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RESEARCH ARTICLE

FTIR SPECTRAL ANALYSIS OF THE PHYTOCONSTITUENTS IN THE MACE OF *MYRISTICA FRAGRANS* FOR THE DETECTION OF POSSIBLE ADULTERATION WITH THE MACE OF *MYRISTICA MALABARICA* AND *MYRISTICA BEDDOMII*

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ABSTRACT

Myristica fragrans is an evergreen tree commercially cultivated for their valuable essential oils present in the mace and nutmeg. India is a leading producer and exporter of these spices. Unfortunately, the Indian market and world market markets have been flooded with the mace and nutmeg obtained from their wild relatives with inferior qualities. There should be some checks for the detection of adulterants from the original one. The present investigation explores the possibility of utilizing FTIR spectral analysis for detecting the possible adulteration. The results indicate that a set of biochemical markers which are unique in the mace of *Myristica fragrans* can be used for the detection of original mace from adulterant.

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INTRODUCTION

*Myristica fragrans*is an evergreen tree belonging to the family Myrtaceae, indigenous to the Moluccas in Indonesia but it has been successfully grown in other Asian countries. The species has been cultivated in many parts of the world for their nutmeg and mace rich in valuable essential oils. Nutmeg contains about 10% essential oils chiefly composed of terpene hydrocarbons (sabinene and pinene), myrcene, phyllandrene, camphene, limonene, terpinene, pcymene and other terpnene derivatives (Qiu *et al.,* 2004; Yang, 2008; Jaiswal, 2009). The various pharmacological activities like hepatoprotective, anti-oxidant, memory enhancing, cytotoxicity, aphrodisiac, anti-diabetic, anti-depressant, anti-microbial, anti-inflammatory, anti-carcinogenic, hypolipidemic and hypocholesterolemic effect are due to the presence of these secondary compounds in nutmeg and mace. Mace is technically the dry, outer aril that firmly enveloping around the nutmeg kernel. It is a strong, aromatic substance and warm in taste. Nutmeg and mace are two separate spice products of same nutmeg fruit. However, mace has higher concentration of certain essential oils with intense aroma than nutmeg.

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Mace aril contains more vitamin A, vitamin C, calcium, copper, iron and magnesium than nutmeg. Mace oil has been used in dentistry for toothache relief and it is used a local massage to reduce muscular pain and rheumatic pain of joints. Indonesia and Grenada are the dominant producers and exporters of nutmeg and mace and India is also a leading producer. However, Indian market and world markets have been flooded with adulterants of nutmeg and mace. The Indian mace is adulterated with the mace of *Myristica malabarica*, commonly called Bombay mace. While in other countries, the mace of *Myristica argentea* is used as an adulterant of the mace of *Myristica fragrans*. Fourier-Transform Infra-Red Spectrometry (FTIR) is an analytical technique is used for the quality analysis in agro-food products (Downey, 1998). It focuses on the MIR region of (4000-400cm^{-1}) of the electromagnetic spectrum and monitors the fundamental vibrational and rotational stretching modes of molecules, which produce a chemical profile of the sample (Sun, 2009). The biological molecules can be identified based on the characteristic vibration of their functional groups. Most of the relevant information that is used to interpret MIR spectra is usually obtained from the functional group region. This molecular fingerprinting can be used as a reliable technique for the detection of adulteration in food products (Brindet *et al.,* 1996; Herringshaw, 2009). The present investigation explores the possibility of utilizing FTIR for detecting the possible adulteration in the mace of *Myristica fragrans*.

MATERIALS AND METHODS

The mature fruits of *Myristica fragrans*, *Myristica malabarica* and *Myristica beddomii* were collected from their natural habitats and the mace (aril) was collected from the seeds. The aril was surface sterilized with 2.5% sodium hypochlorite for 30 minutes and washed extensively with distilled water.A sample of ten replicates of mace from each treatment was taken for dry weight and moisture content analysis. 5g of mace from each treatment was chopped and ground in organic solvents such as chloroform, methanol, methanol (50%) using mortar and pestle. The homogenized sample was centrifuged and the supernatant was taken. The organic solvents were evaporated in a rotary evaporator and the sample was dispersed in dry potassium bromide. The mixture was mixed in a mortar and pressed at pressure of six bars to form a KBr thin disc. The disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectra were obtained using IRPrestige-21, Shimadzu Infrared spectrophotometer. The sample was scanned from 400 to 4000cm^{-1} for sixteen times to increase the signal to noise ratio. Samples were run in triplicate and data were collected within one day (Manigandan *et al.,* 2015; Marimuthu and Gurumoorthi, 2013).

