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# Phytochemical investigation of therapeutic important lichen: *Parmelia perlata*

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## ABSTRACT

*Phytochemical examination on the petroleum ether extract of lichen Parmelia perlata led to the isolation of tridecyl myristate **(I)**, 3-ketooleanane **(II)**, icosan-1-ol **(III)** and (+) usnic acid **(IV)**. Compounds **I-III** are isolated first time from this lichen. The structure of these compounds was elucidated on the basis of different spectroscopic techniques.*

**Key words:** *Parmelia perlata*, Lichen, Phytochemical, Biological activities, Symbiotic.

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## INTRODUCTION

A considerable number of species of lichen forming fungi have wide geographical distribution and are used in the traditional system of medicine. Lichens are symbiotic combination of algae and fungi. They are well known to produce a variety of compounds with remarkable biological activities [1-3]. The beautiful hills of Uttarakhand (In India) are the best source of lichens due to varied temperature difference in day and night. They have used in diarrhoea, dyspepsia, spermatorrhoea, ammenrrhoea, dysentery, and as wound healer [4]. Lichens are used as traditional food by Rai and Limbu communities of east Nepal [5]. Some of the species of the lichens are being used by cosmetic industries as skin lightening agent [6]. *Parmelia perlata (*lichen) belongs to family Parmeliaceae and commonly known as Charila in India. Many species of genus *parmelia* exhibited strong antimicrobial activity [7]. Diffractic acid [8] and caperatic acid [9] were isolated from various species of lichen *parmelia* and are well known for their analgesic [8], antipyretic [8] and antispasmodic activities [9] (*i.e.*). Another compound gyrophoric acid isolated from *Parmelia* species has potent inhibitor of the growth of human keratinocytes and also having beneficial effects against hyperproliferative skin disease such as psoriasis [8]. Usnic acid, the major constituent was isolated from various species of *Parmelia* exhibited antimitotic effects, antitumor and antimycobacetrial activities [8].

## MATERIALS AND METHODS

***General experimental procedures***

Melting points were determined in soft glass capillaries in an electrothermal melting point apparatus and are uncorrected. Qualitative and quantitative TLC were conducted on aluminium sheet Kieselgel 60 $F_{254}$ (E. Merck). Silica gel (E. Merck, 60-120 mesh, 550 gm) used for column (1.5 m × 4.0cm) chromatography. The IR spectra were recorded on FTIR SHIMADZU 8400S spectrometer with KBr pellets. The $^{1}H$ and $^{13}C$ NMR spectra were recorded in $CDCl_3$ at 300 MHz and 75 MHz on a Brucker NMR instrument, respectively, using TMS as internal standard. FAB mass spectra were recorded on JEOL SX 102 /DA-6000 mass spectrometer using Argon /Xenon as FAB gas.

***Plant material***

The plant material *Parmelia perlata* (lichen) was collected from the hills of Uttarakhand (India) and the authenticity of the lichen was confirmed by Mr. R. Singh, Incharge of Harberium, Department of Botany, University of Rajasthan, Jaipur.

***Extraction and Isolation of the Constituents***

The shade dried plant material (1.5 kg) was finely powdered and extracted with petroleum ether for 72 hrs on water bath. The extract was filtered hot and solvent was removed under reduced pressure where a semi-solid yellowish mass (7.0 gm) was obtained. The solvent free extract was chromatographed over silica gel column and gave four compounds **(I to IV)**. For this purpose a column of 1.5 m in height with 4.0 cm in diameter was used and it was charged with 550gm silica gel (60-120 mesh). The column was eluted with different solvents in order of increasing polarity where following compounds were isolated, purified and characterized.

