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EVALUATION OF PHYTOCHEMICAL SCREENING AND ANTI-INFLAMMATORY ACTIVITY OF PARMOTREMA PRAESOREDIOSUM

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Abstract

There are numerous medicinal plants in Indonesia. With the development of science and technology, the general people has been using this medicinal plant extensively in an effort to combat health issues like slow wound healing. The vital significance of secondary metabolites of plants as therapeutic raw materials has been further clarified by the discovery of numerous new pharmaceutical molecules derived from natural sources. The plant *Parmotrema praesorediosum*, also known as the black stone flower and rathi potha, is a member of the *parmeliaceae* family, Subfamilia: *Parmelioideae*, Genus: *Parmotrema*, Species: *Parmotrema praesorediosum*. The current study examines the preliminary phytochemical and anti-inflammatory screening of *Parmotrema praesorediosum*. The flower has antibacterial, anti-oxidant, and anti-microbial properties. Initial phytochemical analysis found flavonoids, glycosides, alkaloids, tannins, etc. in the sample. To identify the components in the ethanolic floral extract, TLC was used. The anti-inflammatory properties of the crude ethanolic extract of *Parmotrema praesorediosum* flower were examined.

Index Terms

Parmotrema praesorediosum, rathi potha, phytochemical screening, ethanolic floral extract, Anti-inflammatory activity.

Introduction

Lichen is a symbiotic organism between a mycobiont (a fungus) and a photobiont (an algae or cyanobacteria), according to *Parmotrema praesorediosum* (T. H. Nash and R. S. Egan, 1998). Lichen is thought of as a type of fungi because of the fungus' special composition and ability to regulate the symbiotic process of the lichen. More than 10% of the world's terrestrial ecosystems are covered by an ecosystem in which lichen and moss predominate, especially on higher ground (G. H. Denton and W. Karlen 1973). Balaji College of Pharmacy, Department of Pharmacology "Fig.1" The *Parmotrema Praesorediosum* Flower Lichen requires energy and nutrients to flourish, just like all other living things. The environment, which includes dust, water, and other substrates present on the habitats it is produced on, provides the necessary nutrients. The energy needed to complete photosynthesis is produced by cyanobacteria and algae. Lichens' symbiotic relationship with their surroundings allows it to withstand harsh conditions. Lichen has the potential to live for several thousand years despite competition from other plants (M.E Hale 1984-K. Buaruang, P. Mongkolsuk and L. Manoch, 2009). The *Parmeliaceae* family, which includes the genus *Parmotrema* of lichen, is the largest lichen family in the world and is thought to include more than 1000 species in 60 or more genera. Due in part to the limited generic notion, the number of genera in the *Parmeliaceae* family greatly grew some decades ago (B. Ronkovic, M. Misic and S. Sukdolak 2007). The foliose growth that develops on the lichens in this family distinguishes them (D. H. S. Richardson 1991). Traditional treatments have employed lichens for millennia, and in many parts of the world, it continues to be of significant interest as an alternative

treatment(D. Gayathri and C. T. Swamy- L. B. Din, G. Ismail and J. A. Elix2012). Researchers have been looking at the secondary substances that lichens produce for more than a century. Lichens produce a wide range of distinctive secondary metabolites that are known to have a variety of biological functions(K. Bombuwala, V. Subramaniam,1999). Many pharmacological effects, including antibacterial, antiviral, antiprotozoal, enzyme inhibitory, insecticidal, antitermite, cytotoxic, antioxidant, wound healing, antiherbivore, and analgesic, have been linked to lichens and their secondary metabolites(S. V. P. Kumar, P. T. R. Kekuda2010). Due to their low concentration in nature, lichens represent a diversity of novel bioactives, the majority of which have yet to be fully described and their potential found.



Figure 1.; *Parmotrema Praesorediosum*

Phytochemistry

Plant phytoconstituents can be used for a variety of medicinal purposes. *Parmotrema praesorediosum* contains a variety of phytoconstituents Table.1 including flavonoids, tannins, alkaloids, glycosides, steroids, terpenes, and lichen acid.

