RESEARCH ARTICLE

FTIR Spectroscopic Analysis of *Anethum graveolens* L. Leaves Extract

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Abstract

Anethum graveolens L. (Dill) is an aromatic annual herb of Apiaceae family, it is easily available and frequently grown in herb, vegetable and flower garden. It is used as a medicine because of its high therapeutic value. The objective of this study is to identify biologically important functional groups present in aqueous, acetone, ethanol, methanol, hydroalcoholic, dichloromethane and chloroform of *Anethum graveolens* L. leaves by using Fourier Transform Infrared Spectroscopy (FTIR). Absorption spectra obtained from *Anethum graveolens* L. leaves FTIR analysis were used to determine the prominent peaks, vibrational assignments, intensities and wave number. FTIR spectra of *Anethum graveolens* L. leaves indicates the presence of alcohol, aldehyde, carbohydrates, ester, phenol, ether and amine groups. The functional groups observed in the analysis were major component of secondary metabolites found in *Anethum graveolens* L. leaves. The determination of functional groups by FTIR helps to identify the potential bioactive constituents of *Anethum graveolens* L. leaves. The findings of present study suggest that the plant extracts have significant medicinal potential, due to the presence of various biologically important functional groups as a chemical composition. It will be eventually utilized to treat a variety of diseases.

Keywords: Dill, functional groups, secondary metabolites, bioactive constituents

Introduction

Medicinal plants serves as valuable raw material for manufacturing numerous traditional and modern medicine as well as vital therapeutic agents. These plants are significant part of natural wealth and the main bioresources of medicine for prevention of diseases (Nayak and Krishna 2007, Muruganantham *et al.* 2009, Maobe and Nyarango 2013). Now a day's number of chemicals are identified from medicinal plants and used as an essential drug (Debnath *et al.* 2006). *Anethum graveolens* L. (dill) family Apiaceae grows in Mediterranean region, Europe and Asia (Ebrahim *et al.* 2017).In Ayurveda *Anethum graveolens* L. is commonly used as a medicine to improve digestion, colic and abdominal discomfort. *Anethum graveolens* L. dried &fresh leaves has been utilized in sandwiches, fish sauces and fried meats and also used as ingredient of sour vinegar. Essential oil of *Anethum graveolens* L. extracted from seed, leaves and stem has been used as flavoring agent in food industry (Jana and Shekhawat 2010). The *Anethum graveolens* L.is specifically grown for its volatile chemicals. The primary components of dill seed oil are carvone and limonene, but the aroma of the *Anethum graveolens* L. is primarily derived from α–phellandrene, dill ether and myristicin (Dhir *et al.*2014). *Anethum graveolens* L. has a history of being used to made pesticides, perfumes and traditional medicines (Jana and Shekhawat 2011).

Anethum graveolens L. has an extensive variety of naturally occurring secondary metabolites with a wide range of structural diversity and biological activity (Bulchandani and Shekhawat 2020). *Anethum graveolens* L. has phytochemical bioactive molecules and antioxidants that can slow down the action of many prolonged diseases connected with reactive oxygen species and oxidative stress. Phytochemicals like flavonoids, cardiac glycosides, tannins and terpenoids are rich in leaves, stems, and roots of *Anethum graveolens* L. (Manal *et al.* 2013, Ahmed *et al.* 2014). Cardiovascular disease, cancer, disease associated with aging and diabetes are decreased by the intake of natural antioxidants (Ani *et al.* 2006). *Anethum graveolens*

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L. has antihypercholesterolemic, antioxidant, anticancer, antifungal, antisecretory, antiulcer, anti-inflammatory and antimicrobial properties (Nair and Chandra 2007, Madani *et al.* 2005, Huda *et al.* 2017).