Statistical analysis

All statistical analyses were carried out using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The biomass and moisture content of mace showed significant differences among *Myristica fragrans*, *Myristica malabarica* and *Myristica beddomii* (Table 1). The biomass was higher in *Myristica malabarica* (72.39%) followed by *Myristica beddomii* (57.16%) and *Myristica fragrans* (52.06%). The mace was reddish in *Myristica fragrans* but yellow in *Myristica malabarica* and yellowish brown in *Myristica beddomii*. Considerable differences have been observed in the seed shape in which the seeds were small and round in *Myristica fragrans* while medium seized and round in *Myristica beddomii* and oblong in *Myristica malabarica.* The FTIR spectral analysis of phytochemicals in the mace of *Myristica fragrans*, *Myristica malabarica* and *Myristica beddomii* has been shown (Fig. 1-9; Table 2-3). The chloroform extract of the mace of *Myristica fragrans* showed the presence of alkanes, aromatic rings, aldehydes, quinones, esters and lactones, carboxylic acids and salts, ammonium salts, alcohols, primary/secondary/tertiary alcohols, phenols, carbohydrates, ethers, primary/secondary amines, nitriles, sulphides, disulphides, sulphur oxy compounds, peroxides, organic phosphates, aromatic phosphates, aliphatic phosphates, fluoro/chloro/bromo/iodo compounds, silicon oxy compounds, aromatic nitro compounds and aliphatic nitro compounds. However, the chloroform extract of the mace of *Myristica malabarica* showed the presence of alkanes, aldehydes, esters and lactones, carboxylic acids and salts, alcohols, carbohydrates, ethers, primary/secondary amines, sulphides, sulphur oxy compounds, peroxides, organic phosphates, aromatic phosphates, aliphatic phosphates, fluoro/chloro/bromo/iodo compounds, silicon oxy compounds, aromatic nitro compounds and aliphatic nitro compounds.

Plate 1. Left - Mature fruit of *Myristicamalabrica*,
Right – Fruit splits open to expose arillated seed. Yellow aril (mace) is an adulterant of common valuable mace of *Myristicafragrans*

Plate 2. Oblong seeds of *Myristicamalabarica*, an adulterant of common nutmeg of *Myristicafragrans*

