Phytochemical, GC-MS and FT-IR Analysis of *Papaver somniferum* L

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Abstract
The present study was aimed to analysis of bioactive constituents of *Papaver somniferum* (Poppy seed). The ethanol extract of the seeds were subjected to Phytochemical Screening, Gas chromatography- mass spectroscopic (GC-MS) and Fourier transform infrared spectroscopy (FTIR) analysis. GC-MS analysis of the seeds was performed using a Scion 436- GC Bruker model nd Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) and IR spectrum was recorded in spectrophotometer (Shimadzu, IR Affinity1, Japan). Phytochemical screening for seeds extracts indicated the presence of various secondary metabolites like Alkaloid, Cardiac Glycosides, Flavonoid, Phytosterols and Terpenoids. GC-MS analysis of compounds with totally, Thirty Nine volatile compounds major chemical compounds were identified, such as 9-Octadecynoic acid(30.72%), 9-Tetradecen-1-ol, acetate, (E)- (24.02%), 9,12-Octadecadienoic acid, methylester, (E,E)- (7.82%), cis-9,10-Epoxyoctadecan-1-ol (7.43%) and Undec-10-ynoic acid(4.36%). FT-IR analysis of peak values with various functional compounds such as alcohols, phenols, carboxylic acids, aldehydes, amides, amino acids, anhydrides, esters, ketones, Unsaturated aliphatics, aromatics, unsaturated heterocycles, amines, Nitro compound, Alkanes, alkenes, sugars,Sulphur, phosphorus, and fluorine compounds. The present results concluded that the phytochemicals was observed in ethanol extract which revealed that the *Papaver somniferum* (Poppy seed) is potential use in different fields namely medical and pharmaceuticals and greatly valuable in medicinal practice for the treatment of several human aliments.

Keywords: GC-MS, FT-IR, Papaver somniferum L and NIST.

Introduction
Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods (Pundir *et al.,* 2010). Herbs and spices have been used for flavoring, food preservation, and/or medicinal commitments. Currently many ethnic cuisines are familiar for their reliance on "signature" herbs and spices. Several readings have endorsed the antimicrobial, antioxidant and pharmaceutical properties of spices and herbs to their phenolic compounds (Shan *et al.,* 2005). Several studies have shown that spices are able to counteract oxidative stress in in vitro and in vivo systems (Ahmed *et al.,* 2000). They extend the storage life of foods by preventing rancidity and oxidation of lipids (kelen and Tepe, 2008) or through bacteriostatic or bactericidal activity (Nazef *et al*., 2008) and they execute the antifungal activity (Kotzekidou *et al*., 2008). Spices and their extracts were had various therapeutic properties (Ayodele et al., 2009), they are affect digestion processes differently. Most of them stimulate the secretion of saliva.

Papaver somniferum L. belongs to the *Papaveraceae* family, and is commonly known as "Opium poppy." The plant is found wild in various parts of Europe, northern Africa, and western Asia (GRIN database 2009). It is traditionally used as an herbal medicine against coughing, bronchitis, sore throat, minor sleep problems, and possesses a sedative effect (Soulimani *et al*.,2001). Previous investigations on this plant have revealed its nutritional composition (Trichopoulou *et al*.,2000), content of alkaloids, (Kalav,and Sarıyar, 2007) and ethnobotanical studies (Scherrer *et al*., 2005, Kultu, 2007) and (Cornara *et al*., 2009). Poppy seeds are used in traditional cuisine of several nations, mostly in confectionary and bakery food products such as fillings in cakes and desserts, or sprinkled on bread or rolls. (Erinç *et al*,. 2009). Moreover, they are a source of highly valuable oil, which is used not only for culinary purposes but also as an adjuvant for pharmaceutical and medical diagnostics, or as a component of cosmetic products and high-class oil-paints or varnishes (Krist *et al.,* 2005).

