



J Ayu Med Sci

Quarterly Journal for Rapid  
Publication  
of Researches  
in Ayurveda  
and Other Traditional  
Medicines

# Journal of Ayurveda Medical Sciences

[www.jayumedsci.com](http://www.jayumedsci.com)

ISSN 2456-4990

## Preliminary Phytochemical and FT-IR analysis of *Murraya koenigii* and *Phyllanthus amarus*

Preliminary phytochemical analysis depicted the presence of alkaloids, flavonoids, carbohydrates, saponins and tanins in alcoholic extracts of *Murraya koenigii* and *Phyllanthus amarus* leaves. FTIR is simple, rapid and sensitive approach to characterize presence of different functional groups present in leaves extract, *Murraya koenigii* and *Phyllanthus amarus*. Both extracts are known to possess medicinal properties because of presence of functional groups like alkane, alcohol, carboxylic acid, ketone and others. However, further advance studies are required to identify presence of active compound in afore side plant extract.

*Kumari et al.*



# Preliminary Phytochemical and FT-IR analysis As A Herbal Standardization Tool – A Trial with *Murraya koenigii* and *Phyllanthus amarus*

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## ABSTRACT

**Background:** Importance of traditional medicinal plants are increasing day by day due to presence of numerous bioactive chemicals in their extracts. *Murraya koenigii* and *Phyllanthus amarus* are common plants that are used as folk medicine since ancient time.

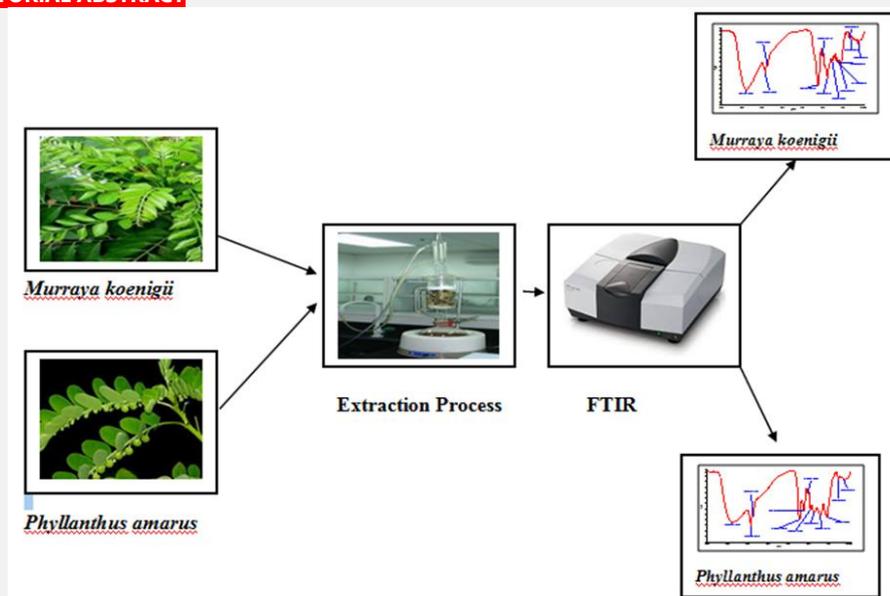
**Method:** Phytochemical screening and FTIR analysis of ethanolic extract of *Murraya koenigii* and *Phyllanthus amarus* were carried out.

**Results:** Phytochemical analysis revealed presence of alkaloids, carbohydrates, saponins, tannin and flavonoids in both the extracts. Total 13 spectral peaks and 12 spectral peaks were observed in FTIR analysis of *Murraya koenigii* and *Phyllanthus amarus* respectively.

**Conclusion:** These results will help to understand whether preliminary phytochemical analysis and Fourier-transform infrared spectroscopy be used as an authentication tool or not.

**KEYWORDS** Phytochemicals, FTIR fingerprinting, *Murraya koenigii*, *Phyllanthus amarus*

## PICTORIAL ABSTRACT



**ARTICLE HISTORY** Received 20.08.2018 Accepted 05.10.2018

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**CITE THIS RESEARCH AS** Kumari N, Gupte S, Sharma A, Yadav D, Tomar RS, Shrivastava S. Preliminary Phytochemical and FT-IR analysis As A Herbal Standardization Tool – A Trial with *Murraya koenigii* and *Phyllanthus amarus*. J Ayu Med Sci 2018;3(2): 396-401.

