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PHYTOCHEMICAL DETERMINATION OF A POLYHERBAL EXTRACT USING FTIR AND GC-MS ANALYSIS

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ABSTRACT

The present study was carried out to characterize the bioactive constituents present in the polyherbal extract using FTIR and GC-MS. From the crude extracts for GC-MS analysis, 10 g sample is extracted with 30 ml ethanol, filtered in ash less filter paper with 2 g sodium sulphate and the extract is concentrated to 1 ml by bubbling nitrogen into the solution. The compound detection employed the NIST Ver.2.0 Year 2005 library. The FTIR spectrum confirmed the presence of aromatic substituted alkaloid functional groups. The results of the GC-MS analysis provide different peaks determining the presence of 16phytochemical compounds. The major phyto constituents were **1H- pyrrolo (2,3c) pyridine-3-propanoic acid, 5(4H)oxo-6,7, dihydro (100%), pentadecanoic acid (100%),5-5'Biphthalide(100%)**. Hence, this study proves the various active phyto constituents present in the polyherbal crude extract.

KEYWORDS: Polyherbal (PH) extract; GC-MS; FTIR.

1. INTRODUCTION

In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources.[17] Medicinal plants are expensive gift from human to nature. The approval of traditional medicine as an alternative form of health care and the improvement of microbial resistance to the existing antibiotics has lead researchers to scrutinize the antimicrobial compounds.[1] Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway. Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health facilities and services are inadequate.[2] Traditional medicine is an important source of potentially useful compounds of chemotherapeutic agents. A wide range of medicinal plant parts are used for extraction of raw drugs and they possess varied medicinal properties. Various plants have been used for many years in daily life to treat disease in all over the world. Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Natural products provide crucial, unmatched chemical diversity to modern drug discovery programs. Natural products play an important role in drug development programs in the pharmaceutical.[3] The role of traditional medicines in the solution of health problems is invaluable on a global level. Medicinal plants continue to provide valuable therapeutic agents, both in modern and traditional medicine.[4] With the associated side effects of the modern medicine, traditional medicines are gaining importance and are now being studied to find the scientific basis of their therapeutic actions.[5]

Plant based natural constituents can be derived from any part of the plant like bark, leaves, roots, flowers, seeds, fruits, rhizome etc.[6] Plants are the traditional sources for many chemicals used as a pharmaceutical biochemicals, fragrances, food colours and flavours.[7] Medicinal plants are at great interest to the researcher in the field of biotechnology, as most of the drug industries depend in part on plants for the production of pharmaceutical compounds. Thus, plants have been used in treating human diseases for thousands of years. The use of medicinal plants is not just a custom of the distant past. Perhaps 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines.[8] A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials.[9] It has been shown that *in-vitro* screening methods could provide the needed preliminary observations to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations.[10] The determination of phyto constituents is largely performed by relatively expensive and often laborious techniques such as gas (GC) and liquid (LC) chromatography combined with specific detection schemes.[11-12] Analysis of small amounts of chemicals has become easier and more cost-effective owing to the development of hyphenated chromatographic techniques such as GC or LC-MS. GC-MS analysis can identify pure compounds present at less

than 1gm.[13] However, simple, cost-effective and rapid tests for detecting phyto components are necessary. Spectroscopic (UV-Vis, FTIR) methods together or separate can be used in this sense as well as conventional methods.[14-16]

Hence, nine new medicinal plants of each different part were taken and prepared a polyherbal formulation. The primary medicinal components such as glycosides, flavonoids, tannins were studied.[18] Hence the present study was aimed to explore the remaining phytochemical constituent of PH extract sing FTIR and GCMS analysis.

2. MATERIALS AND METHODS

2.1Preparation of polyherbal formulation and solvent extraction

Each one gm of a poly herbal (PH) formulation contains equal amount of ***Punica granatum(rind), Catharanthus roseus, Gymnema sylvestre, Cissus quadrangularis, Garcinia cambogia, tinospora cordifolia, Terminalia Arjuna, Urginea indica, Ficus racemosa***. The plants were authentified in Botanical Survey of India, Coimbatore. 10g of the dried powder of each plant was taken and cold macerated with hydro-ethanolic solvent with occasional stirring for 3 days.

