Pharmacogn J. 2025; 17(1): 1-6
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Evaluation of Antioxidant Activity and Flavonoid and Phytocompounds Content in Pongamia Pinnata Seed

Honnesh NH[1], Santanu Saha[2]*

ABSTRACT

Introduction: Pongamia pinnata is a woody shrub and tree found in entire India mainly in dry areas. The seeds of P. pinnata are collected and traditionally oil is extracted and used to kill helminthes. The plant is a remedy for skin diseases, wound, rheumatism & ulcers and also as biofuel, green manure and fodder. This study is an attempt to know antioxidant activity of particular fraction which is having antifungal activity and flavonoid and fatty acids content which is responsible for the same. **Materials and methods:** In this investigation, an effort was made to know the antioxidant activity of PPEAF by DPPH assay, FRAP assay and H_2O_2 assay methods and Optimization of mobile phase by TLC method and supporting major flavonoid content quantification by HPTLC method **Results:** The result shown the ethyl acetate fraction has significant antioxidant activity of IC_{50} value of **212.09** (DPPH assay), **235.87** (FRAP assay) and **268.62** (H_2O_2 assay). The TLC method optimum mobile phase was found to be Toluene: ethyl acetate (7:3). The standard flavonoids used are Karanjin, Gallic acid and Quercetin. **Conclusion:** The HPTLC method results shown PPEAF was found to contain 1.465% Karanjin. GCMS result shown the PPEAF contain 13 phytocompounds in which Octadec-9-enoic acid at retention time 22.356 was found to be abundant.
Keywords: DPPH, FRAP, GCMS, Karanjin, Pongamia pinnata, PPEAF.

Honnesh NH[1], Santanu Saha[2]*

[1]Department of Pharmacognosy, Nitte College of Pharmaceutical Sciences, 6429, NITTE Campus, Govindapura, Gollahalli, Yelahanka, Bangalore - 560 064, Karnataka, INDIA.

[2]Department of Pharmacognosy, NGSM Institute of Pharmaceutical Sciences, Nitte (Deemed to be University), Panner, Deralakatte, Mangaluru-575018, Karnataka, INDIA.

Correspondence

Santanu Saha

Department of Pharmacognosy, NGSM Institute of Pharmaceutical Sciences, Nitte (Deemed to be University), Panner, Deralakatte, Mangaluru-575018, Karnataka, INDIA.

E-mail: santanusaha@nitte.edu.in

History

- Submission Date: 10-11-2024;
- Review completed: 16-12-2024;
- Accepted Date: 02-01-2025.

DOI : 10.5530/pj.2025.17.1

Article Available online

http://www.phcogj.com/v17/i1

INTRODUCTION

Honge and karanj are traditional names for the plant Pongamia pinnata. The typical oil content of the seeds is said to be between 28 and 34 percent, with a significant proportion of polyunsaturated fatty acids. Pinnatin, pongapin, pongagalabrone, karangin, and kanjone are all found in seed oil. The plant's leaves and stem contain flavones and chalcone derivatives such as galbone, pongone, pongalbol, and ponagallone A and B. The fruits of P. pinnata include pongamoside D, furnoflavanoid and glucosides, and three new pongamosides, A-C. Anticancer substances like paclitaxel, fluorophenylalanine, vinblastine, vincristine, teniposide, fluoxetine, and etoposide derivatives also found in P. pinnata root extract. The ground seeds are used to treat whooping cough, fever, bronchiole inflammation, irritations, chest infections, hemorrhoids, and anemia[1]. hence we designed present study to analyse antioxidant property and standardize the PPEAF for major flavonoid by HPTLC and fatty acid content by GCMS.

MATERIALS AND METHODS

All of the analytical-grade chemicals and reagents used in the research were acquired from Merck Pvt. Ltd. and S.D. Fine Chemicals Pvt. Ltd. in Bombay, India. We bought the phytocompounds from Yucca Enterprises in Mumbai. Vasa Scientific Pvt. Ltd. provided the TLC plates.

Collection and Authentification of Plants

Through Pioneer Agro Industries in Coimbatore, Tamil Nadu, the Pongamia pinnata seeds were purchased. At the Foundation for Revitalization of Local Health Traditions (FRLHT), Bangalore, the gathered plant materials underwent authentication, and the voucher specimen (4717-18/28.0/.2021) was deposited.

