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Research Article

PHYTOCHEMICAL PROFILING OF SELECTED SPICES (*FERULA ASAFOETIDA, PIPER NIGRUM, ELETTARIA CARDAMOMUM, CINNAMOMUM AROMATICUM* AND *SYZGIUM AROMATICUM*) EXTRACT

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ABSTRACT

Spices are a promising source of bioactive compounds since it has been traditionally used for the treatment of various diseases. The present study was aimed at evaluating the phytochemical constituents of different spices extracts by qualitative phytochemical analysis, GC-MS and ATR. The results showed that the used (distilled water) solvent took an important role in the extraction of phytochemical components. Aqueous extract was identified as the most effective solvent for the extraction, resulting in the highest content of polyphenolic compounds in all the spices.

Keywords: Phytochemical constituents, Spices, GC-MS, ATR.

INTRODUCTION

Plants produce secondary metabolites mainly as the products of primary metabolism and as part of the defence mechanisms of plants (Saxena, 2013). The secondary metabolites produced by plants are phytochemicals such as, alkaloids, carbohydrates, glycosides, phenols, triterpenoids, *etc*., which provide their healing properties (Bhandary *et al*., 2012). Saxena, (2013) indicated that the phenolic compounds do possess antioxidant activity due to their free radical scavenging activities. Recent research revealed the significance of dietary intake of antioxidants in the protection of human health. As much as and above 4,000 phytochemicals have been cataloged (Meagher *et al*., 1999) and are classified based on their protective function and physical characteristics (Costa *et al*., 1999; Meagher *et al*., 1999) and (Saxena, 2013)reported that nearly 150 phytochemicals have been studied in detail by many researchers. Fruits, vegetables, seeds, legumes, grains, herbs, nuts and spices are found to possess a wide-range of dietary phytochemicals (Mathai, 2000). Harvey, (2000) indicated the accumulation of phytochemicals in different parts of plants such as root, stem, leaves, seeds, buds and flowers *etc*. The outer layer of various plant tissues concentrates phytochemicals particularly as the pigment molecules. Supplementary forms containing the phytochemicals lacks the evidence of having the same health benefits as dietary phytochemicals (Balasundram *et al*., 2006; Tapas *et al*., 2008; Saxena, 2013). Due to the above mentioned facts, it was planned to assess the phyto-constituents of the selected spice extracts.

MATERIALS AND METHODS

Selection of Indian Spices

Indian spices *viz*., asafoetida (*Ferula asafoetida*), black pepper (*Piper nigrum*), cardamom (*Elettaria cardamomum*), cinnamon (*Cinnamomum aromaticum*) and clove (*Syzgium aromaticum*) were selected based on the literature survey about their medicinal properties. The spices were purchased from supermarket and used for the present study.

Aqueous Extract Preparation of Spices

About 20 g of each spices *viz*., *F. asafoetida*, *P. nigrum*, *E. cardamomum*, *C. aromaticum* and *S. aromaticum* were crushed to powder with kitchen blender and the fine powder was mixed with 100 ml of distilled water and boiled in a water bath for 30 minutes. The boiled mixture was filtered using Whatman No. 1 filter paper and the

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filtered aqueous extract was freeze-dried and the powder was stored at -20ºC until further use. Similarly, mixed aqueous extract spice was prepared by mixing 20 g each of all the spices and mixed with 500 ml distilled water and then the mixture was boiled in a water bath for 30 minutes and the boiled mixture was filtered using Whatman No. 1 filter paper and the filtered aqueous extract was freeze-dried and the powder was stored at -20ºC until further use.

Qualitative Phytochemical Screening

Qualitative phytochemical analysis of aqueous extract of asafoetida, black pepper, cardamom, cinnamon and clove and mixed spice aqueous extract of the above-mentioned spices were subjected to preliminary phytochemical screening for its phyto-constituents according to the method of (Kokate *et al*., 2004).

GC-MS Spectral Analysis of Spice Extracts

GC-MS spectral analysis was carried out to determine the presence of aromatic compounds in all the spice extracts. The model of the GC-MS used for mass spectral identification was an Agilent 7890 interfaced to a 240-mass selective detector with ion trap. The capillary column (30 mm x 0.25 mm x 0.25 µm film thickness) was HP-5MS. The oven temperature was initially maintained at 80°C to 300°C. The carrier gas used was nitrogen (99.999%), at a flow rate of 1.0 ml/ minute, and injection volume of 1.0 µl was employed (split ratio of 10:1). The electron-impact ionization of the mass spectrometry was operated at electron energy of 70 eV. Mass spectra were taken at 70 eV, with a scan interval of 0.5 seconds and fragments from 40 to 450 Daltons. Total GC running time was 61 minutes and the interpretation on GC-MS was conducted using the database of National Institute Standard and Technology (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.

ATR Spectral Analysis of Spice Extracts

Liquid form of aqueous extracts of all the spices and mixed spice extract were subjected to ATR analysis, which were carried out in the range of 500 to 3500 cm^{-1} at a resolution of 4 cm^{-1} using Bruker (Alpha).

RESULTS AND DISCUSSION

The preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, phenols, tannins and triterpenoids in *F. asafoetida*; alkaloids, carbohydrates, glycosides, phenols and triterpenoids in *P. nigrum*; carbohydrates, flavonoids, glycosides, phenols and triterpenoids in *E. cardamomum*; alkaloids, flavonoids, glycosides, phenols, saponins and triterpernoids in *C. aromaticum*; alkaloids, flavonoids, glycosides and phenols in *S. aromaticum* and alkaloids, carbohydrates, flavonoids, phenols, saponins, tannins and triterpenoids in the mixed spices extract as shown in the Table 1.

