

Phytochemical and Biological Studies on *Usnea Orientalis* and *Rhododendron Campanulatum* of Nepal

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Abstract

*Phytochemical and biological screening of *Usnea orientalis* and *Rhododendron campanulatum* were carried out. The brine shrimp bioassay showed that the ethereal and methanolic extract of *Usnea orientalis* and the methanolic extract of *Rhododendron campanulatum* were biologically active. Antibacterial susceptibility studies of various extracts showed that the ethereal extract of *Usnea orientalis* and methanolic extract of *Rhododendron campanulatum* have antibacterial properties towards both gram positive and gram negative bacteria. Two major constituents usnic acid and diffractaic acid from the ethereal extract of *Usnea orientalis* have been isolated and identified by their melting points, Co-TLC and ¹H-NMR spectra comparison with authentic samples. The other two compounds viz. atranorin and eumitrin have been identified by Co-TLC and melting points comparison with the authentic samples. Diffractaic acid was isolated from *Usnea orientalis*. Usnic acid showed inhibition towards gram positive bacteria i.e. *S. aureus*. The brine shrimp bioassay of pure isolated compounds showed that usnic acid, diffractaic acid and atranorin were biologically and pharmacologically active.*

Introduction

Usnea Orientalis is a type of lichen plants which belongs to Usneaceae family. It grows on bark of twigs of trees like *Rhododendron*, *Castanopsis* etc. Lichen as pollution indicator of sulphur dioxide, heavy metals and radioactive metals. These substances are absorbed by lichen from the air very rapidly and accumulate inside the thallus which causes the death of lichen. Thus amount of lichen available in an area indicates the level of environmental pollution of that area.

Lichen is one of the economic plants of Nepal as it can be used in different medicinal and other purposes. Usnic acid obtained from different lichens is used as antibiotic like penicillin^{1,2}. Likewise, *Parmelia* species is used to treat epilepsy. *Lobaria* (lungwort) is used to treat TB and Lung Cancer. *Cladonia* is used to treat whooping cough. *Peltigera* for hydrophobia, *Certraria* for diabetes, etc. *Usnea* spp are used in dyes, cosmetics and medicines. *Certraria juniperina* and *Letharia vulpina* (wolfmoss) are used as poisons. The sea lichens are used for food and fodder eg. *Cladonia* (Reindeermoss) in arctic regions and *Certeria* (Iceland moss) in Iceland³.

Collection and taxonomy of lichens from various parts of Nepal have been done by different botanists, however their chemical investigations still need to be carried out. So, this research intends to conduct phytochemical screening and biological and chemical investigation of *usnea orientalis* and *Rhododendron campanulatum*.

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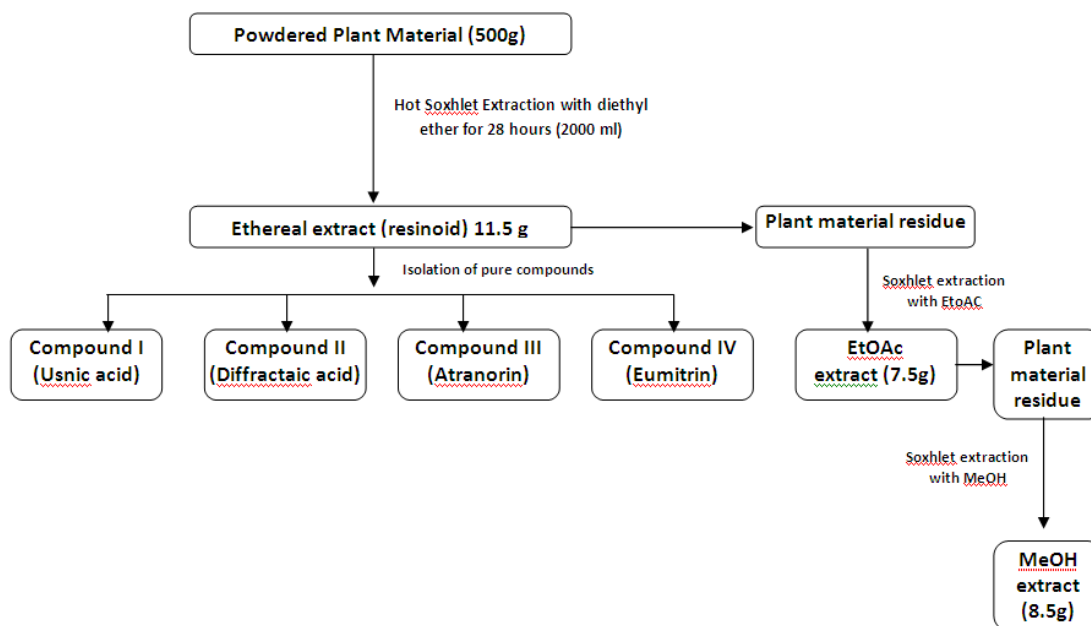
Experimental part

