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Review Paper on Chemical Characterization of Millettia Pinnata of Bhilai –Durg Region of Chhattisgarh

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Abstract: There is a great demand for plant based drugs due to the presence of effective chemical constituents and lesser side effects. This review article attempts to reveal the chemical constituents present in Millettia pinnata and their characteristics determined with the help of proximate analysis, Fourier Transform Infrared Spectroscopy Analysis, High Performance Liquid Chromatography, Atomic absorption spectrophotometer and Thermo gravimetric analysis.

Keywords: Millettia, Pyrolysis, Proximate Analysis, HPLC, FTIR, Thermo Gravimetric

1. Introduction

In recent years Ayurvedic medicines are gaining a sustained portion of global market due to cost effectiveness and lesser side effects[1]. Studying medicinal plants helps to understand the toxins present in the plant and this helps to protect the animals and human beings from the natural poisons which lead to lesser consumption. Cultivation and preservation of medicinal plants protect the biological diversity. Considering the rapid rate of deforestation and loss of biodiversity there is need for accurate scientific documentation of the knowledge and experience of herbalists. For the last few decades, phytochemistry has been making rapid progress and herbal products are becoming popular. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha. The main advantage of using medicinal plants does not produce side effects when compared with synthetic drugs as medicinal plants contain large content of anti oxidants. It has shown protective effects against diseases without reducing their therapeutic efficacy [2].

CG is very rich in medicinal herbs due to supportive climatic conditions, temperature, soil, rainfall. Various types of soil are found here. The soil type of CG plains consists of Entisol 36%, Alfisol 21%, Inceptisol 22%, Vertisol 18%, and Alluvial 3%. Two types of soil are predominant in CG ie Black clayey soil and red to yellow soil. The latter is less fertile and contain substantial amount of sand. The irrigation percentage is 40%.Cropping intensity of the state is 135%. In Durg region red and yellow loamy soil is dominant and the soil is low in nitrogen and humus content.

Pongam tree is found to be one of the richest trees of India. The name Pongamia pinnata is derived from the word pinnata that refers to the pinnate leaves. It belongs to family Fabaceae. It is native in tropical and temperate Asia. It is grown in Indian subcontinent China, Japan, Malaysia, Australia and Pacific islands. It is now called Millettia pinnata as it is moved to the genus Millettia only recently.

Scientific classification
Kindom –Plantae
Order- Fabales
Family- Fabaceae
Genus-Millettia
Species- M. pinnata

Millettia pinnata is named as Karanj in Hindi, Honge in Kannada, Pungai in Tamil, Kanuga in Telugu, Karach in Bengali, Naktanala in Sanskrit.

Millettia pinnata belongs to family fabaceae. It is a medium sized evergreen or briefly deciduous, glabrous tree 15-25 m high with straight trunk 50-80cm or more in diameter. Bark is grey brown, smooth or faintly vertically

fissured. Leaves are alternate, impart pinnate with long slender leaf stalk, hairless, pinkish red when young, glossy darkgreen with prominent veins beneath when mature. It is an ornamental tree and gives shade. It is one of the nitrogen fixing tree and preferred for controlling soil erosion as it has dense network of lateral roots. Karanja oil is extracted from the seeds of this tree.Itis the non edible oil which possess medicinal value as well as it is used as fuel for lamps and cooking stoves. The pressed cake is rich in nitrogen, phosphorus and potassium and used as organic fertilizer and insect repellent.

Figure 1. *Millettia pinnata tree.*

Millettiapinnatais antihelmintic, alexipharmic and useful in the disease of eyes, vagina, skin, tumours, wound ulcers, itching, enlargement of spleen, abdomen urinary discharges etc. Roots and barks of Millettia pinnata are used externally for joint pain. Seeds help in the production of fuel that can serve purpose of alternative fuels. Milletia pinnata leaves are antihelmintic, digestive and laxative for inflammation, piles and wounds. Methanol extract of leaves has wide range of antibacterial activity on the bacterial pathogens than the petroleum ether extract. Phytochemical screening of the plant leaves reveals the presence of carbohydrates, alkaloids, flavonoids, glycosides, steroids, tannins and saponins [3].

2. Materials and Methods

2.1. Collection of Samples

The medicinal plant selected for study was the leaves of Millettia pinnata of family fabaceae. Fresh leaves were collected, dried in shade and subjected to Pyrolysis.

2.2. Pyrolysis Extractof Millettia Pinnata

The dried leaves of Millettia pinnata were subjected to pyrolysis. Pyrolysis reactor consists of Aluminium chamber to which the dried leaves were fed and heated to 360°C. The vapours evolved were condensed in the condenser and the pyrolytic extract was collected in the measuring cylinder.