Table 1. Qualitative phytochemical analysis of selected spices aqueous extract.

Phytochemicals	*F. asafoetida*	*P. nigrum*	*E. cardamomum*	*C. aromaticum*	*S. aromaticum*	Mixed
Alkaloids	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-
Carbohydrates	-	+	+	-	-	+
Flavonoids	+	-	+	+	+	+
Glycosides	+	+	+	+	+	-
Phenols	+	+	+	+	+	+
Quinones	-	-	-	-	-	-
Saponins	-	-	-	+	-	+
Tannins	+	-	-	-	-	+
Triterpenoids	+	+	+	+	-	+
Steroids	-	-	-	-	-	-
Phytosteroids	-	-	-	-	-	-

+ denotes presence, - denotes absence.

The results pertaining to the identification of number of compounds from the GC fractions of *F. asafoetida* aqueous extract showed 8 active phytocompounds, *P. nigrum* aqueous extract showed 11 active phytocompounds, *E. cardamomum* aqueous extract showed 15 active phyto-compounds, *C. aromaticum* extract showed 15 active phytocompounds. *S. aromaticum* aqueous extract showed about 65 active phytocompounds and mixed spices aqueous extract revealed the presence of 75 active phytocompounds. From the spectral analysis, RT, peak name, peak area of the phyto-constituents presented in spices extract were matched with NIST/NBS spectral database. The mass spectra and their mass peaks are given in Figure 1 - 6 for *F. asafoetida*, *P. nigrum*, *E. cardamomum*, *C. aromaticum*, *S. aromaticum* and mixed spices extract.

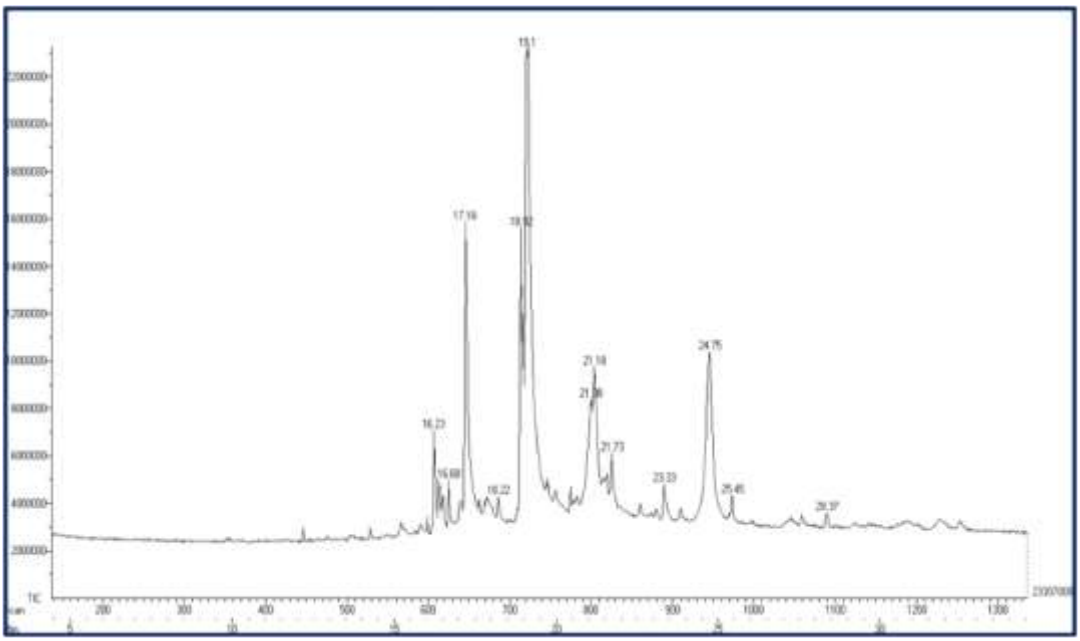

Figure 1. GC-MS chromatogram of asafoetida (*F. asafoetida*).

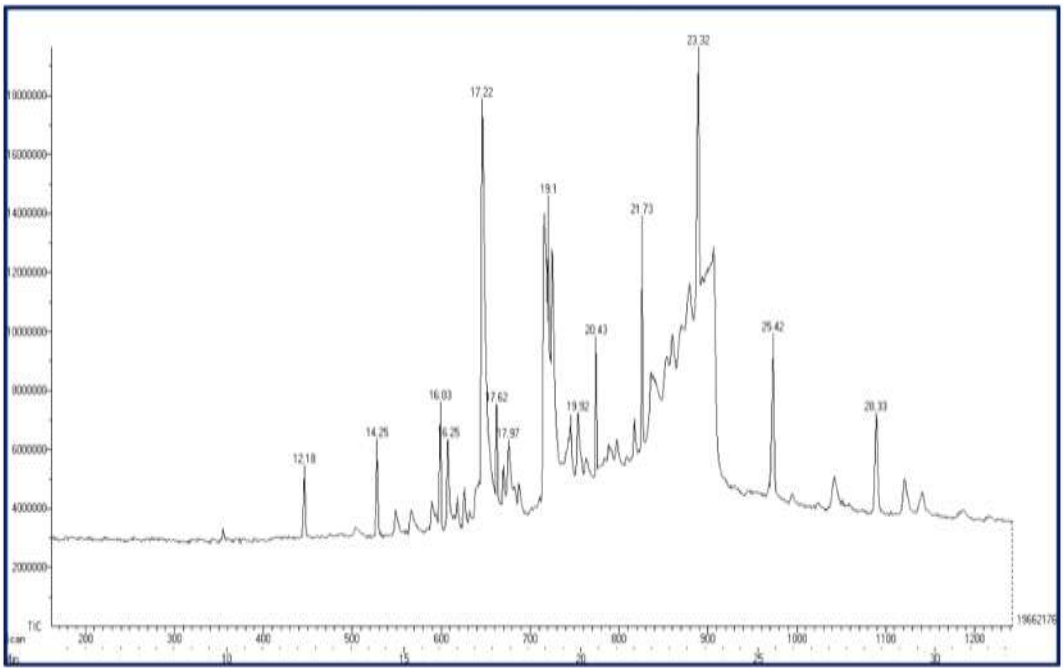

Figure 2. GC-MS chromatogram of black pepper (*P. nigrum*).

