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Determination and comparison of phytochemical in *Sesamum laciniatum* Klein ex Wild by using FTIR spectroscopy

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Abstract
Sesamum belongs to Pedaliaceae, a small family of 17 genera and 80 species of annual and perennial herbs. The biologically active compounds present in plants are called phytochemicals. These phytochemicals are derived from various parts of plants such as leaves, stem and roots. These phytochemicals are used as sources of direct medicinal agents. They serve as a raw material base for elaboration of more complex semi-synthetic chemical compounds. The extraction of active compounds from the various parts of plants, qualitative and FTIR spectroscopic analysis of the phytochemicals. The wavelength of many IR absorption bands are characteristics of specific types of chemical bonds and IR spectroscopy finds its greatest utility for qualitative analysis of organic and organometallic molecules. IR spectroscopy is used to confirm the identity of a particular compound and as a tool to determine the newly synthesized molecule.

Keywords: FTIR, Sesamum, phytochemicals, perennial herbs, organometallic molecules

Introduction
Sesamum belongs to Pedaliaceae, a small family of 17 genera and 80 species of annual and perennial herbs that occur in the old-World tropics and subtropics (Cronquist 1981) [2]. Sesamum was described by Linnaeus in 1754 in Genera Plantarum. This generic name was taken by Hippocrates from the Arabic "Semsin". Other genera that are considered to be related to Sesamum are Ceratotheca, Martyniaceae, Anthadenia, and Volkameria (Nayar and Mehra 1970; Kobayashi 1991) [10, 5]. Various numbers of species have been reported by different workers. Joshi (1961) [4] reported 36 species based on index kewensis. Nayar and Mehra (1970) [10] reported 34 species and Kobayashi (1981) [6] listed 37 species. New species, such as *S. indicum* var. sencottai and var. yanamalai (Devarathinam and Sundaresan 1990) [3] and S. mulayanum Nair (Mehetre *et al*. 1994) [9] were also added. According to Kobayashi (1991) [5], 22 of the species were localized only in Africa, 5 only in Asian countries (India, Sri Lanka or East Indies or in all areas), 7 species were reported to be found in both the African and Asian countries, and one each was reported in Crete and Brazil.
Sesamum laciniatum Klein ex Willd is Perennial, prostrate, profusely branched, having deeply dissected coarse leaves with deep purple flowers with purple anthers where yellow glands absent, small laterally compressed tough capsules and deeply reticulated seeds with thick testa. Several willd Sesamum species have proved resistant to biotic and abiotic stress (Joshi 1961; Nayar and Mehra 1970; Weiss 1971; Brar and Ahuja 1980; Mazzani 1983; Kolte 2018; Prabakaran and Rangasamy 1995) [4, 10, 14, 1, 8, 7, 11]. According to Srinivasulu (1991) [12], *Sesamum laciniatum* Klein ex Willd were resistant to Antigastra catalaunalis (shoot webber) and Thangavelu (1994) [13] reported shoot webber resistance in *Sesamum laciniatum* Klein ex Willd.

Materials and Methods:
Plant Collection and Identification
Fresh leaves, Stem & Root of *Sesamum laciniatum* Klein ex Willd plant were collected from Chorakhali village located in kalamb tehsil of Osmanabad district in Maharashtra, India. The plant identified and authenticated by Senior Professor Arvind S. Dhabe Sir, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, and Aurangabad.
The fresh leaves, Stem & Root of the *Sesamum laciniatum* Klein ex Willd. Plant were washed thoroughly 2-3 times with running tap water.

The plant materials were kept until all the water content evaporated and the dried plant material is stored in the laboratory at room temperature (298K) and in the shade before the extraction then the leaves, stem and roots were crushed into powder and stored in polythene bags for further use.

Fourier transform infrared (FTIR) was used to identify the characteristic functional groups in the plant. Dried powder of leaf, stem and roots was used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of leaf, stem and root specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a scan range from 400 to 4000 cm-1 with resolution of 4 cm-1. The peak value was obtained.