**Tridecyl myristate (I)**

Compound **I** was obtained when column was eluted with petroleum ether and benzene in the ratio of 3 : 1. After removal of solvent, colorless solid was obtained. It showed single spot on TLC examination ($R_f$ = 0.86) in benzene and chloroform (7 : 3) as a solvent system. Melting point of this compound was found to be 63.5°C. IR (KBr, $cm^{-1}$): 2925, 2850 (C-H, stretching), 1730 (>C=O, str.), 1260 (C-O, str.), 715, 710 ; $^{1}H$ NMR (δ ppm, $CDCl_3$): 2.30 (*t*, 2H, *J=7.32, 7.50 Hz*, C-2, -$CH_2$COO-), 1.65 (*q*, 2H, C-3, -$CH_2$-), 4.1 (*t*, 2H, *J=6.75, 6.78 Hz*, C-1', -COO$CH_2$-), 1.55 (*q*, 2H, C-2', -$CH_2$-), 0.90 (*t*, 6H, *J=6.62, 6.93 Hz*, C-14 and C-13', -$CH_3$), 1.24-1.45 (*s*, 40H, C-4 to C-13 and C-3' to C-12'); $^{13}C$ NMR (δ ppm, $CDCl_3$): 175.20 (C-1), 70.10 (C-1'), 34.42 (C-2,), 31.93 (C-2'), 25.93 (C-3), 28.64 (C-3'), 32.11 (C-12), 22.50 (C-13), 14.13 (C-13'), 14.10 (C-14), 29.15-29.70 (C-4 to C-11 & C-4' to C-10'); MS (m/z): 410 ($M^+$) (calcd for $C_{27}H_{54}O_2$ ).

**3-ketooleanane (II)**

It was isolated as colorless solid when elution of column by petroleum ether with benzene in the ratio 1 : 1 and it showed single spot on TLC examination. Its $R_f$ value was observed at 0.63 (benzene as mobile phase). Melting point of this compound was found to be 235°C. IR (KBr, $cm^{-1}$): 1720 (>C=O, stretching), 2930, 2910 (C-H, stretching), 1440 (C=C str.), 1370; $^{1}H$ NMR (δ ppm, $CDCl_3$): 0.71 (*s*, 3H, C-26, -$CH_3$), 0.85 (*s*, 3H, C-25, -$CH_3$), 0.94 (*s*, 3H, C-28, -$CH_3$), 0.98 (*s*, 3H, C-30, -$CH_3$), 1.03 (*s*, 3H, C-29, -$CH_3$), 1.07 (*s*, 3H, C-27, -$CH_3$), 1.16 (*s*, 3H, C-24, -$CH_3$), 1.25 (*s*, 3H, C-23, -$CH_3$), 0.89 (*m*, 2H, C-1, -$CH_2$-), 0.84 (*m*, 1H, C-13, >CH-), 1.32-2.41 (complicated pattern for remaining twenty three protons); $^{13}C$ NMR (δ ppm, $CDCl_3$): 35.10 (C-1), 27.50 (C-2), 213.83 (C-3), 59.41 (C-4), 58.16 (C-5), 40.87 (C-6), 17.93 (C-7), 40.60 (C-8), 53.05 (C-9), 38.26 (C-10), 35.31(C-11), 30.48 (C-12), 38.26 (C-13), 39.22 (C-14), 31.77(C-15),

29.68 (C-16), 32.38 (C-17), 39.48 (C-18), 35.58 (C-19), 29.97 (C-20), 32.07 (C-21), 35.98 (C-22), 28.15 (C-23), 22.26 (C-24), 18.20 (C-25), 14.64 (C-26), 18.65 (C-27), 20.24 (C-28), 35.02 (C-29), 32.74 (C-30); MS (m/z): 426 ($M^+$), 302, 205, 125, 97, 69, 55 (calcd for $C_{30}H_{50}O$).

**Icosan-1-ol (III)**

This compound was isolated when column was eluted with petroleum ether and benzene in the ratio of 1 : 3. It showed single spot on TLC examination ($R_f$ = 0.72) in benzene and chloroform (7 : 3) as a solvent system. Melting point of compound III (colorless solid) was found to be 66°C. IR (KBr, $cm^{-1}$): 3400-3250 (-OH, stretching), 1062 (C-O, stre.), 734, 724; $^1$H NMR (δ ppm, $CDCl_3$): 0.87 (*t*, 3H, *J = 6.2, 6.9Hz*, C-20, -$CH_3$), 2.34 (*t*, 2H, *J = 7.3, 7.6Hz*, C-1, -$CH_2$-), 2.89 (br, *s*, 1H, C-1, -OH), 1.63 (*m*, 2H, C-2, -$CH_2$-), 1.25 (br, *s*, 34H, C-3 to C-19,); MS (m/z): 298 ($M^+$) (calcd for $C_{20}H_{42}O$)