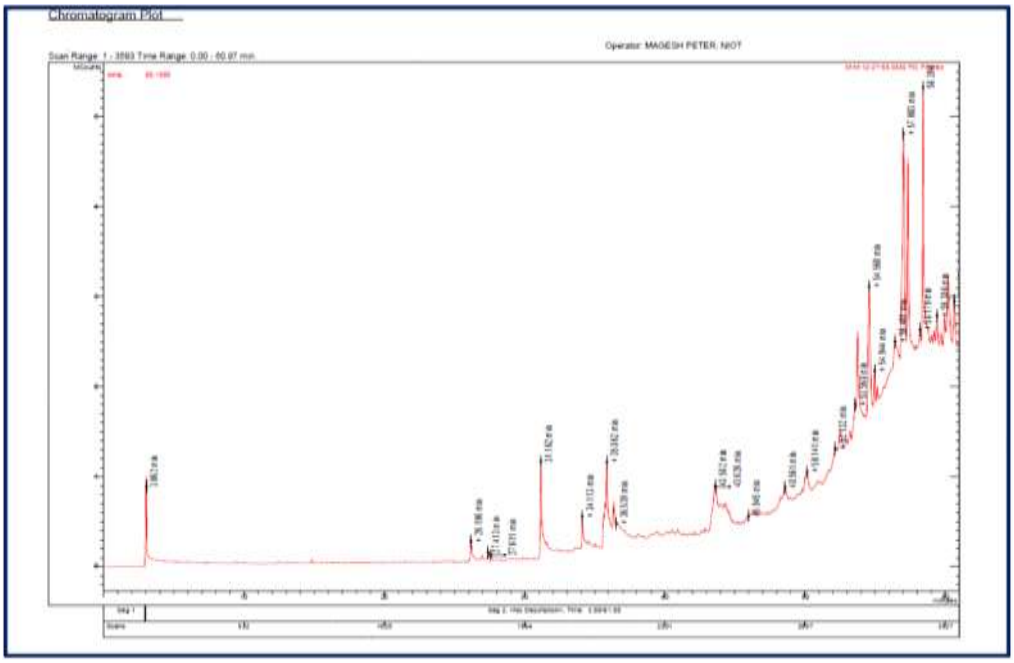

Figure 3. GC-MS chromatogram of cardamom (*E. cardamomum*).

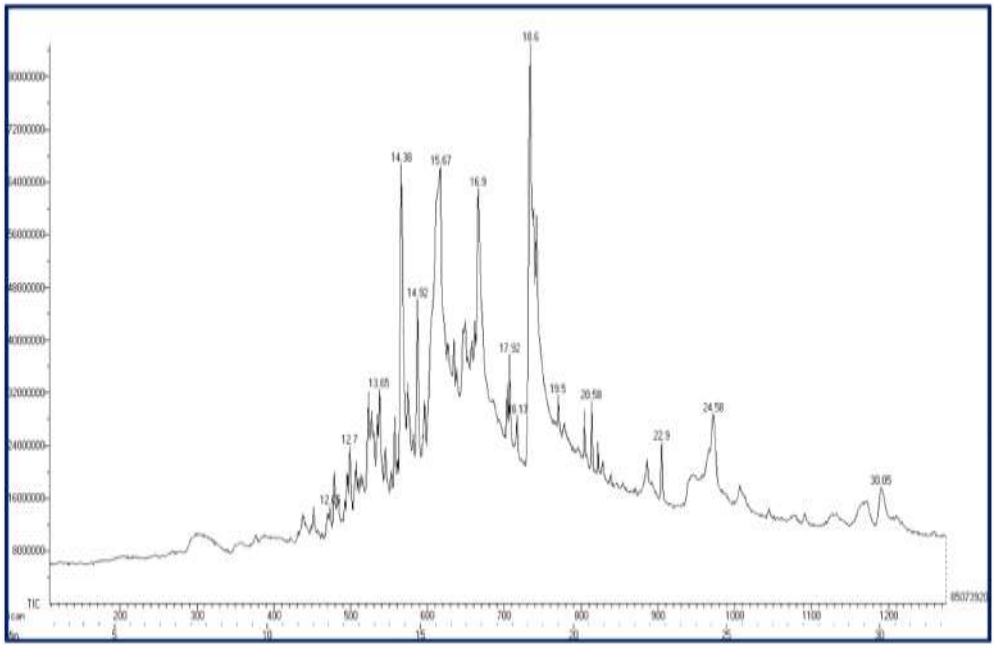

Figure 4. GC-MS chromatogram of cinnamon (*C. aromaticum*).

