

Evaluation of Qualitative and Quantitative Phytochemical Constituents of *Dermatocarpon vellereum* Zschacke

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www.jrasb.com || Vol. 5 No. 4 (2026): August Issue

Received: 10-08-2026

Revised: 24-08-2026

Accepted: 30-08-2026

ABSTRACT

This study examines the qualitative and quantitative phytochemical composition of *Dermatocarpon vellereum* zschacke, a saxicolous lichen from the Melghat forest in India. Compounds were extracted using various solvents and analysed with standard phytochemical methods. Ethanolic extracts contained a wide range of metabolites, including alkaloids, phenolic compounds, tannins, carbohydrates, amino acids, reducing sugars, cardiac glycosides, flavonoids, and coumarins. Petroleum ether, chloroform, and water extracts showed fewer compound classes. Saponins, proteins, and resins were absent in all extracts. Ethanol was the most effective solvent for extracting a range of phytochemicals. Quantitative analysis found 0.03% alkaloids, 2.32 mg QE/g flavonoids, 4.08 mg GAE/g total phenolics, 0.20% tannins, and undetectable saponins. These results suggest *D. vellereum* has potential economic and medicinal value and highlight the need to conserve its natural habitat. This research lays the groundwork for future studies on the bioactive constituents of this understudied lichen.

Keywords- *Dermatocarpon vellereum*, lichen metabolites, phytochemical analysis, qualitative and quantitative constituents.

I. INTRODUCTION

Lichens represent a unique group of organisms with significant ecological and biological importance [1]. They are often the first life forms to colonize newly exposed rock surfaces and are distributed globally [2]. Although lichens are widespread, they are frequently overlooked. Structures that have remained undisturbed for extended periods are commonly colonized by lichens [3]. Lichens are stable, self-supporting symbiotic associations between fungi, most commonly Ascomycota, and photobionts [4]. They serve as primary colonizers in stressful habitats and are major structural components in extremely cold and arid environments. Consequently, lichens are prevalent in the Arctic and Antarctic, and occur as crusts on desert soils [5]. Lichens can be broadly classified based on their substrate: corticolous (growing on tree bark), foliose (on leaf surfaces), saxicolous (on rocks), terricolous (on soil), and muscicolous (on mosses)

[6]. Lichen secondary metabolites possess considerable pharmaceutical value due to their diverse biological activities, including aromatic compounds, amino acid derivatives, dibenzofurans, depsides, and depsidones. The content of these secondary metabolites can reach up to 30% of the dry weight of lichen thalli. To date, over a thousand lichen compounds have been extracted globally for research and are extensively utilised in the pharmaceutical industry and related fields [7].

Lichens have been used from ancient times for medical and edible purposes [8]. Lichens produce a wide array of secondary metabolites, which are extracellular in nature. These metabolites may be in an amorphous or crystalline state. Lichen secondary metabolites are low-molecular-weight aliphatic or aromatic substances [9].

Lichen secondary metabolites are called lichen acids or lichen substances / metabolites. In general, the quantity of lichen secondary metabolites varies up to 10% of the dry weight of whole lichen thalli in most of the

lichen species, and it may reach 30% in some species [10]. Lichen substances are found deposited either in the cortex or in the medulla as crystals or on the outer surface [11]. The commonest cortical compounds are Usnic acid and Atranorin. Anthraquinones and Pulvinic acid derivatives are found in the cortex to a lesser degree. The majority of lichen substances are deposited in the medulla [12,13,14]. It has now been recognised by many lichenologists that some cortical substances are correlated with higher taxonomic ranks. Variations in medullary constituents are used mainly as discriminators at the species level. Most morphological species have a constant chemistry, regardless of their geographical location, substrate or ecology [15,16].

Lichens are probably the earliest colonizers of terrestrial habitats on Earth, with fossil records back to 400–600 million years ago. These fossils indicate that fungi developed symbiotic partnerships with photoautotrophs before the evolution of vascular plants [17]. These lichens produce more than 800 unique secondary metabolites, which are adapted to harsh living conditions [18,17]. Based on their chemical structures, most lichen substances are active against scabies, stomach disorders and many blood disorders [19]. Most of the secondary metabolites present in lichens are produced through the Polyketide Pathway and consist mainly of monocyclic phenols, bicyclic phenols joined by an Ester Bond (depside), both ester and Ether Bonds (depsidones), and Furan Heterocycle [20,21]. It was reported that 18 lichen species were found on an acrylic-coated aluminium roof. Most lichens are terrestrial, but a few species occur in freshwater streams and others in marine intertidal zones [22,23]. Lichens can survive in extreme environmental conditions; they adapt to extreme temperatures, drought, inundation, salinity, high concentrations of air pollutants, and nutrient-poor, highly nitrified environments, and they are the first colonizers of terrestrial habitats [24].

