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Research Article

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Preliminary Phytochemical Screening, GC-MS, FT-IR Analysis of Ethanolic Extracts of *Rosmarinus Officinalis*, *Coriandrum Sativum* L. and *Mentha Spicata*

GC-MS, FT-IR Analizi ile *Rosmarinus Officinalis*, *Coriandrum Sativum* L. ve *Mentha Spicata*'nın etanol ekstraktlarının Ön Fitokimyasal Taraması

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ABSTRACT

Phytochemical and some proximate composition analysis was carried out on three (3) selected edible medicinal plants leaves which were believed to be of medicinal value and have physiological effects as anti-inflammatory, antibacterial, anti-pyretic, antioxidant, laxative etc. The significant aim of the research is to identify the phyto-components as well as compounds presents in the ethanolic extract of *Mentha Spicata* L. (Mint), *Rosemarinus Officinalis* (Rosemary), and *Coriandrum Sativum* (Coriander) using two different analytical methods, GC-MS, along with their functional groups using FT-IR. The GC-MS analysis reveals various compounds identified as major constituents and mostly all the compounds identified was found to possess medicinal properties. The data analysis from FT-IR spectrometry representing most of the strong absorptions bands which further indicates major functional groups such as aliphatic amines, alkanes, aromatic (primary and secondary) and carboxylic acids. While the preliminary phytochemical screening conducted indicates the presence of Flavonoids, Tannins, Triterpenoids, and Saponins from the ethanolic extracts of the plant extracts. Therefore the findings indicate that all the selected plant samples are potential sources of medicinal activities and can be applied in the field of phyto-medicine considering their diverse ethno-pharmacological importance.

Key Words

Medicinal Plants, phytochemical screening, phyto-medicine, phyto-components.

ÖZ

Fitokimyasal ve bazı yakın bileşim analizleri, tıbbi ve fizyolojik değeri olduğuna inanılan anti-inflamatuar, antibakteriyel, anti-piretik, antioksidan, yumuşatıcı vb. etkileri olan üç (3) seçilmiş yenilebilir bitki yaprağı üzerinde gerçekleştirilmiştir. Bu çalışmanın esas amacı *Mentha spicata* L (Nane), R*osemarinus officinalis* (Biberiye) ve *Coriandrum sativum* (Kişniş)'in etanolik ekstraktında bulunan fito-bileşenlerin yanı sıra bileşikleri iki farklı analitik yöntem, GC-MS, ile birlikte fonksiyonel gruplar için FT-IR kullanarak tanımlamaktır. GC-MS analizi, ana bileşenler olarak tanımlanan çeşitli bileşikleri ortaya çıkarır ve çoğunlukla tanımlanan tüm bileşiklerin tıbbi özelliklere sahip olduğu bulunmuştur. FT-IR spektrometrisinden elde edilen veri analizi, alifatik aminler, alkanlar, aromatik (birincil ve ikincil) ve karboksilik asitler gibi ana fonksiyonel grupları da gösteren güçlü absorpsiyon bantlarını göstermektedir. Yapılan ön fitokimyasal tarama, bitkilerin etanolik ekstraktlarından Flavonoidler, Tanenler, Triterpenoidler ve Saponinlerin varlığını gösterir. Bu nedenle bulgular, seçilen tüm bitki örneklerinin potansiyel tıbbi aktivite kaynağı olduğunu ve çeşitli etno-farmakolojik önemi dikkate alınarak fito-tıp alanında uygulanabileceğini göstermektedir.

Anahtar Kelimeler

Tibbi bitkiler, fitokimyasal tarama, bitkisel ilaç, fitokimyasal bileşenler.