Method of plant extraction

Solvent extraction

The coarsely powdered plant material of *Sesamum laciniatum* Klein ex Willd leaves, stem and roots were placed inside a bottle of different extracts of distilled water, ethanol, methanol, chloroform and ether. The menstruum is poured on top until completely covered the plant material. The bottles are kept closed for three days and shaken time to time to ensure complete extraction. At the end of extraction, the micelle is separated from marc by whatmann filtration paper. Subsequently, the micelle is then separated from the menstruum by evaporation on top of water bath.

Methods of qualitative phytochemical analysis

The leaf, stem and root extracts were tested for the presence of phytochemicals by using following standard methods

1. **Test for Alkaloid:** The plant extract is mixed in 1% v/v HCL, warmed and filtered. Now this filtered is used for Mayer's test. The filtrate is treated with Mayer's reagent (Mercuric chloride + Potassium iodide in water). Formation of a yellow-coloured precipitate indicates the presence of alkaloids.
2. **Test for Protein:** The plant extract is treated with a few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.
3. **Test for Flavonoids:** The plant extract treated with 2-3 drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of few drops of sulphuric acid which indicates the presence of flavonoids.
4. **Test for Phenols and Tannin:** The powdered sample of leaves is boiled in 20ml of distilled water in a test tube and then filtered. The 3-4 drops of 0.1% v/v Ferric chloride is added to the filtered sample and observed brownish green or blue colouration, it indicates the presences of phenols or tannins.
5. **Test for Phytosterols:** The plant extract was mixed with chloroform and filtered. The filtrate is treated with 5-6 drops of conc. Sulphuric acid carefully and shaken gently, allowed to stand. A golden yellow colour indicates the presence of triterpens (phytosterol).
6. **Test for Carbohydrates:** Filtrate is treated with Benedict's reagent (Sodium carbonate + sodium citrate and copper sulphate solution), then the mixture was heated on a boiling water bath for 5 minutes and cooled. Orange red precipitate indicates the presence of carbohydrates.

Results and Discussion

From the qualitative analysis of leaves, stem and root of *Sesamum laciniatum* Klein ex Willd plants, the presence or absence of carbohydrates, alkaloids, proteins, flavonoids, phenols and tannin, phytosterol and carbohydrates was investigated. The results of this study are shown in the following Table 1.

Table 1: Preliminary Phytochemical Analysis of leaf, stem and root of *Sesamum laciniatum* Klein ex Willd.

***Sesamum laciniatum* Klein ex Willd.**															
Sr. No.	**Test**	**Aqueous extract**			**Ethanolic extract**			**Methanolic extract**			**Chloroform extract**			**Ether extract**	
		L	S	R	L	S	R	L	S	R	L	S	R	L	S	R
1	Alkaloids	-	+	+	+	+	-	+	+	-	-	-	-	-	-	-
2	Proteins	+	+	-	+	+	+	+	-	-	-	+	-	-	-	-
3	Flavonoids	+	+	-	+	-	-	+	+	+	-	+	-	-	+	-
4	Phenols & Tannins	-	+	+	-	+	+	+	+	+	-	-	-	-	-	-
5	Phytosterol	+	+	-	+	+	-	+	+	+	-	-	-	-	-	-
6	Carbohydrates	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+