**DOI** 10.5530/jams.2018.3.18

Nature is store house of remedies that can cure various diseases<sup>[1]</sup>. Plant based medication are being used to treat illness since ancient time<sup>[2]</sup>. Use of about 1500 plants is well documented in indigenous medical system like Ayurveda, Siddha and Unani<sup>[3]</sup>. Medicinal plants acts as reservoir of chemical constituents that have enormous therapeutic efficacy<sup>[4]</sup>. Nowadays emphasis is given on use of herbal medicines because of presence of active chemical compounds in them<sup>[5]</sup>. According to WHO about 80% of

population still relies on traditional medicines<sup>[1]</sup>. *Murraya koenigii* (L.) Spreng (Rutaceae), a native of East-Asian nations is commonly known as *curry patta* and *meethi neem*. It is an aromatic pretty much deciduous shrub or a little tree growing up to 6 m tall, found through India up to an attitude of 1500 mt. It is utilized a flavoring agent in various kind of food items because of its specific fragrance and flavor. Leaves and bark are used to make indigenous prescription like tonic for stomach ache, stimulant

and carminative since old time<sup>[6]</sup>. In customary system of medicine, it is utilized as antimetic, antidiarrheal, febrifuge, blood purifier, tonic, in stomachache, as seasoning agent in curries and chutneys<sup>[7]</sup>. *M. koenigii* has demonstrated various naturally vital activities like antimicrobial, antioxidant, anti-diabetic, anti-carcinogenic etc<sup>[8]</sup>. *Phyllanthus amarus* (Schumach. & Thonn)(Euphorbiaceae) is one of roughly 800 species of the genus located in tropical and subtropical nations of the world<sup>[9]</sup>. It is commonly known as *ashthumiamla*<sup>[10]</sup>, *jaramla* or *jangliamla*, stone breaker and carry me seed<sup>[11]</sup>. *Phyllanthus amarus* is widespread throughout the tropics and subtropics in sandy regions as a weed in developed and squander lands<sup>[12]</sup>. Various phytochemicals of therapeutic interest present in this herb include alkaloids, flavonoids, tannins, lignans, polyphenolic mixtures and tetracyclic triterpenoids. Lignans like phyllanthin and hypophyllanthin are more important among them<sup>[13]</sup>. The plant is used as astringent, cooling, diuretic, febrifuge and disinfectant. The study was aimed to characterize the antioxidant status of a forest side plant through

primary phytochemical analysis and FTIR. An attempt was made in this investigation to understand phytochemical and functional analysis of plant extract.

### 1. Reagents and Chemicals

All the chemicals used for experiments were of analytical grade and purchased from Sigma-Aldrich, Hi media and SRL chemical substances Pvt.Ltd.

### 2. Preparation of plant extracts

*M. koenigii* and *P. amarus* were collected from Jiwaji University campus and these plants were identified by Dr. Ashok K. Jain, Director, Institute of Ethnobiology Jiwaji University, Gwalior (India). Ten grams of each plant sample were powdered and soaked for 24 hours in 10ml ethanol for extraction by cold percolation followed by filtration.<sup>[14]</sup>

### 3. Phytochemical analysis

Detection of alkaloids, flavonoid, carbohydrates, saponins and tannins was done by slight modification in Harborne's method<sup>[15,16]</sup>.

Table 1. Preliminary phytochemical analysis of ethanolic leaf extract

Tests	Colour if positive	<i>Murraya koenigii</i>	<i>Phyllanthus amarus</i>
<b>Alkaloid</b>			
i. Wagner's test	Reddish brown ppt	+	+
ii. Hager's test	Yellow ppt	+	+
iii. Dragendorff's test	Orange/Red ppt	+	+
iv. Mayer's test	Dull white ppt	+	+
<b>Carbohydrates</b>			
i. Fehling's test	Brick red ppt	+	+
ii. Benedict's test	Red ppt	+	+
iii. Molisch's test	Violet ring	+	+
<b>Saponin</b>			
	Stable froth	+	+
<b>Tannin</b>			
	Dark blue, green or brown	+	+
<b>Flavonoid</b>			
	Red/pink ppt	+	+

