

Research Article**Phytochemical investigation and antioxidant activity of lichen species *Pseudevernia furfuracea* (L) Zopf.****Sharad V. Bhandarkar, Deepak S. Mohale, Anil V. Chandewar, Nitin I. Kochar***P. Wadhvani College of Pharmacy, Yawatal, Maharashtra, India*

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Abstract

This study explores the phytochemical composition, *Pseudevernia furfuracea* (L) Zopf. thin-layer chromatography (TLC) profiles, and antioxidant activity of acetone. Phytochemical analysis reveals that acetone is the most versatile solvent, extracting a wide range of compounds including alkaloids, saponins, glycosides, flavonoids, triterpenes, and steroids. Methanol also extracts multiple phytochemicals but is less comprehensive than acetone. Petroleum ether predominantly extracts alkaloids and glycosides, while chloroform selectively extracts proteins and tannins. Carbohydrates were not detected in any of the extracts. TLC analysis indicates that acetone extracts show the highest mobility of compounds with an Rf value of 0.64, reflecting a strong affinity for the mobile phase. Methanol extracts have the highest Rf value of 0.78, demonstrating the greatest mobility, whereas petroleum ether and chloroform extracts exhibit lower Rf values of 0.54 and 0.71, respectively. Antioxidant activity By DPPH method

Keywords: *Pseudevernia furfuracea*, phytochemical analysis, thin-layer chromatography, antioxidant, lichen

Introduction

Natural products have long held a pivotal role in microbiological research due to their unique and intricate molecular architectures. Over the course of their evolutionary history, natural products have developed traits closely tied to biological processes in both animal and plant organisms. These substances are renowned for their complex structures, which often include unusual functional group configurations, strained ring systems, and other distinctive characteristics that make them highly appealing for scientific exploration (Newman and Cragg, 2016; Harvey, 2017).

The significance of natural products extends beyond their structural complexity. There are several key reasons for the sustained interest in natural product chemistry. First and foremost, natural products serve as invaluable lead compounds in the development of new medications. Their unique molecular frameworks often provide a starting point for the synthesis of novel drugs (Butler, 2017; Chauhan et al., 2018). Additionally, studying natural products offers insights into potential

biological mechanisms, shedding light on the molecular origins and underlying causes of various disorders. Moreover, the isolation and characterization of natural products have driven the advancement of cutting-edge analytical and spectroscopic techniques. Methods such as High-Performance Thin-Layer Chromatography (HPTLC), Nuclear Magnetic Resonance (NMR) spectroscopy, and mass spectrometry have been refined and developed in response to the challenges posed by the study of these complex molecules (Mishra et al., 2017; Mishra et al., 2019). Furthermore, the total synthesis of natural products presents an ongoing challenge that continues to inspire the discovery of new reagents and chemical reactions. Herbal medicine, which has been used for centuries as both food and medicine, remains an area of significant interest. Humans have long relied on medicinal plants to treat various ailments, and the availability of indigenous botanical flora has greatly influenced the development and use of herbal products. The appeal of herbal medicine lies in its affordability, accessibility, and ease of administration. Research has demonstrated that many herbal plants contain a diverse array of chemical constituents, some of which have been shown to inhibit bacterial growth and treat a variety of harmful diseases. The utilization of antimicrobial compounds derived

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from natural vegetation holds the potential to impact pollution control and the management of microbial infections in plants, humans, and animals.

The quest for new therapeutic compounds is an ongoing and urgent endeavor. Despite the vast array of current medications, the need for the discovery and development of new agents remains critical. It is estimated that effective treatments are available for only about one-third of all known human diseases, including cancer, AIDS, autoimmune disorders, and other serious conditions. In developing nations, the enhancement of herbal medicine serves not only to preserve ancient traditions but also as an alternative solution to pressing health concerns. The growing resistance of pathogens to many commonly used antibiotics has intensified the search for novel antimicrobial compounds (Azaizeh et al., 2003; Singh et al., 2018; Mishra et al., 2017). These new agents are essential to combat infections and address the challenges posed by antibiotic resistance and the side effects associated with currently available antimicrobial drugs (Adriana et al., 2015; Bauer et al., 1996; Bisht et al., 2014).