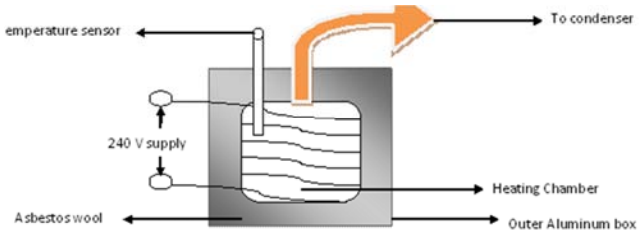


Figure 2. *Pyrolysis reactor.*

2.3. Proximate Analysis of Leaves of Millettia Pinnata [4 to 9]

2.3.1. Extractive Values

About 5g of the dried and finely coursed powder is mixed with 100 ml of 90% ethanol in a closed flask. The flask was frequently shaken during the first 6 hours and allowed to stand for 18 hrs. Then the mixture was rapidly filtered to minimize the loss of ethanol and 25 ml of the filterate was evaporated to dryness in a tarred flat bottomed shallow dish. The residue was dried at 105°C for minutes and then weighed. The procedure was performed twice more from the filtrate.

2.3.2. Ash values

i. Total Ash and Sulphated Ash Values [4]

Preheating of a silica crucible was done for 30 minutes to red hot and then cooled down in a dessicator and weight of the crucible was noted down. Take about 3 g of the powdered medicinal sample and weigh it and dried at 100-105°C for 1 hour and ignited to constant weight in a Muffle furnace at 600-650°C until a carbon free ash were formed. Crucible were allowed to cool and then weighed.

To determine the yield of sulphated ash,the same procedure was carried out with dilute sulphated acid.

ii. Acid Insoluble Ash

About 1g of the total ash (from total ash) was boiled with 25 ml of 2M hydrochloric acid for 5 min. The acid insoluble was separated by filtration on an ash less filter paper in Gooch crucible the content on the ash less filter paper was washed with hot water and ignited and then weighed to obtain the percentage of ash with reference to the air dried samples.

iii. Water Soluble Ash

About 1g of the total ash was boiled with 25 ml of water for 5 min and then filtrated to retain the insoluble matter on ash less filter paper. The content was ignited for 15 min at a temperature not exceeding 450°C then weighed. The difference between the amount of ash subjected and weight of insoluble ash was accounted as the water soluble ash value.

iv. Loss on Drying

About 10 g of each specimen under study were accurately weighed and transferred to a charred china dish which was known for its weight and kept in a hot oven at 100-105°C for an hour. Then the sample was weighed along with china dish to deduct the actual weight of tarred china dish. The weight of the powder was noted to calculate the percentage loss on

drying with reference to air dried specimen.

2.4. HPLC Analysis of Leaves of Millettia Pinnata

HPLC analysis were performed on Water 600 system UV detector with stainless steel flow path features 50 µ l pump head volume, maximum pressure 5000 psi. Methanol extract of the pyrolytic oil of leaves of Millettia pinnata was studied for HPLC finger print. Separation of constituents was done using C-18 (Hypercil 250 x 4 mm, 5 µm particle size) with solvent methanol, the method name is DEFAULT.MTH. The detection wave length was 495 nm and the detector used was Water 600 Controller UV Detector. The column was maintained at ambient temperature and analytical run time was 8 minutes.

2.5. Fourier Transform Infrared Spectroscopy Analysis of Millettia Pinnata

FTIR spectrum helps to identify the functional group present inMillettia pinnata. FTIR spectra are recorded in KBr by sophisticated computer controlled FTIR Perkin Elmer spectrometer with He- Ne Laser as reference. The pyrolysis extract of sample Millettia pinnata were scanned at room temperature and a spectral range of 4000-400 cm^{-1}.

2.6. Determinationof Zinc Concentration in Millettia Pinnata Leaves

Analysis of Zinc concentration in the Pyrolysed extract of Millettia pinnata leaves were determined by Atomic Absorption Spectrophotometer Perkin Elmer 2380 model using suitable hollow cathode lamps.

2.7. Thermogravimetric Analysis of Millettia Pinnata Leaves

TGA were performed with TGA 4000, Pyris 6 TGA. Weight of Millettia pinnata leaves taken were 7.104 mg. These were loaded separately on quartz pan and mounted in instrument. Initial conditions of temperature were 30°C and switch the gas to N_2 at 20 ml/ min. Temperature programming were heating rate from 30°C to 400°C at 10°C/min in nitrogen and hold for 1 min at 30°C.