Phytochemical study

1. Collection of plants materials

The plants materials *Usnea orientalis* and *Rhododendron campanulatum* were collected from Dhankuta district and identified at central herbarium and botanical garden, Godawari. The lichen fragments were made freed from obvious organic substrate materials and species of lichen. The plants materials were dried under shade and powdered 500 gm.

2. Extraction of plants materials



3. Isolation of plant constituents

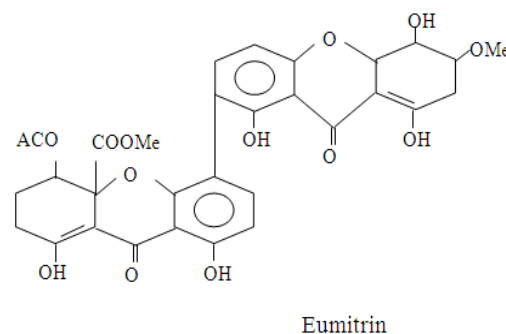
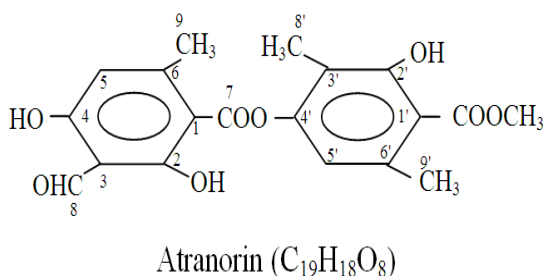
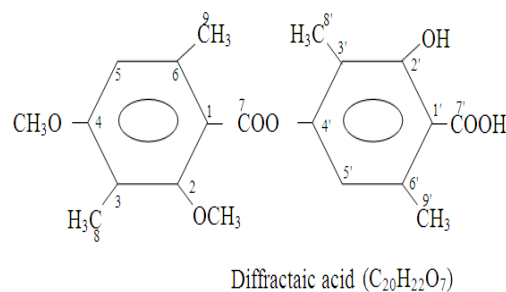
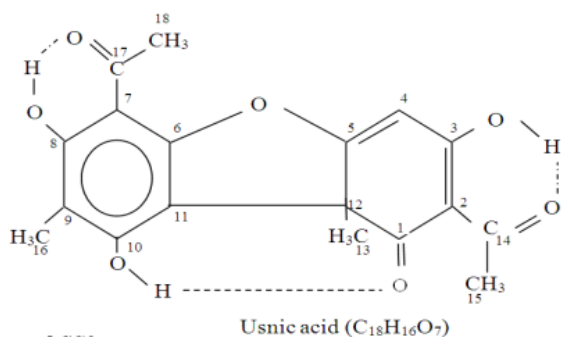
The column (42.5x3.1) was packed with silica gel 100gms 60-120mesh and applied the ether extracts 10.5 gms for chromatography. After loading ether extract, it was eluted sequentially with the solvents of increasing polarity petroleum ether, ethyl acetate and ethanol.

