International Journal of Zoology Studies
ISSN: 2455-7269
Impact Factor: RJIF 5.14
www.zoologyjournals.com
Volume 2; Issue 6; November 2017; Page No. 11-14

Fourier transform infrared spectroscopy analysis of garlic (*Allium*)

D Nagarajan, T Ramesh Kumar
Department of Zoology, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India

Abstract
Identification of secondary metabolic fingerprint by chromatography and spectroscopy tools provides useful information about qualitative, quantitative and the pattern of the composition of there biomolecules. Recently, Fourier Transforms Infrared Spectroscopy (FTIR) reveals to phytochemical profiles containing overlapping signals from a wide array of the compounds.
Allium sativum samples extracted with the help of the solvent ether continuously was subjected to FTIR to determine whether the garlic can we discriminated on the basis of biochemical profiles. This study was carried out to identify the reactive functional groups present in the garlic powder. This preliminary study implied that the garlic (*Allium sativum*) would be used as protection of new drugs to the benefit of mankind.

Keywords: *Allium sativum*, transforms infrared spectroscopy, biochemical profiles, functional groups

Introduction
Medicinal plants are the significant part of natural wealth. They serve as vital therapeutic agents as well as valuable raw materials for manufacturing numerous traditional and modern medicines (Manickam and Veerabahu, 2014) [11]. Today a number of chemicals obtained from plants are used as vital drugs in more countries in the world (Debnath *et al.,* 2006) [2]. The secondary metabolites from plants are referred to as phytochemicals which are naturally occurring and biologically active compounds that have the potential to prevent diseases, (Prabakaran, 2014) [18]. Almost all the medicinal plants available in the world have great potential sources for discovery as well as protection of new drugs of benefit to mankind. Presently, there is a lot of approaches available to reach for new biologically active ingredients in the medicinal plants for the preparation of safe drugs. Scientifically many works have been expended to evaluate and discover new antioxidant, antimicrobial and antifungal ingredients from different kinds of natural sources like soil, microorganisms, animals, and plants. The systematic screening of these available traditional herbs may result in the discovery of novel effective bioactive compounds for the formulation of drugs (Nagesh *et al.,* 2012 and Manickam and Mohan, 2014) [11].
The use of FT-IR spectroscopy demonstrates that this technique is a valuable group belonging to tissue or plants components, such as membranes, proteins, nucleic acids, polysaccharide components as well as for complex biological materials such as plants, tissues, body fluids or cell cultures. It is one of the most widely used methods to identify the chemical constituents and elucidate the structural compounds and has been used as a requisite method to identify medicines in many countries. However, some adulterants come out in the medicinal market along with the high-value medicinal materials (Helm *et al.,* 1991; Janakiraman *et al.,* 2011) [6, 7].
The garlic (*Allium sativum L.)* as a cultured plant, suggesting that its uses as a spice and medicine. (Hahn, 1996; Groppo *et al.,* 2002) [23, 5]. In 1858, Pasteur noted garlic's antibacterial activity, and it was used as an antiseptic to prevent gangrene during World War I and World War II (Murray, 1995; Koch and Lawson, 1996) [13, 10]. The garlic has been used around the world to treat many conditions, including hypertension, infections, snakebites, toxic and some cultures have used it to ward off evil spirits. Currently, garlic is used for reducing cholesterol levels and cardiovascular risk, as well as for its antineoplastic and antimicrobial properties (Koch and Lawson, 1996) [10].
Garlic can rightfully be called one of the nature's wonderful plants with healing power. It can boost the immune system to fight off potential disease and maintain health (Abdullah *et al.,* 1988) [22]. It has the ability to stimulate the lymphatic system which expedites the removal of waste products from the body. It is also considered an effective antioxidant to protect cells against free radical damage. It can help to prevent some forms of cancer, heart disease, strokes and viral infections. Garlic alone can provide us with over two hundred unusual chemicals that have the capability of protecting the human body from a wide variety of diseases. The sulfur-containing compounds found in garlic afford the human body with protection by stimulating the production of certain beneficial enzymes (Mansell and Reckless, 1991) [24]. The garlic has great potential sources for discovery as well as protection of new drugs of benefit to mankind.
Garlic contains sulfur-containing compounds. Alliin is converted to the anti-microbial active alliin when the bulb is cut or bruised. Ajoene which is a secondary degradation product of alliin is presumably the most active compound responsible for the antithrombotic activity of garlic (Padayatty *et al.,* 2003) [15]. Garlic has also been shown to have a productive nature against gastrointestinal neoplasias, against blood clots (antiplatelet action) due to part to the compounds alliin and ajoene, which have fibrinolytic activity, (Rajam *et al*., 2012) [9].

