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Studies on the macronutrient composition in the thallus of *Parmotrema austrosinense*

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Abstract: Based on available data, the macronutrient composition of the lichen *Parmotrema austrosinense* has not yet been documented in scientific literature. To address this gap, the present study focused on analyzing the chlorophyll, carotenoid, sugar, protein, and fat contents within the thallus of *P. austrosinense*. Findings from the analysis confirmed that the thallus is a rich source of these macronutrients, highlighting its potential nutritional significance. However, further research is needed to explore its possible medicinal or economic applications, as such evidence could enhance the value of *P. austrosinense* in therapeutic or commercial contexts.

Key words: Chlorophyll • carotenoid • sugars • protein • fats

Introduction

Lichen is a very diverse biological organism that is found naturally in tropical and temperate rainforests, with an estimated 22,000 species. India is thought to be home to about 2712 different species of lichen (Sinha et al. 2018). Unique secondary metabolites including approximately 1050 chemicals are known to exist in lichens. There are more than 21,500 kinds of lichen in the globe. Lichens include over 550 distinct chemicals. Fatty acids, dibenzofurans, pulvic acid derivatives, zeorins, diphenyl ethers, carotenoids, steroids, anthroquinone derivatives, terpenoids, depsidones, and depsides are among the derivatives of lichen substances. In general, none of the chemicals found in lichens dissolve well in water. The amount of secondary lichen compounds found in lichen varies greatly. Lecanoric acid content in the *Pamotrema tinctorum* thallus has been estimated to be 23.5%. The wolf lichen's thallus contained 10.5% vulpinic acid. Fungal melanins usnic acid, parietin and atranorin, and are present in the cortex of lichen extracts. The medullary layer contains the lichen extracts containing physodic acid physodalic acid and protocetratic acid. Using spot tests, thin-layer chromatography, or high-pressure liquid chromatography, the components of lichen are identified. A consistent methodology for performing lichen chemistry was developed by Walker and James (1980). According to Saklani and Upreti (1992), lichens are used to treat a variety of blood and heart illnesses as well as dyspepsia, bleeding piles, bronchitis, scabies, and stomach disorders. Cetrarin was used for anemia and appetite loss because it sped up peristalsis. Usnic acid, which is produced from lichens, has a broad range of uses in the pharmaceutical industry as analgesics and antipyretics for the treatment of psoriasis, parasite infections, and skin conditions.

Little is known about the macronutrient content of lichens worldwide, despite the fact that a thorough

and comprehensive evaluation of the nutraceutical qualities of most plants has been published. One such lichen is the temperate foliose lichen *Parmotrema austrosinense*. *Parmotrema* species has a fascinating relationship with other spices in cooking, and indigenous communities in Nepal and India frequently use this species to enhance flavor to a variety of foods, including vegetables and meat (Upreti et al., 2005). Thus, in order to document the possible biological activities of these lichens, the proximate chemicals and solvent extract analysis of *Parmotrema austrosinense* were examined in the current study. These lichens have the largest potential for antioxidants, according to the results, which opens up a world of possibilities for more research in the fields of phytochemistry and nutrition.

This study's primary goal was to examine the macronutrient composition of lichens by using the standard protocol.

Materials and Methods

Parmotrema austrosinense was obtained during the winter season in the Kodaikanal hills of Southern India's Western Ghats (Feb 2024). The hills were measured to be roughly 2130 meters in elevation. Lichen identification was done at St. Joseph University in Tanzania, Dar es Salaam (Fig 1). The lichen specimens were recognized using the keys provided in Awasthi's identification key manual (Wolseley 2008). The species were chemically identified using traditional spot tests (K, C, I, KC) (Orange et al., 2001). To remove soil debris, lichen thalli were rinsed in flowing tap water. The air dried samples were placed in a shaded area and ground into powder with a blender before being analyzed

Figure 1 A. Thallus of *Parmotrema austrosinense* B. Medulla KC +ive red
C. TLC plate showing Lecanoric acid labeled 'D" and Atranorin labelled 'E'

The representative sample was authenticated by NBRI, Lucknow. Phytochemical composition of the sample was done using the solvent extracts recovered from the soxhlet extraction method. A standard procedure was used to examine the proximate chemical composition of *Parmotrema austrosinense* such as sugars Gaikwad et al. (2014), proteins (Anupama et al. (2017), carotenoids (Parizadeh and Garampalli 2018), chlorophyll (Wakefield and Bhattacharjee, 2011), and fat contents (Anupama et al. 2017) .