Table 2. Functional groups of ethanolic extract of *Murraya koenigii* leaves

Absorption Peak	Functional group and origin	Intensity
3392.37	O-H stretching of alcohol	Strong
2924	C-H stretching of alkane	Strong
2851.93	C-H stretching of alkane	Medium
1617.52	N-H bending of amine	Medium
1516.14	N-O stretching of nitro compound	Strong
1384.55	S=O stretching of sulfate	Strong
1264.46	C-N stretching of aromatic amine	Strong
1161.79	C-O stretching of tertiary alcohol	Strong
1119.76	C-O stretching of secondary alcohol	Strong
1065.41	C-O stretching of primary alcohol	Strong
834.84	C=C bending of alkene	Medium
768.98	C-Cl stretching of chloro compound	Strong
612.52	C-Br stretching of bromo compound	Strong

Table 3. Functional groups of ethanolic leaf extract of *Phyllanthus amarus*

Absorption Peak	Functional group	Intensity
3362.17	O-H stretching of alcohol	Strong
2926.24	O-H stretching of carboxylic acid	Strong
2854.53	C-H stretching of alkane	Medium
1714.39	C=O stretching of carboxylic acid	Strong
1611.01	C=C stretching of unsaturated ketone	Strong
1515.02	N-O stretching of nitro compound	Strong
1450.59	C-H bending of methyl group	Medium
1350.92	O-H bending of alcohol	Medium
1204.94	C-O stretching of ester	Strong
1038.65	C-O stretching of alkyl aryl ether	Strong
766.87	C-Cl stretching of chloro compound	Strong
621	C-Br stretching of Bromo compound	Strong

#### 4. FT-IR analysis

The biochemical compositions of *M. koenigii* and *P. amarus* were examined using fourier transform infrared spectroscopy (FTIR) operating in 400-4000 $\text{cm}^{-1}$  range to determine functional groups. In ethanolic plant extract different functional groups were observed at different intervals on the basis of peak absorption. For FT-IR analysis ethanolic extract of *M. koenigii* and *P. amarus* were completely dried to powder form. Small amount of dried extract powder was crushed with potassium bromide (KBr) to prepare a translucent sample disc. Then the disc was loaded in FTIR spectroscope with the scan range of 400 to 4000 $\text{cm}^{-1}$ [17]

Phytochemical screening of *M. koenigii* and *P. amarus* plant extract showed presence of various phytochemicals like alkaloids, carbohydrates, saponins, tannin and flavonoids. FT-IR analysis of plant extracts showed presence of different functional groups.

*M. koenigii* showed total 13 spectral peaks showing different functional groups. The 3392.37  $\text{cm}^{-1}$  peaks represent the O-H stretching vibration hinting presence of alcohol. The frequencies ranges from 2924  $\text{cm}^{-1}$  and 2851.93  $\text{cm}^{-1}$  represent the C-H vibrational stretching, mainly alkane. The 1617.52  $\text{cm}^{-1}$  peaks represent the N-H bending vibration hinting presence of amine. The 1516.14  $\text{cm}^{-1}$  peaks represent the N-O stretching vibration shows presence of nitro compound. The 1384.55  $\text{cm}^{-1}$  peaks represent the S=O stretching vibration shows presence of sulfur compound. The 1264.46  $\text{cm}^{-1}$  peaks represent the C-N stretching vibration hinting presence of aromatic amine. The 1161.79  $\text{cm}^{-1}$ , 1119.76  $\text{cm}^{-1}$  and 1065.41 $\text{cm}^{-1}$  peaks represent the C-O stretching vibration showing presence of tertiary, secondary and primary alcohol respectively. The 834.84  $\text{cm}^{-1}$  peak represent the C=C

bending vibration which confirms presence of alkene. The 768.98  $\text{cm}^{-1}$  peak represent the C-Cl stretching vibration which shows presence of chloro-group. The 612.52  $\text{cm}^{-1}$  peaks represent the C-Br stretching vibration which shows presence of bromo group.