After 3 days, the suspensions was filtered through a fine muslin cloth and the filtrate was evaporated to dryness at low temperature ($<40^0$C) under reduced pressure in a rotary evaporator. The yield of crude extract is called as polyherbal (PH) extract which was found to be -9.64% and were stored in an air-tight desiccator's and used for further analysis.

2.2 FTIR Spectroscopic analysis

The extracts were examined under visible and UV light for proximate analysis. For FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200-1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

2.3 GC-MS analysis of a PH extract

10 g of powdered leaf sample is soaked with 30 ml ethanol overnight and filtered through ash less filter paper with sodium sulphate (2 g). The extract is concentrated to 1 ml by bubbling nitrogen into the solution. The extract contained both polar and non-polar phyto components. 2µl of the ethanolic extract of *Stylosanthes fruticosa.* was employed for GC-MS analysis20. The Clarus 500 GC used in the analysis employed a fused silica column packed with Elite-1 [100% dimethyl poly siloxane, 30 nm × 0.25 nm ID × 1µm df] and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The 2µl sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. During the 36th minute GC extraction process, the oven was maintained at a temperature of 110°C with 2 minutes holding.[18-19] The injector temperature was set at 250°C (mass analyser). The different parameters involved in the operation of the Clarus 500 MS, were also standardized (Inlet line temperature: 200°C; Source temperature: 200°C). Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The MS detection was completed in 36 minutes.

2.4 Identification of components

The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The detection employed the NIST (National Institute of Standards and Technology) Ver.2.0-Year 2005 library. The compound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA. Interpretation of GC-MS was conducted using the database of NIST having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

3. RESULTS AND DISCUSSION

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented in (table 1, figure 1). Performing the next advanced phytochemical analysis technique of FTIR the presence of various functional groups of different compounds was found. The solvent had its respective functional group like alkanes, cycloalkanes, carboxylic acids, aromatic compounds, NH group, phenyl *etc*. Hence, the crude extracts subjected to FTIR analysis is used for the identification of chemical constituents present in PH extract. In addition, FTIR spectroscopy is proved to be a reliable and sensitive method for detection of bio molecular composition.[19]

Table 1. The characteristic IR frequencies of some functional groups present in polyherbal crude extract.

TYPE	ABSORPTION FREQUENCY	INTENSITY	REMARKS AN ASSIGNMENT
NH group (Quaternary compouns)	3300±5	S	NH stretching appears as broad band
Methylene group (alkanes)	2931	S	Asym stretching
Methylene group (alkanes)	2854±10	S	Sym stretching
Aromatic C=C Group with phenyl nucleus or condensed systems	1600±12 1650-1600	M ⟶ S M ⟶ S	C=C skeletal stretching, position is slightly affected by nature of substituents C=C skeletal shifted to higher frequencies
NH group Secondary amines group	1350-1280	S	C-N stretching
NH group Primary NH group	1220-1020	W ⟶ M	EN stretching
Cycloalkanes (methylene group)	1030±10	S	With cyclohexyl and alicyclic compounds
Pentasubstituted (phenyl nucleus)	870±1	S	Disubstituted benzene absorption bands
Ortho disubstituted phenyl ring	770-735	V.S	Overlaps with monisubstituted band (Quinoline or Napthalene) Exhibit this absorption.

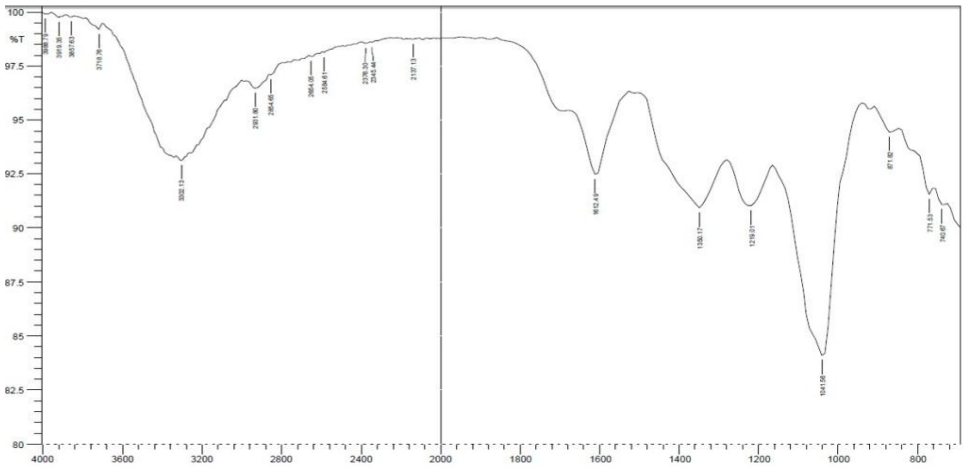

Fig 1: FTIR analysis of a polyherbal extract.