In addition, both fungal and algal cells in the lichen thallus are known for their ability to survive in space. Interactions between the symbiotic partners partially explain this spectacular success of lichens in unusual environments [25].

The presence of secondary metabolites in cultured mycobionts and their absence in algae shows that secondary products of lichens are generally formed by the fungus [26]. Exceptions of strains of lichen-associated Cyanobacteria, which may produce Nostocides, Oligopeptides such as Microcystins or derivatives of amino acids. According to phylogenetic studies, the capacity of ascomycetous fungi to produce diverse and chemically complex compounds appears to have evolved during a major evolutionary radiation that gave rise to several major groups [27]. Differences in the presence of certain classes of substances are sometimes observed among related lichen groups [28]. For example, *Xanthoparmelia* and *Neofuscelia*, two closely related genera of the Parmeliaceae, differ primarily in the pigments of their upper cortex: *Xanthoparmelia* contains

crystallised Usnic acid, whereas the closely related *Neofuscelia* is characterised by melanin-like cortical pigments [29].

Structurally, closely related metabolites often have essentially different biological actions [30]. It was also reported that Barbatic Acid and diffractaic acids, which differ by only one functional unit, exhibit divergent biological effects. Barbatic acid strongly inhibits the growth of *Trebouxia jamesii* and slows down the mitosis rate of the algae at a concentration comparable to the quantity found in the lichen thallus. It can cause cell death in higher concentrations. At the same time, diffractaic acid has no effect on algal growth at all. On the basis of this result, barbatic acid may regulate the growth and mitosis in the lichen thalli [31].

TLC has been used to study specific groups of lichen products. Different authors used different solvent systems and chromatography conditions, making it impossible to compare their results. This problem was solved when a standardised method was developed [32]. In 2014, In Vitro, In Silico and In Vivo Studies on the Biochemical Applications of the Foliose Lichen *Dermatocarpon vellereum* were conducted for clinical debilities; Anti-Bacterial, Anti-inflammatory, Analgesic, Antipyretic, Antiviral and Cytotoxic Effects were tested [33]. They introduced Rf classes that depend only on the relative order of spots and are more reliably constant. This standardised method has been used for routine analyses of lichen products in chemotaxonomic and phytochemical studies, with various updates over time [32].

II. MATERIAL AND METHODS

Collection

The collection of *Dermatocarpon vellereum* was made at Gawilgarh fort in the Melghat forest area, Chikhaldara Tehsil, Amravati.

Identification

The collected *Dermatocarpon vellereum* material was morphologically identified using standard floras and other relevant literature [34,35] and authenticated by experts of our region.

Preparation of powder

After collection, the *Dermatocarpon vellereum* material was washed with tap water, followed by semi-hot water to remove dust and impurities. The cleaned material was shade-dried for one week, then placed in an oven at 45°C for 4 hours to ensure complete dehydration. The dried material was ground into powder using an electric grinder and stored in airtight plastic bags for subsequent experiments.

Cold maceration extraction

Fifteen grams of powdered *Dermatocarpon vellereum* were macerated in 150 ml of each solvent (aqueous, methanol, chloroform, ethyl acetate, and hexane) for 2 to 7 days. The mixtures were filtered through porous fabric, and the process was repeated twice. Extracts were concentrated to remove residual

solvents, then evaporated on a water bath to dryness. The resulting extracts were stored at 4°C. On the day of analysis, crude extracts were dissolved in distilled water to prepare stock solutions and specific dilutions for further evaluation.

III. RESULTS AND DISCUSSION

Morphology

The thallus is foliose and monophyllous, with several irregularly expanding lobes and occasional small secondary lobes at the margins. Lobes are 1-7 cm broad, flat, slightly ripened, and range from light to dark reddish-brown. Both the upper and lower cortex have similar parenchyma thickness. The upper surface is rough and flat, while the lower side is black. Perithecia are immersed and pale, with only the tips appearing brownish. The thallus is densely rhizinate with thick rhizines.

