# **EVALUATION OF ANTI-ULCER ACTIVITIES OF ETHANOLIC EXTRACT OF PARMOTREMA PRAESOREDIOSUM**

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## ABSTRACT

Traditional medicine has traditionally used plants as a therapeutic source to treat both human and animal ailments. Plants are the source of a variety of important secondary metabolites with pharmacological and pharmacognostic consequences that have the potential to become future "super medicines." The biotic and abiotic stressors that affect the in-vivo production of these metabolites lead to a constant accumulation of various phytochemicals and their derivatives, which can be helpful in designing and developing potential medications in the future. The Permeliaceae plant Parmotrema praesorediosum is a rich source of phytochemicals with therapeutic value, including lichen acid, tannins, saponins, flavonoids, terpenoids, alkaloids, and sterols. The current project goal is to evaluate the lichen Parmotrema praesorediosum anti-ulcer properties. In this study, we evaluated the anti-ulcer activity of the ethanol extract and the ability of the invitro approach to neutralise acids and Gastric hydrogen potassium ATPase inhibitory movement in stomach . The extract significantly reduced the neutralise acids to 11.75 at the 1000 mg concentration compared to 15.8 for standard aluminum hydroxide + magnesium hydroxide (500 mg). During Gastric hydrogen potassium ATPase inhibitory movement, the extract showed a maximum

inhibition rate of 62% at 100 µg compared to 69% for standard omeprazole. This study indicates, the ethanol extract contains several compounds with anti-ulcer activity and can therefore be used as an alternative medicine for the disease.

**Key words:** Parmotrema praesorediosum, Permeliaceae, Lichens, anti-ulcer activity, acid neutralizing capacity, neutralise acids and Gastric hydrogen potassium ATPase inhibitory, ethanol extract.

## Introduction

Lichens are commensal fungal and algal organisms that produce their own secondary chemicals. With or without broad leaves, porous epithelium, broad marginal zone, thick-walled hyaline spores, subspinous and marginal cilia, the distinguishing feature of the genus Palmotrema is the giant frond-like fronds. Of the 350 known species, over 220 live in tropical regions (1). Palmotrema seeds have also been found to have characteristics that are antibacterial (2) and antioxidant (3,4). In previous experiments conducted in our laboratory, a methanol extract of Palmotrema praesodiosum showed anti-ulcer activity against acid-neutralizing capacity [ANC] in vitro (5).

ethanolic extract of Parmotrema praesorediosum has also been shown to have antioxidant, antibacterial (6) and antifungal (7) properties.

An uncommon black lichen called Parmotrema praesorediosum grows on trees, rocks, and other solid objects. A subspecies of Parmotrema praesorediosum is Blackstone flowers. [8] Both the northern and southern hemispheres are home to this species. Their chloroplasts have only been found in lichens; they lack any roots, stalks, or leaves. Despite preferring tree trunks, the praesorediosum parmotrema can also be found on rocks. [8] It is frequently utilised in Indian cooking as a component of the masala spice mixture, particularly in recipes that contain meat. It is also a well-liked component in vegetarian recipes. It reduces excessive salivation as well

as the symptoms of bronchitis, vomiting, and other conditions. Flowers can also be used to treat persistent gastritis.

#### **Materials and Methods**

## Plant material and extraction

Plant material Parmotrema praesorediosum (Family: Permeliaceae) was collected from Horsley Hills during December and January and studied by Dr. Raviprasad Rao, Department of Botany, Sri Krishnadevaraya University, Ananthapuram. A copy of the specimen will be retained for future reference (reference number 57418). The lichen of Parmotrema praesorediosum was shade-dried, powdered and in a Soxhlet extractor he extracted with methanol for 16–18 hours (100 g). The extract was concentrated to dryness under reduced pressure and controlled temperature (40° C.-50° C.). The crude ethanol extract [9-10] was a dark brown solid weighing .62 g (62%

yield). Extracts were stored in a refrigerator at 4°C until further use.

# Anti-Ulcer activity:

## The ability of the invitro approach to neutralise acids :

The ability of the invitro approach to neutralise acids was determined for various concentrations (100 mg/ml, 200 mg/ml, 500 mg/ml, 1000 mg/ml) of ethanolic extracts of Palmotrema praesolediosum. was compared to standard antacids, aluminum hydroxide and magnesium hydroxide - 500 mg/mL (AHMH). A precise amount (5 mL) of extract was weighed into a 25 mL beaker and weighed. Pour into a 250ml beaker, make up to 70ml with non-carbonated distilled water and stir for 1 minute. A precise volume of 30 mL of 1.0 N HCl was pipetted into the extract while stirring for 15 minutes. Excess HCl was titrated with 0.5N NaOH (VS) to reach a threshold pH of 3.5. Experiments were performed for all concentrations of and each batch of at a temperature of 37 °C  $\pm$  3 °C using a magnetic stirrer. The number of milliequivalents (mEq) of acid consumed per gram of antacid was calculated [12-15]. the ability of the invitro approach to neutralise acids was calculated using (16) Equation 1.2. Moles of acid neutralized = (Vol. of HCl × Normality of HCl) - (Vol. of NaOH × Normality of NaOH) ......1

Neutralise acids per gram of antacid = Moles of HCl Neutaralized/Grams of antacid/extract... 2.