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INTRODUCTION

In the history of man-kind, plants and their derivatives have always been an important source of medicines for our health conditions for ages. The first knowledge and discovery of the various healing properties of plants has been traced back to ancient days. It is also known that plants are the most important rich source of drugs both in traditional system and that of modern medicines [1]. However, over time, humans have developed an interest in knowing and investigating the exact source and constituents responsible for most of the healing properties associated with the plants parts [2, 3]. As a result of these studies conducted on the plants species birth the word phytochemistry which is regarded as the science responsible for the compounds contained in plants extracts. In recent years, we have seen a revival of interest in the use of medicine from herbal sources, which is evidently possible because herbal medicine has been reported to be a safe with no adverse side effects compared with the synthetic drugs. Most of the medicinal values of these plants lies in some chemical substances that produce a definite physiological action on the human body [4-6]. *Coriandrum Sativum* L. (Apiaceace Family) was originated around the Mediterranean region and usually cultivated mainly in tropical areas. Coriander is among one of the edible plants from the Apiaceae family to be considered medicinal plants due to its exceptionally functional qualities and well documented medicinal history. Aside from its nutritional compositions as seen in Table 1, it is also well known for its sanative character for ages and its phytochemical constituents excel in all bioactive compounds which include, phenolic compound Flavonoids and ascorbic acid. Coriander also has curative and preventive properties such as anti-microbial and anti- carcinogenic. Coriander leaves are also a very vital and important crop that represents a unique position in flavoring substances because of its fragrance and it seeds are used in traditional medicine as an immune booster, and also used in the preparation of many household dishes as it is believed to have contained some medicinal curing abilities that help in curing bed cold, fever, and stomach disorders [7-9]

Rosmarinus officinalis (Rosemary) is a perennial edible plants with a specie name *Rosmarinus officinalis* and is a very popular herbs due to its enormous health properties. Table 2 reveals the chemical and mineral composition of the rosemary plant. Rosemary is an indigenous plant of Mediterranean region where it usually grow and being cultivated worldwide. The plant name arises from rosmarinus in Latin meaning 'dew of the sea' and it has been accepted as the plant with the highest

Table 1. Nutrient composition of coriander leaf as per USDA [2].

Nutrients	Amount (per 100 g)
Energy	279 kcal
Protein	21.93 g
Carbohydrates	52.10 g
Total lipids(fat)	4.78 g
Calcium, Ca	1246 mg
Magnesium, Mg	694 mg
Iron, Fe	42.46 mg
Phosphorus, P	481 mg
Zinc, Zn	4.72 mg
Potassium, K	4466 mg
Vitamin C	566.7 mg
Sodium, Na	211 mg
Thiamine	1.252 mg
Niacin	10.707 mg
Riboflavin	1.500 mg
Vitamin A,	293 ug
Vitamin B-12	0.00 ug
Fatty acids (MUFA)	2.232 g

Table 2. Chemical and minerals composition of rosemary plant [10].

Compounds	%Composition
α -Pinenene	9.72
α -Caryophyllene	8.38
Eucalyptol	5.11
Borneol	24.13
Camphor	5.01
Y-Terpinene	5.005
D-Verbenone	4.17
Limonene	2.29
Methyl jasmonate	2.24
α -Muurolene	1.58
Borneyl acetate	1.55
Myrecene	0.204
Eugenol	1.18
β- Germacrene	1.52
α -cardinene	0.63
farnesyl	0.32

Table 3. Chemical constituent of peppermint as per monographs of international pharmacopeia [13].

Compounds	Percentage composition (%)
Cineole	3.5 – 14.0
Limonene	1.0 – 5.0
Menthone	14.0 – 32..0
Isomenthone	1.5 – 10.0
Methyl acetate	2.8 -10.0
Isopulegol	Max 0.2
Pulegone	Max 4.0
Carvone	Max 1.0
Menthfuran	1.9 – 10.0

antioxidants activity and believed to possess phytochemical compounds such as Flavonoid, triterpenes, diterpenes and Steroids [10] Rosemary is a very tolerance plant that can withstand moderately harsh weather it usually grows averagely within temperature range of 25 -30 °C. The plant can adopt and tolerate easily in almost all weather condition [11]. *Mentha spicata* (Mint) Mint is a very fast growing edible perennial plants with many different varieties that usually grow tall up to 3 feet tall which composes of varieties of chemical constituents (Table 3). The *Mentha spicata* species plant replicate well when exposed to sunlight and sometimes in under partial shades, they are usually planted early in raining seasons which is sometimes referred to as growing season, the plant required at least an average temperature of 25 – 35°C (normally under moist soil condition) but excess or too much water will cause some diseases i.e. roots and leaf diseases [12, 13]. The significant goal of this paper is to identify and isolate volatile biologically active compounds from the ethanolic extracts of the plants and determine their pharmacological effects.