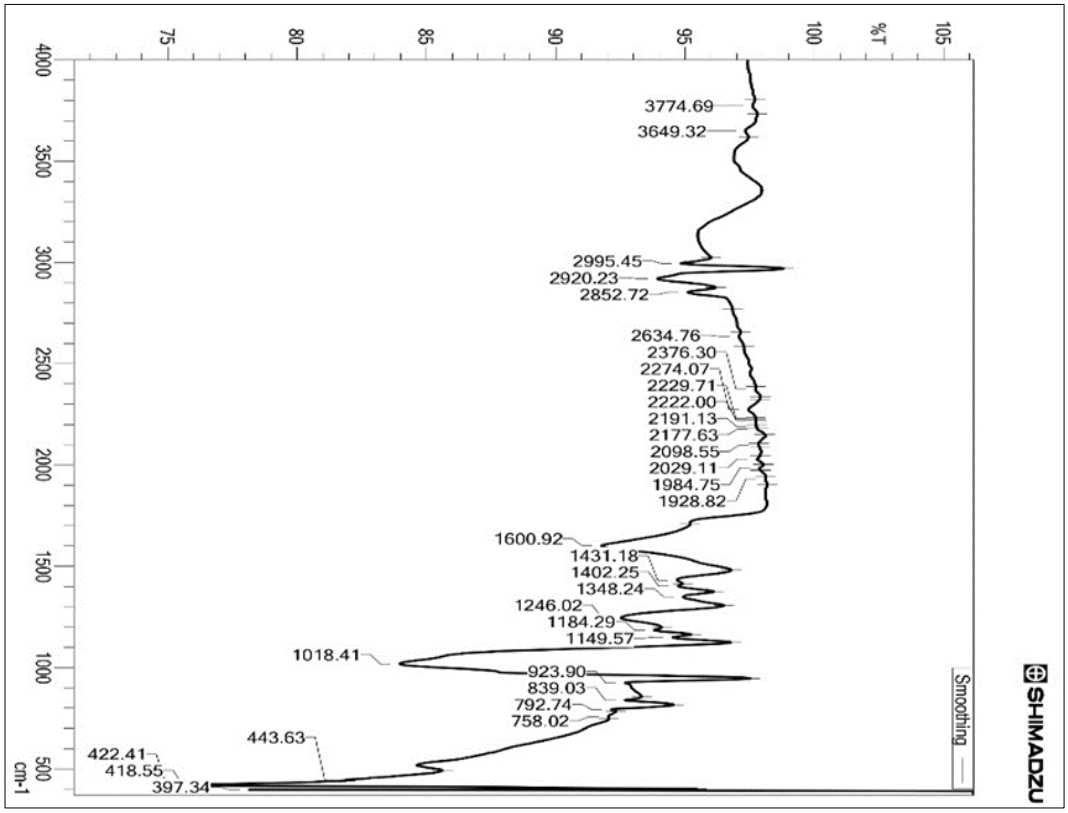
+ Present, - Absent & L- leaf, S- stem, R-root

In Preliminary Phytochemical Analysis of leaf of *Sesamum laciniatum* Klein ex Willd. It has been found that alkaloids are present in ethanolic and methanolic extracts. Proteins are found in all extract except chloroform and ether. Flavonoids and phytosterol are present in all extract except chloroform and ether. Phenols and tannins are absent in all extract except in methanolic extract. Carbohydrates are present in all extract.