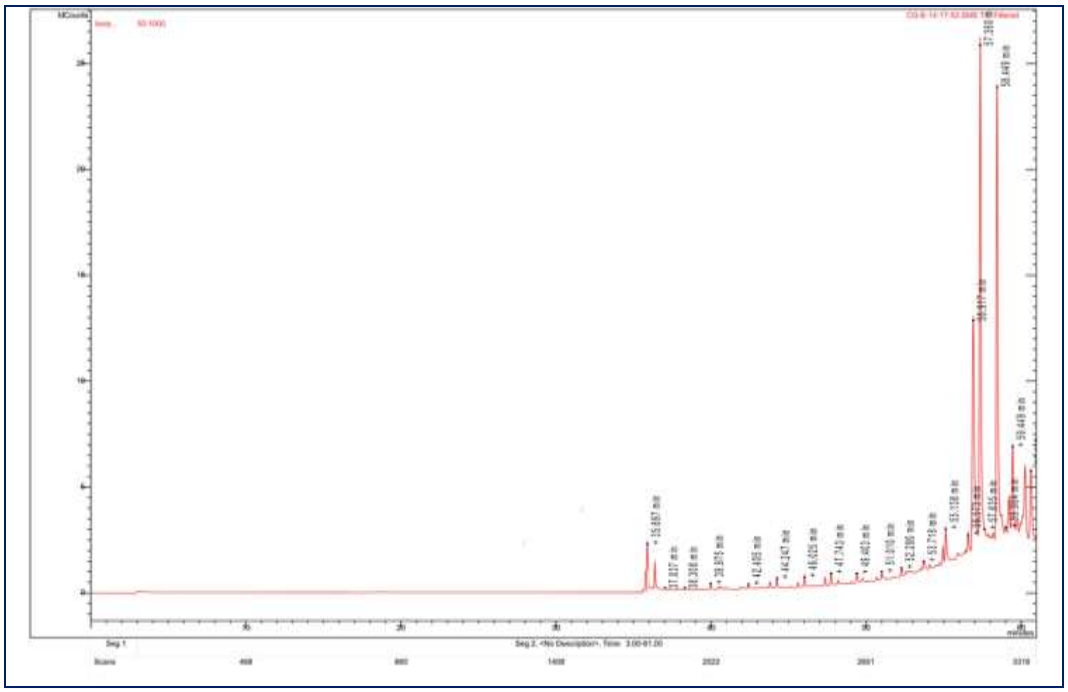

Figure 5. GC-MS chromatogram of clove (*S. aromaticum*).

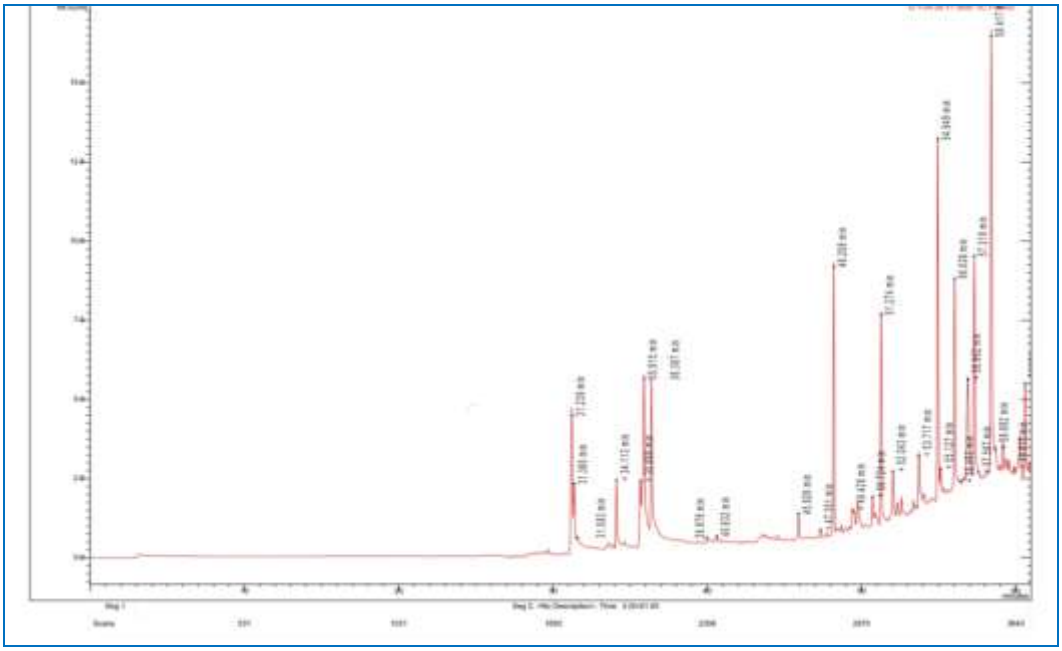

Figure 6. GC-MS chromatogram of mixed spice extract.

ATR spectral analysis of aqueous extract of *F. asafoetida, P. nigrum, E. cardamomum, C. aromaticum, S. aromaticum* and mixed spice extract showed the presence of functional groups namely alcohol, alkene, alkyne, alkane, amine, aromatic, alkyl halides, anhydride, ether and ester as shown in Figure 7 to Figure 12.