Biological investigations

- Brine Shrimp bioassay⁵
- Screening of Antimicrobial activities

Results and Discussion

Four pure compounds were isolated and identified from ethereal extract of *Usnea orientalis* from Column Chromatography as:

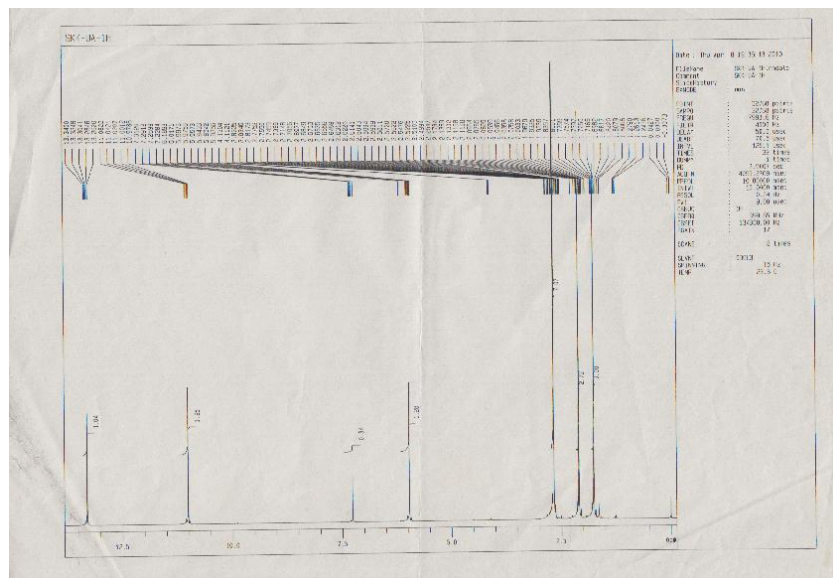


The yellow needle crystal having melting point of 195° C and purple spot under UV light was obtained with 5% EtOAc in ether from column and its R_f value is 0.56 in 25% EtOAc in hexane. The ¹H-NMR spectrum of this compound revealed singlet peak at δ 1.76, 2.11, 2.66, 2.68, 5.98, 11.04 and 13.32. The peaks at 11.0, 13.3 and 18.8 were due to phenolic protons. The peaks appeared at 13.3 was due to -OH of ring at C-8. The peak at 11.0 is due to proton of -OH situated at ring. A flanked with ortho-CH₃ and -COCH₃ group at C-10. On D₂O shaking only peak at 11.0 and 13.3 remains while at peak 18.8 vanished. Therefore, these two phenolic groups at 11.0 and 13.3 must have involved in hydrogen bonding. These spectra data, Co-TLC and its melting point comparison with authentic sample showed that the compound is Usnic acid⁶.