GC-MS and FT-IR has played an important role in pharmaceutical analysis in recent years (Movasaghi *et al*., 2008), recently, spectroscopy has emerged as one of the major tools for biomedical claims and has made noteworthy progress in the field of clinical evaluation. Exploration has been accepted on a number of natural tissues using spectroscopic techniques, including FT-IR spectroscopy. GC-MS analysis is a breakthrough in analysis of phytoconstituents and structure elucidation of these compounds as they have a sensitivity of detecting compounds as low as 1ng (Liebler *et al*., 1996). The present study was carried out the bioactive compounds present in the *Papaver somniferum* L Spice in ethanol extract with the aid of GC-MS and FT-IR techniques, which may offer a perception in its use of out-dated medicine.

Material and Methods
Extraction and Phytochemical Screening
Papaver somniferum L were dried and powdered using a mixer blender to make fine powder. Then 2 grams of the powdered sample was added to 250 mL of solvent was eluted sequentially based on the polarity index of the solvents. Then the extracts were subjected for rotary evaporator and saved at fridge for future uses.

Preliminary qualitative analysis of phytochemical screening was performed with shade dried and powdered of

the spice. The presence and absence of derivative compounds like alkaloids, carbohydrates, Phytosterols, flavonoids, phenolic, tannins, saponins, and terpenoids were confirmed by phytochemical screening using standard protocols (Harborne,1973).

Preparation of Extracts for GC –MS

20 g of the powdered seeds of Papaversomniferum L. were soaked in 100ml of 95% methanol for 12 h and filtered through Whatmann filter paper No. 41 along with 2 g sodium sulfate to remove the deposits and traces of water in the remainder. The filtrate was then concentrated and the extract contained both polar and nonpolar phytocomponents of the plant material used. 2 µl of this solution was used for GC/ MS analysis (Muthukumaran *et al*.,2017).

GC Condition and Identification of Compounds

The sample was examined through Gas Chromatography Mass Spectrometry/Mass Spectrometry Electron Ionization (GC-MS/EI) mode. The GC-MS/MS is a Scion 436- GC Bruker model coupled with a Triple quadruple mass spectrophotometer with fused silica capillary column BR-5MS (5% Diphenyl/95% Dimethyl polysiloxane) and Length: 30m; Internal diameter: 0.25 mm; Thickness: 0.25 µm. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 µl was working (split ratio of 10:1). The injector temperature 250°C; ion-source temperature 280°C.

The oven temperature was automated from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then5°C/min to 280°C, windup with a 9 min isothermal at 280°C and total GC running time was 41 min. This last escalation was to clean the column from any residues. The mass spectrometer was activated in the positive electron ionization (EI) mode with ionization energy of 70eV. The solvent delay was 0-3.0 min. A scan intermission of 0.5 seconds and fragments from m/z 50 to 500 Da was programmed. The inlet hotness was set at 280 °C, source temperature 250 °C. The relative fraction amount of each component was calculated by comparing its average peak area to the total areas. Software approved to handle mass spectra and chromatograms was MS Work station 8.

The NIST Version 2.0 library database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for identifying the chemical components. The GC-MS/MS was performed by Food Safety and Quality Testing Laboratory, Indian Institute of Food Processing Technology, Thanjavur

FTIR Spectroscopic Analysis

Fourier transform infrared spectrophotometer (FTIR) is perhaps the most potent tools for identifying the types of chemical bonds (functional groups) present in compounds. Dry powders of altered solvent extracts of each plant material were used for FTIR analysis. 10mg of the dry extract powder was encapsulated in 100 mg of KBr pellet, in orderto prepare translucent sample disc. The powdered sample of each plant specimen was loaded in FTIR Spectroscope (Shimadzu, IR Affinity1, Japan), with a scan range from 400 to 4000 cm-1 with a resolution of 4cm-1.

Result and Discussion

Phytochemical Analysis

Spices have been supplementary to foods since ancient times as flavoring agent, also as food preservers and folk medicines. Spice is a natural compound that is extracted from the seeds, fruits, flowers or trunks (skin, roots, leaves) of several plants and add to food to provide taste, smell or flavor. Spices are staple dietary additives consumed all over the world (Farrell, 1990). Each spice has a unique aroma and flavor that derive from compounds known as phytochemicals or secondary metabolites. In the present study, the investigation of phytochemical screening was done by ethanol extract of *Papaver somniferum* L.The result revealed that the ethanolic extract of *Papaver somniferum* L recorded the presence of Alkaloid, Cardiac Glycosides, Flavonoid, Phytosterolsand Terpenoids whereas the CarbohydratesSaponins, Tannins were absent in the extract (Table 1).