Material and methods

Collection and authentication of plant material

Specimens of *Pseudevernia furfuracea* L Zopf were collected from Wild life Sanctuary in Gandia District, Maharashtra, India in February 2023. This location, known for its rich biodiversity, provided an ideal environment for sourcing the lichen. The collected specimens were carefully preserved and sent to the CSIR-National Botanical Research Institute in Lucknow, Uttar Pradesh, for authentication. The institute, a recognized authority under the Council of Scientific and Industrial Research (CSIR), conducted a thorough examination of the samples. Upon verification, the voucher specimen numbers PDSH/LWG/Authentication/2023/24/03 and PDSH/LWG/Auth

entication/2023-24/14, both dated 29/05/2023, were assigned and duly deposited at the institute for future reference and further scientific study.

Preparation of plant extract

For the extraction of *Pseudevernia furfuracea* L Zopf sixteen grams of air-dried thallus, including both vegetative and fruiting parts, were initially washed thoroughly with tap water followed by distilled water to remove any impurities. After washing, the thalli were carefully pat-dried. The dried thalli, weighing 16.04 grams, were subjected to successive solvent extraction using a Soxhlet apparatus. The first extraction was carried out using 500 mL of petroleum ether (60-80°C) for 25 to 26 cycles. Following this, the remaining plant material was dried and extracted sequentially with chloroform, acetone, and methanol, each solvent being used until exhaustive extraction was achieved. Finally, water was added to the remaining plant material, and it was macerated for three days to extract any remaining compounds. After the extraction process, the solvent from each extraction step was filtered through Whatman No. 1 filter paper. The crude extract was then obtained by evaporating the solvents under reduced pressure using a rotary evaporator. The final yield of the dried crude extract was 15.04 grams. Per cent yield of crude extract was calculated according to the equation below:

Per cent yield (%) = (Dry weight of extract/Dry weight of sample) X 100.

$$= 15.04/16.04 = 93.76$$

Preliminary phytochemical analysis of lichens extract

The extract of *Pseudevernia furfuracea* L Zopf under went a comprehensive qualitative phytochemical analysis to

Table 1: Preliminary Phytochemical Screening

Test	Procedure	Observation	Result
Test for Alkaloids (Dragendorff's Test)	2 mL crude extract added to 1% HCl, steamed for 10 minutes, then 6 drops of Dragendorff's reagent added.	Reddish-brown precipitate formed.	Indicates the presence of alkaloids.
Test for Carbohydrates	2 mL crude extract mixed with 2 mL Benedict's reagent and boiled.	No reddish-brown precipitate formed.	Indicates the absence of carbohydrates.
Test for Glycosides	2 mL crude extract mixed with 2 mL glacial acetic acid containing 1-2 drops of 2% FeCl ₃ solution, then poured into another test tube containing H ₂ SO ₄ .	Brown ring at the interphase.	Presence of cardiac glycosides.
Test for Tannins	2 mL crude extract mixed with a few drops of 5% ferric chloride solution.	No formation of blue colour.	Absence of hydrolysable tannins.
Test for Triterpenoids	2 mL crude extract shaken with 1 mL chloroform, then a few drops of concentrated sulphuric acid added along the side of the test tube (Salkowski test).	Reddish-brown colour formed.	Positive for triterpenoids.
Test for Steroids	2 mL crude extract added to 2 mL acetic anhydride, and then a few drops of concentrated H ₂ SO ₄ added (Liebermann-Burchard reaction).	Blue-green ring formed.	Steroids are present.
Test for Flavonoids	2 mL crude extract added to 2 mL of 10% NaOH solution.	Yellow to orange colour developed.	Indicates the presence of flavonoids.
Test for Proteins	2 mL crude extract added to 2 mL HNO ₃ , then boiled in a water bath (Xanthoproteic test).	No orange colour observed.	Indicates the absence of proteins.
Test for Saponins (Frothing Test)	2 mL crude extract mixed with 5 mL distilled water, shaken vigorously.	Stable foam formation observed.	Indicates the presence of saponins.