Extraction

After being dried in a hot air oven, the authenticated seeds were ground into a coarse powder and sieved. (5 kg) Following an ethanol maceration, the powders were subsequently separated using methanol, petroleum ether, chloroform, and ethyl acetate, in that order. The extract and fraction that were kept in the desiccator were obtained by filtering and drying.

Fractionation of ethanol extract

The 540g ethanol extract of Pongamia pinnata was extracted in stages using 8x500ml of petroleum ether 60 to 80°C, 8x500ml of chloroform, 8x500ml of ethyl acetate, and 8x500ml of methanol. Following a 30-milliliter wash with distilled water, the fractions were dried over anhydrous sodium sulfate and the solvent was removed by distillation.

Antioxidant activity

Method 1: Diphenyl Picryl Hydrazyl (DPPH) Radical Scavenging Assay[2]

Procedure

The basis of this assay is the evaluation of antioxidants' capacity to scavenge the stable radical (DPPH). In the reaction with hydrogen donors, the free radical DPPH is converted to the equivalent hydrazine. The discolouration assay is a more widely used method to assess this capability. It measures the absorbance drop at 518–528 nm that occurs when an antioxidant is added to a DPPH solution in ethanol

% Antioxidant activity is equal to {Absorbance of Control - Absorbance of Sample/ Absorbance of Control} times 100.

Cite this article: Honnesh NH, Santanu S. Evaluation of Antioxidant Activity and Flavonoid and Phytocompounds Content in Pongamia Pinnata Seed. Pharmacogn J. 2025;17(1): 1-6.

Method 2: FRAP (Ferric Reducing Antioxidant Power) Assay[2]

Procedure

Only fresh samples should be used for the most accurate and repeatable analysis. Aqueous, methanol, acetone, and ethanol are some of the solvents that can be used for plant extractions. The kind of particular material being extracted determines the extraction technique to use.

- Materials from plants were used to prepare the dilutions
- All of the tubes were filled with 2.5 ml of phosphate buffer (0.2 M, pH 6.6).
- All of the components were well combined in each tube.
- Afterwards, all of the samples were mixed with 2.5 milliliters of 1% potassium ferricyanide K3F(CN)6 solution.
- Using a vortex shaker, each reaction mixture was then thoroughly vortexed.
- The samples spent around 20 minutes in an incubator set at 50°C.
- Each sample received 2.5 ml of 10% trichloroacetic acid (TCA) after the incubation period was over.
- For ten minutes, the test tubes were centrifuged at 3,000 rpm. 2.5 ml of the supernatant was taken from these centrifuged samples and placed in different test tubes.
- Next, 0.5 ml of ferric chloride (FeCl3) was added to the same new, distinct test tubes.
- As a result, a bluish color formation appeared.
- At 700 nm, the absorbance was then measured.
- Higher absorbance was observed in a sample with higher concentration, and vice versa.

% Antioxidant activity is calculated as follows: {Absorbance of Control - Absorbance of Sample/ ~ x 100

Method 3: H2O2 (Hydrogen Peroxide) Free Radical Scavenging Activity Assay[2]

Procedure

For the dilution series, powdered plant extract was used.

A total of 600 microliters of H2O2 were then put into the tubes (control, sample, and standard).

A standard (ascorbic acid) or plant extract (100 µl) was applied to each test tube.

• Following that, phosphate buffer (pH 7.4) was added to each test tube until the volume reached 4 mL. As the standard, the same Ascorbic acid series (phosphate buffer, H2O2, and Ascorbic acid) was used without the sample.

• Each test tube was left at room temperature for ten minutes.

• The H2O2 solution's absorbance was measured at 300 nm in comparison to the blank.

• The following was used to compute the IC50 and the percentage of H2O2 scavenging activity in comparison to the standard.

% Antioxidant activity = {Absorbance of Control – Absorbance of Sample/

Absorbance of Control} x 100

Qualitative- Thin layer chromatography (TLC)

The plant fractions PPEAF were subjected to thin layer chromatographic studies to optimize the mobile phase.(Figure 1). The Ellagic acid, Luteolin and Diosmin standards were used for standardization of Pongamia fraction. The different mobile phases were prepared by using different solvents Toluene, ethyl acetate, diethyl amine, formic acid. The standardized mobile phase is used for quantification of flavonoid by HPTLC at Manipal College of pharmaceutical sciences, Manipal, Karnataka.