These compounds involved in plants to protect against herbivorous insect vertebrates, fungi pathogens and parasites (Walker, 1994). For centuries the inherent value as well as potential; toxicity of phytochemicals to human health has been recognized (Charaka, 1994). Spices are used as the substances that increase the taste and variation of food (Bulduk, 2004). The spices, herbs, plant extract and their phytoconstituents have been informed for anti-inflammatory, antidiarrheal, antimicrobial, antioxidant and insecticidal activities (Chouhan and Singh, 2011)

Table1: Phytochemical screening of *Papaver somniferum* L

Phytochemical	Poppy seed
Alkaloids	+
Carbohydrate	-
Cardiac Glycosides	+
Flavonoids,	+
Phytosterols	+
Saponins	-
Tannins	-
Terpenoids,	+

+ Present - Absent

GC MS Analysis

The compounds present in the ethanolic extract of *Papaver somniferum* L, were identified by GC-MS analysis (Fig. 1). Thirty Nine volatile compounds from ethanolic extract of *Papaver somniferum* L were separated and identified by GCMS. The components identified, molecular formulae, molecular weight and the time of elution with peak area were delivered in Table 2.

The GC-MS analyses of Papaver somniferum L established the identification of 39 volatile compounds in the ethanolic extract. The composition are as follows: 9-Octadecynoic acid (30.72%), 9-Tetradecen-1-ol, acetate, (E)- (24.02%), 9,12-Octadecadienoic acid, methyl ester,

(E,E)- (7.82%), cis-9,10-Epoxyoctadecan-1-ol (7.43%) and Undec-10-ynoic acid (4.36%). The chemical group classifications are as follows: Monoterpenes (1.33%), Aromatic (0.47%), Amino acid (1.42), Fatty acid (51.03%), Acetate (24.31%), Nitrogen compounds (0.14%), Alcohol (0.73%), Aldehyde (0.33%), Alkanes (1.22%), Alkenes (1.07), Esters (0.94%), Epoxy compounds (2.23%), naphthalene (0.71%) and ketones (0.75%).