identify the presence of various bioactive compounds. This screening aimed to detect key phytochemicals, including alkaloids, tannins, saponins, flavonoids, triterpenoids, steroids, glycosides, proteins, and carbohydrates. Each of these classes of compounds is known for its potential biological activity and therapeutic value.

The analysis was conducted using standard and widely accepted methodologies. These methods involve specific chemical tests that react with the compounds present in the extract, producing observable changes, such as color shifts or precipitate formation, which indicate the presence of the targeted phytochemicals (Evans and Trease, 2009; Handa et al., 2017; Kokate et al., 2018; Rashmi and Rajkumar, 2014). The successful identification of these bioactive compounds highlights the potential pharmacological significance of *Pseudevernia furfuracea* L Zopf and provides a foundation for further research into its medicinal properties.

Thin Layer Chromatography of *Pseudevernia furfuracea* L Zopf Extract

Begin by preparing the TLC plate, ensuring it is clean and activated by briefly heating it in an oven. Dissolve a small amount of *Pseudevernia furfuracea* L Zopf extract and usnic acid standard in methanol to create concentrated solutions. Using a capillary tube or micropipette, spot both the extract and standard onto the baseline of the TLC plate.

Prepare the mobile phase by mixing toluene, ethyl acetate, and formic acid in a 6:3:1 ratio, and pour it into the TLC development chamber (Rao and Rao, 2015). Place the TLC plate in the chamber, making sure the baseline stays above the solvent level. Allow the solvent to rise up the plate by capillary action, carrying the compounds with it. Once the solvent front nears the top, remove and mark the plate. After drying, visualize the developed plate under UV light or with a staining reagent to reveal the spots. Calculate the retention factor (Rf) values by comparing the distances travelled by the spots relative to the solvent front. Match the Rf value and spot appearance of the

Usnea orientalis extract with the usnic acid standard to confirm the presence of usnic acid.

Results and discussion

Preliminary phytochemical analysis

The table provides an overview of the phytochemical composition of *Pseudevernia furfuracea* (L) Zopf extracts obtained using various solvents, including acetone, petroleum ether, chloroform, and methanol. It details the presence or absence of specific phytochemicals in each extract. Tannins are absent in both acetone and petroleum ether extracts but are present in chloroform and methanol extracts, indicating their higher solubility in polar solvents. Alkaloids are found in the acetone, petroleum ether, and methanol extracts but are absent in the chloroform extract, suggesting that these compounds have varying solubility across polar and non-polar solvents. Saponins appear exclusively in the acetone extract, indicating their preference for acetone as a solvent. Glycosides are present in all extracts, reflecting their solubility in a wide range of solvents and their diverse chemical nature. Flavonoids are found only in the acetone extract, showing that acetone is particularly effective in extracting these compounds. Proteins are detected solely in the chloroform extract, suggesting that chloroform is selective for proteinaceous compounds. Triterpenes, similar to flavonoids, are present only in the acetone extract, reinforcing acetone's suitability for extracting these compounds. Carbohydrates are absent in all extracts, indicating that the solvents used may not be appropriate for their extraction. Steroids are found in both acetone and methanol extracts, but not in the petroleum ether or chloroform extracts, suggesting their solubility in moderately polar solvents.