Dermatocarpon vellereum is a saxicolous lichen that attaches to rocks and accumulates nutrients and minerals from its substrate, influencing its metabolite composition. Research on this lichen is particularly valuable due to its unique ecological adaptation [41]. Consequently, studies of the *Dermatocarpon* thallus have emphasised phytochemical analysis and evaluation, highlighting potential economic applications.

Investigation of *Dermatocarpon vellereum* provides a foundation for future research.

Quantitative phytochemical analysis

Qualitative phytochemical analysis was carried out for the ethanol, petroleum ether, chloroform and aqueous extracts of *Dermatocarpon vellereum* Zschacke using standard methods [36-40]. The extracts were subjected to specific qualitative test for various classes of secondary metabolites. Quantitative analysis of some selected phytochemicals such as flavonoids, alkaloids, saponins and phenolic compounds were carried out using appropriate precipitation and spectrophotometric methods. All quantitative estimations were performed in triplicate to ensure reliability and reproducibility.

Table 1 showed considerable variation in the phytochemical constituents of the different extracts. In more than one extract were found alkaloids, phenolic compounds, carbohydrates and flavonoids, while tannins, amino acids, reducing sugars, coumarins and cardiac glycosides had solvent specific distribution. Petroleum ether, chloroform and aqueous extracts contained steroids/terpenoids but saponins, proteins and resins were absent. Overall results indicate that the polarity and extraction properties of solvents strongly affect the phytochemical composition of *D. vellereum*.

Table No. 1. Qualitative phytochemical analysis of *Dermatocarpon vellereum* in different Solvents

Phytochemical constituents	Test	Solvents			
		ET	PE	CHL	AQ
Alkaloids	Mayer's	-	-	-	-
	Dragendroff's	+	-	+	+
Saponins	Foam Test	-	-	-	-
Phenolic compounds	Lead acetate	+	-	+	+
Tannin	Gelatin test	+	-	-	-
Carbohydrate	Molisch's	+	-	-	+
Amino acids	Ninhydrine	+	-	-	-
Reducing sugars	Fehling's test	+	-	-	-
Cardiac Glycosides	Keller- Killani	-	+	-	+
Flavonoids	Alkaline reagent	+	-	+	+
Protein	Biuret test	-	-	-	-
Coumarins	NaOH test	+	-	-	-
Resins	Acetic anhydride	-	-	-	-
Steroids/Terpenoids	Salkowski's	-	+	+	+

+ : Present ; - Absent
ET: Ethanol; PE: Petroleum ether; CHL: Chloroform; AQ : Aqueous

Quantitative phytochemical analysis

Based on the quantitative phytochemical analysis of *Dermatocarpon vellereum* it was found that total phenolics showed highest content (4.08 mg GAE/g) followed by total flavonoids (2.32 mg QE/g) and total tannins (0.20% w/w). Total alkaloids were

found in low quantity (0.03% w/w) whereas saponins were not detected (0.00% w/w). Overall, the results indicate a considerable abundance of phenolic and flavonoid compounds in *D. vellereum* as shown in table 2.

Table No. 2. Quantitative phytochemical analysis of different phytochemicals in *Dermatocarpon vellereum*.

Compound	Method	Unit	<i>Dermatocarpon vellereum</i>
Total Alkaloids	Acid-Base Titration	% w/w	0.03
Total Flavonoids	AlCl3 Colorimetry	mg QE/g	2.32
Total Phenolics	Folin-Ciocalteu	mg GAE/g	4.08
Total Tannins	Vanillin-HCl Method	% w/w	0.20
Total Saponins	Gravimetric Method	% w/w	0.00

IV. CONCLUSION

This study establishes a comprehensive baseline for the phytochemical and ethnobotanical profile of *Dermatocarpon vellereum* from the Melghat forest area, Chikhaldara Tehsil, Amravati, at Gawilgarh fort. Key secondary metabolites were identified, and ethanol was the most effective solvent for extracting a wide range of chemical constituents. The documentation of traditional knowledge highlights the cultural significance of this species for therapeutic uses, including antioxidant, anti-inflammatory, and anticancer properties. These findings emphasise the importance of lichens in the local pharmacopoeia. Conservation of these underexplored regions is essential to preserve biodiversity and indigenous knowledge. Further research using advanced quantitative techniques such as HPTLC, GC-MS, and NMR is recommended to identify the specific bioactive molecules responsible for the observed medicinal effects.

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