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Bioactive compounds identification through GC-MS Analysis and identify functions groups over FT-IR of *Pongamia pinnata* Seed oil

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Abstract

Due to the widespread use of plant products for medical purposes in the biotechnology and pharmaceutical sectors, phytochemical analysis of medicinal plants has become a more important and challenging task. In this present study, GC-MS was performed, and the findings showed that 17 bioactive phytochemical components were present. According to peak area and molecular weight, the 3H-Indole, 3, 3-Dimethyl-2[2-(2-hydroxy-5-nitrophenyl) ethenyl]-; 4H- chromeno [3,4-c]pyridine-4,5(3H)-Dione,4a, 10b-dihydro-2-(4-methoxyphenyl) were observed as the major constituents, although at different amounts. Absorption spectra were used to determine the prominent peak's wave number, intensities, and vibrational assignments. Numerous functional groups were found, including alkane, alkene, tertiary alcohol, secondary alcohol, carboxylic acid, alcohol, ester, phenol, halo compound, and nitro compounds. Therefore, this oil can be used in the antibacterial, stimulant, therapeutic, and pharmaceutical industries.

Keywords: GC-MS, FTIR, Phytochemical constituents and *P. pinnata*, Seed oil

Introduction

All over the world, people have been using various plants medicinally for many years. Plants are an excellent source of many drugs because they produce a wide variety of bioactive compounds. For contemporary drug development projects, natural products offer unparalleled chemical diversity. In the process of developing new pharmaceutical drugs, natural components are crucial (Basker *et al*., 1995). Throughout the world, traditional medicine is a significant part in the treatment of many health problems. Both traditional and contemporary medicines still rely on medicinal plants as important sources of medicinal products (Krentz and Bailey, 2005). Traditional treatments are becoming increasingly important due to the shortcomings of modern medicine. The

current focus of research is to ascertain the rationale provided by science for certain therapies beneficial effects (Gupta and Briyal 2004).

Pongamia pinnata Linn. (b) belongs to Fabaceae family. It is recognized as karanj in many traditional medicinal systems and is used in the treatment of many human diseases. (Chopade *et al*., 2008). The seeds are abundant in omega 3 fatty acidsand have an oil content of 28-34% on average. For many years, P. pinnata has been applied in conventional medicine. Especially in Indian medicinal systems like Ayurveda and Siddha.

The entire plant is used as an herbal cure for tumors, hemorrhoids, skin diseases, scabies, boils, arthritis, joint ulcers, diarrhea, etc (Meera *et al.,* 2003; Shobha and Thomas, 2001). Recently, research has demonstrated that *P. pinnata* is effective as a source of medication (Brijesh *et al.,* 2006). Many chemical compounds found in nature have drawn the attention of organic chemists who are interested in studying their structure and characteristics (Harborne 1973).

Materials and Methods

Study area and collection of oil extract:

The present study was conducted from March 2022 to July 2022 at the P.G. and Research Dept. of Zoology, RDGA College, Sivagangai, Tamil Nadu, India. *P. pinnata* seed oil was processed by a government-approved oil shop in Madurai. Tamilnadu, India GC-MS and FTIR analyses of bioactive compounds were performed in the Instrumentation Centre, ANJA College, Sivakasi, Viruthunagar, Tamil Nadu, India.

Gas chromatography mass spectroscopy

The Agilent chromatography GC (Model 7820A series) fitted with the VL-MSD detector (Model 5977E) was used to perform GC-MS analysis of the sample. For one minute, the temperature of the GC oven was set to 100^{o}C. Then, at a rate of 10°C per minute, it was increased to 270°C, where it stayed for thirty minutes. The carrier gas, helium, moved at a constant 2 ml/m. A 1.0 µl sample injection was automatically performed into the column (DB-5) with the injector temperature set to 270 - 270^{o}C. The injection method employed was split-less. Compounds were identified by comparing retention indices (RI), retention times (RT), WILEY mass spectra, NIST library data for the GC-MS instrument, and literature data.