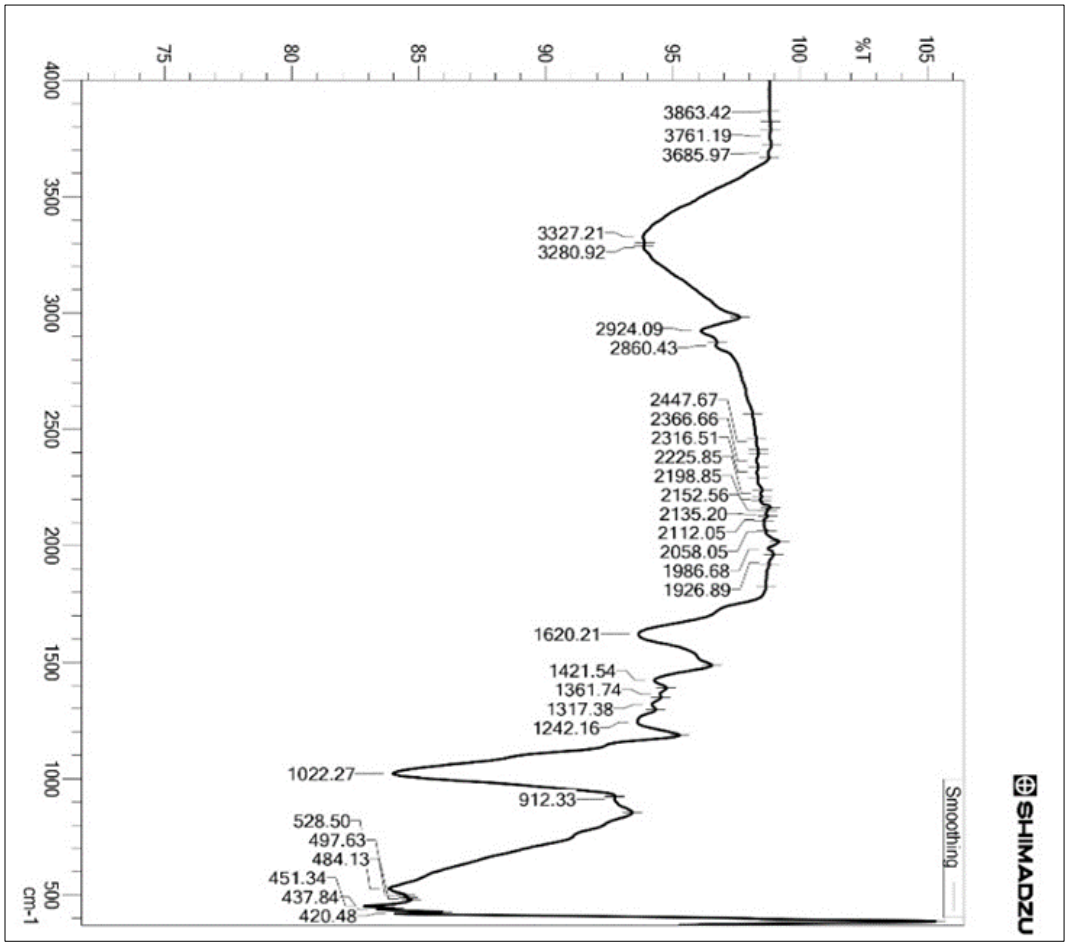
In stem it has been found that alkaloids and phytosterol are present in aqueous, ethanolic and methanolic extracts. Proteins are absent in methanolic and ether extracts. Flavonoids are absent only in ethanolic extract. Phenols and tannins are absent in chloroform and ether extract. Carbohydrates are present in all extract except aqueous extract.

In root it has been found that alkaloids are present in aqueous extracts. Proteins are absent in all extract except ethanolic. Flavonoids and phytosterol are present only in methanolic extract. Phenols and tannins are absent in all extract except in chloroform and ether extract. Carbohydrates are present in all extract.