Plate 3. Mature fruit of *Myristicabeddomii*

Plate 4. Fruit splits open to expose the arillated seed of *Myristicabeddomii*

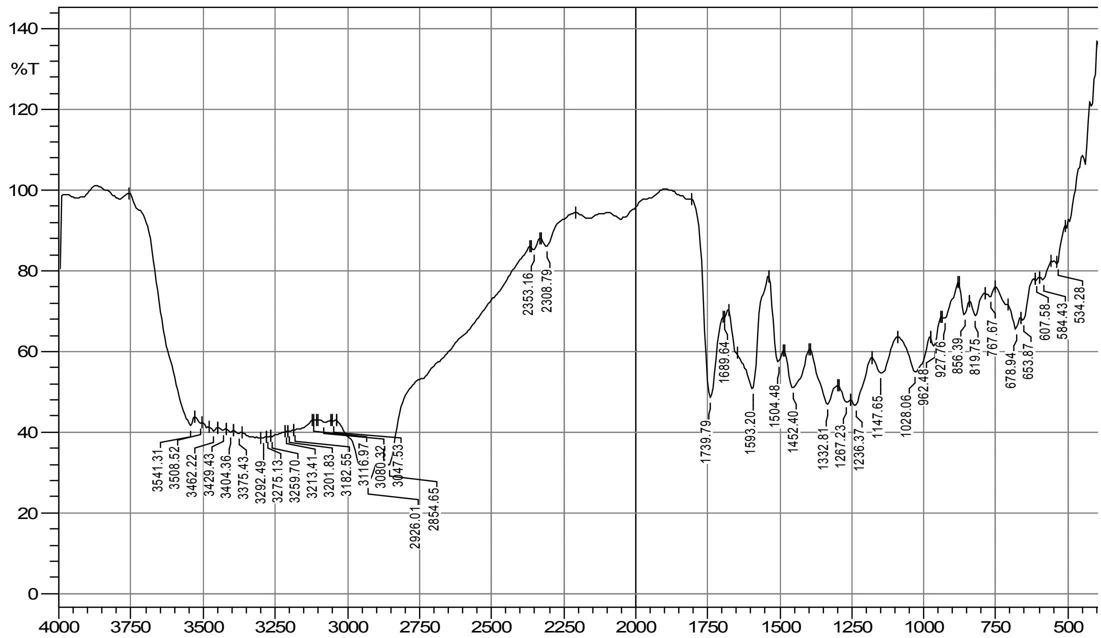

Fig. 1. FTIR analysis of mace of *Myristica fragrans* (chloroform extract)

The chloroform extract of the mace of *Myristica beddomii* showed the presence of alkanes, aromatic rings, quinones, esters and lactones, carboxylic acids and salts, ammonium salts, alcohols, carbohydrates, ethers, primary/secondary amines, nitriles, sulphur oxy compounds, organic phosphates, aromatic phosphates, aliphatic phosphates, fluoro/chloro compounds, silicon oxy compounds, aromatic nitro compounds and aliphatic nitro compounds.

The mace of all these species showed similarity in most of phytochemicals such as alkanes, esters and lactones, carboxylic acids and their salts, alcohols, carbohydrates, ethers, primary/secondary amines, sulphur oxy compounds, organic phosphates, aromatic phosphates, aliphatic phosphates, fluoro/chloro compounds, silicon oxy compounds, aromatic nitro compounds and aliphatic nitro compounds. The mace of *Myristica fragrans* showed difference with the mace of

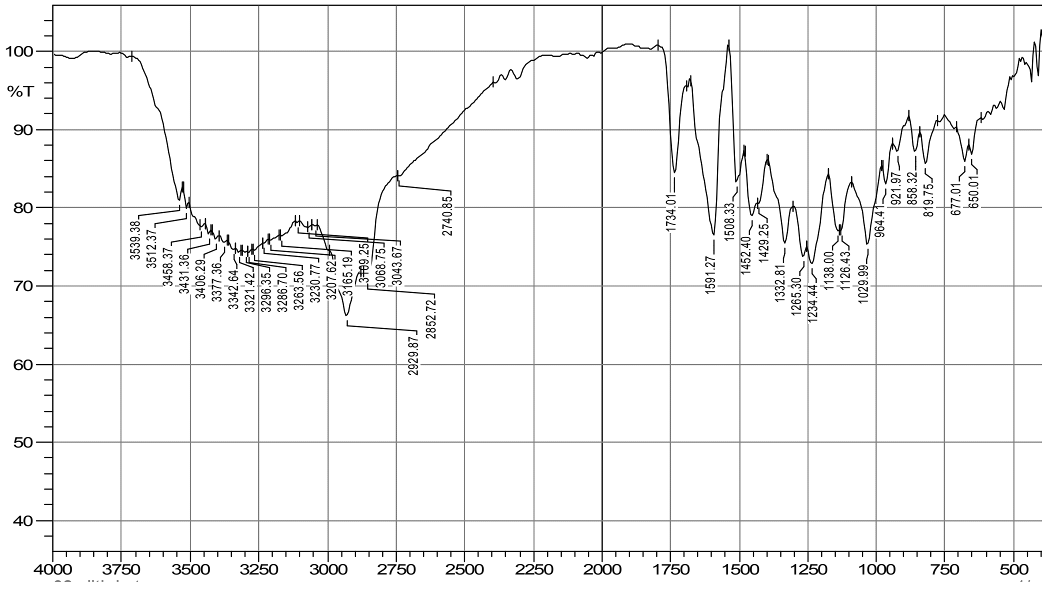

Fig. 2. FTIR analysis of aril of *Myristica fragrans* (50%methanol extract)