Plant collection and authentication

From the Horsley hills, fresh *Parmotrema praesorediosum* flowers were procured in the months of December and January. Dr.Raviprasad Rao, Department of Botany, Sri krishnadevaraya University, Ananthapuram, authenticated and taxonomically recognised the flower material. The floral gift card number is 57418.

Experimental methodology

Preparation of powdered flower for extraction:

In order to prevent chemical contents (Hale ME. 1961) from degrading, the *parmotrema praesorediosum* flowers that have been harvested are cleaned and dried at room temperature. After being ground into a coarse powder, these leaves are used in the study.

Soxhlet (Hot continuous -Extraction)

By employing a hot continuous extraction process (Soxhlet-extractor) and ethanol “Fig 2” as a solvent, 25 gm of *Parmotrema praesorediosum* flower powder was obtained. A thimble is filled with a powdered sample using this procedure(Nur Asnah Sitohang,2022). A heating source, or heating mantle, is used to warm the extraction solvent (ethanol), which is placed in the RBF. The extractor's side tube is where the evaporated solvent travels before condensing in the condenser that is attached to the top of the thimble. The powdered substance is soaked by the hot, condensed solvent as it extracts the chemical components(Mohini Upadhye, Parikshit Gandhi 2014- S. J. Daharwal, Rajendra K. Jangade, 2013) from the heated solvent and streams into the thimble. The liquid content of the chamber syphons down into RBF when the level of liquid in the chamber reaches the top of the syphon tube. Up until a certain point, this process is ongoing. The clear solution in the syphon tube serves as a sign that the procedure is complete because a drop of solvent from the syphon tube evaporates without leaving any residue behind.



Figure 2: Soxhlet assembly appature

Anti- inflammatory activity

Evaluation of in vitro anti-inflammatory efficacy(Budi Arief Waskito, Djanggan Sargowo 2022- Kiran Madhawai, Dinesh Rishipathak2017) Using egg albumin to stop protein denaturation: The reaction mixture contained 2 ml of different quantities of the test extract at concentrations of 100, 200, 300, 400, and 500 g/ml, 0.2 ml of fresh hen's egg albumin, 2.8 ml of phosphate buffered saline (pH 6.4), and 2 ml of egg albumin Table 4. As a control, the same volume of double-distilled water was used(Kiran Madhawai, Dinesh Rishipathak 2017- Prathap Kumar Kothapalli, S. Jagadeesh Sanganal, 2014). The mixes were then heated for 5 minutes at 70°C after 15 minutes of incubation at 37°C with 2°C in a biological oxygen demand incubator. Their absorbance at 660 nm was measured after cooling, using a vehicle as a reference. For the purpose of determining absorbance, diclofenac sodium was handled similarly and used as a reference at a concentration of 100 ((g/ml. Test samples were selected so that, they continued to operate as close to the typical treatment mode as they could. The following formula was used to determine how much protein denaturation was inhibited(K. Teja, T. Satyanarayana , B. Saraswathi-2019- K. Teja, T. Satyanarayana , B. Saraswathi2016).

Inhibition percentage = (Abs Control – Abs test) Abs Management× 100

Results and Discussion

Table 1.: Phytochemical screening of Parmotrema praesorediosum

S.NO	Phytochemicals	Tests	Results
1	Tannins	Gelatin solution test; Ferric chloride	Positive; Positive
2	Flavanoids	Sulphuric acid test; Alkaline reagent test	Positive; Positive
3	Glycosides	Keller killiani test	Positive
4	Alkaloids	Tannic acid test	Positive
5	Carbohydrates	Benedict's test	Negative

Thin layer chromatography

R_f values of flavonoid compounds and their colours was identified under the UV light Table:2

$$R_f = \frac{\text{Abs Control travelled by the compound}}{\text{Distance moved by the solvent}}$$

R_f = 0.68

Table 2: R_f values

Flavanoids	R _f value	Colour under UV 365 nm
Orientin	0.65	Violet

Infrared Spectroscopy

Table 3: IR Spectra and standard values:

S.no	Function al group	Standard value	erved value
1.	C-O	1000-1300	1128.83
2.	C=O	1650-1750	1728.26
3.	C-H	2840-3000	2926.73
4.	C=C	1626-1662	1645.98