Quantification of fat content

A standard method described by Anupama et al. (2017) was employed in the present study for the estimation of crude fat content. Fat content was extracted from the lichen sample using petroleum ether (P-Et). Five grams of dried material were added to the thimble and stored in the Soxhlet

apparatus's body tube. The extractor was filled with the solvent P-Et and attached to the apparatus for the soxhlet extraction. It was then slowly heated to 70°C for 15 reflexes. After transferring the extract to a Petri dish (W1 initial weight) and drying it in a dessicator. Following drying, the final weight W2 was determined. The lipid content has been computed using the formula computed by Yashoda Kambar et al. (2014).

$$\text{Fat content \%} = \frac{\text{(W2) Final weight} - \text{(W1) Initial Weight}}{\text{(W2) Final Weight}} \times 100$$

Quantification of protein

Microkjeldhal method was used for the estimation of protein present in the lichen sample. About 10 gms of lichen sample was taken in a round bottom flask containing concentrated sulphuric acid and potassium sulphate and copper sulphate. The mixture was acid digested by heating in a Hood until the colour of the solution turns to colourless. The digest was distilled in a distillation apparatus by adding 10 mL of the mixture to 10 mL of sodium hydroxide (40%). The solution was heated to trap nitrogen in a 10 mL of boric acid (2%). The trapped nitrogen was titrated against sulphuric acid (0.1 N). The amount of nitrogen was calculated by the following formula

$$\text{N (\%)} = \frac{\text{The volume of titrate} \times \text{Normality of sulphuric acid} \times \text{Sample dilution after the digest} \times 100}{\text{Volume of the sample used for distillation} \times \text{Sample weight}}$$

Quantification of sugars

About 100 mg of lichen sample was transferred to a test tube containing 5 mL of 2.5 N-HCl. The setup was left in a boiling H_2O for 3 hours. The reactant was neutralized by adding a pinch of sodium carbonate salt. The reactant was made up to 100 mL and centrifuged. To prepare the working standard, five test tubes were filled with 0.2, 0.4, 0.6, 0.8, and 1 mL of sugar. About 0.1 and 0.2 mL of sample solution were added to the test tubes labeled "Sample 1 and 2, respectively." Each tube was made up to 1 mL using distilled water. A separate test tube was filled with 1 mL of distilled water and labeled "blank." All of the tubes were added with approximately 1 mL of phenol solution. It was followed by adding 5 mL of 96% sulfuric acid. After 10 minutes, all tubes were placed in a boiling water bath at 25-30 °C for 20 minutes. Color was developed and measured at 490 nm. The sample's total sugar content was determined using the standard graph (Gaikwad et al. 2014).

$$\text{The test sample contains mg/gm dwt} = \frac{\text{Glucose in mg}}{100\mu\text{L}} \times 100$$

Quantification of carotenoid

Parizadeh and Garampalli (2018) procedure was used to measure the concentration of total carotenoids in the thallus of *P.austrosinense*. About 20 milliliters of acetone were used to homogenize one gram of lichen, and the supernatant was then decanted. Until a colorless solution was obtained, this process was repeated. Following filtering, 30 mL of acetone were used to wash each solution, and 60 mL of petroleum ether was used to dissolve it

once it had evaporated. Petroleum ether was used to filter and dilute this solution to a volume of 100 milliliters. The absorbance at 475 nm was measured after mixing two milliliters of this solution with eight milliliters of petroleum ether. Using a calibration curve for β-carotene, the TCC (total carotenoid content) of every sample was determined.

Quantification of Chlorophyll

The subsamples of about 0.02 gm dry weight were left in 10 milliliters of dimethyl sulfoxide (DMSO) overnight. A spectrophotometer was then used to detect the optical densities (Absorbance) at 645 and 665 nm. Using the formula proposed by Wakefield and Bhattacharjee, (2011), the chlorophyll content was computed.

$$\text{Chlorophyll content in mg} = \frac{(A_{645} - A_{665}) \times 10\ \text{mL}}{10^3\ \text{mL} \times 0.02\text{g of sample used}}$$

All tests were conducted in three replicates. Data are presented as mean ± standard error. IBM SPSS was employed to prepare the graphical image.