In plant taxonomy, during this molecular era, the morphological characters also play a vital role in plant systematic study and used as a tool for the classification of a taxon. In recent times, in addition morphological markers, anatomical, cytological, biochemical and molecular markers are also being used to classify the organisms. Gas Chromatography-Mass Spectrometry (GC-MS) is a valuable tool for reliable identification of phytocompounds.[21, 22]

The results pertaining to GC-MS analysis (table 2, fig 2) leads to the identification of number of compounds from the GC fractions of the hydroethanolic extract of *PH formulation*. These compounds were identified through mass spectrometry attached with GC. These observations may be due to the nature of biological active components and the stronger extraction capacity of hydro ethanol could have been produced number of active constituents responsible for medicinal activity.[20] The biological activities based on Dr. Duke's Phytochemical and Ethnobotanical Databases were tabulated in table 2.

Table 2: Phytocomponents identified in polyherbal extract using GC-MS.

NAME OF THE COMPOUND	RT	% PEAK AREA	MOLECULAR WEIGHT	MOLECULAR FORMULA
n-Hexadecanoic acid	17.93	88.1	256.421	$C_{16}H_{32}O_2$
octadecenoic acid	19.1	75.6	282.46	$C_{18}H_{34}O_2$
pentadecanoic acid	17.25	100	270.45	$C_{17}H_{34}O_2$
1H- pyrrolo (2,3c)pyridine-3-propanoic acid,5(4H)oxo-6,7, dihydro,	14.2	100	-	-
3 Butene-2-one,4(2,5,,6-tetramethy 1-2cyclohexene-1-yl)	12.1	100	192.3	$C_{13}H_{20}O$
Ethyl oleate	19.65	33.5	310.51	$C_{20}H_{28}O_2$
N-(4-Benzyloxy-phenyl)-2 iodo-benzamide	21.73	61	-	-
5-5'Biphthalide	16	100	266.248	$C_{16}H_{10}O_4$
Dihydroergokryptine	25.45	26.8	577.715	$C_{32}H_{43}N_5O_5$
2(1H)- Napthalenone, 1-dimethoxymethyl)-3,4,5,6,7,8-hexahydro	13.47	17.7	224.29	$C_{13}H_{20}O_3$
5-Methoxy carbonyltubercidin	20.45	36.6	324.28	$C_{13}H_{16}N_4O_6$
Naphth(1,2-b)oxirene,1a,2,3,7b-tetrahydro	11.23	96	176.211	$C_{11}H_{12}O_2$
1,3,12-Nonadecatriene	16.48	13.9	262.47	$C_{19}H_{34}$
2Methyl-E-7 hexadecene	14.5	39.2	238.45	$C_{17}H_{34}$
8 Hexadecenal,14-methl (Z)	15.6	35.6	252.44	$C_{17}H_{32}O$

GC-MS analysis was done using the organic solvent ethanol and it shows the presence of 16 different chemical compounds namely.

- **Hexadecanoic acid,**
- **octadecenoic acid,**
- **pentadecanoic acid,**
- **1H- pyrrolo (2,3c)pyridine-3-propanoic acid,5(4H)oxo-6,7, dihydro,**
- **3 Butene-2-one,4(2,5,,6-tetramethy 1-2cyclohexen-1-yl),**
- **2(E)Heptenoic aci,4(s)-4-[(t-butoxycarbonyl-(R)- phenylalanyl-(s)-alanyl)amino)-6-methyl,**
- **Ethyl oleate,**
- **N-(4-Benzyloxy-phenyl)-2 iodo-benzamide,**
- **5-5'Biphthalide,**
- **Dihydroergokryptine,**
- **2(1H)- Napthalenone, 1-dimethoxymethyl)-3,4,5,6,7,8-hexahydro,**
- **5-Methoxy carbonyltbercidin,**
- **Naphth(1,2-b)oxirene,1a,2,3,7b-tetrahydro,**
- **1,3,12-Nonadecatriene,**
- **2Methyl-E-7 hexadecene,**

- **8 Hexadecenal, 14-methl (Z)** present in the plant sample. The spectrum profile of GC-MS confirmed the presence of 16 major components with the retention time 17.93, 19.1, 17.25, 14.2, 12.1, 23.32, 19.65, 21.73, 16, 25.5, 13.47, 20.45, 11.23, 16.48, 14.5, 15.6 respectively. This gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in PH extract. The individual fragmentation patterns of the components were illustrated.