## Gastric hydrogen potassium ATPase inhibitory(17):

#### Ability to inhibit Gastric hydrogen potassium ATPase inhibitory:

Gastric hydrogen potassium ATPase inhibitory enzyme Preparation: Fresh goat stomach was prepared by excising and opening the gastric mucosa at the fundus and scraping the stomach lining for parietal cells, The den I bought at a local butcher shop. Gastric parietal cells were homogenized in 16 mM Tris buffer pH 7.4, 10% Triton X-100 and centrifuged at 6000 rpm for 10 minutes. The supernatant was then used to inhibit Gastric hydrogen potassium. Determine the protein content using Bradford's method and use BSA as a reference. Evaluation of Gastric hydrogen potassium-ATPase Inhibition For sample reaction mixtures containing 20 g, 40 g, 60 g, 80 g, and 100 g of plant extract and 0.1 ml of enzyme-containing extract, sample reaction mixtures were incubated at 37°C. 60 minutes was spent incubating (300 grams). A 2 mM ATP substrate, 200 ml of 2 mM MgCl 2, and 10 ml of each KCl were added to start the reactions. 4.5% ammonium molybdate was used to stop the reaction after 30 minutes at 37 degrees Celsius. The mixture was then centrifuged at 2000 rpm for 10 minutes to release the inorganic phosphate, which was discovered at 660 nm using the Fiske-Subbarrow method after the addition of 60% perchloric acid. Briefly, 1 ml of supernatant, 4 ml of Millipore water, 1 ml of 2.5% ammonium molybdate, and 0.4 ml of ANSA were added after 10 minutes at room temperature. For various extract dosages, the inorganic phosphate absorption at 660 nm was determined. Micromoles of Pi emitted each hour were used to measure enzyme activity and were examined at various dosages of the extract Omeprazole, a well-known anti-ulcer PPA inhibitor, was used as a comparison drug. The results were presented as a mean SEM enzyme inhibition of 16%.

Percentage of inhibition = [Activity (control) - Activity (test)/Activity (control)] × 100 ......3

## Thin Layer Chromatography(18)

TLC is a method for analyzing mixtures by separating the compounds in the mixture.TLC can be used determine the number of components in a mixture, and also to identity of compounds and its purity

A factor in quantifying migration of a compound on a particular sorbent and solvent system is

the Rf value. This is defined as(19)

Rf=distance moved by the compound /distance moved by the solvent

# **RESULTS AND DISCUSSION**

## Thin Layer Chromatography

The Rf value in the chromatographic analysis of ethanolic extract of Parmotrema Praesoredisum was found to be 0.61 which was nearer to the standard Rf value of Orientin (0.65). The mobile phase we have chosen is chloroform:methanol:water(4:3:1)

## Invitro anti-ulcer effect of ethanolic extract of parmotrema praesorediosum

# The ability of the *invitro* approach to neutralise acids:

The neutralizing effect of ethanol the extract was measured at four concentrations (100 mg, 500 mg, 1000 mg) and standard aluminum hydroxide + magnesium hydroxide [Al (OH) 3+Mg (OH) 2] (500 mg). was studied about. The results obtained show that extract concentrations of 100 mg, 500 mg and 1000 mg showed a significant decrease in acid capacity .Compared to standard Al (OH) 3+Mg (OH) 2 (500 mg), 96, 62.25, 29.80, and 11.75, or 15.8. The extract at a concentration of 1000 mg was found to neutralize acids significantly more than the standard Table 1

significantly more than the standard. Table 1.

Concentration [mg/ml]	Volume of NaOH Consum ed [ml]	mEq of Acid Co nsumed	ANC per gr am of extract
PP - 100mg	40.8	9.6	96
PP- 200mg	35.1	12.45	62.25
PP- 500mg	30.2	14.9	29.80
PP- 1000mg	39.6	9.74	11.75
AL(OH) <sub>2</sub> &M g(OH) <sub>2</sub> 500 m g	45.2	7.86	15.80

## Table:1 The ability of the invitro approach to neutralise acids of ethanolic extract.

#### Gastric hydrogen potassium

hydrogen potassium ATPase inhibitory of ethanol extracts was tested using omeprazole as the gold standard at various concentrations (20 g, 40 g, 60 g, 80 g, and 100 g). The extract showed a significant dose-dependent effect. The maximum percent inhibition was 62,180.54% for the 100 g concentration extract and 69,561.72% for conventional omeprazole. The who counted the results in Table 2

ATPase inhibitory: Gastric

## TABLE 2: ETHANOL EXTRACT OF ON IN-VITRO Gastric hydrogen potassium ATPase inhibitory

S.NO	Concentration (µg)	Amount of Inhibition (%)	
		Standard Omeprazole	Ethanol extract
1	20	-51.2	-30.2
2	40	-56.2	-18.9
3	60	36.8	31.7
4	80	58.7	55.4
5	100	69.6	62.8

Using the proton pump, the parietal cells of the stomach mucosa secrete excessive amounts of hydrochloric acid, which is what is known as hyperchlorhydria. An essential enzyme for producing acidity is Gastric hydrogen potassium ATPase, which is found on the parietal cell's apical secretory membrane. At a concentration of 100 g, the extract exhibited a maximum percentage inhibition of 62.8% in Gastric hydrogen potassium - ATPase activity.