MATERIAL and METHODS

Collection and extraction of plant extracts

All the fresh plants samples were obtained from a farm garden in Lefkosa district of TRNC, and the plants samples were identified by a botanist from the TRNC ministry of agriculture and natural resources, all leaves are se-

parated from the main plant stalk, soaked and wash in water, air dried at room temperature 25°C. The dried sample were further weighed and crushed into powder. All the plants samples for the phytochemical analysis were extracted in ethanol and distilled water, the ethanolic extract of the plants leaves were prepared by soaking 50g of each of the dried powder of every sample individually in 500 mL of absolute ethanol solution and further allowed to stay for 48 hrs under room temperature condition in other to achieved thorough extraction. After two days, the resulting content was then filtered through a whatmann filter paper (No. 42, 125 mm) and kept for evaporation of the solvent to get the dried concentrated extracts. The dried portion of the extract was then frozen and stored at 5°C and the crude residue was weighed and used for all the preliminary phytoconstituent analysis.

Preliminary phytochemical screening

The preliminary phytochemical analysis for the ethanolic extract were performed to identify different phytochemical content in the plants samples by using standard procedure by Sofowora [14], Trease and Evans [15] and Ayoola et al. [16].

Test for Saponins (frothing test)

0.5 g of the crude ethanolic sample was weighed and added to 10 mL of distilled water in a test tube, the resulting solution was vigorously shaken and stable froth is observed if formed. Followed by adding 3 drops of olive oil to the froth formed sample and shaken vigorously after which the sample is further observed for the formation of emulsion.

Test for Tannins

Two qualitative analysis are used in testing for the presence of Tannins in any given samples.

KOH Test: 10mL of freshly prepared 10%KOH solution in a clean beaker, and 0.3 g of the crude ethanol extract was added and vigorously shaken to dissolve. And partial or dirty precipitate observed indicate the presence of Tannins.

Ferric chloride Test: 0.5 g of extract was dissolve in 10mL distilled water and boiled in a test tube and a few drops of ferric chloride solution was added to the boiled sample a formation of brownish green color is observed for the confirmation of Tannins.

Test for Flavonoids

2mL of sodium hydroxide (NaOH) was mixed with the crude extract of the plant a formation of concentrated yellow color was observed with further addition of 2 drops of diluted H_2SO_4 which later become colorless.

Test for Steroids

2mL of chloroform solution was added to 5mL of aqueous crude sample of the plant extract follow by 4mL of concentrated H_2SO_4 and indication of reddish color indicate the presence of Steroid in the sample.

Test for Terpenoids

5mL of aqueous ethanolic extract of the plant sample was mixed in 4 mL of chloroform and 1mL of acetic anhydride was added to the mixed solution follow by addition of 2 mL of concentrated H_2SO_4 formation of reddish violet color indicates the presence of triterpenoids in the sample.

Test for Glycosides

4 mL of concentrated acetic acid was mixed with 2mL of chloroform and aqueous extract of the plant sample the mixture was then cooled and concentrated H_2SO_4 was added and a formation of green color indicate the presence of glycoside in the given sample.

FTIR Spectroscopic Analysis

Fourier Transform Infrared Spectrophotometer (FTIR) is one of the most important analytical tools for the determination and identifying the types of functional group presents in a given compounds. FTIR method and technique was used for the determination and identification of various types of functional groups in each powdered leave extract of all the three sample used in the analysis as described by Yadav et al. [9]. The infrared spectroscopy spectrum (IR) was obtained using FTIR Shimadzu Japan in the range 400–4000 cm^{-1} with which 10mg of dried extract powdered sample of each of the plant sample was grounded in a mortar and pestle in order to obtain a fine powdered sample and the obtained extracts was subsequently used for the FTIR analysis.