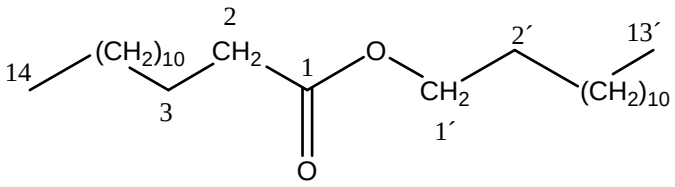
**(+) Usnic acid (IV)**

Removal of solvent afforded yellow solid, by eluting the column with petroleum ether and benzene (1 : 3). This mass was redissolved in acetone and acetone soluble part was purified and analysied. Its Rf value was 0.80 in chloroform and methanol (95% + 5%) system. The m.p. of this crystalline yellow compound was 204°C. IR (KBr, $cm^{-1}$): 3150 (O–H stretching), 1800 (C=O, str.), 1550 (C=C, str.); $^1$H NMR (δ ppm, $CDCl_3$): 2.67 (*s*, 3H, -$COCH_3$ at C-2), 5.97 (*s*, 1H, C-4, -H), 2.66 (*s*, 3H, -$COCH_3$ at C-6), 13.30 (*s*, 1H, C-7, -OH), 2.11 (*s*, 3H, C-8, -$CH_3$), 11.01 (*s*, 1H, C-9, -OH), 1.76 (*s*, 3H, C-9b, -$CH_3$), 18.84 (*s*, 1H, C-3, -OH, enolic form); $^{13}$C NMR (δ ppm, $CDCl_3$): 198.02 (C-1), 109.24 (C-2), 200.38 (-$\underline{C}OCH_3$ at C-2), 32.08 (-$CO\underline{C}H_3$ at C-2), 163.90 (C-3), 101.58 (C-4), 179.32 (C-4a), 155.31 (C-5a), 98.37 (C-6), 200.50 (-$\underline{C}OCH_3$ at C-6), 31.22 (-$CO\underline{C}H_3$ at C-6), 163.56 (C-7), 105.33 (C-8), 7.50 (-$CH_3$ at C-8), 157.38 (C-9), 104.03 (C-9a), 59.13 (C-9b), 27.83 (-$CH_3$ at C-9b); MS (m/z): 345 ($M^+$+H), 344 ($M^+$), 261, 260, 234, 233, 232, 217, 215 (calcd for $C_{18}H_{16}O_7$)

## RESULTS AND DISCUSSION

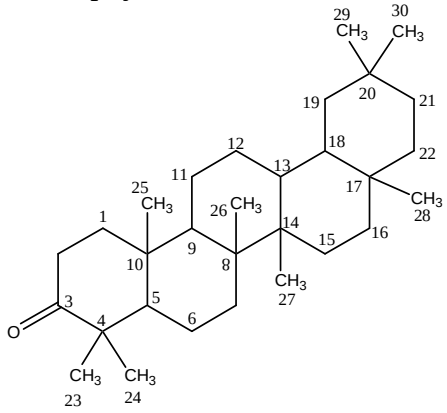
**Compound I,** the mass spectrum of compound I exhibited the molecular ion peak at m/z 410 [$M^+$]. On the basis of $^1$H NMR and $^{13}$C NMR the molecular formula was calculated as $C_{27}H_{54}O_2$. The IR spectrum ($cm^{-1}$, KBr) displayed the significant absorption at 2925, 2850 for C-H stretching, 1730 for C=O stretching and 1260 for C-O stretching of the ester group. The other important absorptions were observed at 715 and 710 (–$(CH_2)_n$- bending). On the basis of these observations, compound I seems to be a long chain saturated aliphatic ester. The $^1$H NMR spectrum (δppm, $CDCl_3$) showed two triplets at 4.1 (*J= 6.75, 6.78 Hz*) $\left(-\underset{\underset{O}{\|}}{C}-O-C\underline{H}_2-\right)$ and 2.30 (*J=7.32, 7.50 Hz*) $\left(-C\underline{H}_2-\underset{\underset{O}{\|}}{C}-O-\right)$ which corresponded to the methylene group attached at ester oxygen (C-1') and ester carboxyl group at (C-2) respectively. The signals observed at 1.65 (*m*, 2H) and 1.55 (*m*, 2H) were assigned for two protons each at C-3 and C-2´ position respectively. The presence of six protons of terminal methyl groups were assigned as a triplet at 0.90 (*J = 6.62, 6.93 Hz*). Methylene protons were observed at 1.24 as a broad singlet and calculated for forty protons. In the $^{13}$C NMR spectrum (δppm, $CDCl_3$), the ester linkage was confirmed by the absorption at 175.20 and 70.10 for carboxyl function of the ester C-1 and methylene proton attached at C-1´ to the ester oxygen. The signals for terminal methyl groups were observed at 14.10 and 14.13. The absorptions for other carbon atoms were assigned as 34.42 (C-2), 25.93 (C-3), 32.11 (C-12), 22.50 (C-13), 31.93 (C-2´), 28.64 (C-3´) and besides these signals many other

overlapped peaks were observed at 29.15 to 29.70 for remaining carbon atoms (C-4 to C-11 & C-4´ to C-10´ positions) in the title compound. On the basis of the above evidences, compound I was identified as tridecyl myristate (tridecyl tetradecanoate). This compound is isolated first time from this lichen.