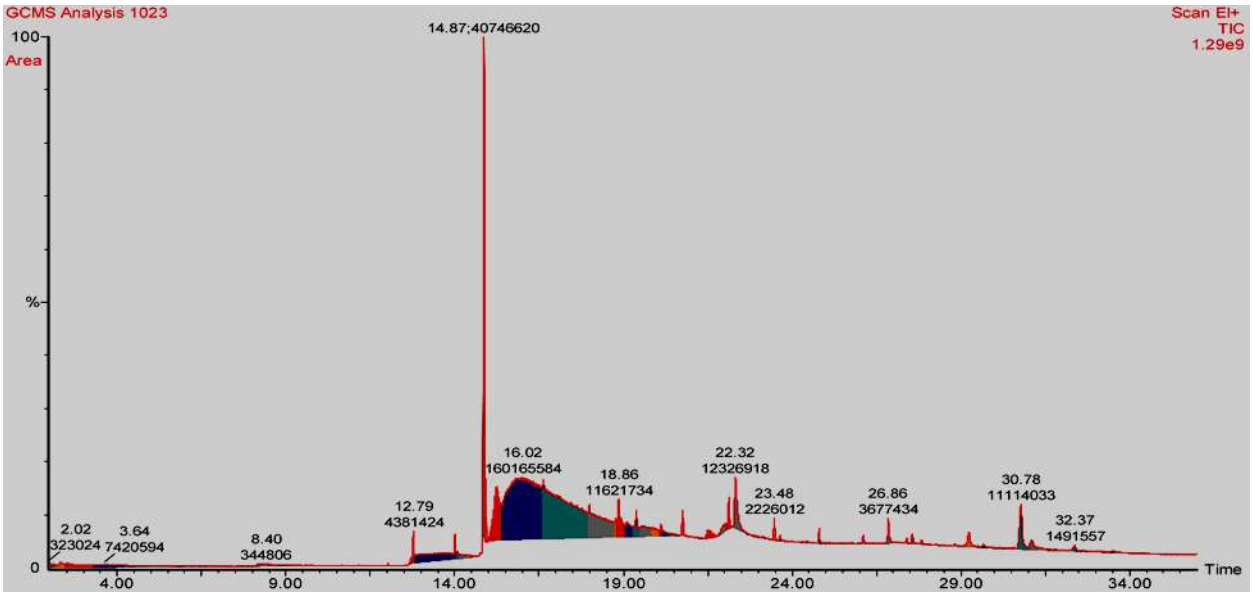

Fig. 1: Peak area percentage of (GC-MS) Gas column mass spectrometry in *Papaver somniferum* L

Table 2: GC-MS analysis revealed the presence of bioactive compounds in the Papaversomniferum L (Poppy seeds).

S. No	Identified Compound Details	Activity
1	á-Pinene(RT-2.06),Molecular Formula- $C_{10}H_{16}$, MW -136, Peak Area% -0.12, CompoundNatureMonoterpene	Anti-inflammatory, Sedative, Anticancer, Antitumor, Antibacterial, Antiflu, Nematicide, Insecticide, Pesticide, Herbicide. Flavor, Immunomodulator, Fungistat, Antiobesity, Detoxicant, Chemo preventive, Expectorant, Photo sensitizer
2	Benzene, 1-methyl-3-(1-methylethyl) (RT-2.33),Molecular Formula- $C_{10}H_{14}$, MW -134, Peak Area% -0.47, CompoundNature-Aromatic compound	No activity reported
3	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-,(RT-2.53),Molecular Formula- $C_{10}H_{16}$, MW -136, Peak Area% -1.21, CompoundNature-Monoterpene	Anti-inflammatory, Sedative, Anticancer, Antitumor, Antibacterial, Antiflu, Nematicide, Insecticide, Pesticide Herbicide, Flavor, Immunomodulator, Fungistat, Antiobesity, Detoxicant, Chemo preventive, Expectorant, Photo sensitizer
4	Butanoic acid, 4-(dimethylamino)-3-hydroxy,(RT-3.64),Molecular Formula- $C_6H_{13}NO_3$, MW -147, Peak Area% -1.42, CompoundNature-Amino compound	Antimicrobial
5	3-Ethylheptanoic acid,(RT-6.01),Molecular Formula- $C_9H_{18}O_2$, MW -158, Peak Area% -0.04, CompoundNature- Fatty acid compound	No activity reported
6	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-,(RT-8.40),Molecular Formula- $C_4H_9NO_5$, MW -151, Peak	Antimicrobial

	Area% -0.07, CompoundNature- Nitrogen compound	
7	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-,(RT-8.40),Molecular Formula- $C_4H_9NO_5$, MW -151, Peak Area% -0.07, CompoundNature- Nitrogen compound	Antimicrobial
8	Cyclopentaneundecanoic acid, methyl ester-,(RT-12.04), Molecular Formula- C17H32O2, MW -268, Peak Area% -0.05, CompoundNature- Fatty acid ester	No activity reported
9	Tetradecanoic acid, ethyl ester-,(RT-12.79),Molecular Formula- $C_{16}H_{32}O_2$, MW -256, Peak Area% -0.84, CompoundNature-Myristic acid ester	Nematicide,Antioxidant,Cosmetic Cancer preventive,Hypercholestrolemic Lubricant
10	Undec-10-ynoic acid-,(RT-14.08),Molecular Formula- $C_{11}H_{18}O_2$, MW -182, Peak Area% -4.36, CompoundNature- Unsaturated fatty acid	No activity reported
11	n-Hexadecanoic acid-,(RT-14.10), Molecular Formula- $C_{16}H_{32}O_2$, MW -256, Peak Area% -0.22, CompoundNature-Palmitic acid	Antioxidant HypocholesterolemicNematicide Pesticide, Anti androgenic FlavorHemolytic 5-Alpha reductase inhibitor
12	Undecanoic acid-,(RT-14.17), Molecular Formula- $C_{11}H_{22}O_2$, MW -186, Peak Area% -0.38, CompoundNature- Saturated fatty acid	No activity reported
13	9,12-Octadecadienoic acid, methyl ester, (E,E),(RT-14.87),Molecular Formula- $C_{19}H_{34}O_2$, MW -294, Peak Area% -7.82, CompoundNature- Linoleic acid ester	Hypocholesterolemic,Nematicide,Antiarthritic,Hepatoprotective, Anti androgenic, 5-Alpha reductase inhibitor Antihistaminic,Anticoronary, InsectifugeAntieczemic,Antiacne
14	9,12-Octadecadienoic acid (Z,Z)-(RT-14.93),Molecular Formula- $C_{18}H_{32}O_2$, MW -280, Peak Area% -0.78, CompoundNature- Linoleic acid ester	HypocholesterolemicNematicideAntiarthriticHepatoprotective Anti androgenic 5-Alpha reductase inhibitor Antihistaminic AnticoronaryInsectifugeAntieczemicAntiacne
15	11,14-Eicosadienoic acid, methyl ester(RT-15.26),Molecular Formula- $C_{21}H_{38}O_2$, MW -322, Peak Area% -5.81, CompoundNature- Unsaturated fatty acid ester	Cardio protective
16	9-Octadecynoic acid(RT-16.02),Molecular Formula- $C_{18}H_{32}O_2$, MW -280, Peak Area% -30.72, CompoundNature- Unsaturated fatty acid ester	No activity reported
	9-Tetradecen-1-ol, acetate, (E)- (RT-16.63),Molecular Formula- $C_{16}H_{30}O_2$, MW -254, Peak Area% -24.02, CompoundNature- Acetate compound	No activity reported