Quantitative- High performance thin layer chromatography (HPTLC)

HPTLC Instrumentation[3,4]

A PC running WinCATS software under MS DOS and a TLC scanner were the two components of the HPTLC system (Camag, Muttanz, Switzerland). The Linomat IV sample applicator used 100µl syringes and was coupled to a nitrogen tank. The following parameters were applied to each plate: band width 6 mm, distance between bands 10 mm, application volume 10 µl, and gas flow 150 nl/s. Each plate had one track of sample and various standards. A twin trough glass chamber that had been pre-stimulated with mobile phase was used to create the plates. Following development, a densitometer scanner was used for scanning.

The data generated was captured by WinCATS Planar chromatography version 1.1.5.0 software (Figure 2).

Gas chromatography mass spectroscopy (GCMS) analysis

The PPEAF sample was sent to National chemical laboratory, Pune for analysis. Gas Chromatography 7890B GC (Agilent Technologies) instrument connected with mass spectrophotometer was used.

RESULTS

The *Pongamia pinnata* seeds were found to contain 10.8 % ethanol extract. The fractions obtained from ethanol extract are Petroleum ether fraction (17%), Chloroform fraction (12.59%), Ethyl acetate fraction (10%) and methanol fraction (6.66%) respectively. The antioxidant activity results shown that PPEAF has IC50 value of 212.09, 235.87 and 268.62 at 500µg per ml concentration by DPPH scavenging of free radicals method, FRAP free radical scavenging method and H_2O_2 free radical scavenging method respectively (Table 1). The IC50 values for standard ascorbic acid in the DPPH, FRAP, and H2O2 test techniques are 12.17, 12.64, and 14.68, respectively. The optimized mobile phase for Pongamia pinnata ethylacetate fraction by TLC is Toluene: ethyl acetate (7:3)**.** The main flavonoid in *Pongamia pinnata* ethylacetate fraction was determined and estimated using HPTLC analysis. The optimized mobile phase was used to separate flavonoids from the 10µL spots that were applied and developed in the TLC chamber. For the estimation of flavonoids in P pinnata the standard Karanjin, Gallic acid and Quercetin were used. The sample and standard spots were developed using the mobile phase Toluene: ethyl acetate (7:3). Karanjin spot was clearly obtained in sample PPEAF at retardation factor 0.75. No Gallic acid and Quercetin spots observed in sample for the selected mobile phase. The area under the curve (AUC) of karanjin in sample PPEAF was 6918.1(100mg/ml concentration) and Standard Karanjin was 4721.2(1mg/ml concentration) at 254nm respectively. By calculation determined that PPEAF was found to contain1.465 % Karanjin (Table 2). The PPEAF GCMS results shown that ethyl acetate fraction contain 13 Phytocompunds, in which Octadec-9-enoic acid was found to be highest abundance other major phytocompound was Flunixin methyl ester (Table 3).

Table 1: **Results of Antioxidant activity of *Pongamia pinnata* ethylacetate fraction.**

SL NO	Concentration (µg/ml)	DPPH scavenging activity for free radicals	FRAP scavenging activity for free radicals	H_2O_2 scavenging activity for free radicals
	5	12.30	8.42	4.23
	10	16.54	13.63	8.54
	25	23.12	20.30	16.12
	50	30.56	28.42	22.56
	100	36.50	36.04	30.50
	250	68.42	62.38	58.42
	500	84.22	80.68	76.22
	IC50	**212.09**	**235.87**	**268.62**

*Standard Ascorbic acid has shown IC50 value of 12.17, 12.64, 14.68 in DPPH, FRAP and H2O2 assay methods respectively

Table 2: **HPTLC results of *Pongamia pinnata* ethylacetate fraction for phytochemicals.**

