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Phytochemical screening and FT-IR analysis in the seeds of *Majidea zanguebarica*, J. Kirk

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Abstract
The main aim of the study is to investigate the presence of phytoconstituents in the seeds of *Majidea zanguebarica* along with FT-IR analysis. Phytochemical screening in methanolic and chloroform extracts revealed positive results for alkaloids, flavonoids, steroids, tannins, saponins, fatty acids, carbohydrates and cardio glycosides. The FT- IR analysis of the methanolic extract evidenced that the presence of alcohols, phenols, carboxylic acids, amines, nitro compounds, alkyl halides, esters, ethers, aromatic and aliphatic compounds. The frequency (cm^{-1}) of *Majidea zanguebarica* ranges from 3745.76 to 532.35 which shows strong, medium and weak bonds.

Keywords: phytochemical screening, seeds of *Majidea zanguebarica*, methanolic extract and FT-IR analysis

1. Introduction
Phytochemicals are naturally occurring, biologically active chemical compounds in plants. The presence of certain types of phytochemicals in some plants can acts as natural defense system providing protection against such things as attack from insects and grazing animals. It is believed that there may be about 4,000 phytochemicals contained in plants that can be used to prevent, minimize and remedial actions like strokes, cancer or metabolic syndrome. The evidence obtained through current live scientific research does not appear to demonstrate that, the use of phytochemical supplements supports long term health as well as consuming the natural fruits, grains and vegetables from which they were taken.

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond can be seen in annotated spectrum by interpreting the infrared absorption spectrum can be determined. FT-IR spectroscopy is a highly sensitive analytic technique that can potentially reveal a wealth of quantitative and qualitative information about a given biological sample. In addition, it makes possible to simultaneously monitor changes in the structure and properties of biomolecules such as proteins, lipids and carbohydrates in biological tissues and cells.

Majidea zanguebarica is a small tree belonging to the Sapindaceae. The tree is native to East Africa and grows up to 5 meters (16 ft) tall. The seeds are highly ornamental referred to as black pearl tree or velvet seed tree. It blooms with dense cluster of small green-red, fragrant flowers at the end of panicles. Fruit is spherical with three lobes 3cm (1.2 inches) long. The fruit splits open, showing the bright red interior, with three spherical, velvety blue -black seeds. Plant is used to treat fever, wound infections and intestinal disorders in traditional medicine as the pods and leaves have antibacterial properties. Hence, the present investigation was made an attempt to analyze the chemical compound present in the seeds of *Majidea zanguebarica*. Still now there is no literature available in this *Majidea zanguebarica* regarding FT-IR analysis.

2. Materials and Methods
2.1 Collection of plant materials
The selected plant *Majidea zanguebarica* were collected in our college campus PSG College of Arts & Science, Coimbatore district, Tamil Nadu.

2.2 Preparation of Extract
The seeds were cleaned and shade dried. The dried seeds were grounded into fine powder. The powder was subjected to extraction with chloroform and methanol.

2.2.1 Methanolic Extract
25 gms of seed powder was subjected to extraction with methanol using Soxhlet apparatus for 24 hours and extract was condensed to remove solvent. The residues from the extracted were using for phytochemical analysis.

2.2.2 Chloroform Extract
10gms of seed powder was weigh and mix well with 150ml of chloroform and subjected for 48 hours with intermittent shaking and the crude extract was filtered using whattman No.1 filter paper.

2.3 Preliminary Phytochemical Studies
The chloroform and methanol extracts were subjected to preliminary phytochemical test using commonly standard methods (Trease and Evans, 1989 ; Tiwari *et al.*, 2011; Harborne, 1998) for the identification of major naturally occurring Bioactive compounds such as alkaloids, flavonoids, phenols, saponins, steroids, triterpenoids, carbohydrates, anthraquinones, glycosides, proteins and amino acids.

Qualitative phytochemical analysis
Phytochemical screening of plants extracts was carried out to assess the bioactive compounds composition qualitatively. General reactions of these tests forming the

precipitation or changing in the color of the test solution revealed the presence or absence of these compounds in the plant extracts tested using specific standard reagents.