18	cis-9,10-Epoxyoctadecan-1-ol(RT-18.00)Molecular Formula- $C_{18}H_{36}O_2$, MW -284, Peak Area% -7.43, CompoundNature- Alcoholic compound	Antimicrobial
19	1,2-15,16-Diepoxyhexadecane(RT-18.86)$C_{16}H_{30}O_2$, MW -254, Peak Area% -2.23, CompoundNature-Epoxy compound	No activity reported
20	Dodecane, 2,6,10-trimethyl,(RT-19.39)Molecular Formula- $C_{15}H_{32}$, MW -212, Peak Area% -1.01, CompoundNature- Alkane compound	No activity reported
21	9,12-Octadecadienal-(RT-20.11)Molecular Formula- $C_{15}H_{32}$, MW -264, Peak Area% -0.33, CompoundNature- Aldehyde compound	Antimicrobial Anti-inflammatory
22	Methoxyacetic acid, 4-tetradecyl ester-(RT-20.76)Molecular Formula- $C_{17}H_{34}O_3$, MW -286, Peak Area% -0.51, CompoundNature Ester compound	No activity reported
23	E,E-1,9,17-Docasatriene,(RT-21.51)Molecular Formula- $C_{22}H_{40}$, MW -304, Peak Area% -0.70, CompoundNature Alkene compound	No activity reported
24	cisZ-11,12-Epoxytetradecan-1-ol,(RT-22.13)Molecular Formula- $C_{14}H_{28}O_2$, MW– 228, Peak Area% -0.68, CompoundNature–Alcoholic compound	Antimicrobial
25	(Z)6,(Z)9-Pentadecadien-1-ol,(RT-22.32)Molecular Formula- $C_{15}H_{28}O$, MW -224, Peak Area% -2.36, CompoundNature Alcoholic compound	Antimicrobial
26	Methoxyacetic acid, 3-tetradecyl ester,(RT-23.48)Molecular Formula- $C_{17}H_{34}O_3$, MW -286, Peak Area% -0.43, CompoundNature- Ester compound	No activity reported
27	1,E-11,Z-13-Octadecatriene,(RT-23.65)Molecular Formula- $C_{18}H_{32}$, MW -248, Peak Area% -0.13, CompoundNature-Alkene compound	No activity reported
28	trans-2-Undecen-1-ol,(RT-24.81)Molecular Formula- $C_{11}H_{22}O$, MW -170, Peak Area% -0.31, CompoundNature- Alcoholic compound	Antimicrobial
29	E-2-Tetradecen-1-ol,(RT-26.11)Molecular Formula- $C_{14}H_{28}O$, MW -212, Peak Area% -0.21,	Antimicrobial

