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Identification of vital phytoconstituents in *Moringa oleifera* (L.) through FTIR Spectroscopy

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A B S T R A C T

Moringa oleifera L. is grown throughout the Indian subcontinent. It is widely cultivated because of its diverse uses, especially for therapeutic and culinary purpose. All the plant parts especially the leaves, seed etc., are useful because of the presence of vital plant constituents which are being used since ages for treatment of various ailments. FTIR analysis indicated the presence of phytoconstituents which have the unique properties for acting against oxidative stress and inflammation.

Introduction

Various pharmacological investigations revealed the importance of plant based phytoconstituents for the treatment of diseases. With the advent of advanced equipment for the identification of phytoconstituents, a solid base has been established for the identification and characterization of the molecules which forms the solid base to popularize Ayurveda among the Indian community so as to avoid the risk of side effects seen with the intake of many modern medications.

Moringa oleifera L. is popularly known for its multipurpose usage. Previous studies indicated that it is rich in calcium, iron and vitamins A and C, alkaloids, flavonoids, steroids, glycosides, terpenoids, saponins, amino acids, anthocyanins and catecholic tannins in aqueous leaf extracts (Rockwood *et al.*, 2013). Aqueous extract of *Moringa oleifera* leaf significantly restored the antioxidant potential, and reduced the oxidative stress–induced DNA damage via amelioration of NF-kB and TNF-α which kept hepatocyte integrity and reduced serum hepatic enzyme activities (Fattah *et al.*, 2020). Almost all parts of the plant have got good therapeutic potential and hence called as Miracle tree that God has created for the

welfare of the humans on the earth. The plant contains white flowers with long and dark green fruits. The fruits are generally used in culinary purpose, both in urban and rural areas of India. Roots are useful as laxative, diuretic, against piles and in treatment of throat infections. The presence of flavonoids such as quercetin and kaempferol, vitamin A, and ascorbic acid provides defense against free radicals and is known for its antioxidant potential (Toppo *et al.*, 2015). Hence, the identification and characterization of compounds is essential to delineate its medicinal and therapeutic uses. The present investigation was focused on identifying the phytoconstituents using Fourier transform infrared (FTIR) Spectroscopy.

Materials and Methods

Collection, identification and processing of plant material

The *Moringa oleifera* leaves were collected from the local market, Rajendranagar, Hyderabad, India. The plant species were authenticated by the Botanist. The leaf samples of *Moringa oleifera* were washed and removed the dust particles. Water in the plant material was removed gently using a paper towel. The leaves were open-dried by keeping them in the air under shade for three weeks till constant weight was achieved. The dried samples were ground to semi-powder form (10 to 20 meshes) using a commercial grinder and were stored in airtight polythene bags in the refrigerator at 4°C for further analysis.

Preparation of extract

200 g. of *Moringa oleifera* leaf powder were packed in thimbles made up of thick filter paper, placed in Soxhlet extraction apparatus and subjected to continuous hot percolation at different temperatures using the solvent 80% ethanol for about 15-16 hrs. (10-12 cycles) until the solution appeared clear. The extracts were then vacuum-concentrated under reduced pressure in a rotatory evaporator at 400C at 30rpm.

The concentrated extracts were then air-dried, transferred to an airtight container, and stored at 40Cuntil further use. The yield of the extract was determined concerning the original weight of plants. After extraction, the contents of the receiver flask were subsequently transferred to sterilized evaporating bowls, already weighed and placed under the fan for evaporation of the solvent.

The residue left in the bowls was again weighed to know the exact amount of extract. The extractability percentage of *Moringa oleifera* leaf powder was determined by the following formula. Concentrated *Moringa oleifera* leaf powder extracts were taken in the bowls and stored in the refrigerator for subsequent studies. The dry extract was kept in a vacuum desiccator until use.

$$\text{Percent extractability} = \frac{\text{Total amount of extract obtained}}{\text{Total weight of powder taken for extract}} \times 100$$

Fourier-transform infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) spectroscopic technique was used to identify the presence of functional groups in the leaves of *Moringa oleifera* ethanol extracts. FTIR spectrum of samples was performed at Sapala Organics Pvt. Limited, Hyderabad with the Perkin Elmer spectrophotometer system with 32 scans and the resolution was set at 4 cm^{-1}.