Characterization of secondary metabolites fingerprint by chromatography and spectroscopy provide valuable information about qualitative and quantitative formulation of plant species and their pattern of recognition by chemometry similarly, evaluation of plants by metabolomic fingerprinting can be accomplished by effective analytical tools such as HPLC with UV (DAD), ELSD, MS detection or GC-MS, HPLC densitometry, FT-MIR, NIR, NMR or a combination of these. The UV-visible spectroscopy offers a simple, technique to identify the main phytochemicals, discriminating between the lipophilic and hydrophilic molecules in relation to the polarity. Fourier transforms infrared spectroscopy is a high-resolution analytical technique to identify the chemical constituents and elucidate the structure of compounds (Hashinoto and Kameoka, 2008, Hussian *et al.*, 2009) [17]. FTIR offers a rapid and nondestructive investigation to fingerprint plant extract or powders, therefore the present study focus on to evaluate the functional group of garlic by using the novel technique of FTIR.

Materials and Methods

Preparation of Garlic Extract

The garlic skin was removed and it was cut into fine pieces and kept for shade-dried (at 25ºC) and were powdered in the mechanical grinder, 20gm of garlic powdered was weighed; 150ml of solvent was added and kept for 3 days. The extract was filtered using Whatman (No: 1) filter paper and the supernatant was collected. The residue was again extracted two times (with 3 days of the interval) and the supernatant was collected. The supernatant were pooled and evaporated (at room temperature 28±1ºC). The garlic powder with solvent ethanol were prepared and stored in air tight bottles for subsequent analysis

Fourier Transform infrared Spectroscopy (FTIR)

It is the most powerful tool for identifying the types of chemical bonds or functional groups present in the phytochemicals. The wave length of light absorbed and the salient feature of the chemical bonds can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a compound can be determined. The dried powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample disc. The powder sample of each extract was loaded in FTIR spectroscope (Shimadzu, japan) with a scan range from 400 to 4000cm^{-1} with a resolution of 4cm^{-1}.

Result

In the present study, the biochemical content of garlic was investigated using FT-IR spectroscopy by monitoring different functional groups. Figure 1 shows the representative FT-IR spectra obtained from the garlic in the 4000–400 cm^{-1} region. The frequency ranges from 3423.78 cm^{-1} peaks are represents the O-H stretching vibration, the presence of carbohydrate and amino acids. The frequencies are 2977.78 cm^{-1} and 2937.40 cm^{-1} peaks were represents the C-H stretching mainly: lipids. (Table.1) The frequency ranges from 1571.26 cm^{-1} peaks are represents the Amide I: C=O stretching mainly: proteins and the frequency range from 1424.21 cm^{-1} peaks are represents the CH_2 bending vibration mainly: lipids. Then the frequency ranges from 1374.70 cm^{-1} peaks are represents the C=S stretching mainly: sulfur compounds. The frequency ranges from 1298.26 cm^{-1} peaks are represents the C-N stretching mainly: amino acids.

Table 1: Shows the FT-IR frequency range and functional groups present in the Garlic Powder

Wave number in cm^{-1}		Vibrational Assignment
Peak no.	Garlic	
1.	3423.78	O-H stretching vibration, presence of carbohydrate amino acids
2.	2977.78	C-H stretching mainly: lipids
3.	2937.40	C-H stretching mainly: lipids
4.	1571.26	Amide I: C=O stretching mainly proteins
5.	1424.21	CH_2 bending vibration mainly lipids
6.	1374.70	C=S stretching mainly: sulfur compounds
7.	1298.26	C-N stretching mainly amino acids
8.	1120.14	C-N stretching mainly; amino acids, and symmetric C-H stretching vibration presence of antioxidant enzymes
9.	1077.65	SO_3 symmetric stretching vibration, presence of acid and RSO_3 Ionic sulphones
10.	1044.28	C-N stretching mainly; amino acids
11.	1012.47	SO_3 symmetric stretching mainly sulphones compound
12.	923.78	N-H stretching of proteins
13.	879.00	C-H bending: mainly glycogen
14.	817.10	N-H stretching of proteins
15.	780.31	C-H bending: mainly glycogen
16.	649.34	C-Br stretching of alicyclic axial (Bromo compounds)
17.	620.72	C – C stretching of iodo compounds
18.	463.31	S-S stretching mainly: sulphur compounds