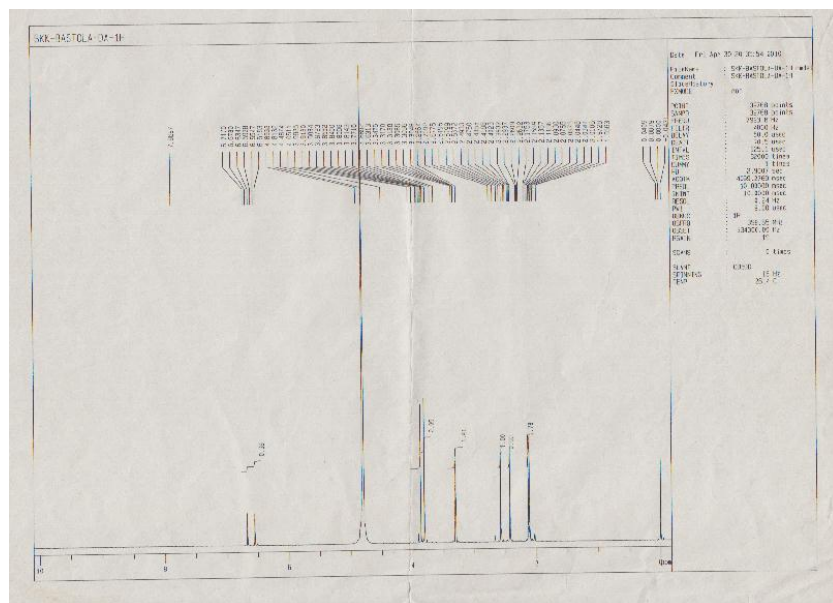
The white crystalline solid having melting point of 188° C was obtained with 10% EtOAc in ether from column and its R_f value was 0.52 in 50% EtOAc. The ¹H-NMR spectrum revealed single peaks at δ 2.165, 2.185, 2.48, 2.61, 3.85, 3.85, 6.54, 6.61. Two single peaks at 2.185, 2.165 were accounted for two methyl groups attached to C-8 and C-8' respectively, whereas singlet peaks at 2.48, 2.61 were due to the methyl groups attached to oxygen atoms at C-9 and C-9'. The singlet peak at δ 3.85 showed the presence of -OMe proton at C-2 and C-4 of the compound. The singlet peak at δ 6.54 and 6.61 accounted for the presence of aromatic proton (H-5) and (H-5'). This spectrum suggested the compound is diffRACTAIC acid⁷. This was further confirmed by its melting point, Co-TLC and comparison with proton NMR spectral data with of authentic sample.

Fraction 70% of EtOAc in ether of column gave a colourless substance which was crystallised out from the concentrated solution. The compound was purified by washing with hexane. Its Co-TLC and having melting point of 194° C showed the compound is Atranorin⁸.

Fraction 10% of methanol in EtOAc gave white crystal after washing with hexane. Its melting point and Co TLC showed the compound is Eumitrin¹.



¹H-NMR of Usnic Acid



¹H-NMR of Diffractaic Acid

From Brine Shrimp bioassay, the ethereal and methanolic extracts of *Unsea orientalis* were found to have LC₅₀ value less than 1x10³ and showed pharmacologically active. Methanolic extract of *Rhododendron Campanulatum* showed LC₅₀ value less than 1x10³ indicating more cytotoxic whereas

EtOAc extract of *Rhododendron campanulatum* showed LC_{50} value more than 1×10^3 indicating less cytotoxic in nature.

From Screening of Antimicrobial activities test, the highest zone of inhibition (ZOI) was found in the ether extract of *Usnea orientalis* with diameter 17mm for *Staphylococcus aureus* and lowest ZOI was seen in EtOAc extract of this lichen with 9mm for *S. typhi*. The highest zone of inhibition (ZOI) of methanolic extract of *Usnea orientalis* was 12mm for *Staphylococcus aureus* and lowest zone of inhibition (ZOI) was of each 10mm for *Pseudomonas* and *Proteus vulgaris*. Similarly, the highest ZOI was found in the Methanolic extract of *Rhododendron campanulatum* with diameter 25mm for *E.coli* and lowest was seen in this plant with diameter 22mm for *Klebsiella spp.* The zone of inhibition (ZOI) of usnic acid for gram positive bacteria *S. aureus* was found to be 18mm.

Conclusion

Four constituents usnic acid, diffractaic acid, atranorin and eumitrin from the ethereal extract of *Usnea orientalis* have been isolated and identified. Ethereal and methanolic extracts of *Usnea Orientalis* were found to have cytotoxic activity. Usnic acid and Diffractaic acid have found to cytotoxic activity. Methanolic extract of *Rhododendron campanulatum* showed more cytotoxic, whereas EtOAc extract of this plant showed less cytotoxic in nature. Antimicrobial activities studies showed the in ethereal extract of *Usnea Orientalis* against *Staphylococcus aureus* as the highest zone of inhabitation and methanol extract of *Rhododendron Campanulatum* showed the highest zone of inhabitation against *E. coli*.

Acknowledgement

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