Fourier Transform Infrared Spectroscopy (FTIR) is essential and widely used analytical technique to classify and identify the chemical constituents. In several countries FTIR analysis is also used to identify the medicines for pharmacopeia (Subashini *et al.* 2015). FTIR is analytical technique which provides structural information of different compounds (Agatonovic-Kustrin *et al.* 2013).This analytical technique is sensitive, considerable and reliable used to analyze the types of functional groups present in sample. FTIR that apply the infrared radiation to absorb molecular bonds within the sample, it allows gaining information on particles of sample (Baker *et al.* 2008). The objective of the current work is to identify the functional groups present in the different extracts of *Anethum graveolens* L. leaves by using FTIR analytical technique which will be helpful to evaluate different medicinal property of *Anethum graveolens* L.

Materials and methods

Leaves of *Anethum graveolens* L. were used for the present study. *Anethum graveolens* L. was cultivated in July and collected the leaves in November from Katraj, Pune. Location map of the study area is shown in Figure 8. The herbarium of *Anethum graveolens* L. (specimen no. JVK-01) was prepared and authentication has been obtained from Botanical Survey of India (BSI) Pune, Maharashtra. *Anethum graveolens* L. leaves were shade dried and finely grinded to form crude powder. This dried powder was used for preparation of various extracts. The aqueous, acetone, ethanol, methanol, hydroalcoholic, dichloromethane and chloroform extracts of *Anethum graveolens* L. leaves were prepared by using maceration method.

Preparation of extracts

10gm of *Anethum graveolens* L. leaves powder was weighted and extracted with 100ml of aqueous, acetone, ethanol, methanol, hydroalcoholic, dichloromethane and chloroform solvent and allowed to stand at room temperature for a period of at least 3 days. The extracts were collected and dried & kept under 4°C for further use.

FTIR Spectrum Analysis

Anethum graveolens L. leaves extracts were analyzed by Fourier Transform Infrared Spectroscopy (FTIR).The leaves extracts were mixed with 100mg of KBr salt, condensed in a small pellet the samples were loaded on FTIR spectrophotometer. The results were recorded on Shimadzu FTIR Spectrophotometer 8000 series with scan range from 400 to 4000 cm^{-1} (Cakmak *et al.* 2006). Absorption of wavelength of light is typical feature of the chemical bond which is illustrated by spectrum (Sahayaraja *et al.* 2015).

The unique chemical bonding and molecular structure of the sample material was displayed in a graph that the FTIR spectrophotometer creates in the form of an absorption spectrum. There were peaks in this absorption spectrum that correspond to the components. These absorption peak represents functional groups, such as alcohol, alkanes, amino acids, phenol, alkenes, carboxyl acid, aromatic ether, ester, hydroxyl groups and ketones. Different functional groups and bond types absorb infrared radiation of different wavelength.

Results

FTIR spectroscopy study revealed the presence of different functional groups in various extracts like aqueous, acetone, ethanol, methanol, hydroalcoholic, dichloromethane and chloroform of *Anethum graveolens* L. leaves which shows variation in peaks depicted in figures, and spectra analysis was given in table which observed the functional groups such as, phenol, alcohol, alkenes, carboxyl acid, amino acids, aromatic amines, halogen compound and more.

Figure 1 and Table 1 shows FTIR spetra and peak value of *Anethum graveolens* L. leaves in aqueous extract, broad peak observed at 3314.07, 3270.68 cm^{-1} shows the presence of alcohols suggest that component of phenol groups present in aqueous extract (O-H stretch).The peak identify at 2748.07 cm^{-1} indicates the presence of aldehyde. Peak observed at 2352.73 cm^{-1} represents the nitrile compound and the peak at 2183.02cm^{-1} indicates alkyne group. The peak shows at 1659.45, 1621.84 cm^{-1} which shows the alkene and amide (non-conjugated N-H bond) respectively. The intensive peak observed at 1498.42 cm^{-1} denotes the C=C aromatic compound. The peak shows at 1297.86 cm^{-1} indicates the aromatic ester (C-O stretch). The band occurring at 1114.65 and 1078.98 cm^{-1} show the presence of (C-O stretch) aromatic cyclic ether and aliphatic compound(C-O-C stretch) respectively. The peak shows at 869.739 &737.639 cm^{-1} indicates the carboxylic acids (C-H bend) and aromatics (C-H).