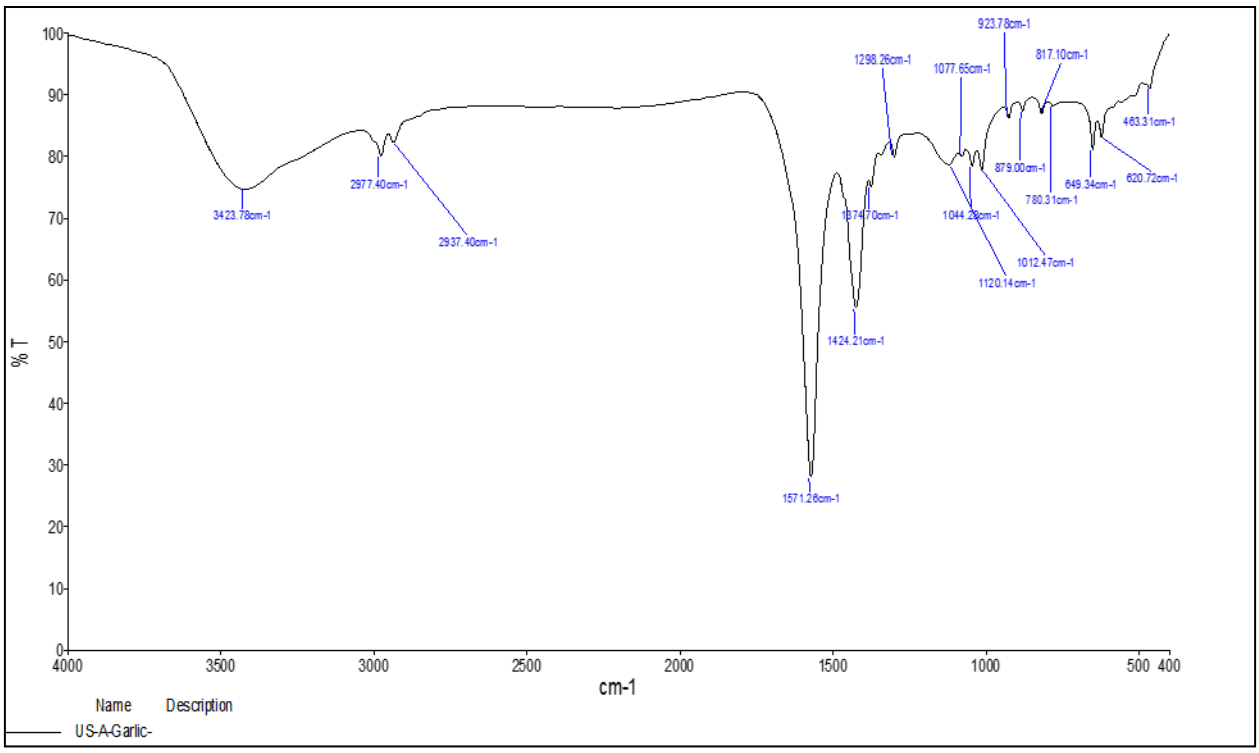

Fig 1: FT-IR Study shows the functional group of the Organic and Inorganic Compounds of the Garlic Powder.

The frequency ranges from 1120.14 cm^{-1} peaks are represents the C-N stretching mainly: amino acids, symmetric C-H stretching vibration, the presence of antioxidant enzymes and the frequency ranges from 1077.65 cm^{-1} peaks are represents the SO_3 symmetric stretching vibration, presence of acid and RSO_3 ionic sulphonates. Then frequency ranges from 1044.28 cm^{-1} peaks are represents the C-N stretching mainly: amino acids. The frequency of 1012.47 cm^{-1} peaks represents the SO_3 symmetric stretching mainly: sulphur compounds and the frequency from 923.78 cm^{-1} peaks are represents the N-H stretching of proteins. The 879.00 cm^{-1} peaks represent the C-H bending mainly: glycogen. The frequency ranges from 817.10 cm^{-1} peaks are represents the N-H stretching of proteins and 780.31 cm^{-1} peaks are represents the C-H bending mainly: glycogen Then ranges from 649.34 cm^{-1} peaks are represents the C-Br stretching of alicyclic axial (Bromo compounds). The 620.72 cm^{-1} peaks represent the C–C stretching of iodo compounds (alkyl halides) and 463.31 cm^{-1} peaks are represents the S-S stretching mainly: sulphur compounds.