GC-MS Specifications

All the plant extracts analyses were performed using a GC-MS system (GCMS-QP2010 SE plus Shimadzu Technology Japan) equipped with an HP-5MS capillary column (30 m x0.25 mm). The injection volume of each sample was 1 µL. And Helium was used as a carrier gas

with flow rate of 1 mL/min, the injection port temperature was 250°C and the program of the sample was set to a temperature ranges from 50°C to 300°C at a rate of 50°C/min and 10 min hold at 300°C for non- volatile constituents.

Peak Identification

The interpretation of mass spectrum of GC-MS and the constituents was identified by comparing their mass spectra with those in the Database of National Institute of Standard and technology (NIST). The spectrum of the unknown component was compared with spectrum of the known components stored in the NIST Library according to the chemical constituents present in the plant extracts.

RESULT and DISCUSSION

Preliminary phytochemical analysis

The Preliminary phytochemical analysis results revealed the presence of medically active compounds in the plants samples studied. As shown in Table 4, it could be seen that almost all the three selected plants contain various secondary metabolites such as Flavonoids, Saponins, Tannins, Triterpenoids and Steroids respectively, with the exception of Mints (*Mentha spicata* L) samples in which its test analysis results for Flavonoids test showed negative result. The positive results of the preliminary tests for Saponins, Flavonoids, and steroids are displayed in Fig .1. All the three (3) plants selected for the study are very vital and mostly used for the treatment of many diseases as a results of their phytochemicals constituents which have been confirmed in so many studies conducted in the field of food and nutrition and have been reported to possess high medicinal value. Flavonoids are very vital bioactive polyphenols that are distributed widely in the plant kingdom and play an important role in photosynthesizing cells [17, 18]. Flavonoids have been reported to possess a wide range of biological activities such as antibacterial, anti-inflammatory and analgesic [19-21]. Furthermore these findings suggest that all the plants samples analyzed contain phytochemical nutrients in them, therefore all the three plants are a potential sources of natural antioxidants that could serve great importance in therapeutic purposes [22-24].

Gas chromatography Mass spectrometry (GCMS) Analysis

From Figure 2 and Table 5, the GC-MS analysis of the ethanolic extract of Rosemary (*Rosemarinus officinalis*) revealed the presence of various bioactive compounds. Some of the biologically active compounds revealed by the GC-MS are camphene a (monoterpenes) at a retention time of 5.33 with peak area of 43.2% which is known for its analgesic and anti- inflammatory property, and Eucalyptol(1,8-cineole) with retention time of 14.67 and peak area of 7.6% is used as antioxidant, anti-cancer and analgesic in drug formulation, while

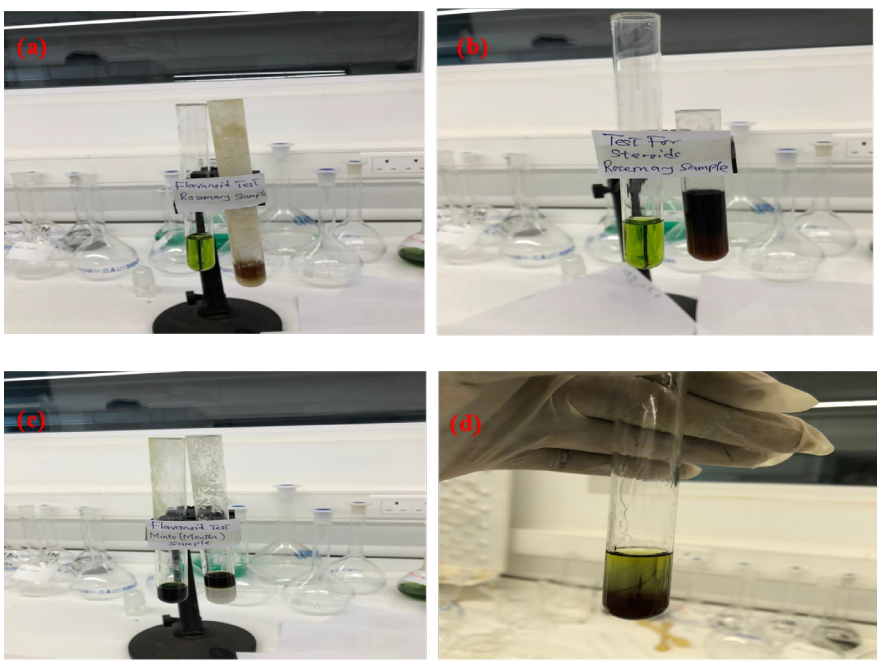

Figure 1. Preliminary phytochemical screening showing (a) Flavonoid test for rosemary, (b) Steroids test for rosemary, (c) Flavonoid test for *Mentha spicata*, (d) Steroids test for *Mentha spicata.*