	CompoundNature- Alcoholic compound	
30	Naphthalene, decahydro-2,2-dimethyl-,(RT-26.86)Molecular Formula- $C_{12}H_{22}$, MW -166, Peak Area% -0.71, CompoundNature- Naphthalene compound	No activity reported
31	2-Hydroxy-(Z)9-pentadecenyl propanoate-,(RT-27.56)Molecular Formula- $C_{18}H_{34}O_3$, MW -298, Peak Area% -0.29, CompoundNature- Hydroxy compound	No activity reported
32	13-Oxabicyclo[10.1.0]tridecane-,(RT-27.84)Molecular Formula- $C_{12}H_{22}O$, MW -182, Peak Area% -0.13, CompoundNature- Alkane compound	No activity reported
33	E,E-1,9,17-Docasatriene-,(RT-28.83)Molecular Formula- $C_{22}H_{40}$, MW -304, Peak Area% -0.07, CompoundNature- Alkene compound	No activity reported
34	Dodeca-1,6-dien-12-ol, 6,10-dimethyl--,(RT-29.24)Molecular Formula- $C_{14}H_{26}O$, MW -210, Peak Area% -0.73, CompoundNature- Unsaturated alcoholic compound	No activity reported
35	Z,Z,Z-4,6,9-Nonadecatriene-,(RT-29.69)Molecular Formula- $C_{19}H_{34}$, MW -262, Peak Area% -0.17, CompoundNature- Alkene compound	No activity reported
36	5à-Androstan-16-one, cyclic ethylene mercaptole-,(RT-30.78)Molecular Formula- $C_{21}H_{34}S_2$, MW -350, Peak Area% -2.13, CompoundNature- Steroid	Antimicrobial Anti-inflammatory Anticancer Diuretic Antiarthritic Antiasthma
37	Oxacycloheptadec-8-en-2-one-,(RT-31.11)Molecular Formula- $C_{16}H_{28}O_2$, MW -252, Peak Area% -0.75, CompoundNature- Ketone compound	No activity reported
38	cis-7,cis-11-Hexadecadien-1-yl acetate-,(RT-32.37)Molecular Formula-$C_{18}H_{32}O_2$, MW -280, Peak Area% -0.29, CompoundNature- Acetate compound	No activity reported
39	12-Methyl-E,E-2,13-octadecadien-1-ol-,(RT-33.51)Molecular Formula- $C_{19}H_{36}O$, MW -280, Peak Area% -0.08, CompoundNature- Unsaturated alcoholic compound	No activity reported

The functional therapeutic activity of the poppy seed compounds were identified through Dr. Duke's Phytochemical Database. The fatty acids which constitute 51.03% possess antioxidant activity and also the anti-inflammatory activity. Compounds namely, á-Pinene, 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-, n-Hexadecanoic acid are having insecticide activity and proven for pesticide activity. Flavors compounds like ketones, aldehydes and alcohols were enriched in poppy seed. The present study indicates that poppy seed is a good natural source of sterols. In addition, the findings in this study are

important for the nutrition sciences, because fatty acids and phytosterols, in particular, seem to have considerable effects on health.

FTIR Analysis of *Papaver somniferum*

The FT-IR spectrum was used to find the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. Once the extract was passed into the FT-IR, the functional groups of the components were separated based on its peaks ratio.

The ethanolic extract of *Papaver somniferum* L showed characteristic absorption bands at 3285.85 cm -1 for O–H stretching vibration presence of alcohols, phenols, 2925.05 cm -1(O–H stretching vibration presence of carboxylic acids), 2855.04 cm -1(CHO Aldehydes (Fermi doublet), 1744.18 cm -1(C=O Acid halides, aldehydes, amides, amino acids, anhydrides, carboxylic acids, esters, ketones, lactams, lactones, quinines), 1637.03 cm -1(C=C, C=N, NH Unsaturated aliphatics, aromatics, unsaturated heterocycles, amides, amines, amino acids), 1545.82 cm -1(NO2 Nitro compound CH3 and CH2 Alkanes, alkenes), 1454.4 cm -1(C–H bend stretching vibration presence of alkenes), 1313.89 cm -1(N–O stretching vibration presence of nitro compounds), 1235.3 cm -1(C-O-C and C-OH Ethers, alcohols, sugars S=O, P=O, C-F Sulphur, phosphorus, and fluorine compounds) and 1049.35 cm -1 for Si-O and P-O Organosilicon and phosphorus compounds (Fig. 2 & Table 3)