Test for alkaloids
- **Dragendroff's Test:** A few drops of Dragendroff's reagent (potassium bismuth iodide solution) were added to 1ml of the Test solutions.
- **Mayer's Test:** 1ml of the test solutions were added with 2-3 drops of Mayer's reagent (potassium mercuric iodide).

Test for flavonoids
- **Alkaline Reagent Test:** 1 ml of the test solutions were treated with few drops sodium hydroxide solution.

Test for cardiac glycosides
- **Keller kilimi test:** To 1ml of the test solutions 0.4 ml of glacial acetic acid containing a trace amount of Ferric Chloride were added to them. Carefully added 0.5 ml of Concentrated sulphuric acid by the side of the test tube and observed.

Test for saponins
- **Foam Test:** 1 ml of extract was taken in a test tube and 5ml of distilled water was added and vigorously shaken.

Test for tannins
- **Ferric chloride Test:** 2 ml of the extracts were diluted with distilled water in separate test tubes, 2-3 drop of 5% ferric chloride ($FeCl_3$) solution was added.

Test for steroids
- 2 ml of the extract were taken in separate test tubes and evaporated to dryness. The residues were dissolved in acetic anhydride and then chloroform was added. Conc H_2SO_4 was added by the side of the test tube.

Test for terpenoids
- **Salkowski's Test:** 1ml of the test solutions were taken in a clean and dried test tubes and 2ml of chloroform followed by a few drops of concentrated sulphuric acid were added into it. Shake well and allowed to stand for some time.

Test for phenols
- **Ferric chloride Test:** 5 ml of concentrated extracts were taken and 2ml of neutral ferric chloride solution was added.

Test for carbohydrates
- **Benedict's Test:** The test solutions were treated with Benedict's reagent and heated gently in water bath for five minutes.

Test for proteins
- **Biuret Test:** To 1ml of the test solutions, few drops of 0.5% of sodium hydroxide solution and 1% copper sulphate solutions were added.

Test for fatty acids
- A small quantity of powdered sample was pressed in between 2 filter paper presence of oil strains on paper indicates the presence of fixed oils.

2.4 FT-IR
FTIR is tool for identifying dried powder of different solvent extract of each plant materials were used for analysis 10mg of the dried extract powder was encapsulation in 100mg of *Majidea zanguebarica* in order to prepare translucent sample discs. The powdered sample of the plant specimen was loaded in FTIR spectroscope with a scan range from 400-4000cm-1 with a resolution of 4cm. The FTIR spectrum of seed extracts (prepared in solvents) if the data on the peak values and probable functional groups obtained by FTIR analysis present in seed extract. One of the advantages of Fourier transform technique over dispersive or continuous instruments is that it can record the signal intensities directly as a function at frequency region 4000-400cm^{-1}.

3. Results
3.1 Preliminary phytochemical screening
The compounds like alkaloids, flavonoids, glycosides, steroids, phenols, tannins, saponins and resins are the preliminary phytochemical compounds present in the seeds, maximum amount of all the compounds in seeds were presented in the methanolic extracts than the chloroform extracts.

3.2 FT-IR
The methanolic extract was passed in to the FT-IR, the functional groups of the components were separated based on its peak ratio was represented in Figure.1. The results of FT-IR analysis confirmed the presence of free hydroxyl alcohols, phenols, aromatic carboxylic acids, alkanes, α, β-unsaturated esters, aliphatic amines, alkyl halides and nitro compounds. The peak values in the IR region was presented in the Table.2.