*P. amarus* showed total 12 spectral peaks showing different functional groups. The 3362.17  $\text{cm}^{-1}$  peak represent the O-H stretching vibration, hinting presence of alcohol. The 2926.24  $\text{cm}^{-1}$  peak depicts the O-H vibrational stretching, mainly carboxylic acid. The 2854.53  $\text{cm}^{-1}$  peak depicts the C-H vibrational stretching hinting alkane. The 1714.39  $\text{cm}^{-1}$  peak depicts the C=O stretching vibration showing the presence of carboxylic acid. The 1611.01  $\text{cm}^{-1}$  peak represent the C=C stretching vibration showing the presence of unsaturated ketone. The frequency ranges from 1515.02  $\text{cm}^{-1}$  spectral peak represent the N-O stretching vibration shows presence of nitro compound. The 1450.59  $\text{cm}^{-1}$  peak represent the C-H bending vibration shows presence of methyl group. The 1350.92  $\text{cm}^{-1}$  peak represent the O-H bending vibration which confirms presence of alcohol. The 1204.94  $\text{cm}^{-1}$  peak represent the C-O stretching vibration which shows presence of ester. The 1038.65  $\text{cm}^{-1}$  peak represent the C-O stretching vibration which shows presence of alkyl aryl ether. The 766.87  $\text{cm}^{-1}$  peak represent the C-Cl stretching vibration which shows presence of chloro- group. The 621  $\text{cm}^{-1}$  peak represents the C-Br stretching vibration which shows presence of bromo- group.

The plants are known to possess various phytochemicals which acts as antioxidants and promote healing. The presence of phytoconstituents provides scientific explanations for the long-term use of herbal medications<sup>[18]</sup>. Phytochemical estimation of ethanolic extract of *M. koenigii* and *P. amarus* showed presence of various compounds like alkaloids, flavonoids, saponins, tannins

and carbohydrates. Alkaloids are one of the major group of phytochemicals associated with antimicrobial and antifungal activity<sup>[19]</sup>. Ethanolic extract of *M. koenigi* and *P. amarus* showed presence of alkaloids<sup>[20,21]</sup> which is very similar to results of this study. Flavonoids are low molecular weight metabolites that interacts with various cellular components and manifests its actions. It acts as strong antioxidant agent and have ability to scavenge hydroxyl radicals and superoxide anions<sup>[22]</sup>. Various studies reported presence of flavonoids in ethanolic extract of *M. koenigi* and *P. amarus* which resembles findings of the current study<sup>[23,24]</sup>. Tannins are best known for their astringent properties. The plants containing tannins are used for treatment of inflammation, liver and kidney injury, hypertension, arteriosclerosis and etc<sup>[18]</sup>. Tannins were reported from ethanolic, aqueous and acetone extract of *M. koenigi*<sup>[25]</sup>. Characterization of ethanolic extract of *P. amarus* revealed the presence of tannins<sup>[26]</sup>, there by supporting the current work. Saponins are secondary metabolites of plants and they possess various biological activities like hepatoprotection, anti-tumor, anti-ulcer and anti-inflammatory<sup>[27]</sup>. Ethanolic extract of *M. koenigi* and *Phyllanthus amarus* showed positive result for saponins and carbohydrates<sup>[26,28,29]</sup>. Identification of various phytochemicals and their chemical nature is of great concern to understand its medicinal values, this is achieved by analysis of FTIR spectra<sup>[30]</sup>. The present study revealed the presence of 13 peaks in *M. koenigi* extract with strong peaks at 3392.37, 2924, 1516.14, 1384.55, 1264.46, 1161.79, 1119.76, 1065.41, 786.98 and 612.52  $\text{cm}^{-1}$  showed total 12 spectra with strong peak values at