Standard/Sample	Initial Concentration	Rf (Retardation factor)	AUC(Area under curve) at 254nm
Karanjin	1mg/ml	0.75	4721.2
Gallic acid	1mg/ml	0.67	281.5
Quercetin	1mg/ml	0.67	1350.2
PPEAF(*Pongamia pinnata* ethyl acetate fraction)	100mg/ml	0.76	6918.1
% of Karangin			**1.4653%**

Table 3: **Phytocompounds identified by GCMS.**

Name of the compound	Retention time (min)	Abundance
Hexadecanoic acid, methyl ester	18.126	
n-Hexadecanoic acid	19.035	
Hexadecanoic acid, ethyl ester	19.222	
Methyl 10-trans,12-cis-octadecadienoate	20.885	
10-Octadecenoic acid, methyl ester	21.001	
6-Octadecenoic acid	22.252	
Octadec-9-enoic acid	**22.356**	**Highest**
Pentadecanoic acid	22.522	
Butyl 11-eicosenoate	24.989	
Eicosanoic acid	25.364	
Docosanoic acid	28.570	
Flunixin methyl ester	29.622	
2H-Indol-3-one, 2-(1,3-dihydro-1-methyl-3-oxo-2H-indol-2-ylidene)-1,3-dihydro-1-methyl-	29.837	

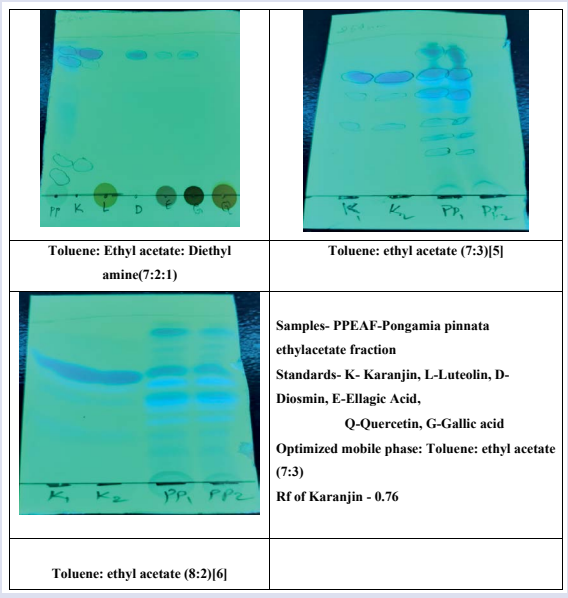
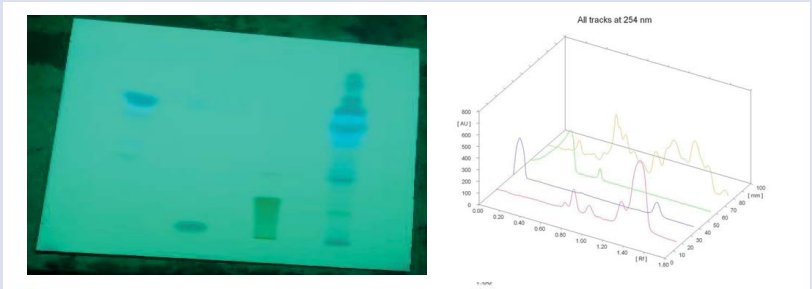
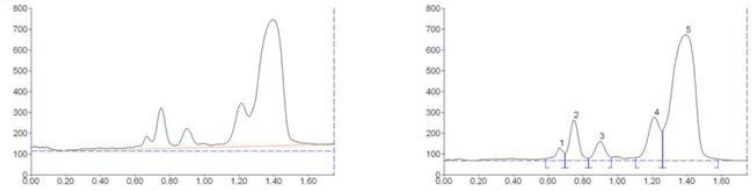


Figure 1: Results of TLC studies of *Pongamia pinnata* ethylacetate fraction for phytochemicals.