The information provided here suggests that the presence of chemicals in the mixture may be the cause of the ethanol extract's potential antacid, antisecretory, and antiulcer properties. However, more research is needed to determine its precise mechanism of action and the key ingredients responsible for its antiulcer efficacy.

### **CONCLUSION:**

Based on the results, it can be concluded that ethanolic extracts of species can be regarded as the sole source of new anti-ulcer drugs. A detailed study of the isolate and the mechanism of action responsible for its anti-ulcer activity is currently under investigation.

#### **Reference:**

1. Balaji P, Hariharan GN. In vitro antimicrobial activity of Parmotrema praesorediosum. Res J Bot. 2007;2:54-9.

2.Sati SC, Joshi S. Antibacterial activity of the Himalayan Lichen Parmotrema nilgherrense extracts. Br Microbiol Res J. 2011;1:26–32.

3. Stanly C, Hag Ali DM, Keng CL, Boey PL, Bhatt A. Comparative evaluation of antioxidant activity and total phenolic content of selected lichen species from Malaysia. J Pharm Res. 2011;4:2824.

4. Ghate NB, Chaudhuri D, Sarkar R, Sajem AL, Panja S, Rout J, et al. An antioxidant extract of tropical lichen, *parmotrema reticulatum*, induces cell cycle arrest and apoptosis in breast carcinoma cell line MCF-7. *PLoS One*. 2013;8:e82293.

5. Devhare, L., & Gokhale, N. (2021). Acid neutralizing capacity and antimicrobial potential of selected solvent extract from various indigenous plants. *Journal of Advanced Scientific Research*, *12*(04), 175-179. https://doi.org/10.55218/JASR.202112423

6 Mie R, Samsudin MW, Din LB, Ahmad A, Ibrahim N, Adnan SN. Synthesis of silver nanoparticles with antibacterial activity using the lichen *Parmotrema* praesorediosum. Int J Nanomedicine. 2014;9:121-7. doi: 10.2147/IJN.S52306. Epub 2013 Dec 19. PMID: 24379670; PMCID: PMC3872223.

7 Grinshpan, D.D., Nevar, T.N., Savitskaya, T.A. *et al.* Comparison of Acid-Neutralizing Properties of Anti-Acid Preparations of Various Compositions. *Pharm Chem J* **42**, 400–404 (2008). https://doi.org/10.1007/s11094-008-0139-

8. Hale ME. (1961). "The typification of Parmeliapraesorediosum (Huds.) Ach". Brittonia.

9. F. Chemat and M. A. Vian. Alternative Solvents for Natural Products Extraction. Heidelberg: Springer-Verlag Berlin. 2014

10. Mai Anugrahwati, Extraction of Ethanolic Extract of *Red Betel Leaves* and Its Cytotoxicity Test on HeLa Cells, Procedia Engineering, Volume 148, 2016, Pages 1402-1407,

11. Dr. K Jagadesh, Dr. Chidananda K N, Study of Acid Neutralizing Capacity of Various Antacid Formulations, Asian Journal of Pharmaceutical Technology & Innovation, 03 (12); 2015.

12. J. Shery, S. Annie, A. Shijna, K. Reham, I. Mariyam, N Anroop, Acid neutralization capacity and cost effectiveness of antacids sold in various retail

13. United States Pharmacopoeia (USP) and National Formulary (NF), Acid neutralization capacity. rockville, MD: US Pharmacopoeial Convention Inc

14. K.N. Chidananda, K. Jagadesh, Study of acid neutralising capacity of various antacid formulations, AJPTI 03 (12) (2015) 113-120.

15. Isaac Ayensu, et al ..., Evaluation of acid neutralizing and buffering capacities of selected antacids in Ghana, Scientific African .Volume 8,2020

16. Dr. K Jagadesh, Dr. Chidananda K N, Study of Acid Neutralizing Capacity of Various Antacid Formulations, Asian Journal of Pharmaceutical Technology & Innovation, 03 (12); 2015.

17. Yadav P, Ganeshpurkar A and Rai G: In-vitro H+/K+ - ATPase inhibitory potential of methanolic extract of Cissus quadrangularis Linn. Pharmacognosy Research 2012; 4(2): 123-26.

18. Krishna Murthy Naik et al.Extraction, Isolation and Phytochemical Investigation of Natural Products by Using Chromatographic (TLC) Method IJPPR, August 2016 Vol.:7, Issue:1

19. Thinlayer chromatrography; A complete guide to TLC .chemistry hall.2020-01-02.retrieved 2020-01-30