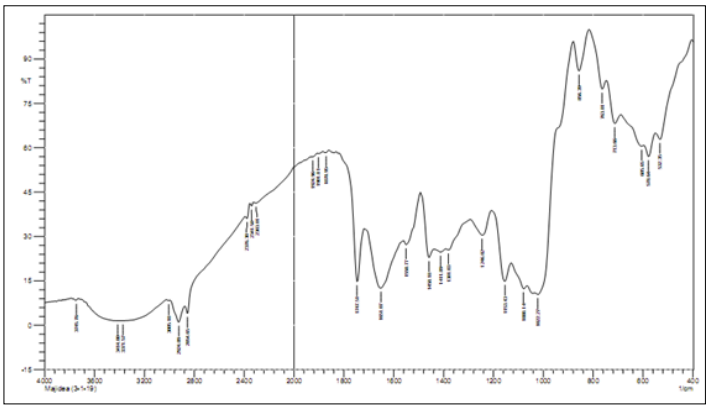

Fig 1: FT-IR Spectrum Analysis in Methanolic extract of *Majidea zanguebarica*

Table 1: Preliminary phytochemical analysis in seeds of *Majidea zanguebarica*

S. No.	Detection	Test	Response	Chloroform Extract	Methanol Extract
1	Alkaloids	i)Dragendroff's reagent ii)Mayer's reagent	Orange red precipitate White precipitate	+	+
2	Flavonoids	Alkaline reagent	Brown color	+	+
3	Steroids	Chloroform &Sulphuric acid	Upper layer red & lower layer green fluorescence	-	-
4	Tannins	Ferric chloride	Bluish black	+	+
5	Triterpenoids	Salkowski Test	Yellow color changes to red	-	-
6	Phenols	Sulphuric acid & sodium hydroxide	Blue color	+	+
7	Saponins	Foam Test	Formation of foam	+	+
8	Fatty acid	Filter paper test	Immersion of oily glands	+	+
9	Carbohydrates	Benedict's reagent	Bluish green color	-	+
10	Proteins	Biuret Test	Red color	-	-
11	Cardiac Glycosides	Salkowski test	Reddish brown ring	+	+

+ : Presence of bioactive compounds
- : Absence of bioactive compounds

Table 2: FT-IR Spectrum Analysis in Methanolic extract of *Majidea zanguebarica*

S. No.	Sample	Frequency cm^{-1}	Bond	Functional group name
1.	Methanol extract	3745.76 (m)	C=O	Free hydroxy alcohols and phenols
		3414 (s,b)	O-H stretch	Alcohols
			H- bonded	Phenols
		3371.57 (s,b,m)	O-H Stretch	Alcohols
			H- bonded	Phenols
			N-H stretch	1°,2°amines,amides
		3005.10 (m,s)	O-H stretch	Carboxylic acids
			-C=C-H	Alkynes (terminal)
			C-H stretch	Aromatics
			= C-H stretch	Alkenes
		2924.09 (m)	C-H stretch	Alkanes
			O-H stretch	Carboxylic acid
		2854.65 (m)	C-H stretch	Alkanes
			O-H stretch	Carboxylic acid
		2376.30 (m)	C≡N stretch	Nitrites
		2341.58 (m)	-C≡C- stretch	Alkynes
		1924.96 (m)	-C≡C- stretch	Alkynes
			C=O stretch	α,β - unsaturated
		1747.51 (s)	C=O stretch	Carbonyls
			C=O stretch	Carboxylic acid
			C=O stretch	Esters, Saturated Aliphatic
		1651.07 (m)	-C=C-stretch	Alkenes
		1550.77 (s)	N-O asymmetric stretch	Nitro compounds
		1458.18 (m)	C-C stretch (in-ring) C-H bond	Aromatics Alkanes
		1381.03 (s)	N-O asymmetric stretch	Nitro compounds
		1246.02 (s,m)	C-O stretch	Alcohols, Carboxylic acids, Ethers
			C-H wag (-CH_2X)	Alkyl halides
			C-N stretch	Aliphatic amines
		1153.43 (s,m)	C-O stretch	Alcohols, Carboxylic acids, Ethers
			C-H wag (-CH_2X)	Alkyl halides
			C-N stretch	Aliphatic amines
		1080.14 (s,m)	C=O stretch	Alcohols, Carboxylic acids, Ethers
			C-N stretch	Aliphatic amines
		1022.27 (s,m)	C-N stretch	Aliphatic amines
			C=O stretch	Alcohols, Carboxylic acids, Ethers
		856.39 (s,s.b,m)	=C-H bend	Alkenes
			N-H wag	1°,2°amines
			C-H "o op"	Aromatics
		763.81 (s,s.b,m)	=C-H bend	Alkenes
			N-H wag	1°,2°amines
			C-H "oop"	Aromatics
			C-Cl stretch	Alkyl halides
		713.66 (b,s)	-C=C-H: C-H bend	Alkynes
		605.65 (m)	C-Br stretch	Alkyl halides
			C-Cl stretch	Alkyl halides
		578.64 (m)	C-Cl stretch	Alkyl halides
			C-Br stretch	Alkyl halides
		532.35 (m)	C-Br stretch	Alkyl halides