Fourier Transform Infrared Spectrophotometer

FTIR is an extremely useful technique for distinguishing the sorts of functional groups or chemical bonds present in substances. According to the annotated spectrum, the chemical bond is indicated by the wavelength of light that is absorbed. An analysis of a molecule's infrared absorption spectra can reveal the chemical bonds. FTIR study was performed on *P. pinnata* seed oil extract. To create transparent sample discs, 10 mg of seed oil extract and 100 mg of KBr

particles were combined. Using a scan range of 500 to 4000 cm^{-1} and a resolution of 4cm^{-1}, the seed oil sample was placed into an FTIR spectroscope (Shimadzu, 8400S).

Results

The GC-MS characterization of seed oil extract of *P. pinatta* was analyzed and presented in Table 1 and Figure1. Totally, seventeen chemical compounds were identified, such as 7-Octadecenoic acid, methyl ester (0. 97 %), Oleic Acid (0.72 %), 9,12-Octadecadienoic acid (Z,Z)-(1.19 %), 1H-Pyrrole-2,5-dione,1-(4- ethylphenyl)--(5.39%), 1H Isoondole-1,3(2H)-dione, 2- (2-bromoethyl)-(9.21%), 3H-Indole, 3,3-Dimethyl-2[2-(2-hydroxy-5-nitrophenyl) ethenyl]- (17.35 %), Quinazolin-4(3H)-one, 2-[2-(4-methoxyphenyl)ethenyl]- (10.76 %), Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- (3.85 %), 1 H-Indole, 2-methyl-3-(2-pyridin-4-yl) thiazol-4yl (1.09%), Acetic acid [4-(1,1- dimethylethyl) phenoxyl]-methyl ester (3.84%), 4H- chromeno[3,4-c]pyridine-4,5(3H)-Dione,4a, 10b-dihydro-2-(4-methoxyphenyl) (13.25%), Molybdenum [1,2,3,3a,7a-eta)-2-methoxy-1H-inden-1-yl]bis(eta 3-2-propenyl (2.32%), 1,3-Bis(3-aminophenoxy)benzene (1.90 %), beta.-Sitosterol (8.83 %), 2,4-Diamino-7-[2-[3,4-methylenedioxyphenyl]vinyl] pteridine (2.78 %), 6-Hydroxy-2-methylcyclohepta(b)pyridin-7-one (5.24 %) and Triphenyl phosphate (8.31 %) were found in P. pinnata essential seed oil. Retention time, peak area, molecular formula and molecular weight all helped to identify the phytochemical substances.

Fig. 1: GC-MS analysis of essential oil of *P. pinnata*

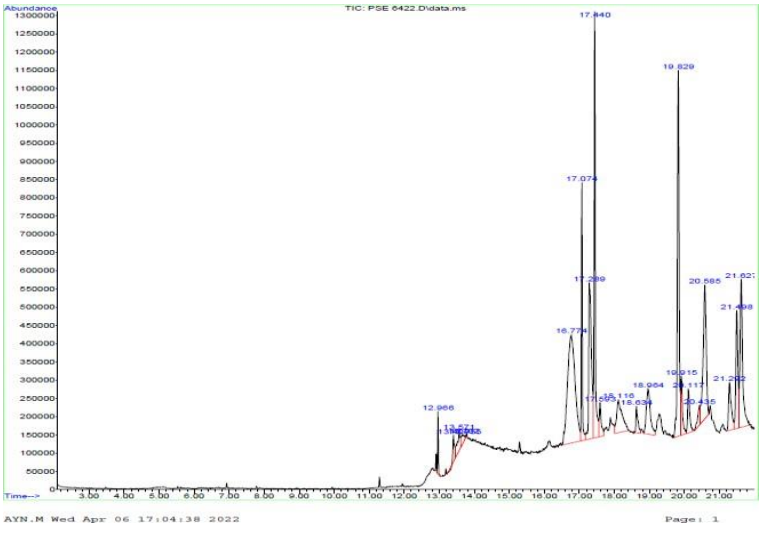
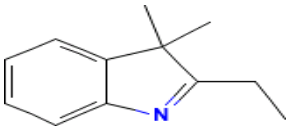


Table 1: GC-MS analysis of seed oil of *P. pinnata*

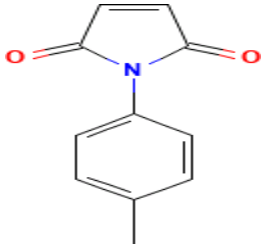
Sl.No	Retention time	Area	Compounds	Formula	Molecular Weight
1	12.97	0.97	7-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.48