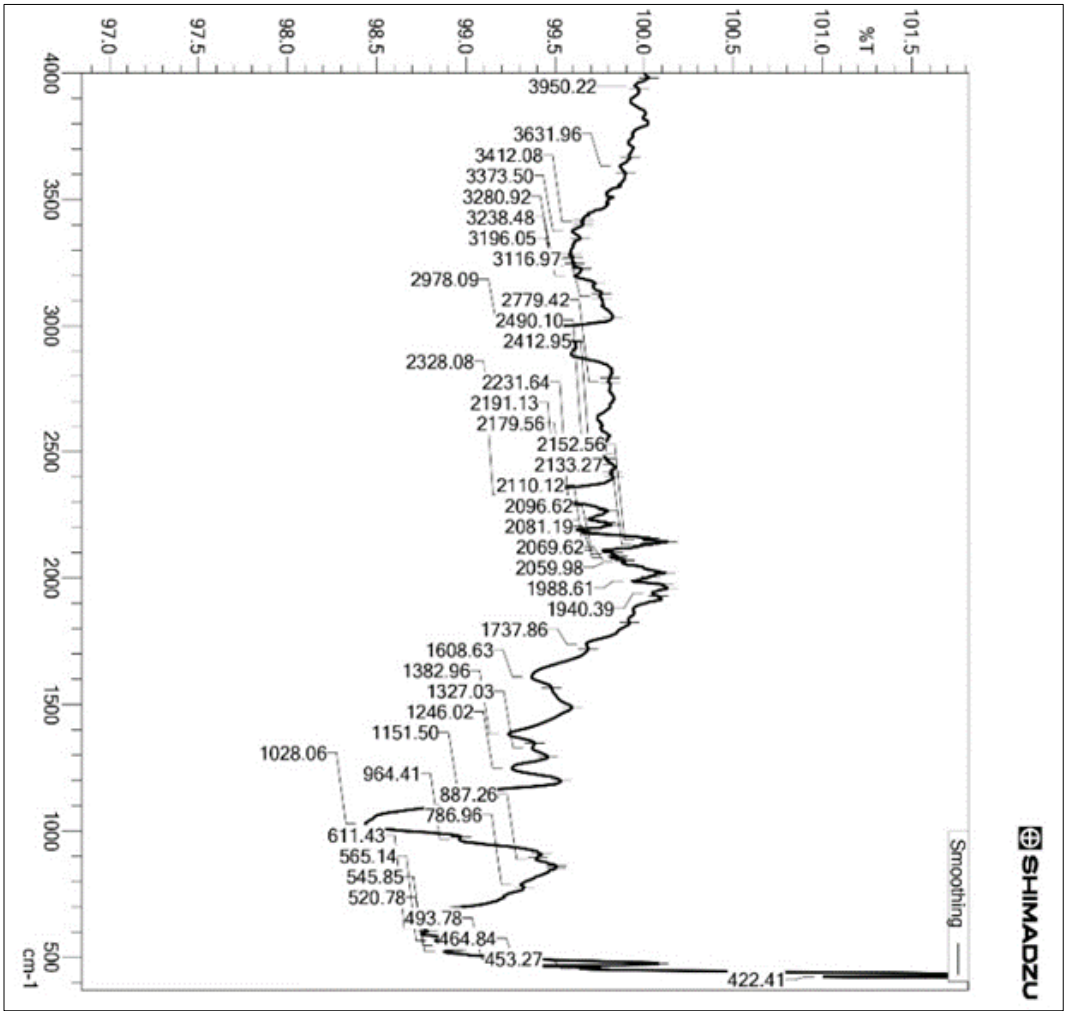
The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The interpretation of FTIR compounds given in following graphs and tables.


%T
cm-1
3774.69
3649.32
2995.45
2920.23
2852.72
2634.76
2376.30
2274.07
2229.71
2222.00
2191.13
2177.63
2098.55
2029.11
1984.75
1928.82
1600.92
1431.18
1402.25
1348.24
1246.02
1184.29
1149.57
1018.41
923.90
839.03
792.74
758.02
443.63
422.41
418.55
397.34
Smoothing
SHIMADZU

A


%T
cm-1
3863.42
3761.19
3685.97
3327.21
3280.92
2924.09
2860.43
2447.67
2366.66
2316.51
2225.85
2198.85
2152.56
2135.20
2112.05
2058.05
1986.68
1926.89
1620.21
1421.54
1361.74
1317.38
1242.16
1022.27
912.33
528.50
497.63
484.13
451.34
437.84
420.48
Smoothing
SHIMADZU

B



C

Fig 1: FTIR Interpretation of A) Leaf B) Stem and C) Root compounds.

Table 2: Wave number (cm-1) of dominant peak obtained from absorption spectra.

