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 its in vitro method as an acid-neutralizing capacity (ANC) and H+/K+-ATPase inhibitory activity. The extract significantly reduced the ANC to 11.75 at the 1000 mg concentration compared to 15.8 for standard aluminum hydroxide + magnesium hydroxide (500 mg). During H + /K + - ATPase inhibitory activity, the extract showed a maximum inhibition rate of 62.18% at 100 µg concentration compared to 69.56% 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, H+/K+ - ATPase inhibition, 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 antimicrobial (2) and antioxidant (3,4) properties. 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). Methanolic 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:

Acid Neutralizing Capacity (ANC)

ANC 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 ± 3 °C using a magnetic stirrer. The number of milliequivalents (mEq) of acid consumed per gram of antacid was calculated [12-15]. Acid-neutralizing capacity (ANC) was calculated using (16) Equation 1.2. Moles of acid neutralized = (Vol. of HCl × Normality of HCl) - (Vol. of NaOH × Normality of NaOH) 1

ANC per gram of antacid = Moles of HCl Neutaralized/Grams ofantacid/extract… 2.

H+/K+ - ATPase Inhibition Activity(17):

Ability to inhibit H+/K+ - ATPase:

H+/K+ - ATPase 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 H+/K+ ATPase. Determine the protein content using Bradford's method and use BSA as a reference. Evaluation of H+/K+-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). Reactions were initiated by adding 2 mM ATP as substrate along with 200 ml each of 2 mM MgCl 2 and 10 ml each of KCl. After 30 minutes at 37 degrees Celsius, the reaction was quenched with 4.5% ammonium molybdate. 60% perchloric acid was then added and the mixture was centrifuged at 2000 rpm for 10 minutes to liberate inorganic phosphate, which was detected at 660 nm using the Fiske-Subbarrow method. Briefly, after 10 minutes at room temperature, 1 ml supernatant, 4 ml Millipore water, 1 ml 2.5% ammonium molybdate, and 0.4 ml ANSA were added. The absorbance of inorganic phosphate at 660 nm was measured for different doses of extract. Enzyme activity was calculated as micromole Pi released per hour evaluated at different doses of extract. Results were expressed as a mean SEM enzyme inhibition of 16% and compared to omeprazole, a known anti-ulcer PPA inhibitor.

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

Acid Neutralizing Capacity:

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 (ANC). H. 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.

Table:1 Acid Neutralizing Capacity [ANC] of ethanolic extract.

Concentration [mg/ml]	Volume of NaOH Consumed [ml]	mEq of Acid Consumed	ANC per gram 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
$AL(OH)_2$&$Mg(OH)_2$500 mg	45.2	7.86	15.80

H+/K+ - ATPase Inhibition Activity: The H+/K+-ATPase inhibitory activity 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 inTable 2

TABLE 2: ETHANOL EXTRACT OF ON *IN-VITRO* H+/K+ - ATPase INHIBITION ACTIVITY

S.NO	Concentration **(µg)**	**Percentage Inhibition (%) (Mean ± SEM)**	
		Standard Omeprazole	**Ethanol extract**
1	20	-51.25±0.78	-30.12±0.26
2	40	-56.32±1.24	-18.84±1.86
3	60	36.58±1.58	31.64±0.68

4	80	58.62±0.24	55.36±1.54
5	100	69.56±1.72	62.18±0.54

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 H+/K+ - ATPase, which is found on the apical secretory membrane of parietal cells. At a concentration of 100 g, the extract exhibited a maximum percentage inhibition of 62.18% in H+/K+-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.

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