. The dose has a substantial impact on the amount of activity demonstrated by the extract. At a dosage of 100 mg, the extract demonstrated a maximum percentage inhibition of 62.14 0.56 percent and 60.14 0.45 percent, whereas the standard Pantoprazole showed 69.54 1.45 percent. Table 3 and Figure 7 contain the tabulations of the results, respectively.

Table: 3 H⁺/K⁺ - ATPase Inhibition Activity

Con ^c	% Inhibition (Mean ± SEM)		
	Standard drug	Ethanol extract RT	Ethanol extract MP
20	-52.25 ± 0.78	-28.15± 0.25	-25.14±0.62
40	-55.31±1.45	-20.87±1.45	-21±1.78
60	34.47±0.36	32.62±1.8	28.36±1.96
80	55.96±0.47	52.12±0.56	52.31±1.5
100	69.54±1.45	62.14±0.56	60.14±0.6

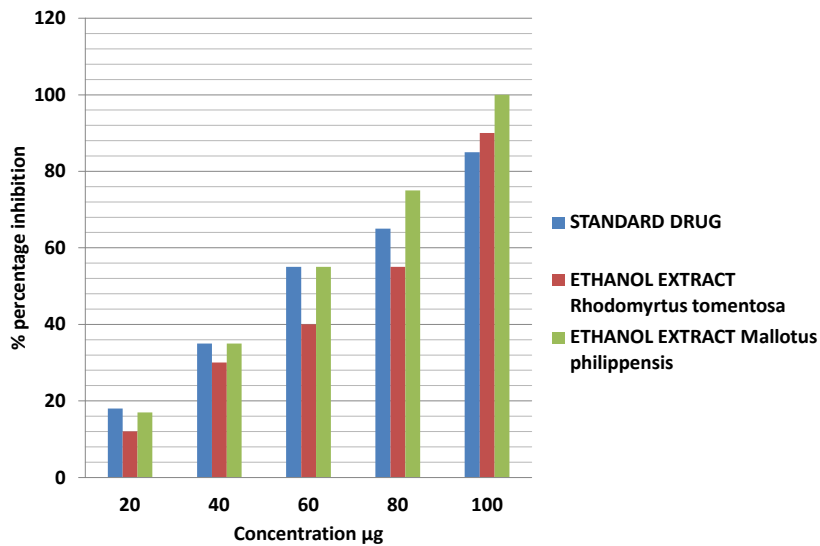


Fig: 7 Shows the Effect that Ethanol Extract Had on the In vitro H⁺/K⁺ Atpase Activity

Conclusion

Peptic ulcer disease is the most common GI condition, characterised by frequent flare-ups and multiple complications. And it's allopathic medicines that are employed in Some people who undergo this treatment may experience unpleasant side effects that are harmful to their health. Inhibition of stomach H⁺/K⁺ ATPase will be the main focus of my research as I compile data on the gastroprotective properties of *Rhodomyrtus tomentosa* and *Mallotus philippensis* leaves. It will take more research to determine the active principles involved in this effect and clarify the mechanisms underlying this activity.

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