Overall, this phytochemical analysis underscores the differential solubility of bioactive compounds in *Pseudevernia furfuracea* L Zopf based on the solvent used. Acetone proves to be the most versatile solvent,

Table 2: Phytochemical studies of *Pseudevernia furfuracea* L Zopf extracts

Phytochemicals	Acetone Extract	Petroleum Ether	Chloroform	Methanol
Tannins	-	-	+	+
Alkaloids	+	-	-	-
Saponins	+	-	-	-
Glycosides	+	+	+	+
Flavonoids	+	-	-	-
Proteins	-	-	+	-
Triterpenes	+	-	-	-
Carbohydrates	-	-	-	-
Steroids	+	-	-	+

+ Indicates Presence, - Indicates Absence

extracting a wide array of phytochemicals such as alkaloids, saponins, glycosides, flavonoids, triterpenes, and steroids. In contrast, petroleum ether is less effective, primarily extracting alkaloids and glycosides. Chloroform and methanol extracts are rich in tannins and glycosides, with chloroform also selectively extracting proteins.

TLC studies of *Pseudevernia furfuracea* L Zopf extracts

The retention factor (Rf) values for the different extracts are determined by dividing the distance travelled by the compound by the distance traveled by the solvent front, which is 5.7.

The results reveal that the acetone extract has an Rf value of 0.64, indicating that its compounds exhibit relatively high mobility in the TLC system. This suggests that acetone is an effective solvent for extracting *Pseudevernia furfuracea* L Zopf components, as these compounds have a strong affinity for the mobile phase. In contrast, the petroleum ether extract has an Rf value of 0.54, which is lower, signifying reduced mobility of its compounds

Table 3: TLC results for *Pseudevernia furfuracea* L Zopf extracts

Extracts	Distance Travelled by Compound (cm)	Distance Travelled by Solvent Front (cm)	Rf Value
Acetone Extract	3.7	5.7	0.64
Petro. Ether Extract	3.1	5.7	0.54
Chloroform Extract	4.1	5.7	0.71
Methanol Extract	4.5	5.7	0.78

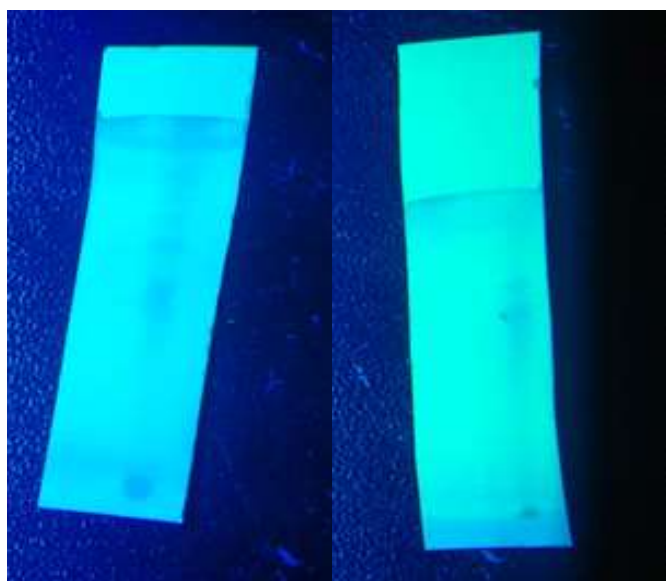


Figure 1: TLC plate of *Pseudevernia furfuracea* L Zopf (acetone extract)

compared to those in acetone. The chloroform extract shows an Rf value of 0.71, indicating better mobility than petroleum ether but still lower than acetone. The methanol extract, with the highest Rf value of 0.78, demonstrates the greatest mobility of its compounds among the solvents tested.