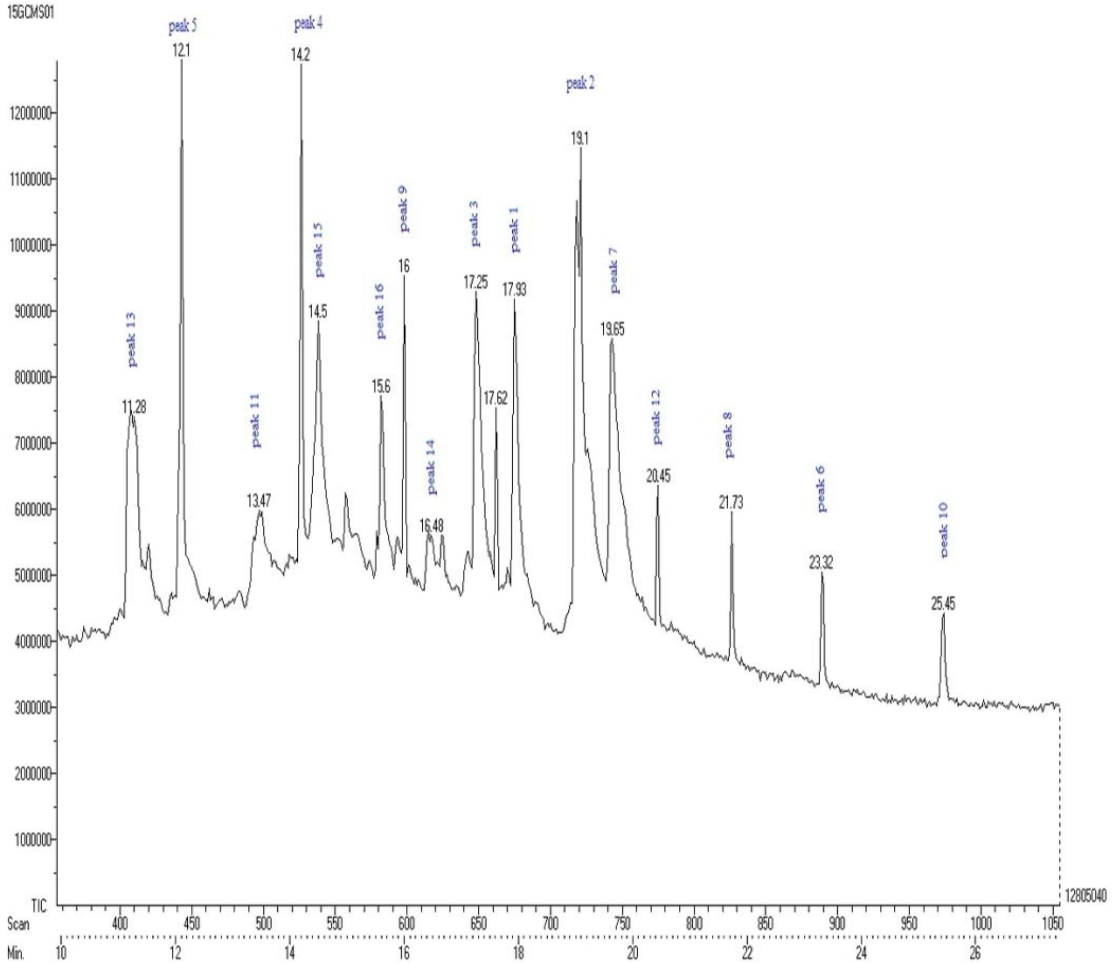

Fig 2: Gas chromatography and Mass spectroscopy analysis of a Polyherbal extract.

4. CONCLUSION

These mass spectra are fingerprint of the compound which can be identified from the NIST data library. Hence, the identified phyto components using GC-MS can be used as a pharmacognostical tool for the identification of active constituents. It paves the way for the development of several treatment regimens based on this extract. In addition, these active constituents may be responsible for the medicinal characteristics o the polyherbal extract.