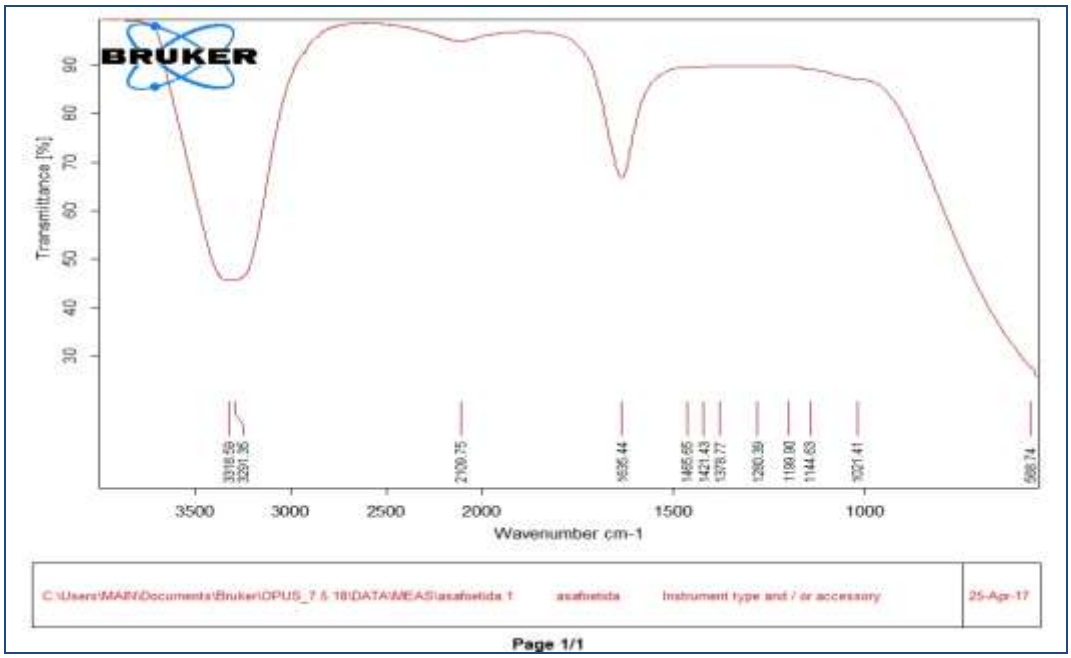

Figure 7. ATR spectrum of asafoetida (*F. asafoetida*) aqueous extract.

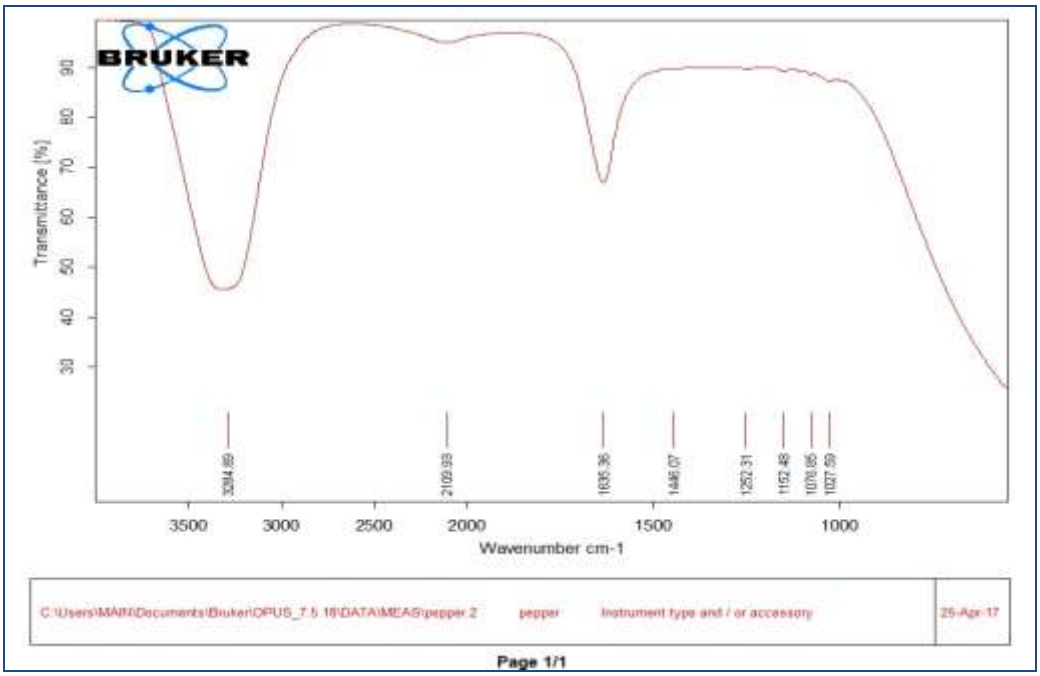

Figure 8. ATR spectrum of black pepper (*P. nigrum*) aqueous extract.