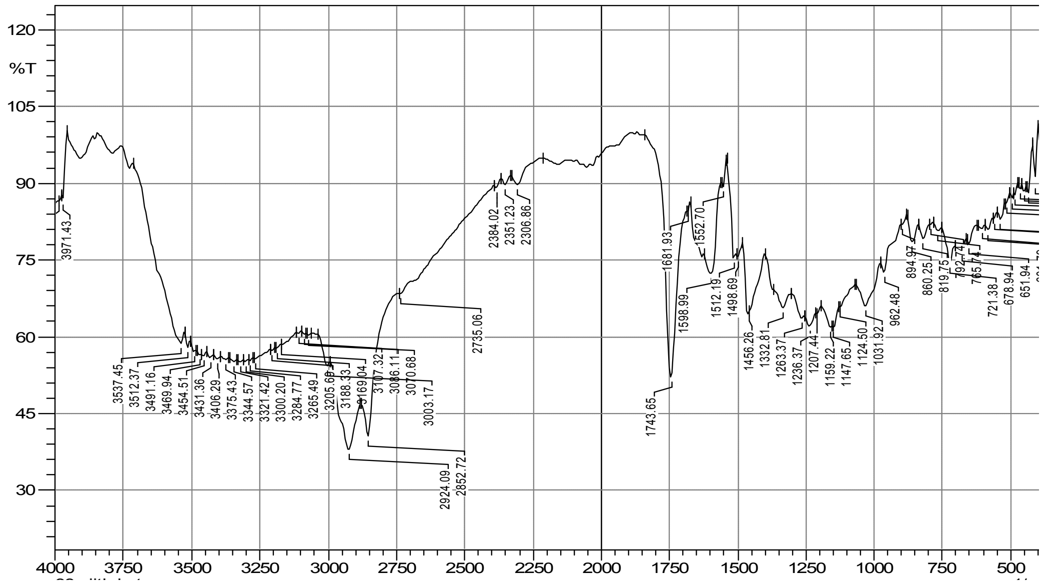

Fig. 3. FTIR analysis of mace of *Myristica fragrans* (methanol extract)

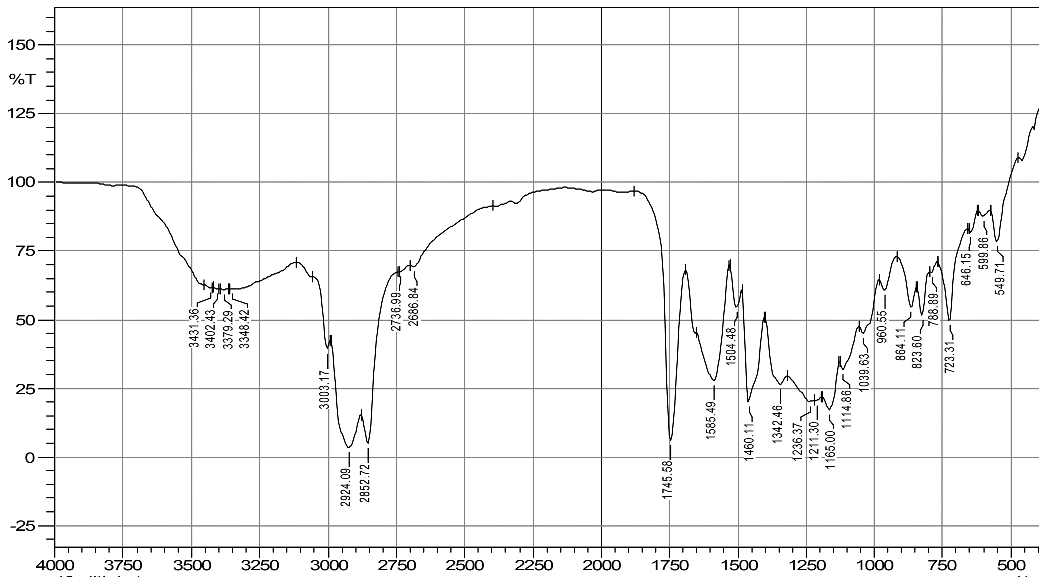

Fig. 4. FTIR analysis of mace of *Myristica malabarica* (chloroform extract)