**Compound I**

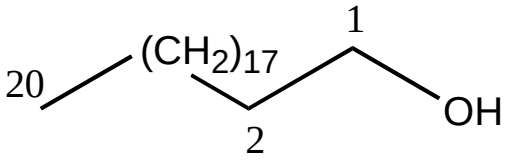
**Compound II,** the mass spectrum of compound II displayed [$M^+$+H] at 427 and [$M^+$] at m/z 426. Other characteristic ions were observed at m/z 302, 205, 125, 97, 69, 55 etc. The compound gave positive test with Libermann–Burchard reagent which indicated its triterpenoid nature. It also responded Zimmermann's test suggesting the presence of keto group at position C-3 (3-ketone), which was further confirmed by the fragment at m/z 205 in the mass spectrum. Thus the molecular formula for compound II was established as $C_{30}H_{50}O$. In the IR spectrum [$cm^{-1}$, KBr] characteristic strong absorption was observed at 1720 suggested the presence of a carbonyl group. Strong absorptions observed at 1440 and 1370 were characterized for bending vibration of gem dimethyl group (-$CHMe_2$). The $^1$H NMR spectrum [δppm, $CDCl_3$], showed the presence of eight methyl groups, at 0.71 (*s*, 3H, C-26), 0.85 (*s*, 3H, C-25), 0.94 (*s*, 3H, C-28), 0.98 (*s*, 3H, C-30), 1.03 (*s*, 3H, C-29), 1.07 (*s*, 3H, C-27), 1.16 (*s*, 3H, C-24) and 1.25 (*s*, 3H, C-23). The signals at 0.89 (*m*, 2H) & 0.84 (*m*, 1H) were observed for C-1 and C-13 protons respectively. A complicated pattern from 1.32 to 2.41 was observed and accounted for remaining twenty three protons in the title compound. In $^{13}$C NMR spectrum [δppm, $CDCl_3$] an absorption at 213.83 confirmed the presence of keto group at C-3 position. Other absorptions at 14.64, 18.20, 18.65, 20.24, 22.26, 28.15, 32.74 and 35.02 were due to eight methyl groups and their positions was assigned as C-26, C-25, C-27, C-28, C-24, C-23, C-30 and C-29 respectively. The assignments of other carbon atoms and their position were established as 35.10 (C-1), 27.50 (C-2), 59.41 (C-4), 58.16 (C-5), 40.87 (C-6), 17.93 (C-7), 40.60 (C-8), 53.05 (C-9), 38.26 (C-10), 35.31 (C-11), 30.48 (C-12), 38.26 (C-13), 39.22 (C-14), 31.77 (C-15), 29.68 (C-16), 32.38 (C-17), 39.48 (C-18), 35.58 (C-19), 29.97 (C-20), 32.07 (C-21), 35.98 (C-22), by comparing the data with the literature values. The identity of the title compound was further confirmed by comparing the spectral data with reported values [10]. On the basis of above spectral analysis compound II was characterized as 3-ketooleanane. This phytoconstituent is identified first time in this lichen.




**Compound II**

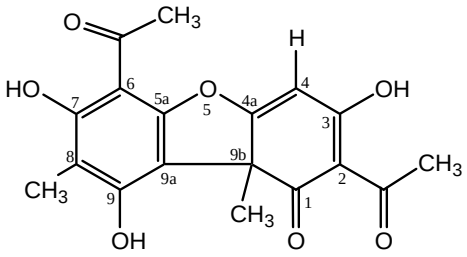
**Compound III,** the molecular formula of compound III was found to be $C_{20}H_{42}O$ by mass spectral studies [$M^+$, 298]. An intense peak was observed at m/z 31 corresponding to the