In Figure 2 and Table 2 observe the spectra and peak value in acetone extract of *Anethum graveolens* L. leaves. The peak between 3000-2800 cm^{-1} range indicates the C-H stretching frequency which shows alkane compound (3020.94, 2925.48, 2853.17 cm-1).The peak at 2391.3 cm^{-1} shows the presence of nitrile. The strong peak observed at 1736.58 cm^{-1} represents (C=O stretch) cyclic aromatic ketone. The peak observed at 1619.91,1458.89,1375,1270.86,1170.58, and 971cm^{-1} showed the presence of amide (N-H), vinyl aromatic compounds (C-H bend),aliphatic compounds (C-H bend), ester carbonyl (C=O stretch),ester (C-O stretch) and carboxylic acids(O-H bend) respectively.

The peak value observed in ethanolic extract of *Anethum graveolens* L. leaves at 2924.52, 2852.2 cm^{-1} shows asymmetric and symmetric C-H stretching frequency it indicates lipids and proteins. The peak at 2389.37 cm^{-1} indicates the nitrile compound. And other functional groups such as aldehyde, amide, amines, vinyl compound, aliphatic compound,
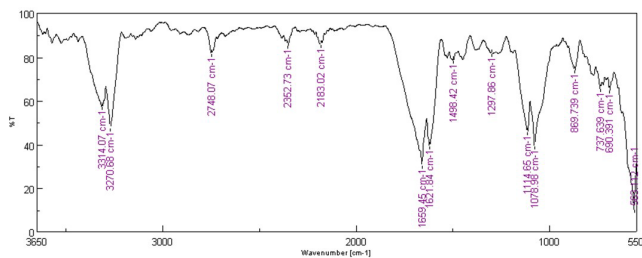
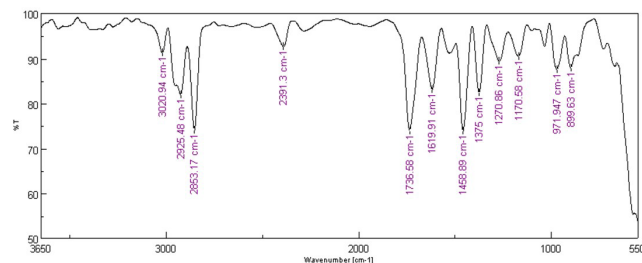**Figure 1:** FTIR spetra of *Anethum graveolens* L. leaves in Aqueous extract.

Figure 2: FTIR spetra of *Anethum graveolens* L. leaves in Acetone extract.
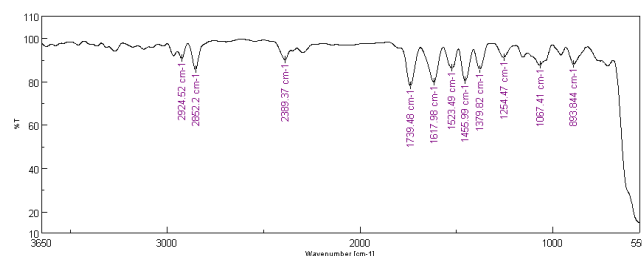

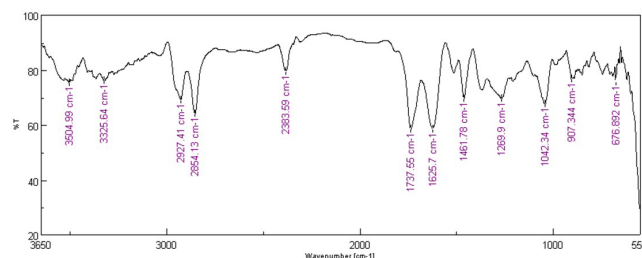**Figure 3:** FTIR spetra of *Anethum graveolens* L. leaves in Ethanol extract

Figure 4: FTIR spetra of *Anethum graveolens* L. leaves in Methanol extract.JPG

aromatic ester, aliphatic compound and, halogen are observed at 1739.48, 1617.98, 1523.49, 1455.99, 1379.82, 1254.47, 1067&893.844 cm^{-1} respectively in Figure 3 and Table 3.

