ASSESSMENT 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's anti-ulcer properties..In this study, we assessed for anti-ulcer activities with ethanol extract and in-vitro method as the acid neutralizing capacity (ANC), H+/K+ - ATPase inhibition activity method. The extract significantly reduced ANC to 11.75 at a concentration of 1000 mg as compared to 15.8 with standard Aluminium hydroxide + Magnesium hydroxide (500mg).While in H+ /K+ - ATPase inhibition activity, the extract showed maximum percentage inhibition of 62.18% at the concentration 100µg as compared to 69.56% with standard Omeprazole. The study reveals that the ethanol extract may contain some compounds possessing anti-ulcer activity and thus can be used as an alternative medicine for diseases.

Key words: Parmotrema praesorediosum, Permeliaceae, Lichens, anti-ulcer activity, acid neutralizing capacity, H+/K+ - ATPase inhibition, ethanol extract.

Introduction

Lichens are fungal and algae symbiotic organisms that produce distinctive secondary chemicals. The huge foliose thalli with broad lobes, pored epicortex, broad marginal zone, thick-walled hyaline spheroid ascospores, sublageni shape, and with or without marginal cilia are prominent characteristics of the genus Parmotrema. Out of the 350 species that are known, more than 220 species are found in tropical areas (1). Species of Parmotrema have also been discovered to exhibit antibacterial (2) and antioxidant(3,4) characteristics. In a prior experiment conducted in our lab, Parmotrema praesorediosum methanol extracts demonstrated invitro anti-ulcer action against acid neutralising capacity[ANC] (5). The Parmotrema praesorediosum methanol extracts have also been found to have antioxidant, antibacterial(6), and antifungal(7) activities.

A 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 on 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

The plant material Parmotrema praesorediosum (Family: Permeliaceae) was collected in Horsley hills in the month of December-January and was authenticated by Dr. Raviprasad Rao,Department of Botany,Sri Krishnadevaraya university,Ananthapuram. A speciman voucher is preserved for future reference (Voucher No 57418). The lichen of Parmotrema praesorediosum was shade dried, powdered, and extracted (100 g) with methanol in a Soxhlet extractor for 16 – 18 h. 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 62g (yield, 62%). The extract was preserved in a refrigerator at 4°C until further use.

Anti-Ulcer activity:

Acid neutralizing capacity (ANC)

The ANC was determined for ethanolic extract of Parmotrema praesorediosum in different concentrations (100mg/ml,200mg/ml,500mg/ml,1000mg/ml) were compared with the standard antacid Aluminum hydroxide+nMagnesium hydroxide -500mg/ml(AHMH) . An accurate volume(5 mL) of the extract was measured into a 25 ml beaker and weighed. Then transferred into a 250 ml beaker and made up to 70 ml with carbon dioxide-free distilled water and stirred for one minute.[11] An accurate volume of 30 ml of 1.0 N HCl was pipetted into the extract whiles stirring for 15 mins. The excess HCl was titrated with 0.5 N NaOH (VS) to attain a threshold pH of 3.5. The experiment was carried out for the all concentration and their respective batches at a temperature of 37 °C ± 3 °C on a magnetic stirrer. The number of milliequivalent (mEq) of acid consumed per gram of antacid was calculated [12-15].The 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):

The ability to inhibit H+/K+ - ATPase: H+/K+ - ATPase Enzyme Preparation: The gastric mucosa of the fundus was cut off and opened, and the inner layer of the stomach was scraped out for the parietal cell in order to prepare the fresh goat stomach that had been obtained from the neighbourhood butcher. The stomach parietal cell was homogenised in 16 mM Tris buffer with a pH of 7.4, 10% Triton X-100, and centrifuged at 6000 rpm for 10 minutes. The supernatant solution was then used to inhibit H+/K+ ATPase. Bradford's technique is used to determine protein content, and BSA is used as a reference. Evaluation of the H+/K+ ATPase inhibition 60 minutes were spent incubating the reaction mixture of the sample at 37 ºC for the reaction mixture of the sample, which contained 20g, 40g, 60g, 80g, and 100g of plant extract and 0.1ml of enzyme extract (300g). 2 mM ATP was added as the substrate, along with 200 mL each of 2 mM MgCl2 and 10 mL each of KCl, to start the reaction. After 30 minutes at 37 degrees Celsius, the reaction was halted with 4.5% ammonium molybdate. Then, 60% perchloric acid was added, and the mixture was spun at 2000 rpm for 10 minutes to liberate the inorganic phosphate, which was then detected at 660 nm using the Fiske-Subbarow technique. In a nutshell, 1ml of supernatant, 4ml of Millipore water, 1ml of 2.5% ammonium molybdate, and 0.4ml of ANSA were added after 10 min at room temperature. Inorganic phosphate absorption at 660 nm has been measured at various doses of the extract;The enzyme activity has been computed as micromoles of Pi released per hour, assessed at varied extract dosages. The results were expressed as Mean SEM 16% enzyme inhibition and compared to the well-known anti-ulcer PPA inhibitor Omeprazole.

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 the ethanol extract was studied for four concentration (100mg, 500mg, 1000mg,) and standard Aluminium Hydroxide + Magnesium Hydroxide [Al(OH)3+Mg(OH)2](500mg). The results obtained envisage that the extract at concentration 100mg, 500mg, 1000mg, showed a significant reduction in acid capacity (ANC), i.e., 96, 62.25, 29.80, and 11.75,respectively, as compared to standard Al(OH)3+Mg(OH)2 (500 mg) which is 15.8. The extract at a concentration of 1000mg has been found to neutralize acid more significantly as compared to 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 extract has been tested with omeprazole as the gold standard at various concentrations (20g, 40g, 60g, 80g, and 100g). Significant dose-dependent action was demonstrated by the extract. The maximum percentage inhibition was 62.180.54% for the extract at a concentration of 100g, and 69.561.72% for conventional omeprazole. The outcomes have been talliedin Table 2

TABLE 2: EFFECT OF 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 the ethanol- extract of species can be regarded as the sole source of new anti-ulcer drugs. A detailed study of the isolates and the underlying mechanism of action responsible for their anti-ulcer effects will be explored.

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