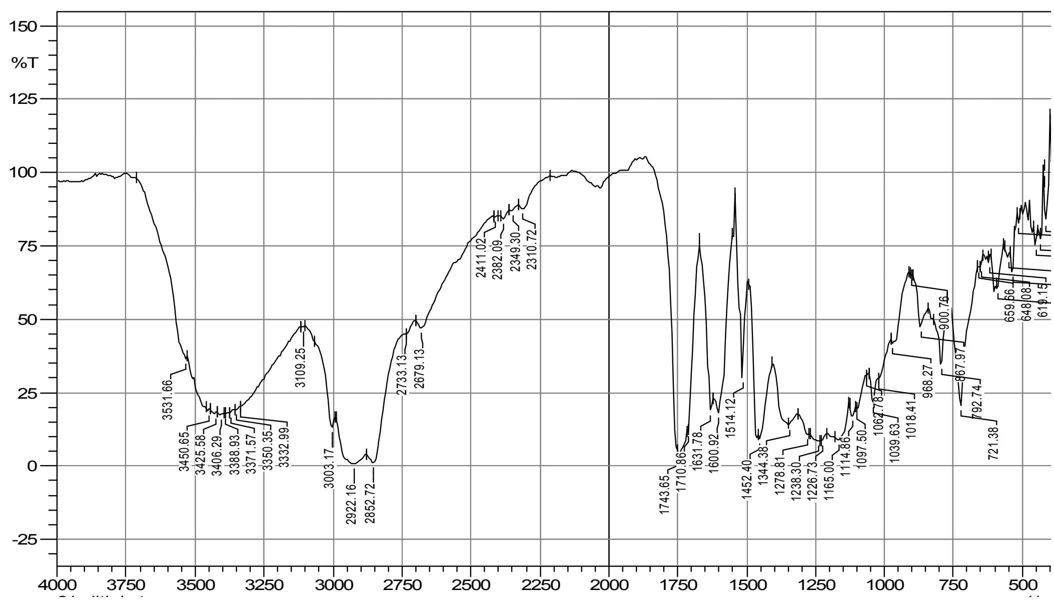

Fig. 5. FTIR analysis of mace of *Myristica malabarica* (methanol extract)

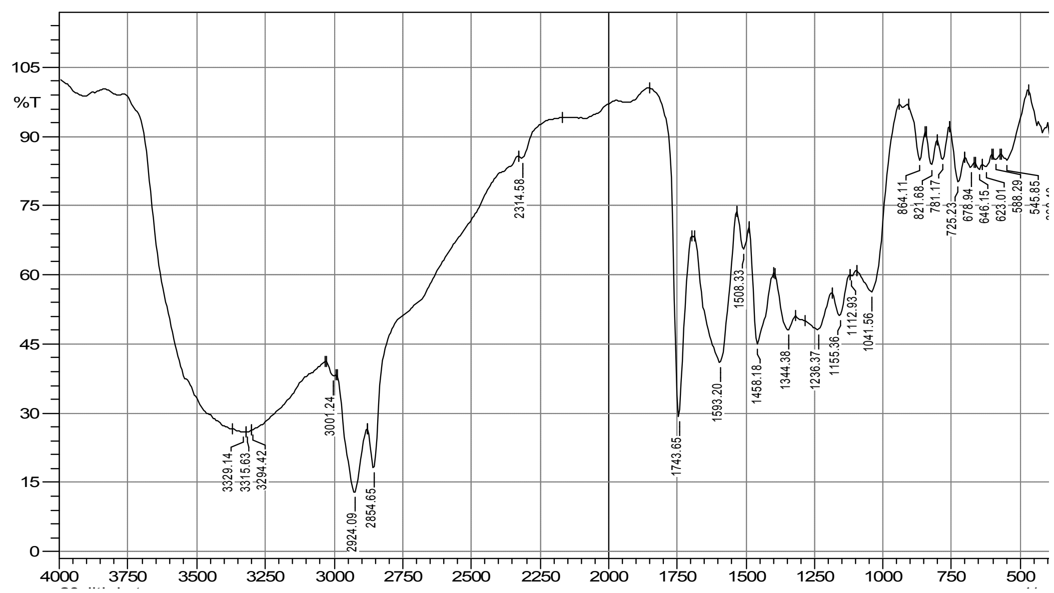

Fig. 6. FTIR analysis of mace of *Myristica malabarica* (50%methanol extract)