2	13.05	0.72	Oleic Acid	$C_{18}H_{34}O_2$	282.46
3	13.57	1.19	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O$	280.44
4	16.77	17.35	3H-Indole, 3,3-Dimethyl-2[2-(2-hydroxy-5-nitrophenyl) ethenyl]-	$C_{12}H_{15}N$	173.25
5	17.07	5.39	1H-Pyrrole-2,5-dione,1-(4- ethylphenyl)-	$C_{11}H_9NO_2$	187.19
6	17.29	9.21	1H Isoondole-1,3(2H)-dione, 2- (2-bromoethyl)-	$C_{10}H_8BrNO$	254.08
7	17.42	10.76	4H-Benzo(def)naththo(2,3-b)carbazole	$C_{12}H_{13}N$	291.35
8	18.11	3.85	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{10}H_{50}O_7Si_8$	579.25
9	18.63	1.09	1H- Indole, 2-methyl-3-(2-pyridin-4-yl) thiazol-4yl	$C_{17}H_{13}N_3S$	291.37
10	18.96	3.84	Acetic acid [4-(1,1- dimethylethyl) phenoxyl]-methyl ester	$C_{13}H_{18}O_3$	222.28
11	19.82	13.25	4H- chromeno[3,4-c]pyridine-4,5(3H)-Dione,4a, 10b-dihydro-2-(4-methoxyphenyl)	$C_{19}H_{13}NO_4$	319.32
12	19.91	2.32	Molybdenum [1,2,3,3a,7a-eta)-2-methoxy-1H-inden-1-yl]bis(eta 3-2-propenyl	$C_{15}H_8Mo$	284.96
13	20.11	1.90	1,3-Bis(3-aminophenoxy)benzene	$C_{18}H_{16}N_2O_2$	292.30
14	20.58	8.83	beta.-Sitosterol	$C_{29}H_{50}O$	414.70
15	21.29	2.78	Pyrimidine, 4,5,6-triphenyl-	$C_{22}H_{16}N_2$	308.38
16	21.49	5.24	6-Hydroxy-2-methylcyclohepta(b)pyridin-7-one	$C_{11}H_9NO_2$	187.19
17	21.63	8.31	Triphenyl phosphate	$C_{18}H_{15}O_4P$	326.30

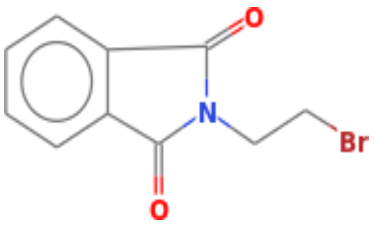
Figure: 2 GC-MS analysis of major bioactive compounds in oil of *P. pinnata*



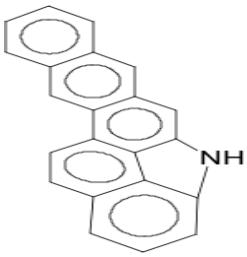
3H-Indole, 3, 3-Dimethyl-2[2-(2-hydroxy-5-nitrophenyl) ethenyl]-



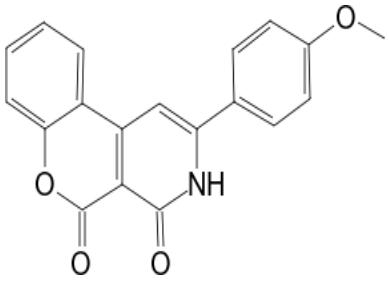
1H-Pyrrole-2, 5-dione,1-(4- ethylphenyl)-



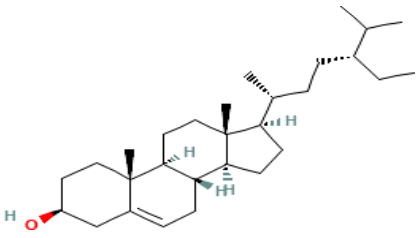
1H Isoondole-1,3(2H)-dione, 2- (2- bromoethyl)-