Results and Discussion

The findings of the following tests validated the *Pamortema asutrosinese* species (Fig 1A). The spot test revealed that the cortex was K positive yellow, while the medulla was KC and C positive red (Fig 1B). TLC analysis revealed that lecanoric acid was detected in Rf class 3 and atranorin was detected in Rf class 7 (Fig 1C). Soredia were discovered on the gray-colored thallus after being examined under a microscope. Rhizines were spreading across thallus on the basal surface.

The proximate composition of lichen extracts of *P. austrosinense* had the highest total carbohydrates content (4.0 ± 0.5 mg/gm sample) (Figure 2). The result was further validated by comparing the present study values to those in a prior article for the same assay. The results are in agreement to the report given by Gaikwad et al. (2014). who reported the total carbohydrate content in the methanol extract of *P.asutrosinense* was 4.57±0.31 mg/gm.

The fat content of the extract in the present study was found to be 1.4 ± 0.34 %. The results of the present study are found to be comparable to the report given by Anupama et al. (2017) who reported the fat content of *P.tinctorum* was 1.28%. The lichen extract of *P.austrosinense* had shown the highest carotenoid content (Fig 2). The present study had shown that the carotenoid content of *P.austrosinense* was 1.1±0.1 mg/gm. The results are comparable to the work conducted by Parizadeh and Garampalli (2018) who reported that the B carotene content in the *P.tinctorum* was 1.82 mg/gm while the *Ramalena celestri* was 11 mg/gm of the solvent extract.

P. austrosinense produced a high amount of chlorophyll (3.00±0.5 mg/gm) in the solvent extract tested (Fig 2). Differences in the activity of the standards employed in each antioxidant assay were compared to previously published research. The results are not in agreement with the findings of Wakefield and Bhattacharjee (2011) who reported that the chlorophyll content of *Ramalina stenospora* was 7.4 mg/gm at D'Arbonne NWR h and 14.23 mg/gm at Restoration Park, Louisiana. The discrepancy in phytochemical composition of *P.austrosinense* is most likely related to variations in abiotic and biotic factors such as habitats, lichen age, solvent employed for extraction, and lichen material provenance.

The estimation of protein in the *P.asustrosinese* solvent extract had shown the highest total protein content (18.6±0.5%) (Fig 3). Similar work conducted by Yashoda Kambar et al. (2015). reported that the amount of protein content was 11.3% in *Parmotrema tinctorum*. The lichen extracts of *P.austrosinense* had the high nitrogen content (3.0

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± 0.5) (Fig 3). The results of the present study are found to be comparable to the report given by Anupama et al. (2017).

The results of crude fat content in the solvent extract of *Parmotrema austrosinense* were found to be 1.4±0.34 % in the present study. The results are comparable to the earlier reports. Anupama et al. (2017) had reported that the total fat content in the *P.tinctorum* was 1.28±0.152 %.

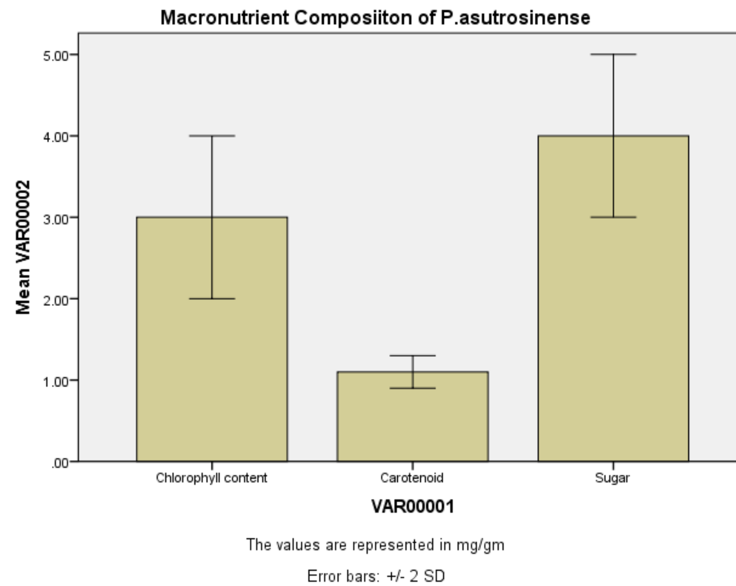

Fig 2: Chlorophyll, Carotenoid and Sugar composition of *P.austrosinense* thallus