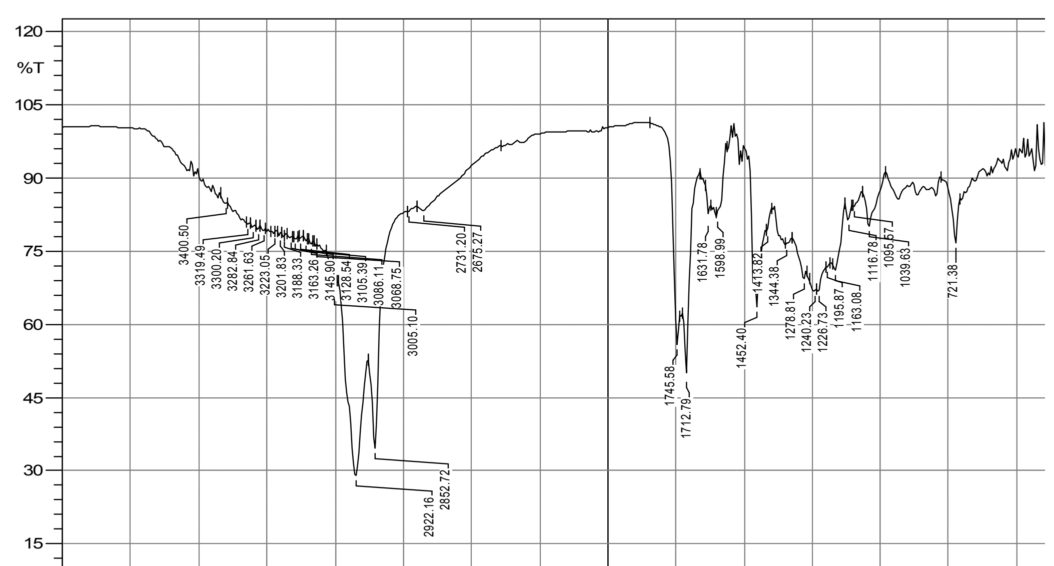

Fig. 7. FTIR analysis of mace of *Myristica beddomii* (chloroform extract)

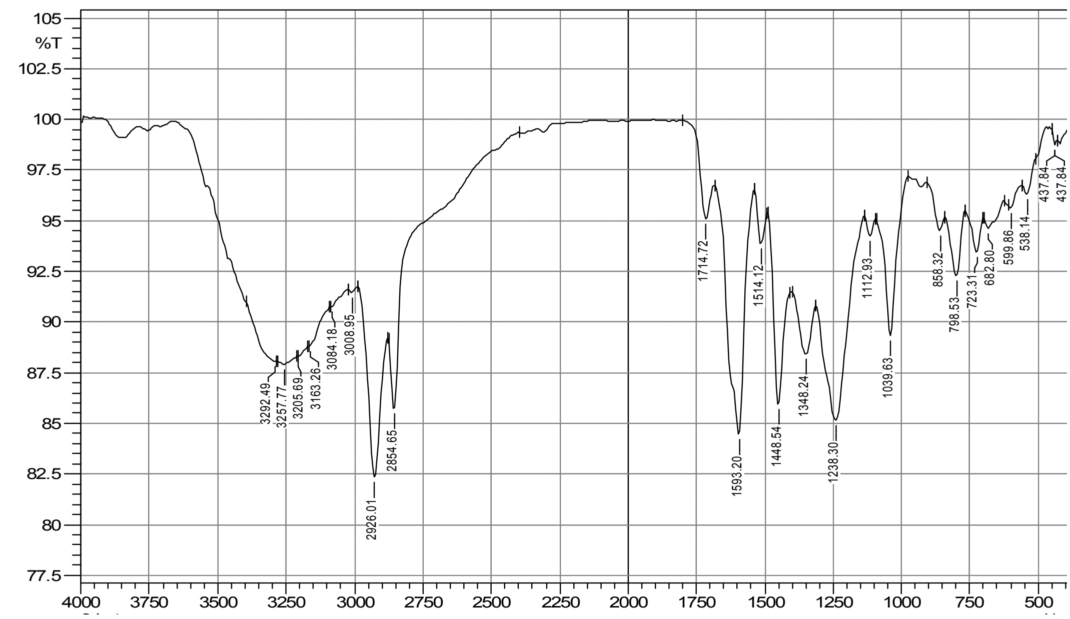

Fig. 8. FTIR analysis of mace of *Myristica beddomii* (50%methanol extract)