4. Discussion

Preliminary qualitative phytochemical analysis of *Aesculus indica* crude extract showed presence of flavonoids, coumarins, saponins and tannins, sterols and anthraquinones were absent in ethyl acetate and methanol extracts was reported by Yamin Bibi *et al.*, 2012. Similar results obtained in methanolic and chloroform extract of *Majidea zanguebarica*.

In the present study, the various tests of phytochemical analysis for testing the presence of the bioactive compounds such as alkaloids, phytosterols, tannins, terpenoids, phenols, saponins, anthraquinones, carbohydrates, glycosides, cardiac glycosides, proteins and flavonoids were carried out in the chloroform extracts and methanolic extracts of seeds of *Majidea zanguebarica*.

The FTIR spectroscopic analysis showed the presence of phytoconstituents. It gives a broad peak of 3745.76, 3414, 3 371.57, 3005.10, 2924.09, 2854.65, 2376.30, 2341.58, 1924.96, 1747.51, 1651.07, 1550.77, 1458.18, 1381.03, 1246.02, 1153.43, 1080.14, 1022.27, 856.39, 763.81, 713.66, 605.65, 578.64, 532.35. It was identified the functional groups of the active components present in methanolic extract based on the peak values in the region of IR radiations. The peak obtained at the methanol 3414,3371.57, 3005.10, 2924.09, 2854.65cm^{-1} indicates the presence of O-H stretching, the peak obtained at 3005.10, 2924.09, 2854.65cm^{-1} indicates the presence of C-H stretching, the peak obtained at 1924.96, 1747.51, 1246.02, 1153.43, 1080.14,1022.27cm^{-1} indicates the presence C=O stretching. The peaks obtained at 1246.02, 1153.43, 1080.14, 1022.27cm^{-1} indicates the presence of C-N stretching. The peak obtained at 2341.58, 1924.96, 1651.07cm^{-1} indicates the presence of -C$\equiv$C- stretch. The peak obtained at 763.81, 605.65, 578.64cm^{-1} indicates the presence of C-Cl stretching. The peak obtained at 605.65, 578.64 and 532.25cm^{-1} indicates the presence of C-Br stretching in *Majidea zanguebarica*.

Murugesh *et al.*, 2017 investigated the presence of alcohols, alkenes, phenols, aromatic carboxylic acids, esters, aliphatic amines, primary and secondary amines indicated in the peaks obtained 3411.91, 2920.52, 2850.52, 1602.95, 1402.92, 1261.85, 1119.08, 1074.86, 859.13 and 832.09 cm-1 of ethanolic extract of *Pisonia grandis* and similar peak ranges was also obtained in *Majidea zanguebarica*.

5. Conclusion

Worldwide traditional system of medicines has always an important role in global health. Herbal plants are effective source of traditional and modern medicines, useful for primary health care. This helps to getting increase knowledge of medicinal plants. In the present study, the methanol and chloroform extract were used for extraction of *Majidea zanguebarica*, preliminary screening given an idea about the presence of different phytochemical compounds. The study concluded that the phytochemicals with the present of so many compounds. So, it contains high therapeutic content. Based on the conclusion, trace elements present in *Majidea zanguebarica* has a lot of biological activities to prevent diseases was analyzed by FT-IR. In future, *Majidea zanguebarica* could be uses as good pharmaceutical and therapeutic agents. Further integrated investigation using HPTLC, antioxidant and antimicrobial activities will lead to purification and structural elucidation of active principles. Therefore, it will serve as a basic knowledge for applying in the future studies.

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