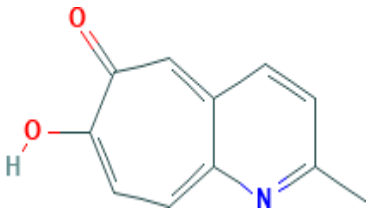
4H-Benzo(def)naththo(2,3-b)carbazole



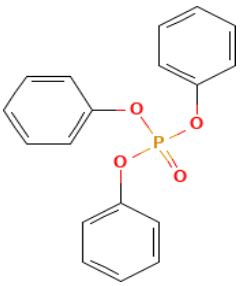
4H- chromeno [3,4-c]pyridine-4,5(3H)-Dione,4a, 10b- dihydro-2- (4-methoxyphenyl)



beta.-Sitosterol



6-Hydroxy-2-methylcyclohepta(b)pyridin-7-one



Triphenyl phosphate

FTIR analysis of the essential oil extract of *P. pinatta* was carried out and presented in Fig. 2. The compounds indicated show that the band is at 3478.38, 3076.25, 2904.60, 2674.12, 2308.63, 2039.58, 1747.39, 1597.91, 1532.34, 1377.08, 1243.04, 1147.57, 1096.46, 1035.70, 967.23, 815.83, 722.29, 586.31, and 516.89 cm^{-1}. The broad band at 3478.38 cm^{-1} O-H is stretching in alcohol groups. The presence of peaks at 3076.25 cm^{-1} and 2904.60 cm^{-1} corresponds to the alkenes and C-H stretching of alkane groups. The band at 2674.12 cm^{-1} is an O=H stretch in carboxylic acid. The peaks at 1747.39 cm^{-1} correspond to the C=O stretch esters and N=H bend in amine groups. The peak is peak is at 1532.34 cm^{-1}, 1377.08 cm^{-1}, and 1243.04 cm^{-1} in N-O stretch nitro compounds, O-H bend alcohol, and C-N stretch amine groups. The broad bands at 1147.57 cm^{-1} and 1096.46 cm^{-1} correspond to the C-O stretch in tertiary alcohol groups and the C-O stretch in secondary alcohol. The peak is at 1035.70 cm^{-1}, 815.83 cm^{-1}, and 586.32 cm^{-1} in S=O stretch sulfoxide, C=C bend alkene, and C-Br stretch in halo compounds.