Discussion

FT-IR spectroscopy is capable of providing strong insight into the structural and functional alterations induced by various factors due to its high sensitivity (Toyran *et al.*, 2005; Dogan *et al.*, 2007) [3]. FT-IR technique was used for assessment the type of organic and inorganic complexes in plants. The present study, the FTIR spectroscopic analysis showed the presence of phyto constituents in the garlic extract. The preliminary phytochemical screening of garlic powder showed (Table.1) the presence of phytochemicals screened such as amino acids, alkaloids, carbohydrates, amines, carboxylic acid, alkenes, proteins, sulphur compounds, and lipids.

The results shows, the presence of a phytochemical compound in the extract of garlic. This was correlated with the work done by (Rajam *et al.*, 2013) [9], reported that the whole plant extracts of *Allium Sativum* (Garlic) revealed the presence of phytochemicals such as alkaloids, proteins, lipids, oils, flavanoids, gums, phenols, saponins, steroids, tannins, and terpenoids. The FTIR spectral analysis of medicinal plants such as *Eclipta alba* and *Eclipta* prostrate and reported that the very strong absorption band appearing in the region 2933–2922 cm^{-1} for whole plant parts is due to N–H stretching and also reported the presence of functional groups like carboxylic acids, amines, polysaccharides, nitrates and carbohydrate (Murugananthan *et al.*, 2009) [14].

Fourier transform infrared spectroscopy (FTIR) can provide fundamental information on the molecular structure of organic and inorganic components and is one of the most versatile analytical techniques for the non-destructive, chemical characterization of geological samples, such as coal, shale, fluid and melt inclusions, silicate glass, minerals, and microfossils (Iglesias *et al.*, 2011; Angelo and Zodrow, 2011) [8]. The underlying mechanism of the FTIR technique is associated with transitions between quantized vibrational energy states (Griffiths and Haseth, 2007) [4]. In FTIR analysis, absorption of IR radiation occurs when a photon transfers to a molecule and excites it to a higher energy state (Parikh and Chorover, 2005) [16]. The crude powder and dry ethanolic extracts of *Albizia lebbeck* leaves. FTIR analysis results proved the presence of alcohols, phenols, alkanes, carboxylic acids, aromatics, ketones, and alkyl halides (Bobby *et al.*, 2012) [1]. The more intense bands occurring at O–H/N–H, C– H, C–O, and C–CI/C–CS stretching/bending vibrations respectively indicate the presence of amino acids, alkenes, nitrates, ethers, organic halogen compounds and

carbohydrates in plants (Manoj and Ragothaman, 1999).
Lu *et al.* (2004) [11] used this method to identify the species in Hypericum and Triadenum (Gorgulu *et al.* 2007) [3] revealed FTIR spectroscopy could be successfully applied to differentiate genera *Ranunculus*, Astragalus and Acantholimon. Zavoi *et al.*, (2011) [21] investigated hepatoprotective action using FTIR analysis of the polyphenolic composition of medicinal herbs viz., Cynara scolimus, Taraxacum officinalis, Chelidonium majus, Hypericum perforatum, Silybum marianu and Lycopodium clavatu) from the wild flora of Romania. Recently, Csernatoni *et al.*, (2013) [20] applied FTIR screening to characterize and identify the main biomarkers of food supplement PROMEN by analysis of plant ingredients comparatively with the final product.

Conclusion

The result in the present study showed that FTIR spectroscopy is a valuable technique to fingerprint and to analyze the different biomolecules from garlic extract which contains 8 peaks. Based on the peak values more functional groups were obtained from the garlic. In order to validate the FTIR method as a good tool to investigate fingerprint and to predict the composition of the garlic and to evaluate the quality and its detoxifying capacity of garlic.