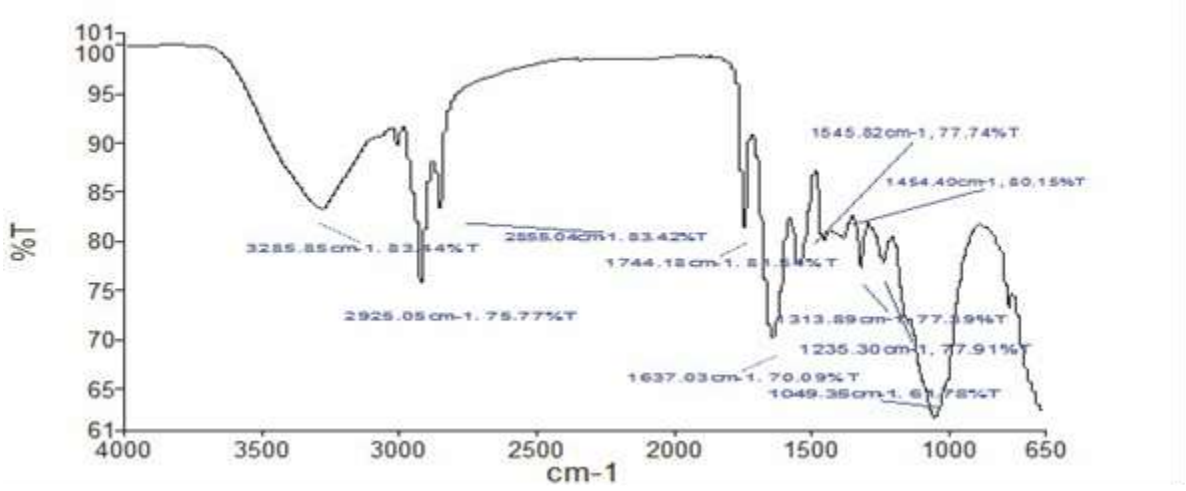

Fig. 2: FTIR- Spectrum wave numbers of *Papaver somniferum L*

Table 3: FTIR Analysis of *Papaver somniferum* L

S. No	Peak values	Frequency ranges(cm^{-1})	Functional groups and Possible compounds
1	3285.85	3500–3200	O–H stretchingvibrationpresenceof alcohols, phenols
2	2925.05	3300–2500	O–H stretchingvibrationpresenceof carboxylicacids
3	2855.04	2800-2600	-CHOAldehydes (Fermi doublet)
4	1744.18	1870-1650	C=O Acid halides, aldehydes,amides, amino acids, anhydrides, carboxylic acids, esters, ketones, lactams, lactones, quinines
5	1637.03	1650-1550	C=C, C=N, NH Unsaturated aliphatics, aromatics, unsaturated heterocycles, amides, amines, aminoacids
6	1545.82	1550-1300	NO2NitrocompoundCH3andCH2Alkanes, alkenes, etc
7	1454.4	1470–1450	C–H bend stretchingvibrationpresenceof alkenes
8	1313.89	1400–1290	N–Ostretchingvibrationpresenceofnitro compounds
9	1235.3	1300-1000	C-O-C and C-OH Ethers, alcohols,sugars S=O, P=O, C-F Sulphur, phosphorus, and fluorine compounds
10	1049.35	1100-800	Si-O and P-O Organ silicon andphosphorus compounds

Conclusion

The presence of naturally active compounds also contributes to its healthy value and thus proved to be potential sources of useful foods. Additionally, isolation, purification and characterization of the phytochemicals will make remarkable studies. The result of this study would lead to discovery of some compounds which are very useful for the manufacturing of new drugs. This primary information will simplify in leading further studies on discovery of bioactive

ingredients, resolve of their efficacy by in vivo studies and demonstration of their safety and efficacy in clinical trials.

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Conflict of Interest: None.

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