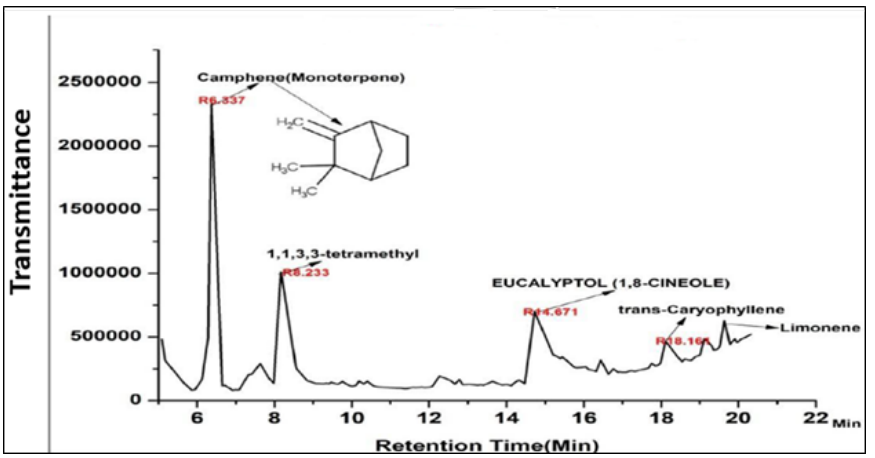

Figure 2. GC-MS Spectral Analysis of Ethanolic extract *Rosmarinus officinalis*.

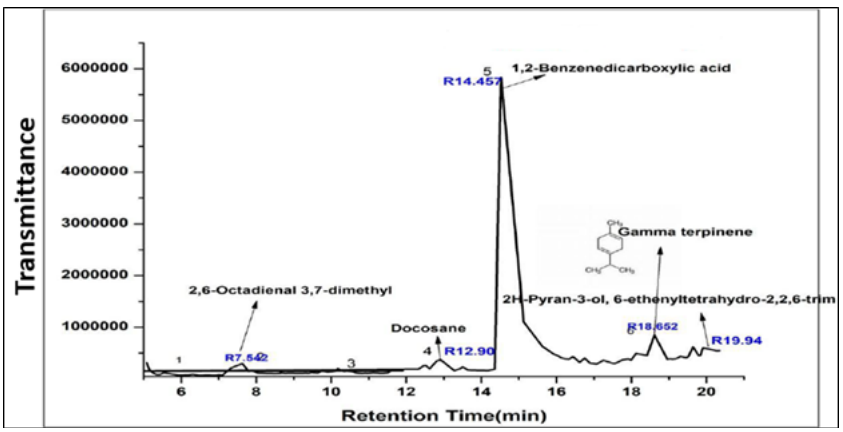

Figure 3. GC-MS Spectral Analysis of Ethanolic extract of *Coriandrum sativum*.

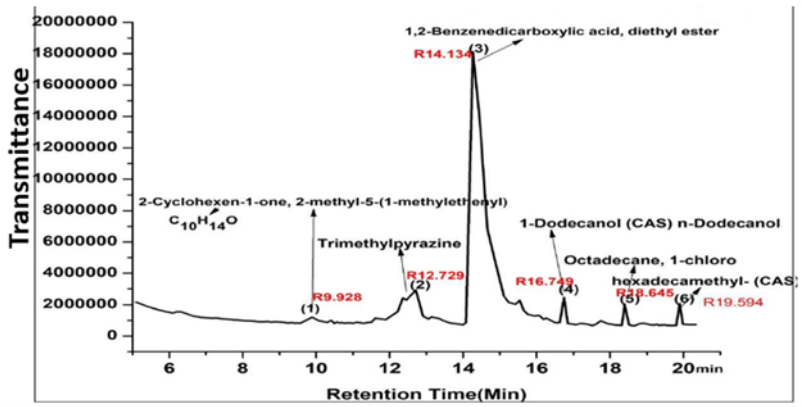

Figure 4. GC-MS Spectral Analysis of Ethanolic extract of Mint plant.