References

1. Bobby MA, Wesely EG, Johnson M. FT-IR studies on the leaves of Albizia lebbeck Benth. Int J Pharm Pharm Sci. 2012; 4(3):293-6.
2. Debnath Malik CP, Bisen PS. Micropropagation M. a tool for the production of high-quality plant-based medicines. Curr Pharm Biotechnol. 2006; 7:33-49.
3. Gorgulu ST, Dogan M, Severcan F. The characterization and differentiation of higher plants by Fourier transform infrared spectroscopy. Appl Spectrosc. 2007; 61:300-308.
4. Griffiths PR, de Haseth JA. Fourier Transform Infrared Spectrometry, 2nd ed.; John Wiley & Sons, Inc.: New York, NY, USA, 2007.
5. Groppo FC, Ramacciato JC, Simoes RP, Florio FM, Sartoratto A. Antimicrobial activity of garlic, tea tree oil, and chlorhexidine against oral microorganisms. Int Dent J. 2002; 52:433-7.
6. Helm D, Labischinski H, Schallehn G, Naumann D. Classification and identification of bacteria by Fourier transform infrared spectroscopy. J Gen. Microbiol. 1991; 137:69–79.
7. Janakiraman N, Sahaya Sathish S, Johnson. M. UV-VIS and FTIR Spectroscopic Studies on Peristrophe bicalyculata (Retz.) Nees. Asian Journal of Pharmaceutical and Clinical Research. 2011; 4(4):125-129.
8. Iglesias MJ, Jiménez A, Laggoun-Défarge F, Suárez-Ruiz I. FTIR study of pure vitrains and associated coals. Energy Fuels. 1995; 9:458-466.
9. Rajam k, Rajendran S, Saranya R. Allium Sativum (Garlic) Extract as Nontoxic Corrosion Inhibitor Hindawi Publishing Corporation Journal of Chemistry Volume Article ID 743807, 2012; 4. http://dx.doi.org/10.1155 /2013/743807.
10. Koch HP, Lawson LD. Garlic the science and therapeutic application of Allium sativum L. and related species. 2d ed. Baltimore: Williams & Wilkins, 1996.
11. Lu HF, Cheng CG, Tang X, Hu ZH. FTIR spectrum of Hypericum and Triadenum with reference to their identification. J Integr Plant Biol. 2004; 46:401-406.
12. Manickam, Veerabahu, Ramasamy Mohan. Phytochemical, FT-IR and antibacterial activity m M of whole plant extract of Aerva lanata (L.) Juss. Ex. Schult. Journal of Medicinal Plants Studies Year. 2014; 2(3):51-57. ISSN: 2320-3862.
13. Murray MT. The healing power of herbs: the enlightened person's guide to the wonders of medicinal plants. 2d ed. Rocklin, Calif: Prima, 1995.
14. Muruganantham S, Anbalagan G, Ramamurthy N. FT-IR and SEM-EDS comparative analysis of medicinal plants, Eclipta alba Hassk and Eclipta prostrata Linn. Rom J Biophys. 2009; 19:285-94.
15. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee JH. Vitamin C as an antioxidant: evaluation of its role in disease prevention. J Am CollNutr. 2003; 22:18-35.
16. Parikh SJ, Chorover J. FTIR spectroscopic study of biogenic Mn-oxide formation by Pseudomonas putida GB-1. Geomicrobiol. J. 2005; 22:207-218.
17. Hashimoto A, Kameoka T. Applications of infrared spectroscopy to biochemical, food, and agricultural processes. Applied Spectroscopy Reviews. 2008; 43:416-451.
18. Hussain K, Ismail Z, Sadikun A, Ibrahim P. Evaluation of Metabolic Changes in Fruit of *Piper armentosum* in various Seasons by Metabolomics using Fourier Transform Infrared (FTIR) Spectroscopy.International Journal of Pharmaceutical andClinical Research. 2009; 1(2):68-71.
19. Prabakaran S. Hepatoprotective Efficacy of Pisonia Alba and *Cardiospermum halicacabum* and their Antioxidant Potential in the freshwater fish *Labo rohita* exposed to atrazine. The thesis of Annamalai University, 2004.
20. Csernatoni F, Socaciu C, Pop RM, Fetea F, Bunghez F. Application of FT-IR Spectroscopy for Fingerprinting Bioactive Molecules in a Nutraceutical PROMEN, comparatively with Plant ingredients. Bulletin UASVM Food Science and Technology. 2013; 70(1):68-69.
21. Zavoi S, Fetea F, Ranga F, Pop RM, Baciu A, Socaciu C. Comparative Fingerprint and Extraction Yield of Medicinal Herb Phenolics with Hepatoprotective Potential, as Determined by UV-Vis and FT-MIR Spectroscopy. Notulae Botanicae Horti Agrobotanici Cluj –Napoca. 2011; 39(2):82-89.
22. Abdullah TH, Kandil O, Elkadi A, Carter J. Garlic revisited: therapeutic for the major diseases of our times? J Natl Med Assoc. 1988; 80:439-445.
23. Hahn G. In: Koch HP, Lawson LD, eds. Garlic: the science and therapeutic application of Allium sativum L and related species (2nd edn). Baltimore Williams and Wilkins. 1996, 1-24.
24. Mansell P, Reckless J. Effects on serum lipids, blood pressure, coagulation, platelet aggregation, and vasodilation. BMJ. 1991; 303:379-380.