3. Result &Discussion

Table-1 and Table-2 shows proximate analysis of the leaves of Millettia pinnata. The colour of the residue of the alcoholic extractives of leaves of Millettia pinnata was found to be green in colour and the extractive % w/w were 5.41. The results reveal how far they differ in their qualities and it gives a finger print out of the sample purity. Ashes give us the idea of mineral matter contained in the plant which is responsible for pharmacological effect. The total ash value and the moisture content is low for the leaves of Millettia pinnata which shows high calorific value and high energy value so it is a good bio fuel.

Table 1. *Proximate analysis of Millettia pinnata.*

Extractive values	Specimen	Colour of the residue	Extractive % w/w
Alcoholic extractives	Millettia pinnata-leaves	Green	5.41

Table 2. *Proximate analysis on leaves of Millettia pinnata.*

S. No.	Experimental studies	Millettia pinnata leaves %w/w
1.	Total ash value	6.97
2.	Water soluble ash	45.92
3.	Acid insoluble ash	10.08
4.	Sulphated ash	6.86
5.	Loss on drying	4

*HPLC peaks:*of methanolic leaf extract of Millettia pinnata were shown in Figure 3.

Millettia pinnata showed three peaks with retention time 2.254, 3.686 and 7.096 min. Out of these, one prominent peak were identified as Karanjin which is responsible for curative effect in skin diseases. Clinical experts indicate that it is free from highly irritating and inflammatory effects of Caimarin compounds and its application with other vegetable oil such as coconut, ground nut oil is reported to be better than when incorporated in paraffin base.

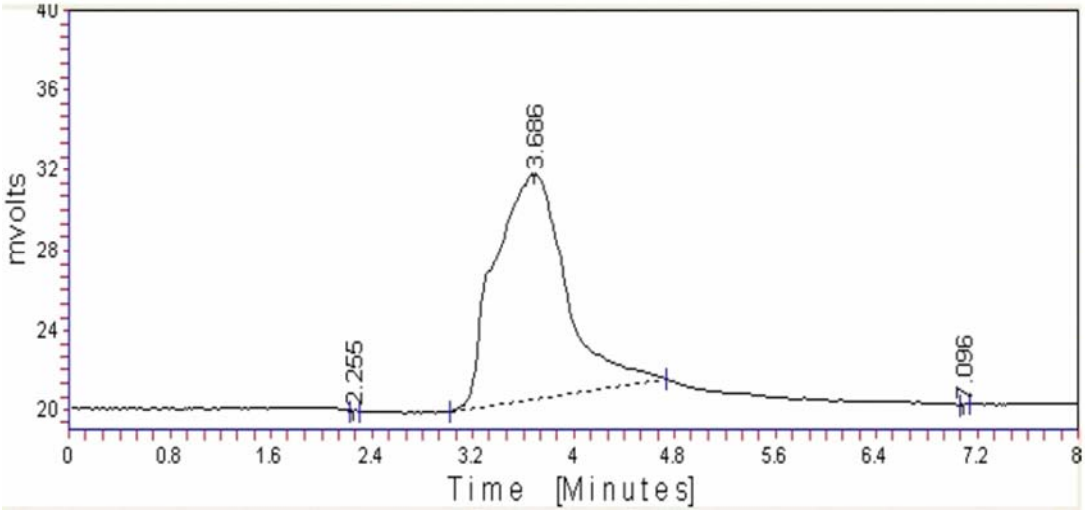


Figure 3. *HPLC of leaves of Millettia pinnata.*

*FTIR spectrum:*of leaves of Millettia pinnata were obtained and depicted in Figure 4 which confirmed the presence of functional group.The intense bands occurring at 3289.40 cm^{-1} ,2923.79 cm^{-1},1742.40 cm^{-1} ,1637.71 cm^{-1}, 1385.18 cm^{-1}, 1260.78 cm^{-1} and 1055.81 cm^{-1} corresponding to N-H/ O-H str / C=O/phenol C-O str / stretching. This confirms the presence of functional groups in Millettia pinnata like carboxylic acids, amines, amides, phenol, ether etc. Strong absorption band observed around 3373 to 3422 cm^{-1} indicates the presence of amines and amides [10]. Absorption band observed around 3200-3400 cm^{-1} shows the presence of polymeric hydroxyl derivatives. Presence of primary amines is indicated by the vibration of N-H band [11]. Strong absorption band observed near 2848 cm^{-1} shows C-H symmetric stretching of methylene group [12]. Strong absorption band observed near 1621-1635 cm^{-1} were due to the presence of chelated C=O stretching [13]. Carboxylic acids present in the medicinal plants is responsible for treatment of diseases like headache, fever, rheumatic joint pain[14].