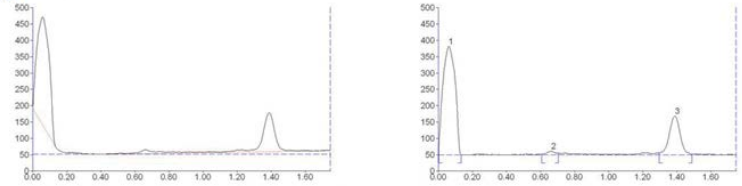
Track 1, ID: Karanjin



winCATS Planar Chromatography Manager

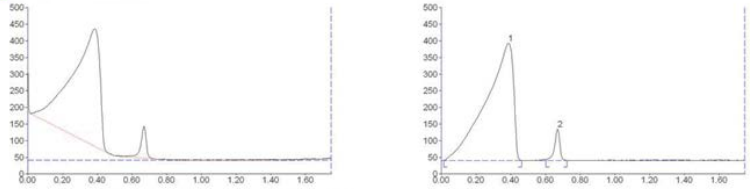
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.58	9.1	0.67	62.3	5.34	0.70	35.1	1385.0	2.63	unknown *
2	0.70	35.1	0.75	195.2	16.75	0.83	13.1	4721.2	8.95	unknown *
3	0.83	13.2	0.90	93.6	8.03	0.97	16.1	2664.1	5.05	unknown *
4	1.11	14.2	1.22	208.9	17.92	1.26	144.2	7057.1	13.38	unknown *
5	1.26	145.3	1.40	605.8	51.96	1.58	8.1	36904.3	69.98	unknown *

Track 2, ID: Gallic acid



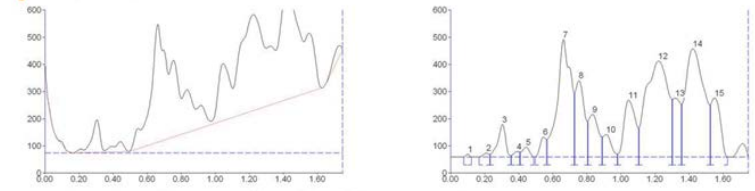
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	21.9	0.06	332.6	71.83	0.14	0.2	11020.1	74.18	unknown *
2	0.61	2.4	0.67	11.4	2.45	0.70	5.5	281.5	1.89	unknown *
3	1.30	6.4	1.39	119.1	25.72	1.49	2.7	3553.8	23.92	unknown *

Track 3, ID: Quercetin



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	0.3	0.39	352.9	78.79	0.47	0.2	25665.3	95.00	unknown *
2	0.60	3.8	0.67	95.0	21.21	0.72	0.6	1350.2	5.00	unknown *

Track 4, ID: Pongamia



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.08	0.1	0.10	10.8	0.41	0.12	0.1	100.8	0.11	unknown *
2	0.17	0.0	0.21	15.1	0.58	0.23	10.2	226.1	0.25	unknown *
3	0.23	10.6	0.30	120.0	4.57	0.35	6.1	2552.6	2.77	unknown *
4	0.36	6.3	0.39	20.6	0.78	0.41	18.8	333.5	0.36	unknown *
5	0.41	19.0	0.45	36.8	1.40	0.49	0.4	727.4	0.79	unknown *
6	0.50	0.1	0.55	73.9	2.81	0.56	65.1	1226.6	1.33	unknown *
7	0.57	65.4	0.66	432.6	16.47	0.73	235.3	15989.3	17.36	unknown *
8	0.73	236.3	0.76	281.6	10.72	0.81	131.7	6918.1	7.51	unknown *
9	0.81	133.0	0.83	157.1	5.98	0.89	74.3	4251.5	4.62	unknown *
10	0.89	74.6	0.92	83.0	3.16	0.98	11.3	1907.8	2.07	unknown *
11	0.98	11.6	1.05	209.3	7.97	1.11	106.3	6555.2	7.12	unknown *
12	1.11	106.8	1.22	353.2	13.44	1.30	213.3	21218.9	23.04	unknown *
13	1.30	213.4	1.32	217.2	8.27	1.36	194.4	4610.9	5.01	unknown *
14	1.36	194.7	1.43	398.5	15.17	1.52	192.3	19929.3	21.64	unknown *
15	1.53	193.4	1.55	217.6	8.28	1.63	0.4	5536.8	6.01	unknown *

Figure 2: HPTLC study of *Pongamia pinnata* ethylacetate fraction and selected phytocompounds.