The results of FTIR analysis shows the presence of functional groups present in methanol extract of *Anethum graveolens* L. leaves in Figure 4 and Table 4 such as at 3504.99,3325.64 cm^{-1} represents the alcohol group(O-H) and 2927.41,2854.13 cm^{-1} represents the alkane group. The peak observed at 2383.59 cm^{-1} which shows the nitrile group. The

Table 1: FTIR peak value of Anethum graveolens L. leaves in Aqueous extract

Functional Group	Component (Peak)	Wave number (cm-1)
Alcohols	O-H Stretch	3314.07
Alcohols	O-H Stretch	3270.68
Aldehydes	C=O Stretch	2748.07
Nitrile	C=N Stretch	2352.73
Alkyne	C=C Stretch	2183.02
Alkene	C=C Stretch	1659.45
Amide	N-H Stretch	1621.84
Aromatic ring	C=C Stretch	1498.42
Aromatic ester	C-O Stretch	1297.86
Cyclic Ether	C-O Stretch	1114.65
Aliphatic compound	C-O-C Stretch	1078.98
Halogen	C-H bend	869.793

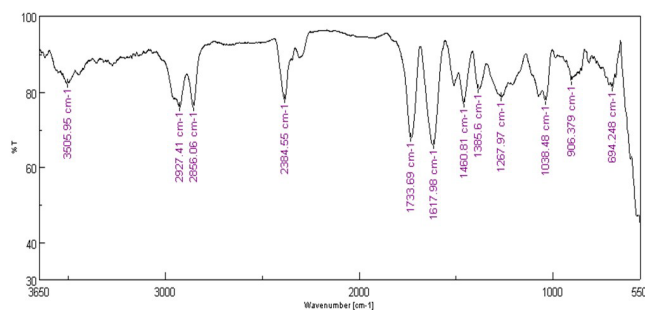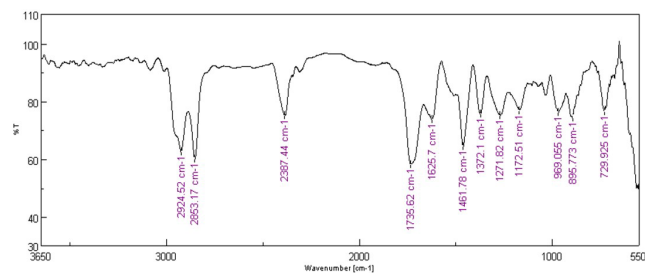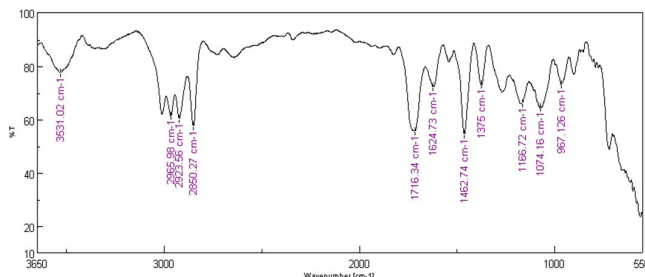**Table 2:** FTIR peak value of Anethum graveolens L. leaves in Acetone extract

Functional Group	Component (Peak)	Wave number (cm-1)
Alkane	C-H Stretch	3020.94
Alkane	C-H Stretch	2925.48
Alkane	C-H Stretch	2853.17
Nitrile	C=N Stretch	2391.3
Ketone	C=O Stretch	1736.58
Amide	N-H Stretch	1619.91
Venyl	C-H Bend	1458.89
Aliphatic compound	C-H bend	1375
Ester Carbonyl	C=O Stretch	1270.86
Ester	C-O Stretch	1170.58
Carboxylic acid	O-H bend	971.947
Halogen	C-H bend	899.63

Table 3: FTIR peak value of Anethum graveolens L. leaves in Ethanol extract

Functional Group	Component (Peak)	Wave number (cm-1)
Alkane	C-H Stretch	2924.52
Alkane	C-H Stretch	2852.2
Nitrile	C=N Stretch	2389.3
Aldehyde	C-H Stretch	1739.48
Amide	N-H Stretch	1617.98
Amines and amides	N-H Stretch	1523.49
Venyl	C-H Bend	1455.99
Aliphatic compound	C-H bend	1379.82
Aromatic ester	C=O Stretch	1254.47
Aliphatic compound	C-O-C Stretch	1067.41
Halogen	C-H bend	893.844