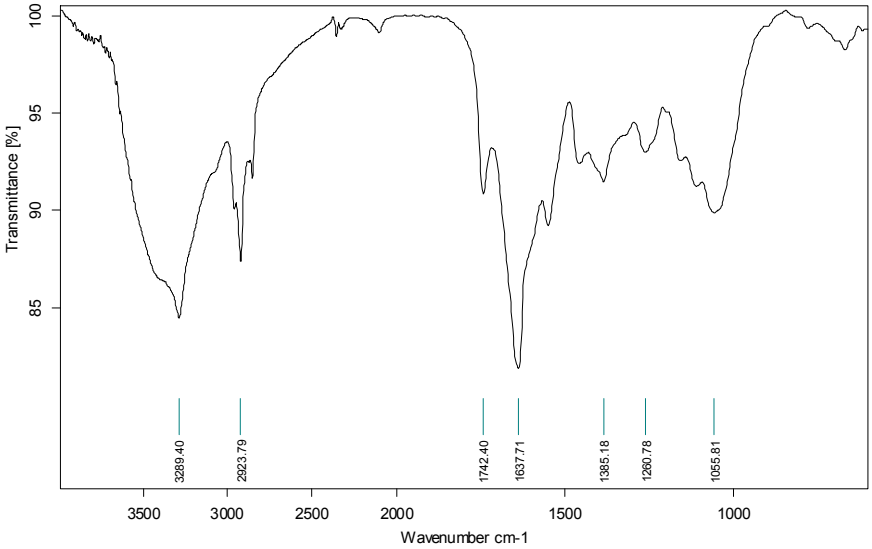


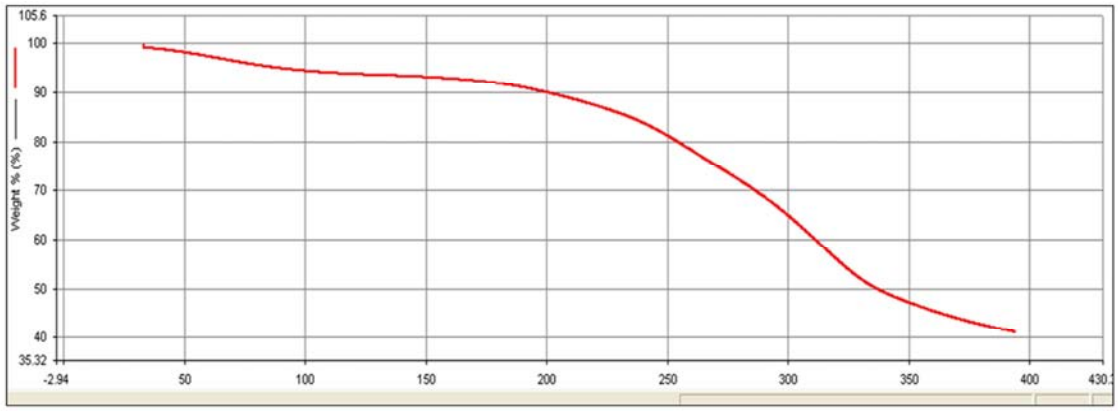
Figure 4. *FTIR Spectra of leaves of Millettia pinnata.*

Concentration of Zinc in Millettia pinnata leaves: Table 3 shows the concentration of Zincin the leaves of Millettia pinnata which was found to be high 26. Zinc is very effectic antioxidant and it helps in the brain development in young children [15]. It is also helpful in DNA synthesis, cell division and protein synthesis [16]. It help in enzyme catalysis [17].

Table 3. *Concentration of Zinc in leaves of Millettia pinnata.*

S.No.	Medicinal plant sample	Concentration of Zinc(mg/kg)
	Millettia pinnata leaves	26.0

TGA curve: Figure 5 depicts the thermogravimetric analysis of leaves of Milletia pinnata. Maximum thermal degradation was observed at 250°C. This is due to decomposition of organic moiety. TGA curve was plotted between temperature and weight of the sample and Z shaped curve was obtained. Initial degradation was observed at 100 ^{o}C which was due to the loss of water molecules. Millettia pinnata shows minimum initial temperature decomposition which supports its stability and active role in its theraupatic action [18].



Plot between weight and temperature of leaves of Millettia pinnata

Figure 5. *TGA of Millettia pinnata.*

4. Conclusion

The leaves of Millettia pinnata were collected, dried and pyrolysed in Pyrolysis reactor. The pyrolytic extract were subjected to proximate analysis, HPLC, FTIR, TGA and concentration of Zinc were determined by Atomic absorption spectrophotometer. This analysis creates a platform to screen many bio active chemical constituents present in Millettia pinnata to treat various diseases.

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