DPPH free radical scavenging activity

The results of free radical scavenging activity study demonstrate that the lichens extracts were exhibits significant antioxidant activity, as evidenced by its potent free radical scavenging ability in various assays. Lichens reported to present various flavonoids that may have

Table 4: Antioxidant Activity (DPPH) of Ascorbic Acid

Concentration	Sample (Std)	% Inhibition	IC 50
0	0.312	0	214.5162
50	0.282	9.62	
100	0.226	27.56	
150	0.198	36.54	
200	0.165	47.12	
250	0.124	60.26	
300	0.106	66.03	

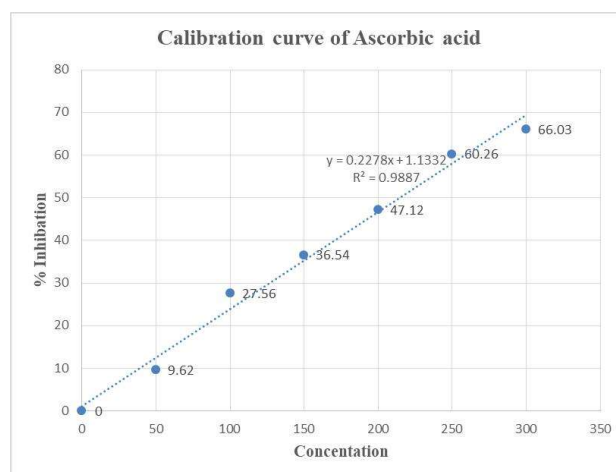


Figure 2: DPPH radical scavenging activity of Ascorbic acid

Table 5: Antioxidant Activity (DPPH) of *Pseudevernia furfuracea* L Zopf

Concentration	Sample (Std)	% Inhibition	IC 50
0	0.312	0	
50	0.291	6.73	
100	0.24	23.08	
150	0.199	36.22	257.13
200	0.182	41.67	
250	0.169	45.83	
300	0.139	55.45	

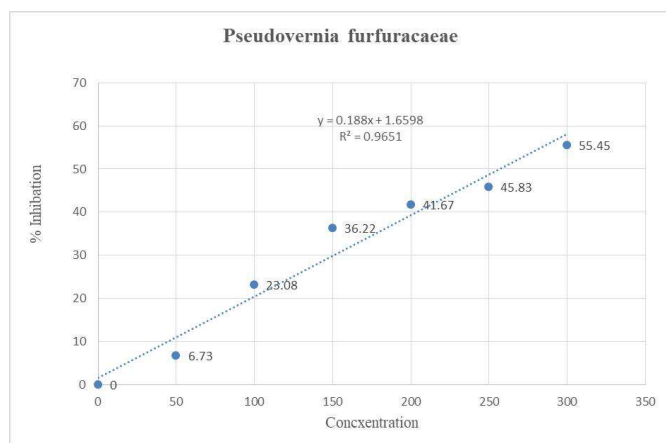


Figure 3: DPPH radical scavenging activity of *Pseudevernia furfuracea* L Zopf

antioxidant and antimicrobial potential in various reports. Results showed in table 4 and 5.

Discussion and Conclusion

The comprehensive analysis of *Pseudevernia furfuracea* L (Zopf) extracts reveals important insights into their phytochemical composition, TLC profiles, and antioxidant activity. The preliminary phytochemical analysis demonstrated that acetone is the most effective solvent for extracting a broad range of bioactive compounds, including alkaloids, saponins, glycosides, flavonoids, triterpenes, and steroids. In contrast, petroleum ether primarily extracts alkaloids and glycosides, while chloroform and methanol are effective for tannins and glycosides, with chloroform also selectively extracting proteins. The absence of carbohydrates in all extracts suggests that the solvents used may not be suitable for their extraction.

The TLC studies further support the efficacy of acetone, which showed a high Rf value of 0.64, indicating that its compounds have strong mobility and affinity for the mobile phase. Methanol, with the highest Rf value of 0.78, also demonstrated effective extraction properties, but acetone remains superior in terms of overall phytochemical extraction. The results of this study demonstrate that the lichens extract from exhibits significant antioxidant activity, as evidenced by its potent free radical scavenging ability in various assays. This suggests its potential to protect against oxidative stress and may contribute to the prevention of diseases associated with oxidative damage. Further research is necessary to elucidate the underlying mechanisms.

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