Table 4: FTIR peak value of Anethum graveolens L. leaves in Methanol extract

Functional Group	Component (Peak)	Wave number (cm-1)
Alcohols	O-H Stretch	3504.99
Alcohols, Phenols	O-H Broad	3325.64
Alkane	C-H Stretch	2927.41
Alkane	C-H Stretch	2854.13
Nitrile	C=N Stretch	2383.59
Aldehyde	C-H Stretch	1737.55
Amide	N-H Stretch	1625.7
Aromatic ring	C=C Stretch	1461.78
Ester Carbonyl	C=O Stretch	1269.9
Aromatic and vinyl	C-O Stretch	1042.34
vinyl	C-H bend	907.344
Halogen	C-H bend	667.892

Figure 5: FTIR spetra of **Anethum graveolens** L. leaves in Hydroalcoholic extract.

Figure 6: FTIR spetra of **Anethum graveolens** L. leaves in Dichloromethane extract.JPG

Figure 7: FTIR spetra of **Anethum graveolens** L. leaves in Chloroform extract

Table 5: FTIR peak value of *Anethum graveolens* L. leaves in Hydroalcoholic extract

Functional Group	Component (Peak)	Wave number (cm-1)
Alcohols	O-H broad	3505.95
Alkane	C-H medium	2927.41
Alkane	C-H Stretch	2856.06
Nitrile	C=N stretch	2384.55
Cyclic aromatic Ketone	C=O Stretch	1733.69
Amide	N-H Stretch	1617.98
Vinyl aromatic	C-H Bend	1460.81
Aliphatic compound	C-H bend	1385.6
Ester carbonyl	C=O Stretch	1267.97
Non conjugated alkene	C-O-C	1038.84
vinyl	C-H bend	906.379
Halogen	C-H bend	694.248

Table 6: FTIR peak value of *Anethum graveolens* L. leaves in Dichloromethane extract

Functional Group	Component (Peak)	Wave number (cm-1)
Alkane	C-H Stretch	2924.52
Alkane	C-H Stretch	2853.17
Nitrile	C=N stretch	2387.44
Cyclic aromatic Ketone	C=O Stretch	1735.62
Amide	N-H Stretch	1625.7
Vinyl aromatic	C-H Bend	1461.78
Aliphatic compound	C-H bend	1372.1
Ketone aromatic ester	C=O Stretch	1271.82
Ester	C-O Stretch	1172.51
Alkene	C-H bend	969.055
Halogen	C-H bend	895.773
Halogen	C-H bend	729.925

Table 7: FTIR peak value of *Anethum graveolens* L. leaves in Chloroform extract

| Functional Group	Component (Peak)	Wave number (cm-1)
Alcohols	O-H Stretch	3531.02
Alkane	C-H Stretch	2956.98
Alkane	C-H Stretch	2923.56
Alkane	C-H Stretch	2850.27
Cyclic aromatic Ketone	C=O Stretch	1716.34
Amide	N-H Stretch	1624.73
Venyl aromatic	C-H Bend	1462.74
Aliphatic compound	C-H bend	1375
Ester	C-O Stretch	1166.72
Aromatic and vinyl	C-O Stretch	1074.16
Alkene	C-H bend	967.126
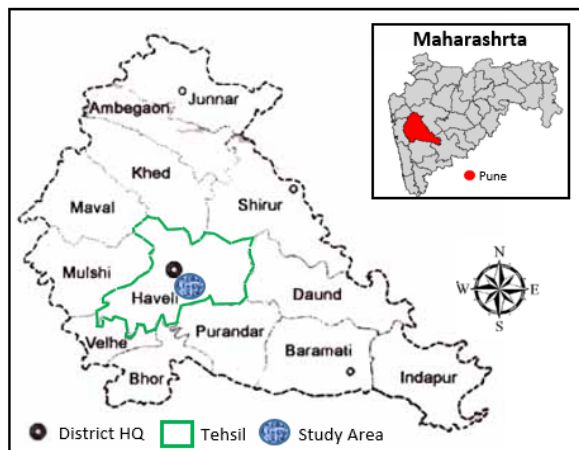
Figure 8: Location map of the study area

