Research Article

Functional Group Analysis for Methanolic Extracts of Root, Fruit and Callus of *Myxopyrum* Smilacifolium Blume.

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ABSTRACT

The purpose of present study was to analyze and compare the functional groups present in the crude methanolic root, fruit as well as callus extracts of *Myxopyrum smilacifolium* Blume which is known to possess many medicinal properties. FTIR spectroscopy was conducted for this purpose. The FTIR spectrum generated in the present study revealed that all the three extracts have almost similar functional groups *viz*: alcohol, alkanes, alkenes, aldehydes, ketones, amines, carboxyl groups, alkyl halides etc. The similarity in the functional groups between callus and other extracts is promising for future prospects that could replace the exploitation of plant material for pharmaceutical applications thereby conserving biodiversity.

Keywords: *Myxopyrum smilacifolium*, FTIR Spectoscopy, Callus, Functional groups

INTRODUCTION

Functional group analysis is prerequisite in any phytochemical studies which helps in determining the chemical composition of a lead compound. Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases[1]. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts[2]. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances.

The study of plants continues principally for the discovery of novel secondary metabolites which possess many pharmacological properties.

The functional group identification is the key step in the process of determining the chemical constituents and it could be elucidated by FTIR. FTIR is a physico chemical analytical technique providing an idea of the metabolic composition of a tissue at a given time. It measures the vibrations of bonds within chemical functional groups and generates a spectrum that can be regarded as biochemical or metabolic finger print of the sample[3,4]. IR spectrum is simplest and most reliable method for assigning a compound to a particular class[5].

Myxopyrum smilacifolium is a large woody climbing shrub belonging to the family Oleaceae. Its root, stem, leaves are of much medicinally active and is employed in many traditional systems of medicines. The roots are used to treat various diseases like scabies, cough, rheumatism, fever, cuts and wounds[6]. The leaves are astringent, acrid, sweet, thermogenic, anodyne, febrifuge and tonic. They are useful in vitiated conditions of kapha and vata, cough, asthma, rheumatism, cephalalgia, nostalgia, fever, otopathy, neuropathy and cuts and wounds[7]. Pharmacognostical evaluation has been made for the plant and reported for the presence of terpenoids, flavones, anthraquinones, sugars, alkaloids, phenols, tannins, and saponins, antimicrobial study has been carried out in leaves[8,9]. Previous studies have shown the presence of triterpenoid ursolic acid in leaves[10] and the iridoid glycoside myxopyroside[11]. The objective of this study is to identify and compare the functional groups present in root, fruit as well as callus extracts. Identifying and characterizing the functional groups helps to elucidate structure[12].

Literature survey has revealed that FTIR analysis was not done with the root, fruit as well as callus extracts of *M.smilacifolium*. So an attempt has been made in present study to analyze the functional group of phytoactive compounds present in the samples under study.

MATERIALS AND METHODS

Collection and Processing of plant material

Fresh plant roots of *M. smilacifolium* were collected from Botanical garden, Dept. of Botany, University of Kerala, Kariavattom. The roots were washed thoroughly with running tap water followed by sterile distilled water followed by drying under shade. Roots were then crushed to coarse powder and were stored at room temperature in air tight container bottles.

Fresh matured fruits collected were washed thoroughly under running tap water, chopped into pieces, dried in shade and then stored in refrigerator.

For callus induction surface sterilized internode explants were first inoculated in MS medium supplemented with 0.1% 2, 4 Dichlorophenoxy acetic acid (2,4-D) and then sub-cultured on to same medium supplemented with a

combination of 0.1% 2,4-D and 1.0 % Benzyl amino purine (BAP). Four week old mature callus was collected and dried in hot air oven at 50°C. Dried callus were then stored in refrigerator.

Powdered root (15 gms) of M.smilacifolium were extracted using methanol in soxhlet apparatus for 12 hrs. The extracts were then filtered through Whatmann No.1 filter paper and concentrated using vacuum evaporator. The extract value calculated and then stored in refrigerator for further use.

For fruit extraction, dried fruits (15gms) were crushed with mortar and pestle followed by methanolic extraction in soxhlet apparatus and repeated the same procedure as for root extract.

For callus extraction, dried callus (15 gms) was ground with mortar and pestle and then methanolic extract was taken using soxhlet apparatus and repeated the same procedure as for root extract.

FTIR Spectroscopic Analysis

For FTIR analysis of the three extracts NaCl method was adopted for the analysis in an FTIR spectroscope (Jasco6300) with a scan range from 600-4000cm^{-1}. The results were interpreted by computerized methods. Samples were done in triplicate and the whole procedure completed within one day.