Table 4. Phytochemical constituents present in the plants extracts.

Plants	Percentage composition (%)				
	Flavonoids	Tannins	Saponins	Steroids	Triterpenoids
Mint	-	+	+	+	+
Rosemary	+	+	+	+	+
Coriander	+	+	+	+	+

- Negative result

+ Positive result

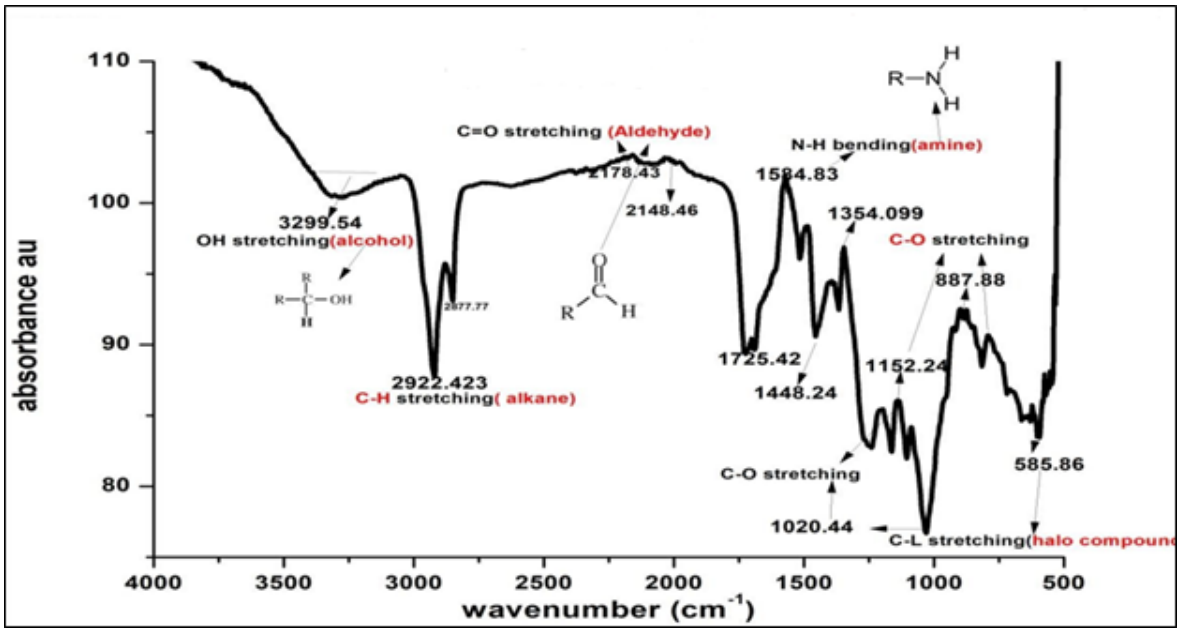

Figure 5. FTIR Analysis of *Rosmarinus officinalis* at solid state.

Table 5. Activity of Phyto-components identified in *Rosmarinus officinalis* by GC-MS.

Retention Time(Min)	Compounds Name	LRI	Peak Area %
5.337	Camphene (monoterpenes)	943	43.2
8.233	1,1,3,3-tetramethyl	1487	11.4
14.671	Eucalyptol (1,8- cineole)	1059	7.6
18.161	Trans-caryophyllene	1224	2.7
19.832	Limonene	1545	1.2
	Others		33.9

Table 6. Activity of Phyto-components identified in *Coriandrum sativum* by GC-MS.