5.	C-C	2100-2150	2025.96
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***In-vitro* anti-inflammatory effect of *parmotrema praesorediosum*(EPP) by protein denaturation method.**

Table 4: protein denaturation method

Treatments	Concentration (µg/ml)	Absorbance at 660nm	%Inhibition of Protein Denaturation
Control	-	0.36±0.03	-
EPP	100	0.28±0.04	22
EPP	200	0.22±0.03	38
EPP	300	0.13±0.02	63
EPP	400	0.11±0.04	69
EPP	500	0.08±0.02	77
Diclofenac sodium	100	0.11±0.01	59.3

Discussion

In the presence investigation, the dried powder flower of *Parmotrema praesorediosum* was extracted using the Soxhlet method with ethanol as the solvent.

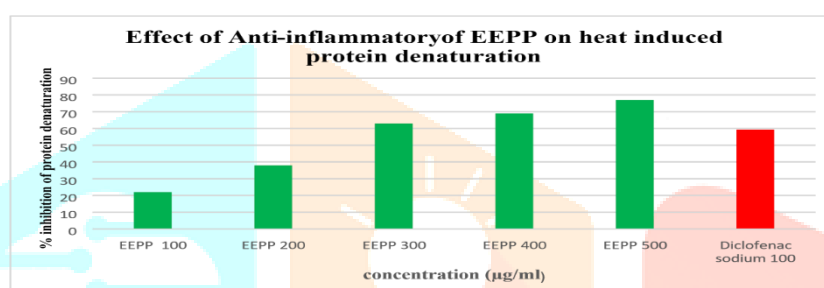
The remainder of the extracts were used for TLC, IR, and pharmacological activity, with a portion set aside for preliminary phytochemical analysis.

Flavonoids, tannins, glycosides, and alkaloids chemicals were found, according to the preliminary phytochemical analysis. Since flavonoid molecules make up the majority of a plant's phytochemical components, they were isolated from an ethanolic flower extract and put through qualitative TLC analysis and IR Spectroscopy.

The ethanolic extract of *Parmotrema Praesorediosum*'s Rf value was determined by chromatographic analysis to be 0.61, which was close to the Orientin standard Rf value (0.65). Chloroform, methanol, and water make up our selected mobile phase (4:3:1)

The vibrations of atoms are measured using IR spectroscopy Table 3, and based on using this, the functional groups can be identified.

Investigated *in-vitro* evaluation of anti-inflammatory activity of *parmotrema praesorediosum* flower.



When a protein is exposed to an external stressor or substance, such as a strong acid or base, a concentrated inorganic salt, an organic solvent, or heat, the protein loses both its primary and secondary structural integrity. This process is known as denaturation. When biological proteins are denaturated, they stop functioning biologically. Protein denaturation is a well-known contributor to inflammation. The increases in absorbance of test samples compared to controls showed that proteins had been stabilised, i.e., that *parmotrema praesorediosum* and the reference medication diclofenac sodium had prevented heat-induced protein (albumin) denaturation. It is clear from the findings for the percentage inhibition of protein denaturation that EEPP was more active than diclofenac sodium and that it was efficacious at lower concentrations.

The *in-vitro* anti-inflammatory action of EEPP in the current study can be attributed to its flavonoids content. Instead of just one of the ingredients, a synergistic impact could be to blame. At a concentration of 500 g/ml, EEPP displayed a maximum inhibition of 77 percent. Standard anti-inflammatory medication diclofenac sodium demonstrated the greatest inhibition of 69 percent as compared to control at a concentration of 100 g/ml.

Conclusion

Based on the findings of the current investigation, it was determined that the ethanolic extract of *Parmotrema praesorediosum* has anti-inflammatory properties. Its flavanoids content is what causes this activity. Instead of being the result of a single element, the effect could be a synergistic one. This research raises the possibility that a lead component from the flower *Parmotrema praesorediosum* may be used to create effective medications that can be used to treat a variety of disorders. However, these investigations do not confirm the traditional significance of the claimed action, so numerous additional pharmacological, phytochemical, and bioanalytical research must be conducted, followed by observational studies in humans.

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