RESULTS AND DISCUSSION

The functional groups of the active components based on the peak value in the region of infrared spectrum were identified using FTIR spectrum. The methanolic root, fruit and callus extracts were passed into the apparatus and the functional group of the components were separated based on its peak ratio. All the three samples under study showed almost same wave numbers which clearly indicate similar functional groups. Alcohol, alkanes, carboxyl groups, alkenes, aromatics, amines and alkyl halides were present in all the three samples. Nitro groups were found to be absent from callus extracts. Callus extracts confirmed the presence of alcohols, alkanes, ethers, alkenes, amino groups, amines, nitro compounds, carboxyl groups, aromatics, and alkyl halides.

The very strong broad band for root extracts at 3414 cm^{-1} indicates the presence of hydroxyl derivatives. Bands at 2943 cm^{-1},1634 cm^{-1}, 1441 cm^{-1} and 1257 cm^{-1} corresponds to N-H, C=O, C-H bending and C-N stretch respectively. Band occurring at 1512 cm^{-1} indicates the presence of Nitro compounds. Vibrations at 1634 cm^{-1} clearly shows the presence of C=C stretch. Bands at 1257 cm^{-1}, 1170 cm^{-1} and 1075 cm^{-1} indicate C-CHO bending[13]. The peak values confirms the presence of alcohols, carboxyl groups, nitro groups, amino compounds, aromatics and alkyl halides. Fruit extract and callus extracts also showed similar banding pattern. Alkyl halide groups were much more prone in this samples with lower wave numbers. Peak at 2856cm^{-1} for fruit sample indicates the presence of ether linkage. There was no absorbance in between 2220-2260 cm^{-1} which indicates that no cyanide group is present in all the three extracts which means it is non toxic [14]. Analysis of the data clearly shows that methanolic root and callus extracts have more or less similar functional groups. From this FTIR spectrum it is easy to determine the constituents of extracts and further analysis for its medicinal properties.

The fact that callus extracts showing similar functional groups with root powder could be taken into advantage that without exploiting the exact plant, callus could be employed for further studies in order to validate the pharmacological application with reference to methanolic root extract. The FTIR spectra of three extracts are as shown in Figure 1-3 and its characteristic peak values and functional groups in table 1. The characteristic peak values and corresponding functional groups clearly indicate the presence of alkaloids, terpenoids and phenolic compounds. Further studies need to be conducted for structure elucidation as well as pharmacological properties of the lead compound.