Retention Time(Min)	Compounds Name	LRI	Peak Area %
5.62	2,6-octadienal 3,7-dimethyl	1024	0.45
12.90	Docosane	2208	0.25
14.457	1,2Benzenedicarboxylic acid	1620	24.7
18.652	Gamma terpene	1137	2.12
19.942	2H-Pyran-3-ol	1278	0.87
	Others		71.61

Table 7. Activity of Phyto-components identified in *Rosmarinus officinalis* by GC-MS.

Retention Time(Min)	Compounds Name	LRI	Peak Area %
9.928	2 Cyclohexen-1-one	873	0.76
12.729	Trimethylpyrazine	1008	0.94
16.749	1 Dodecanol(n-dodecanol)	1461	0.54
19.594	Hexadecamethyl	2097	0.3
18.645	Octadecane	1810	0.23
14.134	1,2,benzene dicarboxylic acid	1620	24.43
	Others		72.8

Trans- caryophyllene at retention time 18.161 with a peak area of 2.7% has an anti-microbial and anti- bacterial as a their biological activity. Also a bioactive compounds Limonene with a retention time of 19.832 and peak area of 1.2% has antimicrobial and antibacterial activity [25, 26].

As revealed in Figure 3 and Table 6 which shows the GC-MS analysis results of *Coriandrum sativum*. The phytoconstituents detected in ethanolic extract Coriander with their retention time and peak area are 2,6-octadienal 3,7-dimethyl with retention time of 5.62 and peak area 0.45%, Docosane with retention 12.9 and peak area 0.25%, 1,2-Benzenedicarboxylic acid (phthalic acid) with retention time of 14.45 and peak area of 24.7, Gamma terpene with retention time 18.65 and peak area of 2.12% and finally 2H-Pyran- 3-ol with retention time 19.94 and peak area of 0.8%. 2, 6-octadienal, 3, 7-dimethyl is used as Anti-fungal agent and also in Anticancer treatments. Biologically active compound Docosane also has antioxidant and anti-microbial properties and 1,2-Benzenedicarboxylic acid (phthalic acid) is used as anti-oxidant and antibacterial agents, also Gamma terpene has been reported to have a potent antioxidant property, and cardiovascular support and also provide neuro-protection [27]. While 2H-Pyran-3-ol serve as Antibacterial, antimicrobial as well as anti-oxidants.

The GC-MS analysis of *Mentha spicata* are shown in Figure 4 and Table 7. The phytoconstituents detected in ethanolic extract of Mints (*Mentha spicata*) sample extract using gas chromatography-mass spectrometry(GC-MS) with their retention time and peak area are 2 Cyclohexen-1-one at a retention time of 9.928 with a peak area of 0.76%, and its biological activities include Antioxidants, antibacterial agents used in drugs formulation. And Trimethylpyrazine (TMP) with retention time of 12.729 min with a peak area of 0.94% and is used in treatment of cardiovascular diseases, headache and vertigo and also used in preventing cell damage [28-30].

FTIR Analysis

Basically in this study the potential of FTIR Spectroscopy as analytical tools is used for identification of various functional groups responsible for medicinal properties in all the plant samples. The FTIR spectra of *Rosmarinus officinalis*, *Coriandrum sativum* and *Mentha Spicata* are revealed in Figure 5, 6 and 7 respectively. The results confirms the presence of many characteristics functional groups detected at different vibrational frequency band in the IR spectrum. The various functional groups observed using FTIR spectrum indicates the presence of O-H group(alcohol), carboxylic acid, amine, sulphur derivatives ,amino acid, and nitro - compounds among others. From the summarized FTIR Spectrum the fingerprint at 3299.54 cm^{-1} in rosemary sample which is the representative for O-H group predict the presence of alcohol in the sample, and the analysis reveals that all the three plants samples contains O-H(alcohol) and CH (alkane) functional group in them [31]. The band at 2922.423, 2832.61, 2993.83, and 2981.63cm^{-1} represent – CH_2 and –CH_3 groups which is due to presence of chlorophyll groups in the samples the weak band at 1476.80 cm^{-1} in parsley sample which is the representative of N-H bending is as a result of the amino acid present in that sample [32, 33]. And also the bands at 1152.24, 1029.98 and 1057.85 cm^{-1} represent stretching vibration of (C-O) and are due to the presence of acid. As OH (alcohol group) group has the ability of forming hydrogen bonding capacity, presence of OH group probably indicates the higher potential of the sample towards inhibitory activity against microorganisms [34].