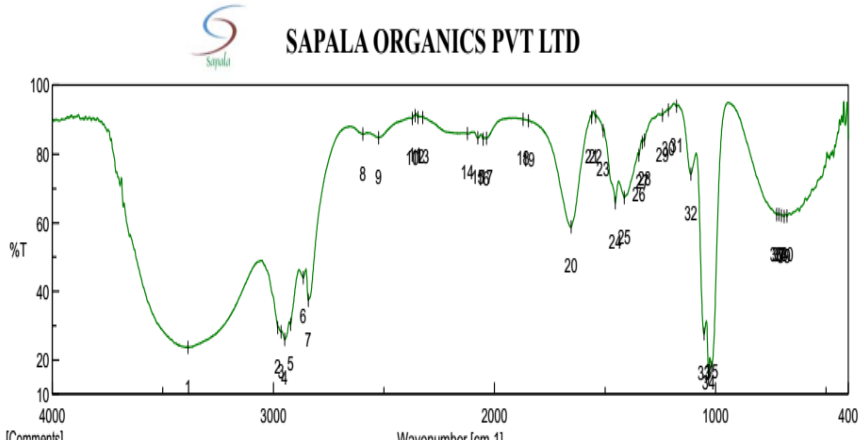
The diffuse reflectance method was used with TGS detectors to detect peaks ranging from 400-4000 cm^{-1} and their functional groups.

Results and Discussion

The FTIR spectrum of the ethanolic extract of MOLE is shown in (Fig.1). It was found that the extracts exhibited different FTIR spectra due to the presence of different natural compounds. Altogether, 40 peaks were identified from FTIR analysis at different wavelengths. Peaks ranging from 2922-3386 cm^{-1} with -OH and -COOH functional groups; peaks at 1541.3 and 1507 cm^{-1} with C=C indicating the aromatic stretching.

The compounds with a hydroxy group, H-bonded -OH and -COOH functional groups at 3700-3000 cm^{-1} indicated the presence of phenolics, primary and tertiary alcohols; peak was observed at 2051.89 represents Isothiocyanates; Peak at 1051.98 cm^{-1} with C-O functional group indicating glycosides; peak at 3386.39 cm^{-1} with -OH group indicating the presence of phenols and flavonoids; peaks ranging at 2922.59 to 2980.45 cm^{-1} indicating aldehydes; peaks from 1412.60 to 1561.09cm-1, peaks from 1213.97 to 1240.0 cm^{-1} with -C=C-H indicated the presence of alkaloids, flavonoids, saponins, polyphenols and phenolic compounds.

Fig.1 FTIR Absorption Spectra for *Moringa oleifera* leaf extract (MOLE)



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3386.39	23.577	2	2980.45	29.4035	3	2965.02	28.0833	4	2948.63	25.8512
5	2922.59	30.2665	6	2864.74	43.8524	7	2841.6	37.2899	8	2594.75	85.7141
9	2524.36	84.68	10	2371.05	90.2429	11	2359.48	91.0807	12	2345.98	90.5927
13	2324.77	90.5945	14	2122.28	85.8148	15	2075.03	84.6049	16	2051.89	84.2525
17	2036.46	84.4323	18	1870.61	89.9194	19	1846.51	89.5426	20	1653.66	58.639
21	1561.09	90.4672	22	1542.77	90.7547	23	1509.03	86.6508	24	1454.06	65.6574
25	1412.6	67.1599	26	1347.03	79.5037	27	1331.61	83.2897	28	1321.96	83.9032
29	1240	91.2201	30	1213.97	92.723	31	1176.36	93.7934	32	1111.76	74.1189
33	1051.98	27.5562	34	1031.73	14.4811	35	1018.23	17.7907	36	724.139	62.3141
37	714.497	62.3065	38	702.926	61.9893	39	690.391	61.684	40	676.892	61.9266

FT IR (KBr Disc): ν_{max} (cm^{-1}) 3700-3000 (br, Phenolic –OH or -COOH), 3000-3100 (Ar-H) 1561 (C=C-H), 1240 (C=C), 1051 (C-O glycoside), 714 (C=C Bendings)

In conclusion *Moringa oleifera* L. being known as miracle tree because of its wide therapeutic potential against several metabolic disorders associated with stress, inflammation. Identification of phytochemicals provides additional information for the development of new compounds in the pharmaceutical industry which can be used for the wellbeing of humans and the animals.

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