intensive peak observed at 1737.55, 1625.7, 1461.78,1269.9, 1042.34, 907.344 cm^{-1} and 676.892 cm^{-1} which indicates the aldehyde, amide, cyclic aromatic ketone, ester carbonyl, aromatic and vinyl compound, vinyl and halogen compound respectively.

FTIR spectroscopic analysis of hydroalcoholic extract was shown in Figure 5 and Table 5 the peak observed at 3505.95, 2927.41, 2856.06, 2384.55, 1733.69, 1617.98, 1460.81, 1385.6, 1267.97, 1038.48, 906.79 & 694.248 cm^{-1} corresponds to the functional groups such as alcohol(O-H bond), alkane(C-H stretch &C-H medium), nitrile compound (C=N), cyclic aromatic ketone, amide, vinyl aromatic compounds, aliphatic compounds, ester carbonyl, alkene, vinyl compounds and halogen compounds respectively.

In Figure 6 and Table 6 functional groups are identified in dichloromethane extract of *Anethum graveolens* L. leaves. The intensive peaks are observed at 2924.52, 2853.17, 2387.44, 1735.62, 1625.7, 1461.78 cm^{-1} which are corresponds to alkane (C-H), nitrile, aromatic ketone, amide (N-H), and vinyl aromatic compounds respectively. The peak shows at 1372.1, 1271.82, 1172.51, 969.055, 895.773, 729.925 cm^{-1} which are assigned to aliphatic compound, ketone, ester, alkene (C-H) and halogen compounds respectively.

Figure 7 and Table 7 shows the spectra of *Anethum graveolens* L. leaves in chloroform extract which observed the peak at 3531.02 cm^{-1} attributes O-H it indicates hydroxyl group. The peak at 2956.98, 2923.56, 2850.27 cm^{-1} which shows the C-H stretching frequency variation it means alkanes are present in chloroform extract. Some other functional groups such as aromatic ketones, amide, vinyl aromatic compound, aliphatic compound, ester, aromatic and vinyl compound, alkenes are observed at 1716.34, 1624.73, 1462.74, 1375, 1166.72, 1074.16, 967.126 cm^{-1} respectively.

Discussion

The objective of the present study was to identify the functional groups present in aqueous, acetone, ethanol, methanol, hydroalcoholic, dichloromethane and chloroform

extracts of *Anethum graveolens* L. leaves by FTIR analysis. FTIR analysis explains chemical structure and help to clarify the important role of functional groups as bioactive components in leaves and seeds of medicinal plants (*Ocimum sanctum*, *Azadirachta idica*, *Pongamia pinnata*, *Celastrus paniculatus and Embelia ribes*) for illness (Nair *et al.*2013). Analysis and identification of phytoconstituents of plant has been carried by using various techniques such as GS-MS, FTIR and HPTLC (Subrahmanian *et al.* 2017).

Alcohols, methyl groups, alkyl groups, esters, ether, carboxylic acid, deoxyribose and anhydrides were all present due to the appearance of C=O, C-H, C=C and C-O, C-C and C-O bonding structures (Dukor 2002, Sohrabi *et al.* 2005). The absorption band seen between 3373 and 3422 cm^{-1} may be due to N-H/C-H/O-H amines and amides being stretched (Mohan 2001). The presence of polymeric hydroxyl derivative is indicated by the high absorption band between at 3200 to 3400 cm^{-1}. The presence of primary amine is indicated by N-H vibration and the presence of amino acid is indicated by the extremely strong absorption seen in 1600-1660 cm^{-1} area. The band seen at around 2848 cm^{-1} represents the methylene groups C-H symmetric stretching in aliphatic molecules observed in *Aerva lanata* L. plant (Raghvendra *et al.* 2011).