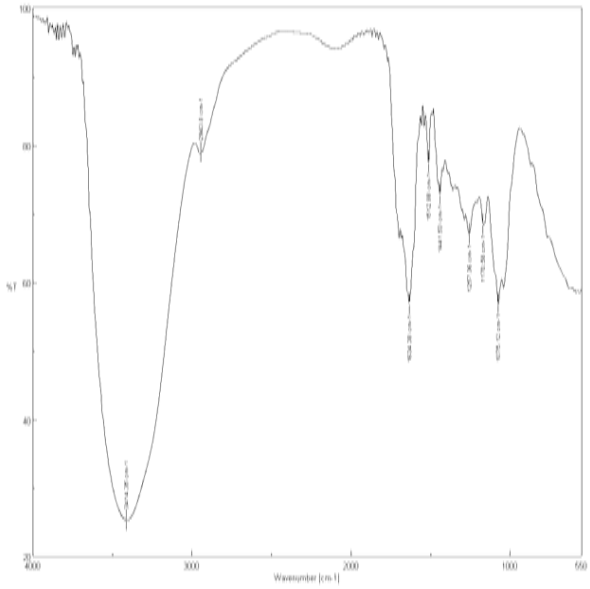

Figure 1: FTIR spectrum of M.smilacifolium- root extract

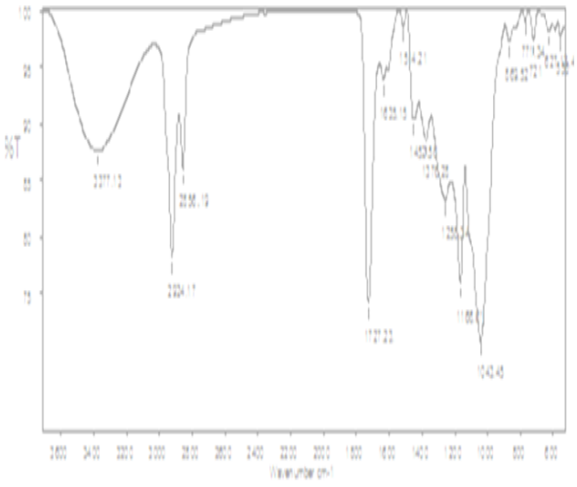

Figure 2: FTIR spectrum of M.smilacifolium fruit extract

Table 1: FTIR peak values of methanolic extracts of root, fruit and callus

Functional groups	Methanolic Extracts		
	Root	Fruit	Callus
Alcohol and Phenol -OH stretch	3414	3377	3393
C-O stretch	1170 1075	1166	1183 1059
Amino groups N-H stretch	3414 2943	3377 2940	3393 2924
C-N stretch	1257	1258	1259
C-O stretch	1634 1170 1075	1635 1166	1648 1183 1059
Nitro group N-O asymmetric	1512	1514	---
Alkanes C-H stretch	2943	2924	2940
C-H bend	1441 1442	2856 1452	1399
Alkenes C=C stretch	1634	1635	1648
=C-H bend		869 771 721	865 820 779
Carboxyl group O-H stretch	3414 2943	3377 2924	3393 2940
C-O stretch	1257	1258	1259
C=O stretch	1634	1727	1648
O-H bend	1441	1452	1399
Aldehyde C-H bending	---	869	865
C-CHO bending	1257 1170 1075	1376 1258 1166 1043	1259 1183 1059
Ketones C-H stretching	2943	2924 2856	2940
C-CO-C stretching	1170 1075	1166	1183
Aromatics	1257	1258	1259
Alkyl Halides	1170	1166 771 721	1183 820 779

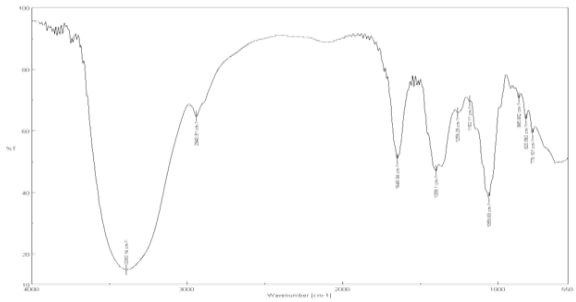

Figure 3: FTIR spectrum of M.smilacifolium-Callus

CONCLUSION

The results of the present study clearly shows that root, fruit and callus of *M. smilacifolium* is a rich source for many phyto-constituents which can be isolated, characterized and tested for its biological and pharmaceutical applications.

REFERENCES

1. Verpoorte R, Chemodiversity and the Biological Role of Secondary metabolites, some thoughts for selecting plant material for drug development. Proc. Phytochem. Soc. Europe, Kluwer Publishers, 43, 1998, 11-24.
2. Sandhya B Thomas S, Isabel W and Shenbagarathai R, Complementary and alternative medicines, 3, 2006, 101-114.
3. Griffiths PR, de Haseth JA. Fourier transform infrared spectroscopy. New York: Wiley, 1986.
4. Surewicz WK, Mantsch HH, Chapman D. Determination of Protein Secondary Structure by Fourier Transform Infrared Spectroscopy: A Critical Assessment. Biochemistry, 32(2), 1993, 389-393.
5. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis, 3, 1998, 22-23.
6. Vaidhyrathnam Varrier's PS, Indian medicinal plants-a compendium of 500 species, Arya vaidya sala, Kottakkal, 4, 1996, 88.
   Orient Longman. Indian medicinal plants, 4, 2006, 98-99.
7. Gopalakrishnan S, Rajameena.R ,Vadivel.E, Phytochemical and Pharmacognostical studies of leaves of *Myxopyrum serrtulum* A.W Hill, Journal of chemical and pharmaceuticalresearch, 4(1), 2012, 788-794.
8. Gopalakrishnan S,A Rajameena.R, Vadivel.E Antimicrobial activity of leaves of *Myxopyrum serratulum* A.W.Hill, International journal of Pharmaceutical sciences and drug research, 4(1), 2012, 31-34.
9. Sudharmini D, Ashalatha SN, Antimicrobial Studies of Triterpenoid Fractions from *Myxopyrum smilacifolium* Blume, Ethnobotanical Leaflets, 12, 2008, 912-15.
10. Franzyk H, Jensen S R, Olsen C E. J Nat Prod, 64, 2001, 632-633.

11. Grube M, Muter O, Strikauska S, Gavare M, Limane B. Application of FT-IR spectroscopy for control of the medium composition during the biodegradation of nitro aromatic compounds. Journal of Indian Microbiology and Biotechnology, 35, 2008, 1545–1549.

12. Mohan J, Organic spectroscopy-principles and applications, Nasrosa publishers, New Delhi, 2001.

13. Ragavenran P, Sophia D, Arul Raj C, Gopalakrishnan VK, Functional group analysis of various extracts of *Aerva lanata* (L) by FTIR spectrum, Pharmacologyonline, 1, 2011, 358-364.

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