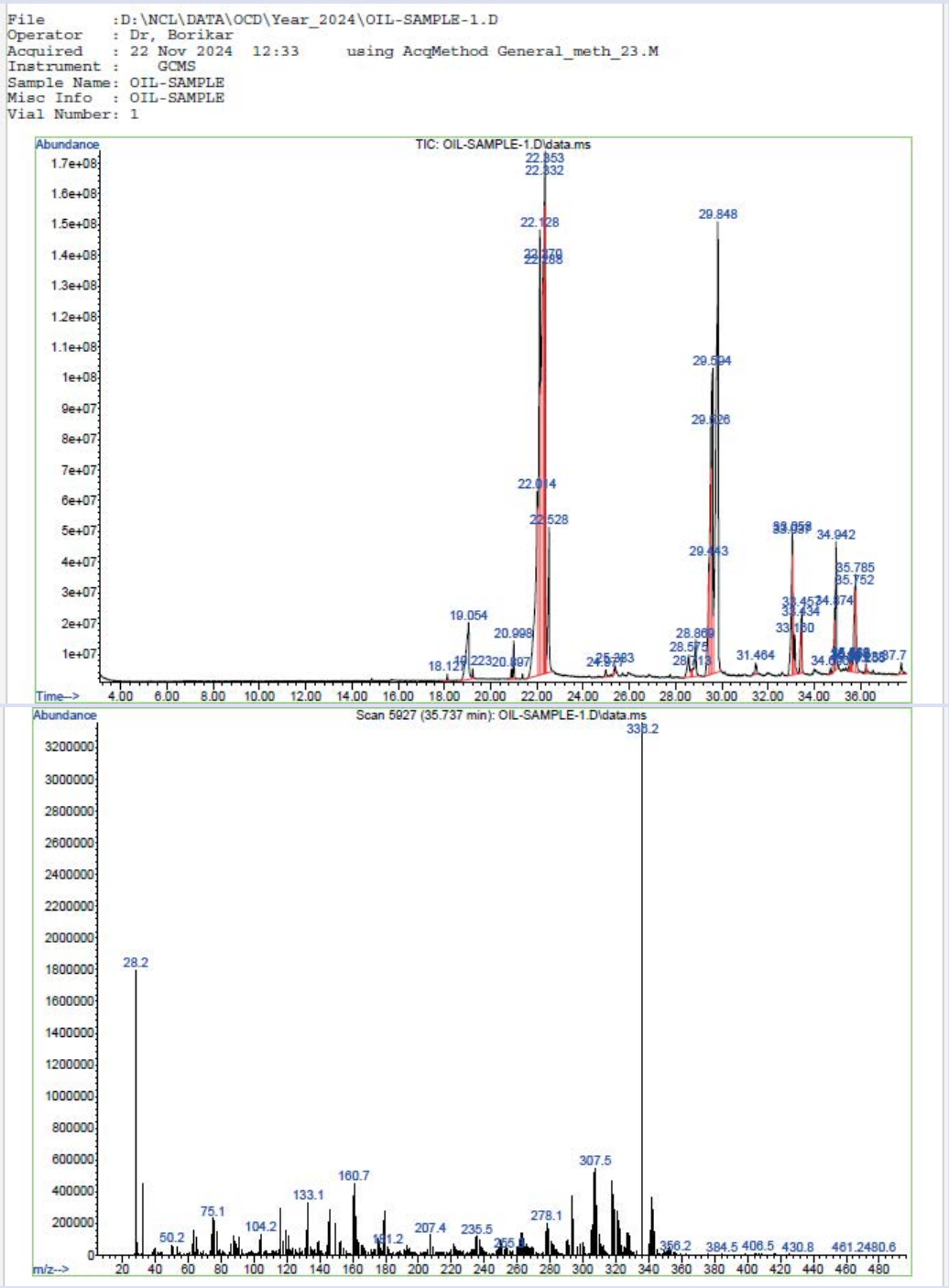


Figure 3: GCMS study of *Pongamia pinnata* ethylacetate fraction.