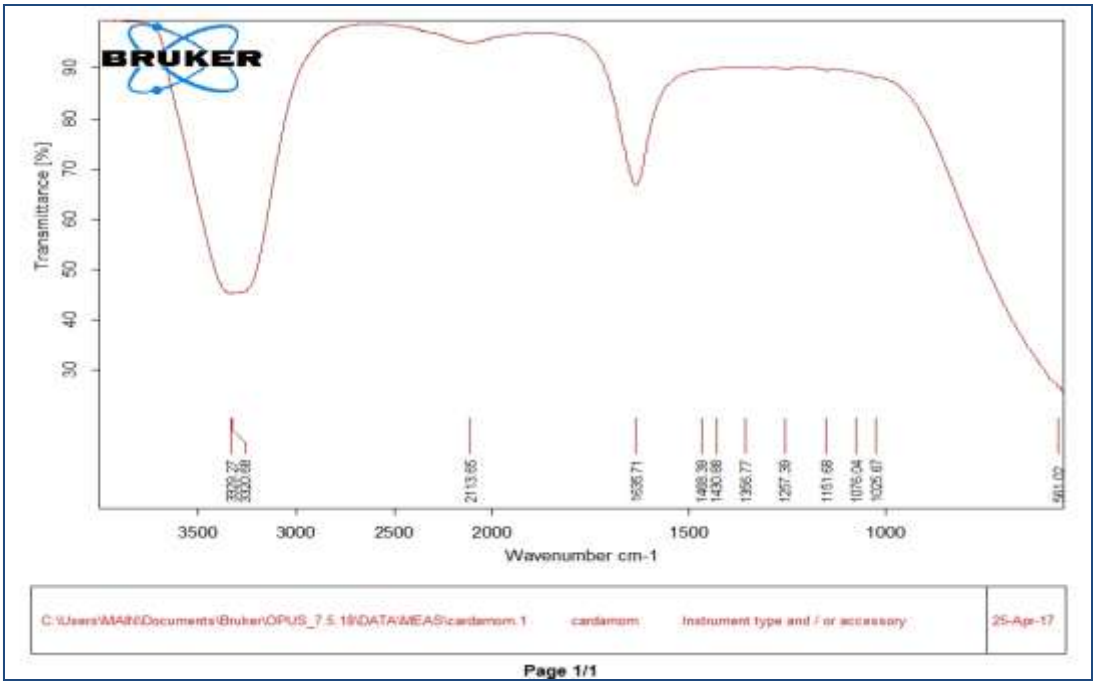

Figure 9. ATR spectrum of cardamom (*E. cardamomum*) aqueous extract.

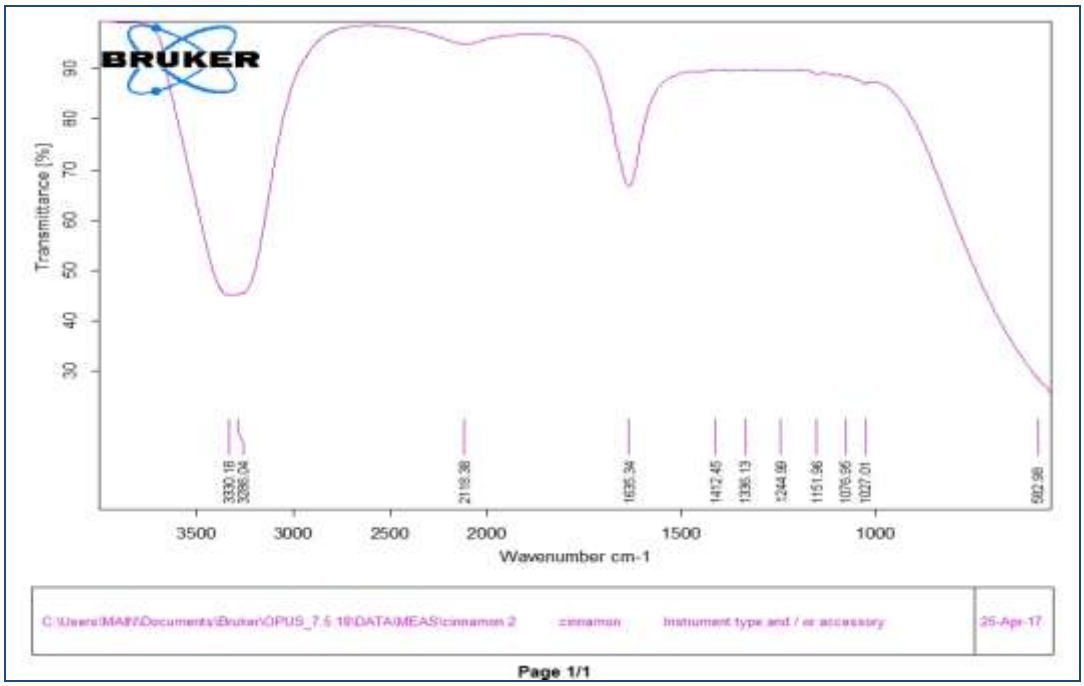

Figure 10. ATR spectrum of cinnamon (*C. aromaticum*) aqueous extract.

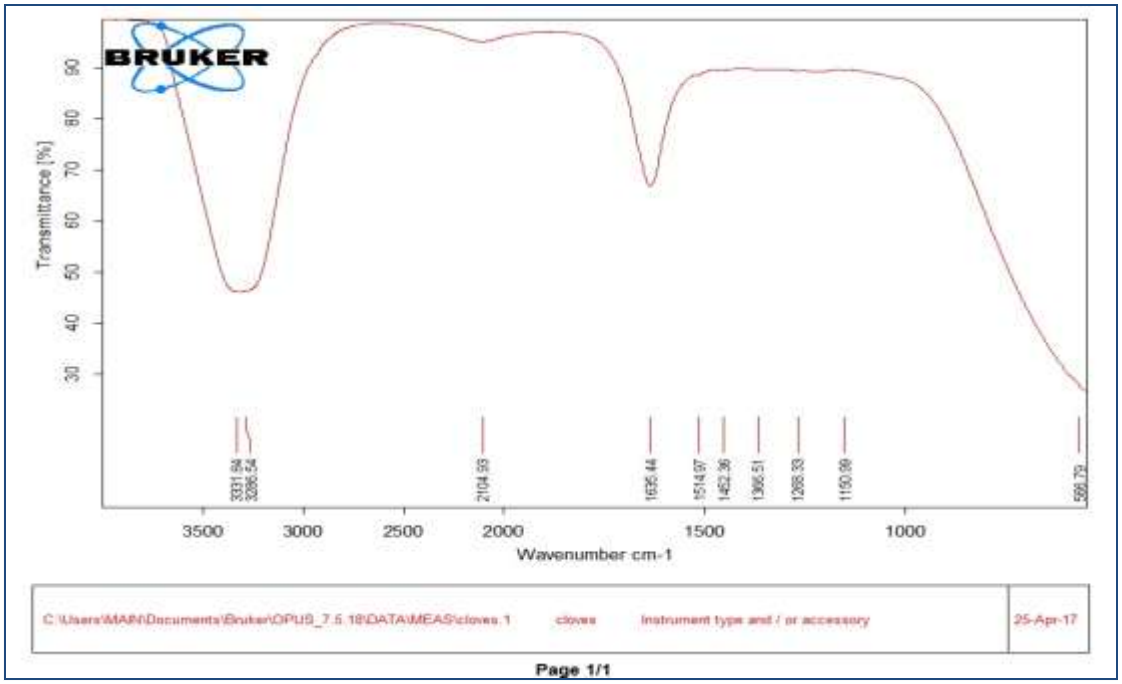

Figure 11. ATR Spectrum of cloves (*S. aromaticum*) aqueous extract.