In present study the broad peak observed at 3314 and 3270.68 cm^{-1} in aqueous and 3325.64cm^{-1} in methanol extract of leaves, it indicates hydroxyl groups. The peak observed at 1659.45, 1621.84, 1619.91, 1617.98, 1625.7, 1617.98, 1625.7, 1624.73 cm^{-1}in all extracts it indicates the presence of amino acid. The peak intensities were observed in present study at 2748.07, 2853.17, 2852.2, 2854.13, 2927.41, 2856.06, 2924.52, 2853.17, 2850.27, 2923.56, 2956.98 cm^{-1} it indicates that methylene, methyl ether, methyl, aldehyde and alkane groups were present in all extracts of leaves. Anthraquinones present as aromatic ethers due to the presence of C-O stretch at 1297.86, 1114.65, 1170.58, 1042.34, 1172.51, 1166.72, 1074.16 cm^{-1} and Carboxylic acid were found due to the presence of C=O stretch at 1736.58, 1733.69, 1735.62, and 1716.34 cm^{-1}. All extracts of *Anethum graveolens* L. leaves showed strong peak between 3000-2800 cm^{-1}, sign of C-H stretching vibration, which indicates lipids and proteins. There is no peak value observed between 2200-2226 cm^{-1}it indicates that no hazardous chemical present in all extracts of *Anethum graveolens* L. leaves.

FTIR analysis of the essential oil of *Anethum graveolens* L. revealed the presence of functional groups such as alcohol, alkane, ketone, hydroxyl group and alkene (Dimov *et al.* 2017 and Dimov *etal.*2018). Nithyadevi and Sivakumar (2015) analyzed the methanolic extract of *Solanum torvumto* by FTIR spectroscopy revealed the presence of alcohol, alkane, aromatics, alkyl halide, carboxylic acid and halogen compound as a functional group.

The presence of amino acids, alkenes, nitrates, ethers, organic halogen compounds and carbohydrates in plants is indicated by stretching and bending vibrations.

Carboxylic acid present in medicinal plants acts as the primary pharmaceutical ingredient in treating headaches, ulcers, stomatitis, jaundice, fever, hemicranias, edema, joint discomfort by rheumatism, liver pain and cow wound (Nair *et al.* 2013). The primary building blocks of protein synthesis are amino acids, amines, amides used as hair tonic and herb oils and control blood pressure, anxiety, nephrosis, acidosis, pneumonia, diabetes, repair tissue, provide energy to body (Muruganatham *et al.* 2009, Husain *et al* 2019). Any change in protein could have a negative impact on the vital and complex groups of biological components, including the nitrogenous compounds of body and dietary intake, which carry out various biological processes to keep the cells homeostasis (Mitra and Bakers 2011). Polysaccharides, nitrates, carbohydrates and chlorates are used as disinfectants (Muruganatham *et al.* 2009). Protein is essential to physiology of living things. Secondary plant metabolites known as polyphenols occur in range of chemical forms, from condensed tannins and highly polymerized compounds to simple molecule like phenolic acids. The positive effects of polyphenols on human health are attributed to their potential role as antioxidants (Merly de Arma-Recard *et al.*2019). In the present study biologically essential functional groups like amides, phenols, alcohol, alkane, aromatic ether, ester, alkene, ketone group, hydroxyl group, carboxylic acid, halogen, amines were detected in different extracts of *Anethum graveolens* L. leaves.

Conclusion

The comparative account of different extracts of *Anethum graveolens* L. leaves represents some variations of peak which reveals different functional groups like phenols, carbonyls, alkanes, ketone, aromatic compounds which may responsible for various medicinal properties of *Anethum graveolens* L. leaves. The corresponding peaks of phenols and flavonoids shows that *Anethum graveolens* L. leaves extracts may have rich source of phytochemical compounds. The determination of functional groups helps to identify the potential bioactive constituents of *Anethum graveolens* L. leaves. It may be used in herbal formulation preparation and also to study additional medicinal properties of *Anethum graveolens* L.

Acknowledgement

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