

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

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 Parmeliaceae 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.

Keywords: Parmotrema praesorediosum, Parmeliaceae, Lichens, anti-ulcer activity, acid neutralizing capacity, neutralise acids and Gastric hydrogen potassium ATPase inhibitory, ethanol extract.

I. INTRODUCTION

Authors

S. Lahari

Department of Pharmaceutical Chemistry
JNTUA-OTPRI
Anantapur, India
lahari.sidde@gmail.com

L. Pranathi

Department of Pharmaceutical Chemistry
JNTUA-OTPRI
Anantapur, India

G. MadhaviLatha

Department of Pharmacy
JNTUA-OTPRI
Anantapur, India

M. Ummer

Department of Pharmacy
JNTUA-OTPRI
Anantapur, India

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 *Parmotrema* is the giant frond-like fronds. Of the 350 known species, over 220 live in tropical regions (1). *Parmotrema* 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 *Parmotrema praesorediosum* 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.

II. MATERIALS AND METHODS

1. 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.

2. 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 *Parmotrema praesorediosum*. 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 ± 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

$$\times \text{Normality of HCl} - (\text{Vol. of NaOH} \times \text{Normality of NaOH}) \quad 1$$

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

3. 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 20g, 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 MgCl₂, 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-Subbarow 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%.

$$\text{Percentage of inhibition} = [\text{Activity (control)} - \text{Activity (test)} / \text{Activity (control)}] \times 100 \quad \dots\dots\dots 3$$

4. Thin layer chromatography(18): TLC is a method for analyzing mixtures by separating the compounds in the mixture. TLC can be used to determine the number of components in a mixture, and also to identify compounds and its purity

A factor in quantifying migration of a compound on a particular sorbent and solvent system is the R_f value. This is defined as (19)

$$R_f = \text{distance moved by the compound} / \text{distance moved by the solvent}$$

III. RESULTS AND DISCUSSION

1. **Thin Layer Chromatography:** The R_f value in the chromatographic analysis of ethanolic extract of Parmotrema Praesorediosum was found to be 0.61 which was nearer to the standard R_f 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

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- 2. 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 aluminumhydroxide + 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.

Table1: The ability of the invitro approach to neutralise acids of ethanolic extract.

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) ₂ &M g(OH) ₂ 500 mg	45.2	7.86	15.80

- 3. Gastric hydrogen potassium ATPase inhibitory:** Gastric 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

Table 2: Ethanol Extract of on *In-Vitro* Gastric hydrogen potassium ATPase inhibitory

Sl. 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.

IV. 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.

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