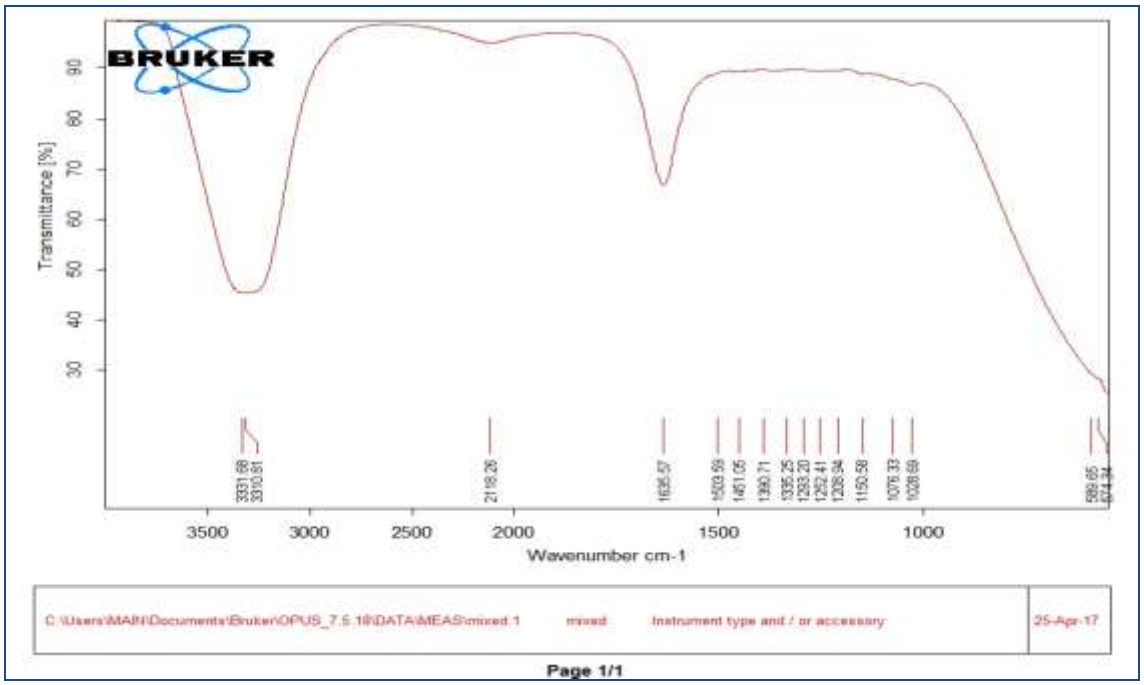

Figure 12. ATR spectrum of mixed spice aqueous extracts (1000 to 3500 cm-1).

India ranks among the 12 mega biodiversity centres by having about 45,000 plant species, with unmatched diversity due to the 16 different agro-climatic zones, 10 vegetative zones and 15 biotic provinces. India has a rich floral diversity of flowering plants (15,000 - 18,000), fungi (23,000), algae (25,000), lichens (1,600), bryophytes (1,800) and microorganisms (30 million). Traditional medicine is the synthesis of therapeutic experience of

generations of practicing physicians of indigenous systems of medicine. Medicinal plants, minerals and organic matters *etc*. comprises the traditional preparation. Herbal drug constitutes only those traditional medicines that primarily use medicinal plant preparations for therapy. Hasan *et al*., (2009) emphasized that the use of plant preparations by Indian, Chinese, Egyptian, Greek, Roman and Syrian dates back to about 5000 years by drawing the evidence from ancient record. Chopra *et al*., (1956) indicated the use of 800 plants in indigenous systems of medicine and also revealed about the record of 500 plants in ancient texts. Indian subcontinent has a vast repository of medicinal plants which is used in traditional medical treatments and also forms a rich source of knowledge. (Rabe & Van Staden, 1997) reported the use of several plant species to treat different ailments in various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy. Recently 20,000 medicinal plant species have been recorded In India (Dev, 1997) and about 800 plant species are used by more than 500 traditional communities for curing different diseases (Kamboj, 2000). Currently 80% of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation because it has no side effects. Plants are important sources of medicines and currently in the world about 25% of pharmaceutical prescriptions contain at least one plant-derived ingredient. Hasan *et al*., (2009) revealed that nearly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources. The qualitative phytochemical profiling revealed the presence of alkaloids, flavonoids, glycosides, phenols, tannins and triterpenoids in *F. asafoetida*; alkaloids, carbohydrates, glycosides, phenols and triterpenoids in *P. nigrum*; alkaloids, carbohydrates, flavonoids, glycosides, phenols and triterpenoids in *E. cardamomum*; alkaloids, flavonoids, glycosides, phenols, saponins and triterpernoids in *C. aromaticum*; alkaloids, flavonoids, glycosides and phenols in *S. aromaticum* and alkaloids, carbohydrates, flavonoids, phenols, saponins, tannins and triterpenoids in the mixed spice extract. Moreover, ATR spectral study confirms the presence of functional groups namely alcohol, alkene, alkyne, alkane, amine, aromatic, alkyl halides, anhydride, ether and ester in *F. asafoetida, P. nigrum, E. cardamomum, C. aromaticum, S. aromaticum* and the mixed spice extract showed the presence of functional groups namely alcohol, alkene, alkyne, alkane, amine, aromatic, alkyl halides, anhydride, ether and ester.