Fig. 3 Functional groups of the components of seed oil extract of *by* FTIR

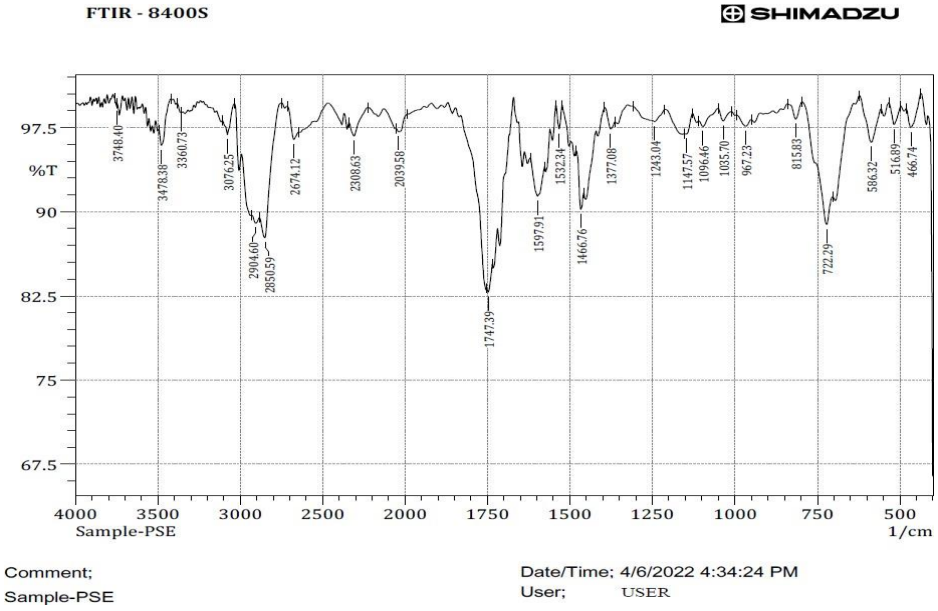


Table 2 Functional groups of the components of seed oil extract of *by* FTIR

Sl. No	Absorption (Cm^{-1})	Functional Groups	Compounds	Intensity
1	586.32	C-Br stretching	Halo compound	Strong
2	815.83	C=C bending	Alkene	Medium
3	1035.70	S=O stretching	Sulfoxide	Strong
4	1096.46	C-O Stretching	Secondary alcohol	Strong
5	1147.57	C-O stretching	Tertiary alcohol	Strong
6	1243.04	C-N stretching	Amine	Medium
7	1377.08	O-H bending	Alcohol	Medium
8	1532.34	N-O stretching	Nitro compound	Strong
9	1597.91	N=H bending	Amine	Medium
10	1747.39	C=O stretching	Esters	Strong
11	2674.12	O-H stretching	Carboxylic acid	Strong
12	2904.60	C-H stretching	Alkanes	Strong
13	3076.25	=C-H stretching	Alkenes	Medium
14	3478.38	O-H stretching	Alcohol	Strong

Discussion

Bioactive phytoconstituents *P. pinnata* seed oil may be responsible for the therapeutic potential of the extract. GC-MS, one of the most used methods for isolating plant components, was utilized to conduct the analysis. According to a GC-MS analysis *P. pinnata* contains 17 phytochemical substances, some of which may be involved in the plant species' therapeutic qualities (Thomas *et al*., 2012; Capra *et al*., 2019). According to our GC-MS analysis of some major organic compounds, 3H-Indole, 3,3-Dimethyl-2[2-(2-hydroxy-5-nitrophenyl) ethenyl]-(17.35%), 4H-chromeno[3,4-c]pyridine-4,5(3H)-Dione,4a, 10b-dihydro-2-(4-methoxyphenyl)- (13.25%), 4H-Benzo(def)naththo(2,3-b)carbazole (10.76%).

Kabir and Usman (2022) Heterocyclic compounds are crucial to biological processes and have a wide range of uses in the pharmaceutical sector. Many heterochemicals are used as medicines to treat various diseases and injuries. 1H-Isoindole-1,3(2H)-dione, 2-(2-bromoethyl)- heterocyclic compound with antifungal, anti-inflammatory, antibacterial, antiviral, antioxidant, anthelmintic, antiallergic, antihistaminic, herbicidal, larvicidal and anticancer activities. (9.21%) A

number of isoindole alkaloids have been identified from *P. oleracea* recently, and several of them have been shown to exhibit biological activities and strong anti-inflammatory properties (Xu *et al*., 2016; Meng *et al*., 2016; Li *et al*., 2017a; Li *et al*., 2017b). Beta-sitosterol (8.83%) is found in some unsaturated fatty acids.

Identification of (unknown) plant components has been an area of research interest (Kedik *et al.,* 2003; Sarveswaran *et al*., 2010). The first step is to apply scientific methods to clarify the advantages of traditionally used medicinal herbs (Tu *et al*., 2008). The GC-MS technique, which is used for the study of seed oils, may be used to assess the quantity of certain active compounds in herbs used in the food, pharmaceutical, cosmetic, or medical sectors (Sangwan 2018). Bio-oil with low calorific value and high acidity is produced by rapid pyrolysis of biomass as it often contains significant amounts of oxidizing substances such as alcohols, esters, carboxylic acids, nitro compounds and alkanes (Mochizuki *et al.,* 2013; Shadanki *et al.,* 2014).

FTIR analysis of the *P. pinnata* was also performed. With its help, chemical elements can be identified and chemical structures can be elucidated. Furthermore, there have been attempts to better understand the importance of functional groupings as bioactive factors in the treatment of many disorders (Nair *et al*., 2013). Carboxylic acid of the medicinal plant is a major drug used to treat arthritic joint pain, wounds, swelling, fever, jaundice, stomatitis, migraine and ulcers in cows. (Muruganatham *et al*., 2009)

Conclusion

Scientific research has demonstrated the beneficial effects of plant-based goods and their active substances on health in recent times. Many medicinal herbs have been prescribed in various medical texts to treat various ailments. *P. pinnata* is the source of many marketed compositions due to its various proven medicinal effects and large number of isolated phytoconstituents. It is a dependable biofuel, and more study in the pharmaceutical sector as well as efforts to make *P. pinnata* a powerful biofuel should be made.

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