3362.17, 1611.01, 1204.94, 1038.65, 766.87 and 621  $\text{cm}^{-1}$ . 3392.37 and 3362.17  $\text{cm}^{-1}$  are  $-\text{OH}$  stretch of *Murraya* and *Phyllanthus* revealed in the present study. Other studies<sup>[31,32]</sup> have also shown the presence of  $-\text{OH}$  stretch in similar range 3446.76  $\text{cm}^{-1}$  is  $-\text{OH}$  stretch for *Murraya* and  $-\text{OH}$  stretch for *Phyllanthus* is seen at 3397  $\text{cm}^{-1}$ . These results indicates presence of polyphenolic or alcoholic group in both the plants.  $\text{C}=\text{O}$  stretching of carboxylic acid at 1714.39  $\text{cm}^{-1}$  can only be seen in *Phyllanthus* extract, other authors<sup>[34]</sup> also reported presence of  $\text{C}=\text{O}$  stretching of carboxylic acid at 1745  $\text{cm}^{-1}$ . 2854.53  $\text{cm}^{-1}$  and 2851.93  $\text{cm}^{-1}$  indicates C-H stretch in *Murraya* and *Phyllanthus* respectively, the similar ranges of C-H are reported by other authors<sup>[35,34]</sup>, these C-H stretch indicates presence of alkanes and aldehydes. Previous study<sup>[33]</sup> reported presence of C-Cl stretch in *Murraya* extract at 850-550  $\text{cm}^{-1}$ , our results also showed presence of C-Cl stretch at 768.98  $\text{cm}^{-1}$  in *Murraya* extract indicating the presence of halo group in extract. N-H bending of amines can be seen at 1617.52  $\text{cm}^{-1}$  spectral peak which is similar to spectral peak for N-H bending of amines observed in previous study<sup>[35]</sup> of *Murraya*. FTIR of both the plants indicates presence of different functional group. C-Br stretching of Bromo compound were found in both as strong bond that was because the preparation of tablets from functional group identification was done in KBr, we have observed that analysis of each plants is almost same each extract so the appearance of this stretch is common in all the plant extracts studied. For identification and quantification of different phytochemicals other advance techniques can be used.

Figure 1. FT IR analysis of ethanolic leaf extract of *Murraya koenigi*

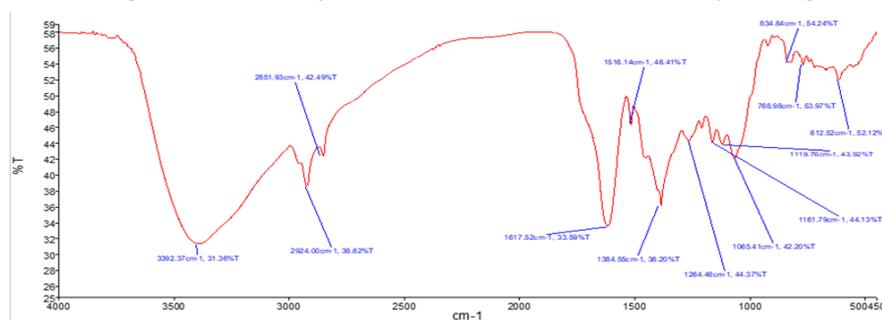
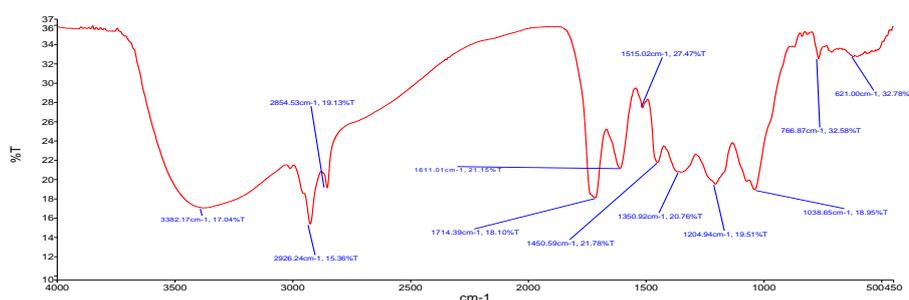


Figure 2. FT-IR analysis of ethanolic leaf extract of *Phyllanthus amarus*



The results obtained from preliminary phytochemical analysis suggested presence of various phytochemical compounds which attributes to antioxidant properties of a foresaid plants. FTIR analysis of both the plants showed presence of strong –OH stretch of alcohol. The –OH group of alcohol renders antioxidant potential to these plants. Preliminary phytochemical analysis coupled FTIR can be used as basic authentication tool, for further quantification and characterization of phyto compounds implementation of advanced techniques are needed.

**Acknowledgement** Authors are thankful to Jiwaji University Gwalior for providing lab facilities. Authors acknowledge Ms Shivani Singh and Ms Arti Rathoure M.Phil. Students for constant support during the study.

**Source of support** Authors are thankful to Department of Health Research (DHR) V.25011/455-HRD/2016-HR.

**Conflict of interest** None

**Contributors** Drafting of manuscript and experimental work by Kumar, Gupte and Sharma. Study conception, standardization and design by Yadav and Shrivastava. Analysis and interpretation of data by Yadav, Tomar and Shrivastava. Critical revision by Yadav, Tomar and Shrivastava.

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