The GC-MS study also confirmed that aqueous extract of *F. asafoetida* aqueous extract contains 8 active phytocompounds, *P. nigrum* aqueous extract contains 11 active phytocompounds; *E. cardamomum* contains about 22 active phytocompounds, *C. aromaticum* aqueous extract contains 15 active phytocompounds, *S. aromaticum* aqueous extract contains 65 active phytocompounds and mixed spice aqueous extract revealed the presence of 75 active phytocompounds. Similar phytochemical profiling studies were also studied by Anjum *et al*., 2017; Hemalatha *et al*., 2020; Manimaran *et al*., 2017; Manimekalai, *et al*., 2016; Rajesh *et al*., 2016; Saranya *et al*., 2017; Rajesh *et al*., 2019; Swamy *et al*., 2017) in various plant parts. (Mena *et al*., 2016) stated that rosemary extract contains 17 active compounds in GC-MS among them most of the compounds flavonoids. Likewise, GC-MS profiling of Malaysian *Plectranthus amboinicus* leaves also showed 115 peaks of which about 46 chemical compounds identified were found to polyphenol in nature (Swamy *et al*., 2017). Earlier studies of Manimekalai *et al*. (2016) and Swamy *et al*. (2017) have identified the presence of polyphenols such as rutin, quercetin, gallic acid, caffeic acid, squalene, phytol, stigmasterol, triacontate, tetrapentacontane, phytol acetate, glycerol, nonacosane, Hexadecanoic acid, amyrin, vitamin-E, benzene, ascorbic acid, 1-methyl-3-(1-methylethyl)-and butanoic acid methyl ester in *G. mangostana* fruit extract and *P. amboinicus* leaves. In a similar way, the present GC-MS result also finds support from the reports of the above authors.

Polyphenols and polyphenol rich foods, especially fruits, vegetables and green tea are widely known for their antioxidant properties; however they also exert anti-inflammatory, anticancer, and neuro-protective properties, which might contribute to their purported benefits also, possibly or not, *via*., their antioxidant properties and they are linked to the maintenance of health *via.,* protection against the development of non-communicable diseases (Mena *et al*., 2016). Antimicrobial, ant-diabetic type-II and anti-asthma activities are the other properties depicted by polyphenols (He *et al*., 2013). Herbs and spices have also been shown to possess anticancer, neuro-protective, anti-inflammatory, arterial dysfunction, oxidative stress with aging, Alzheimer's disease, antimicrobial, cardiovascular diseases, neurodegenerative disorders, prostate cancer, type II diabetes, liver diseases, tumour, digestive disorders, colon cancer, constipation, breast cancer, leukaemia and colorectal neoplasia properties as suggested by Ghawi *et al*., 2014; Manimekalai *et al*., 2016; Mena *et al*., 2016). The antioxidants in spices is their structural diversity, with organosulfides being contained in garlic, carotenoids in saffron, proanthocyanidins in okra, blueberry, cranberry and grapes, B-type proanthocyanidins in *U. tomentosa*, curcumins in turmeric and proanthocyanidins in cinnamon and as a result these spices possess a broad spectrum of human health activities (Huang, 2018). Blumberg *et al*., (2013) reported that Cranberry contains A-type proanthocyanidins responsible for health benefits against urinary tract infection. The presence of polyphenolic and flavonoid compounds in spices in our study also finds support from the above reports that they might also possess protective potential against various ailments.

ATR spectral analysis of aqueous extract of *F. asafoetida, P. nigrum, E. cardamomum, C. aromaticum, S. aromaticum* and mixed spices extract showed the presence of functional groups namely alcohol, alkene, alkyne, alkane, amine, aromatic, alkyl halides, anhydride, ether and ester. Similar results were also observed in the FT-IR finger printings of OH stretched phenol, C=O aryl ketone and C-C aromatic ring in *Aloe vera*, *Mimosa pudica* and *Phyllanthus niruri*, which confirmed that they were flavonoids (Jose *et al*., 2014). Manimekalai *et al*., (2016)also revealed the presence of alkyl halides, phenols,

alcohols, carboxylic acids, amines, amides, acyl chlorides, alkynes, ketones, aromatic rings, nitro compounds and esters in *G. mangostana* extracts. The present study summarizes that the spice extracts are a good source of various metabolites like alkaoids, carbohydrates, flavonoids, glycosides, phenols, saponins and triterpenoids and this could be a potential source of natural antimicrobial to kill the dread full microbes and the GC-MS results also showed the presence vitamin-E, phytol (vitamin-p) and ascorbic acid and this spice extracts can also be used as a natural antioxidant. Further investigation on bioactivity studies might prove their medical applications.

CONCLUSION

Spices are well known for its medicinal properties. A comparative study has been conducted with an aim to achieve the best extraction solvent for the extraction of phytochemicals from the selected spice extracts such as *Ferula asafoetida, Piper nigrum, Elettaria cardamomum, Cinnamomum aromaticum, Syzgium aromaticum* and mixed spices. The results from this study demonstrated that using distilled water as extraction solvent results in the maximum extraction of polyphenolic compounds in our study. To best of our knowledge this is the first report that directly compares six different spice extracts and our results clearly demonstrates that distilled water is the best extraction solvent for the extraction of various phytochemicals from the spices. The medicinal potential of these spices can be explored further.

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