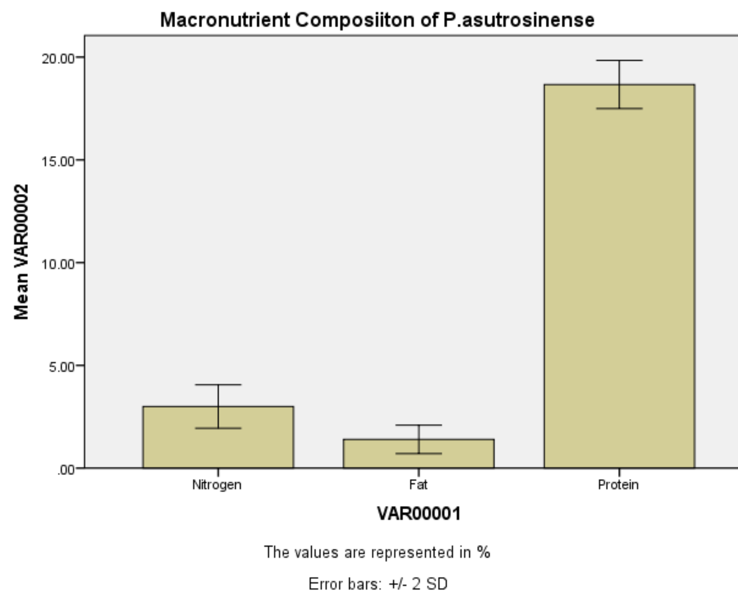

Fig 3: Nitrogen, Fat and Protien composition in the thallus of *P.austrosinense*

Conclusion

The current research has proved that the lichen species *Parmotrema austrosinense* is rich in macronutrient elements. The research done on the lichen *Parmotrema austrosinense* is just to prove its nutraceutical significance. Based on the current research, it can be concluded that the lichen needs further research to prove that it has medicinal value.

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References

Anupama T V, Sheela K B and Saji G (2017). Phytochemical screening and proximate composition of lichen Parmotrema tinctorum (Nyl.) Hale (Parmeliaceae) from Wayanad, Kerala. *International Journal of Chemical Studies*, *5*(5), 1003-1007.

DuBois M, Gilles K A, Hamilton J K, Rebers P T and Smith F (1956). Colorimetric method for determination of sugars and related substances. *Analytical chemistry*, *28*(3), 350-356.

Gaikwad S, Verma N, Sharma B O and Behera B C (2014). Growth promoting effects of some lichen metabolites on probiotic bacteria. *Journal of food science and technology*, *51*, 2624-2631.

Orange A, James P W and White F J (2001). *Microchemical methods for the identification of lichens*. Twayne Publishers. pp.1-101.

Parizadeh H, and Garampalli R H (2018). Chemical composition and correlation study on phytochemicals of some lichen species. *International Journal of Pharmacogency*, *5*, 167-176.

Saklani A, and Upreti D K (1992). Folk uses of some lichens in Sikkim. *Journal of ethnopharmacology*, *37*(3), 229-233.

Sinha G P, Nayaka S and Joseph S (2018). Additions to the checklist of Indian lichens after 2010. *Cryptogam biodiversity and assessment special*, *197*, 206.

Upreti D K, Divakar P K and Nayaka S (2005). Commercial and ethnic use of lichens in India. *Economic botany*, *59*(3), 269-273.

Wakefield J M and Bhattacharjee J (2011). Effect of air pollution on chlorophyll content and lichen morphology in northeastern Louisiana. *Evansia*, *29*(4), 104-114.

Walker F J and James P W (1980). A revised guide to microchemical techniques for the identification of lichen products. British Lichen Society. pp.1-17.

Wolseley P (2008). A Compendium of the Macrolichens from India, Nepal and Sri Lanka. By Dharani Dhar Awasthi. 2007. 580 pages. Hardback 24× 17× 5 cms. ISBN 81 21 106009. Published by Bishen Singh Mahendra Pal Singh, Dehra Dun, India. Available on line at the special price of $96.30 from http://www. bagchee. com. *The Lichenologist*, *40*(3), 267-268.

Yashoda Kambar V, Manasa M, Prashith Kekuda T R and Ramesh Kumar K A (2014). Proximate and elemental analysis of Ramalina conduplicans Vain.(Ramalinaceae) and Parmotrema tinctorum (Nyl.) Hale (Parmeliaceae). *Journal of Chemical and Pharmaceutical Research*, *6*(6), 2668-2674.