$CH_2\overset{+}{O}H$ ion, which confirmed the primary alcoholic nature of the compound. The characteristic ion at m/z 280 appeared due to the loss of water molecule from the molecule ion i.e. ($M^+$ -$H_2O$). Besides this the spectrum showed the peaks at 280, 266, 252 etc. at the interval of 14 mass units i.e. regular loss of methylene ($CH_2$) groups suggesting that this is a long chain alcohol. The IR spectrum [$cm^{-1}$, KBr] showed broad absorption peak at 3400 – 3250 (O-H stretching, due to polymeric association), 1062 (C-O stretching) for primary alcohol and a doublet at 734 and 724 [C-H bending for straight chain methylene groups [-$(CH_2)_s$-] further confirmed it to be a long chain primary alcohol [11-15]. The $^1$H NMR spectrum (δppm, $CDCl_3$) showed a triplet for methyl group at 0.87 (*t*, 3H, *J = 6.2, 6.9 Hz*, C-20) and a broad signal with side bands integrating for thirty four protons of methylene groups were accounted at 1.25 (br, *s*, 34H, C-3 to C-19). A triplet for two protons at 2.34 (*t*, 2H, *J = 7.6, 7.3 Hz*, C-1) was observed along with a broad singlet at 2.89 for one proton of hydroxyl group. A multiplate for two protons was observed at 1.63 for C-2 protons. On the basis of above spectral studies compound III was identified as Icosan-1-ol (m.p. 66$^o$C). This compound is being reported for the first time from the title lichen.




**Compound III**

**Compound IV,** the mass spectrum of this compound showed a molecular ion peak at m/z 344[$M^+$]. Other prominent ions were observed at m/z : 345 [$M^+$+H], 261, 260, 234, 233, 232, 217, 215 etc. The spectral analysis and molecular weight determination established its molecular formula as $C_{18}H_{16}O_7$. In the IR spectrum (KBr, $cm^{-1}$) the presence of phenolic (OH) group was observed at 3150 and the absorptions at 1800 confirmed the presence of carbonyl group whereas the absorption at 1550 was assigned for >C=C< stretching. The $^1$H NMR spectrum (δppm, $CDCl_3$) showed two sharp singlets at 1.76 (*s*, 3H) and 2.11 (*s*, 3H) for two methyl groups and their positions were assigned as C-9b and C-8 respectively as reported [16]. The absorption at 2.66 (*s*, 3H) and 2.67 (*s*, 3H) as two sharp singlets confirmed the presence of two acetyl groups at C-6 and C-2 positions respectively. Two sharp singlets were observed at 11.01 (*s*, 1H) and 13.30 (*s*, 1H) for phenolic groups at position C-9 and C-7 correspondingly. Vinylic proton presents at C-4 position showed the absorption at 5.97 as a singlet for one proton. In solution form this compound exists in two tautomeric forms i.e. ketonic and enolic. The enolic proton showed intense $^1$H NMR absorption at 18.84. The absorptions at 163.90, 163.56 and 157.38 in $^{13}$C NMR spectrum (δppm, $CDCl_3$) also confirmed the presence of three phenolic groups linked at C-3, C-7 and C-9 positions respectively. Vinylic carbon atom was confirmed by the absorption at 101.58 which was assigned to C-4 carbon atom. The absorption appearing at 198.02 clearly indicated the presence of carbonyl group at C-1 position. The values of other carbon atoms in compound IV were established as 109.24 (C-2), 200.38 (-$\underline{C}OCH_3$ at C-1), 32.08 (-CO$\underline{C}H_3$ at C-1), 179.32 (C-4a), 155.31 (C-5a), 98.37 (C-6), 200.50 (-$\underline{C}OCH_3$ at C-6), 31.22 (-CO$\underline{C}H_3$ at C-6), 105.33 (C-8), 7.50 (-$CH_3$ at C-8), 104.03 (C-9a), 59.13 (C-9b), and 27.83 (-$CH_3$ at C-9b). On the basis of above spectral analysis compound D was identified as (+) usnic acid. The identity of this compound was confirmed by comparing its spectral data with reported values [16-23].



**Compound IV**

## CONCLUSION

The objective of the present work was to find the medicinal importance of lichens. By using chromatography techniques, we isolated four phytoconstituents and they are identified and characterized on the basis of different spectroscopic techniques. Compound I, II and III are isolated first time from this lichen. These compounds have very useful medicinal activities. So present work gives a direction for future investigators to carry out research on the lichens, so that they could get some medicinally important drugs.

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