REFERENCES

1. Sumathi, Parvathi. Antimicrobial activity of some traditional medicinal plants. J Med Plant Res., 2010; 4: 316-321.
2. Zaidan MRS, Rain NA, Badrul AR. In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. Trop Biomed, 2005; 22: 165-170.
3. Baker, J. T., Borris, R. P., Carte Cordell, B. G. A., Soejarto, D. D., Cragg, G. M., Gupta, M. P., Iwu, M. M., Madulid, D. R., Tyler, V. E., "Natural products drug discovery and department: New perspective on internal collaboration", Journal of Natural Products, 1995; 58: 1325-1357.
4. Krentz, A. J., Bailey, C. J., "Oral antidiabetic agents: current role in type 2 diabetes mellitus", Drugs, 2005; 65: 385-411.
5. Gupta, Y. K., Briyal, S., "Animal models of cerebral ischemia for evaluation of drugs", Indian Journal of Physiology and Pharmacology, 2004; 48: 379-394.
6. Gordon DM. Geographical structure and host specificity in bacteria and the implications for tracing the source of coliform contamination. Microbiology, 2001; 147: 1079-1085.
7. Leung, A.Y., Encyclopedia common natural ingredients used in food drugs and cosmetics. New York, 1980.
8. Drew AK, Myers SP. Safety issues in herbal medicine implications for the health professions. Med J Aust, 1997; 166: 538-41.
9. Khalaf N.A., Shakya A.K., Al-othman A., Ahbar Z. and Farah H., Antioxdidant activity of some common plants, Turk. J. Biol., 2007; 31: 1-5.
10. Mathekaga A.D. and Meyer J.J. M., Antibacterial activity of South African Helichrysumspecies, South Afr. J. Bot., 1998; 64: 293-295.
11. Uzer A., Ercag E. and Apak R., Selective spectrophotometric determination of TNT in soil and water with dicyclohexylamine extraction, Anal. Chim. Acta, 2005; 534: 307-317.
12. Eisenhauer N., Klier M., Partsch S., Sabais A.C.W., Scherber C., Weisser W. and Scheu S., No interactive effects of pesticides and plant diversity

on soil microbial biomass and respiration, Appl. Soil Ecol., 2009; 42: 31-36.
13. Liebler D.C., Burr J.A., Philips L. and Ham A.J.L., Gas chromatography-mass spectrometry analysis of vitamin E and its oxidation products, Anal. Biochem., 1996; 236: 27-34.
14. Aysal P., Ambrus A.D., Lehotay S.J. and Cannavan A., Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction, J. Environ. Sci. Heal., 2007; 42: 481-490.
15. Ibrahim M. and Abd-El-Aal M., Spectroscopic study of Heavy Metals Interaction with Organic Acid, Int. J. Environment and Pollution, 2008; 35(1): 99-110.
16. Ibrahim M., Hameed A.J. and Jalbout A., Molecular Spectroscopic Study of River Nile Sediment in the Greater Cairo Region, Applied Spectroscopy, 2008; 62(3): 306-311.
17. Perumal samy, R., Gopalkrishnakone P. crrent status of herbal and their future perspectives. Nature proceeding, 2007; 1: 1176-1188.
18. Anonymous. The useful plants of India [M]. New Delhi: Publication and Information Directorate, CSIR, 1992; 132.
19. Patil MV, Patil DA. Ethnobotany of Nasik district, Maharashtra [M]. New Delhi: Daya Books, 2006; 112.
20. Komal Kumar J. and Devi Prasad A.G., Identification and comparison of biomolecules in medicinal plants of Tephrosiatinctoria and Atylosiaalbicansby using FTIR, Romanian J. Biophys, 2011; 21(1): 63-71.
21. Sampath Kumar, S and Rama Krishnan, N. Chromatographic fingerprint analysis of Naringi crenulata by HPTLC technique. Asian Pal. J. Trop. Biomedicine, 2011; 1: S195-S-198.
22. Johnson M, Mariswamy Y and Gnaraj, W.F. Chromatographic finger print analysis of steroids in Aerva lanasa L. by HPTLC technique. Asian Pal. J. Trop. Biomedicine, 2011; 1: 428-433.