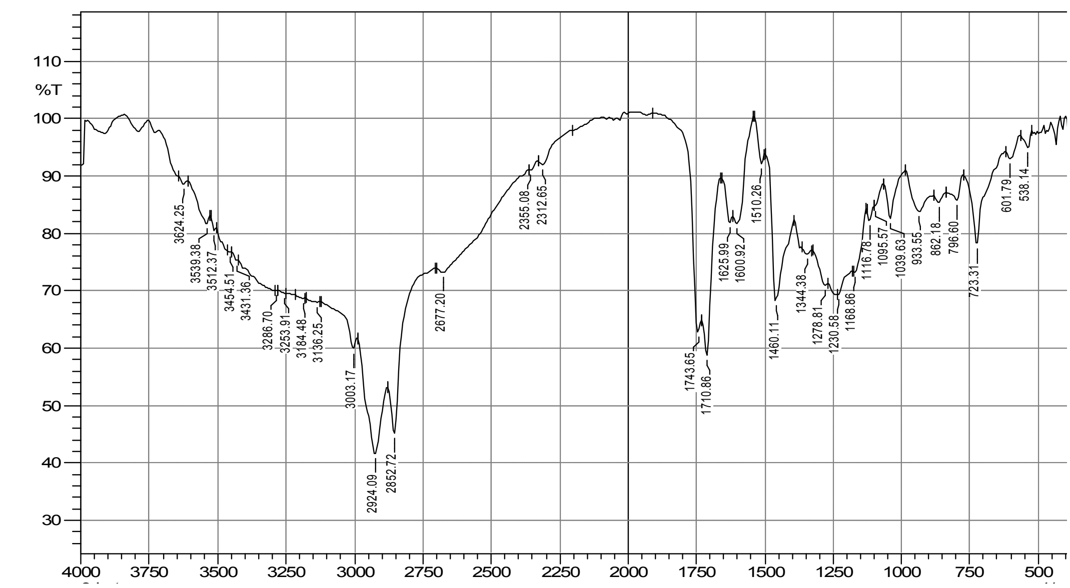

Fig. 9. FTIR analysis of mace of *Myristica beddomii* (methanol extract)

*Myristica malabarica*in the presence of certain phytochemicals such as aromatic rings, quinones, ammonium salts, primary/ secondary/tertiary amines, phenols and primary/secondary amines. However, it showed difference with the mace of *Myristica beddomii* in the presence of aldehydes, primary/secondary/tertiary alcohols, phenols, sulphides, disulphides, peroxides and bromo/iodo compounds. The results indicate that the mace of *Myristica fragrans* is characterized by a unique set of biochemical markers such as aromatic rings, aldehydes, quinones, ammonium salts, phenols, primary/secondary/tertiary alcohols, nitriles, sulphides, disulphides, peroxides and bromo/iodo compounds which can be taken as a fingerprint for the identification of possible adulteration. The methanolic extract of the mace of *Myristica fragrans* showed the presence of alkanes, alkenes, aromatic rings, quinones, esters and lactones, carboxylic acid and their salts, ammonium slats, amides, phenols, carbohydrates, ethers, primary/secondary amines, nitriles, sulphides, disulphides, polysulphides, sulphur oxy compounds, peroxides, organic phosphates, aromatic phosphates, aliphatic phosphates, fluoro/chloro/bromo/iodo compounds, silicon oxy compounds, aromatic nitro compounds and aliphatic nitro compounds (fig 1-9; table 2-3). A specific set of phytochemicals such as alkenes, aromatic rings, aldehydes, amides, sulphides and polysulphides which can be taken as a unique fingerprint of the mace of *Myristica fragrans* so that the adulteration can be detected.

Conclusions

The following conclusions are drawn from the present investigation

- FTIR spectral analysis can be used for molecular fingerprinting of food stuff so as to detect possible adulteration.
- The mace of *Myristica fragrans* can be identified from the mace of adulterants due to their colour and biomass.
- The mace of *Myristica fragrans* is characterized by a unique set of biochemical markers such as aromatic rings, aldehydes, quinones, ammonium salts, phenols, primary/secondary/tertiary alcohols, nitriles, sulphides,

disulphides, peroxides and bromo/iodo compounds which can be taken as a fingerprint for the identification of original sample from the adulterants.

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