CONCLUSION

The Pongamia pinnata seeds found to contain non polar phytocompounds so the significant ethanol extract (10.8%) was obtained. The major fractions reported from ethanol extract are Petroleum ether fraction (17%), Chloroform fraction (12.59%), and Ethyl acetate fraction (10%). The yield obtained by methanol is very less (6.66%). The PPEAF was reported to possess significant antioxidant activity (IC50 value 212.09). The optimised mobile phase for estimation of flavonoids by HPTLC by preliminary TLC method is Toluene: ethyl acetate (7:3. Finally the major flavonoid reported is Karanjin (1.465%) which may be responsible for medicinal use of P. pinnata seed. As per previous research studies flower methanol extract was fractionated with ethyl acetate to obtain fraction which was analysed by GCMS and found to contain 30 phytocompounds in which Dibutylphthalate Rt-18.49) was found to be in significant amount[7]. In other research study it was shown that oil was analysed by GCMS and found to contain 17 fatty acid, in which 3H-Indole, 3,3-Dimethyl-2[2-(2-hydroxy-5-nitrophenyl) ethenyl]- (17.35 %) is reported as significant compound[8]. In other research article pongamia pinnata leaf hydro alcoholic extract[ethanol: water(80:20)] was obtained and TLC was performed using Toluene, ethyl actetae, formic acid mixtures as mobile phase, and flavonoid

content was estimated by aluminium chloride method and reported the presence of flavonoids content as 1.84(mg/100mg dry extract)[9].

ACKNOWLEDGEMENT

We are grateful that the necessary research facilities were made available by Dr. Kusum Devi, Principal of Nitte College of Pharmaceutical Sciences. I also thank the Manipal College of Pharmacy's Dr. Vasudev R. Pai Pharmacognosy department for supporting my HPTLC study. I also thank Dr. Dhatchanamoorthy N, an assistant professor at the Conservation of Natural Resources Trans-Disciplinary University for performing medicinal plant authentication. Additionally, I am grateful to Dr. Borikar National Chemical Laboratory in Pune for supplying analytical data for my research.

REFERENCES

1. Akram M. et al. Pongamia pinnata: an updated review on its phytochemistry, & pharmacological uses. Pharmacy & Pharmacology International Journal. 2021 Sep;9(5):194-9.DOI: https://doi.org/10.15406/ppij.2021.09.00344
2. Madhuranga HDT, Samarakoon DNAW. Methods for determining in vitro antioxidant activity: Methodologies for the DPPH, FRAP, and H2O2 assays. Journal of. Natural and Ayurvedic Medicine. 2023; 7(4): 1-7.doi: https://doi.org/10.23880/jonam-16000431
3. Kagawad P et al. Quality control and standardization of Quercetin in herbal medicines by spectroscopic and chromatographic techniques. Future Journal of Pharmaceutical Sciences. 2021 Aug 28; 7(1): 176. https://doi.org/10.1186/s43094-021-00327-y
4. Ravikanth K et al. TLC based method for standardization of Pongamia pinnata (Karanj) Using Karanjin as Marker. Chromatographia. 2009 Mar; 69(5): 597-9. https://doi.org/10.1365/s10337-008-0922-x
5. Damle M, Choudhari S. Validated stability indicating method for Karanjin using HPTLC and HPLC. Journal of Pharmacognosy and Phytochemistry. 2018; 7(2):2808-15.
6. Kavitha S, Kannan MV, Mani P. Identification of Bioactive Compounds in the leaves extract of Piper longum using GCMS. Research Journal of Pharmacy and Technology. 2020;13(7):3169-70. Doi: http://dx.doi.org/10.5958/0974-360X.2020.00560.0
7. Anuradha R, Krishnamoorthy P. Screening of phytochemicals and identification of chemical constituents of Pongamia pinnata by GC-MS. International Journal of ChemTech Research. 2012 Jan; 4: 16-20.
8. Suganya M et al. Bioactive compounds identification through GC-MS Analysis and identify functions groups over FT-IR of Pongamia pinnata Seed oil. https://www.afjbs.com/uploads/paper/e5387d931d86bb56f5f2948f3c061d55.pdf
9. Chandewar AV et al. Phytochemical screening, chromatographic and quantitative study of phenols and flavonoids in leaves of Oroxylum indicum and Pongamia pinnata. Research Journal of Pharmacy and Technology. 2023; 16(6): 2604-8. http://dx.doi.org/10.52711/0974-360X.2023.00427

Cite this article: Honnesh NH, Santanu S. Evaluation of Antioxidant Activity and Flavonoid and Phytocompounds Content in Pongamia Pinnata Seed. Pharmacogn J. 2025;17(1): 1-6.