Functional Group	*Sesamum laciniatum* J. G. Klein ex Willd		
	Leaf	Stem	Root
Aliphatic ether (C-O stretching)	1149.57	--	--
Amine (C-N stretching)	1246.02	1022.27	--
Phenol (O-H bending)	1348.24	--	1382.96
Conjugated alkene (C=C stretching)	1600.92	--	1608.63
Aromatic compound (C-H bending)	1984.95	1986.68	1737.86
Isocyanate (N=C=O stretching)	2274.07	--	--
Alkane (C-H stretching)	2920.23	2924.09	--
Alcohols (O-H stretching)	3649.32	3685.97	3631.96
Carboxylic acid (O-H bending)	--	1421.54	--
α,β-unsaturated ketone (C=C stretching)	--	1620.21	--
Secondary amine (N-H stretching)	--	3327.21	--
Allene (C=C=C stretching)	--	--	1988.61
Amine salt (N-H stretching)	--	--	2978.09
Aliphatic primary amine (N-H stretching)	--	--	3373.50

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The FTIR spectrum of leaf of *Sesamum laciniatum* J. G. Klein ex Willd plant. The absorption at 1149.57 cm-1 is due to the C-O stretching of aliphatic ether that are present in the Extract. The band at 1246.02 cm-1 is due to the C-N stretching of Amine. The absorption at 1348.24 cm-1 is due to the O-H bending of Phenol that are present in the Extract. The band at 1600.92 cm-1 is due to C=C stretching associated with the conjugated alkene mode of the extracts. The vibrational absorption band at 1984.95 cm–1 was assigned to C-H bending of aromatic compound. A notable band at 2274.07 can be assigned to N=C=O stretching. A band at 2920.23 cm-1 represent the C-H stretching of alkane that are present in the extract. The band at 3649.32 cm-1 is due to O-H stretching

The FTIR spectrum of stem shows absorption at 1022.27 cm-1 is due to the C-N stretching of amine that are present in the Extract. The band at 1421.54 cm-1 is due to the O-H bending of Carboxylic acid. The absorption at 1620.21 cm-1 is due to the C=C stretching of α, β-unsaturated ketone that are present in the Extract. The band at 1986.68 cm-1 is due to C-H

bending associated with the aromatic compound mode of the extracts. The vibrational absorption band at 2924.09 cm–1 was assigned to C-H stretching of alkane. A notable band at 3327.21 can be assigned to N-H stretching. The band at 3685.97 cm-1 is due to O-H stretching.

The FTIR spectrum of root of *Sesamum laciniatum* J. G. Klein ex Willd plant. The absorption at 1382.96 cm-1 is due to the O-H bending of phenol that are present in the Extract. The band at 1608.63 cm-1 is due to the C=C stretching of conjugated alkene. The absorption at 1737.86 cm-1 is due to the C-H bending of aromatic compound that are present in the Extract. The band at 1988.61 cm-1 is due to C=C=C stretching associated with the ALLENE mode of the extracts. The vibrational absorption band at 2978.09 cm–1 was assigned to N-H stretching of N-H stretching. A notable band at 3373.50 can be assigned to N-H stretching. The band at 36.31.96 cm-1 is due to O-H stretching associated with the Alcohol mode of the extracts. All three extracts respectively indicate the presence of amino acids, alkenes, nitrates, ethers, organic halogen compounds and carbohydrates in *Sesamum laciniatum* J. G. Klein ex Willd.

There is no absorbance in between the region 2220-2260 cm-1 indicates that no cyanide groups in all three samples. By this results they do not contain any toxic substances.

Conclusion

The presence of these bioactive compounds in *Sesamum laciniatum* J. G. Klein ex Willd plant lends credence to its use by the human community. It could be concluded that *Sesamum laciniatum* J. G. Klein ex Willd contain various bioactive compounds. So, it is recommended as a plant of phytopharmaceutical importance. However, further studies will need to be undertaken to ascertain fully its bioactivity, toxicity profile, effect on the ecosystem and agricultural products. The presence of characteristic functional groups Carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons, halogens are responsible for various medicinal properties of *Sesamum laciniatum* J. G. Klein ex Willd.

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