Conclusion

This present study has been performed to established different phytochemical parameters on three medically valued plants using two distinctive analytical techniques FTIR, and GC-MS. The GCMS chromatogram conducted on all the selected plant extracts revealed the presence of many bioactive compounds of medicinal value such as Camphene (monoterpenes), Eucalyptol (1,8- cineole), Trans-caryophyllene, Limonene, 2-Cyclohexen-1-one, Trimethylpyrazine(TMP), 1-Dodecanol (n-dodecanol), Octadecane, 1,2-benzendicarboxylic acid (phthalic acid), 2,6- octadienal 3,7-dimethyl, Gamma terpene, 2H-Pyran-3-ol, 1,4 Cyclohexadiene, and Metronidazole (anazole). While the FTIR analysis conducted showed the presence of many characteristics functional groups such as Carboxylic acids, amine amide, and OH group along with sulphur derivatives with some notable alkanes and alkynes. The preliminary phytochemical screening indicates the presence of various secondary metabolites such as Tannins, Flavonoids, Saponins, Triterpenoids and Steroids which is evident in the research carried out by [35, 36, 37]. Therefore, these findings suggest that all the three plants samples analyzed contain many phytochemical compounds in them, and conclusively are a potential sources of natural antioxidants that could serve great importance for varieties of therapeutic purposes.

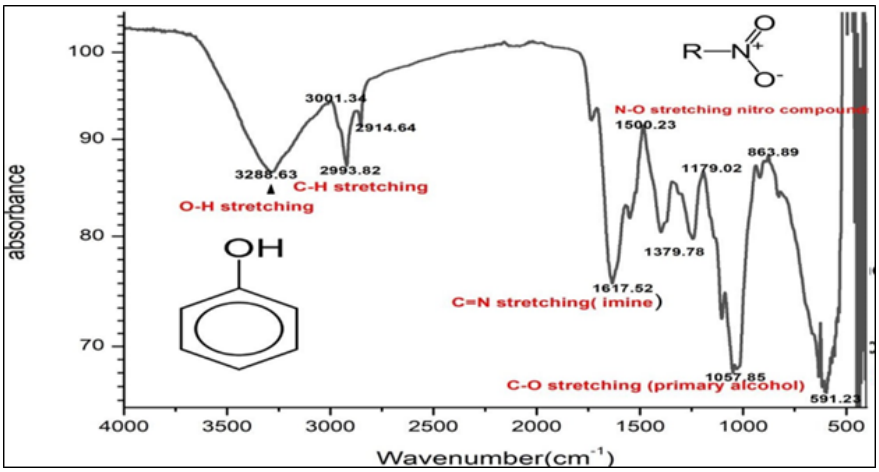

Figure 6. FTIR Analysis *Coriandrum sativum* at solid state.

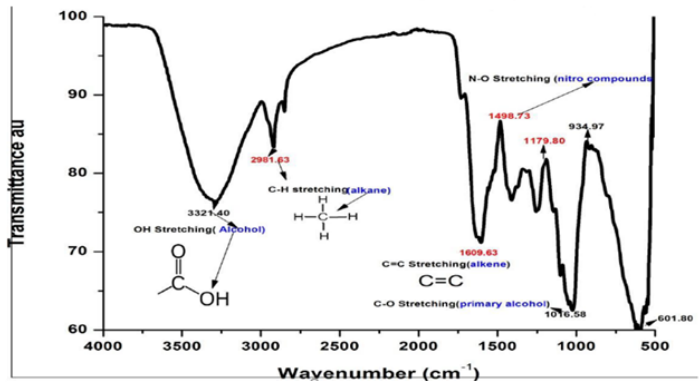

Figure 7. FTIR Analysis *Mentha Spicata* at solid state.

Conflict of Interest

The authors declare that they have no conflict of interest.

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