



## Physicochemical Investigation and Phytochemicals Screening of Karanj Leaves (*Milletia Pinnata* L.)

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### ABSTRACT :

The Present paper deals with the “Physicochemical investigation and phytochemicals screening of karanj leaves (*milletia pinnata* L.)” The results which were obtained showed the presence of various Phytochemicals viz. Alkaloids, Tannins, Resins, Phenols, Carbohydrates, Cardiac Glycosides, Proteins etc. The detailed discussion is given in the paper.

**Keywords :** *Milletia pinnata*, Physicochemical, Phytochemical, Alkaloids, carbohydrates, Proteins.

### Introduction:-

Medicinal plants play an important role in human lives for many years to Treat various diseases all over the world. Plants are the diverse producer of Bioactive compounds that make them a rich source of different types of medicines[1]. Today, there is widespread interest in drugs obtained from natural plants for Their various therapeutic properties. *Pongamia pinnata* Linn Pierre (Fabaceae) is a Fast-growing medium-sized tree that belongs to the Leguminosae family [2]. It is an important non-edible minor oilseed tree. It is also called as “Karum Tree” or “Poonga Oil Tree” in English [3]. It is native mainly to hot arid regions of Asia. The trees *Pongamia* are cultivated commercially in India. It Includes various chemical compounds such as alkaloids, flavonoids, tannins, glycosides, hormones, karangin, glabrin, kanugin, fixed oils, and others [4] that possess various potential anti-inflammatory, anti-nociceptive, antioxidant, anti-diarrheal, anti-fungal, anti-plasmodial, anti-ulcer, anti-hyperglycemic, anti-oxidative, anti-hyperammonemic, and analgesic functions [5,6]. All parts of the plant *P. pinnata* have been considered as a crude drug [7]. The traditional use of *Millettia* species includes antibacterial, anti-tumour, insecticidal, pesticidal, piscicidal antispasmodial, chemopreventive joint pain, rheumatoid arthritis, amenorrhea, tuberculosis, etc. Most of them are used in the production of biodiesel [8,9] The tree Is known for its Multipurpose advantages and as a potential biodiesel source. On average, the seeds are stated to contain around 28-34% Oil with a high percentage of polyunsaturated fatty acids.[10].

### Classification:-

Kingdom	Plantae
Order	Fabales
Family	Fabaceae
Subfamily	Faboideae
Genus	<i>Millettia</i>
Species	<i>m. Pinnata</i>



(A)



(B)

- **\*medicinal use:-**
- Millettia pinnata has laxative properties that can help clear the stomach.
- It is used for the treatment of piles as well as for bleeding disorders.
- The leaf juice is meant for the treatment of cough, colds, leprosy, diarrhoea.
- It is used for the treatment of abdominal ulcers, tumor.
- It has antibacterial and anti-inflammation properties
- Roots and bark of millettia pinnata are used extremely for joint pain.
- Material and method

Materials which were used in the study are given in the table: 1

The list of glassware, instrument, reagents and chemicals which were used in the study are given in table-1

Glass ware	Instrument	Reagent
Conical flask	Water bath	Lead acetone solution
Funnel	Weighting machine	Benedict solution
Glass rod	Hot air oven	Dragendroff's reagent
Petri dish	Heating mental	Hager's reagent
Pipette	Brush	Wagner's reagent
measuring calendar	Desiccators	Molish's reagent
Iodine flask	Filter paper	Cuso <sub>4</sub> . 5H <sub>2</sub> o
Test tube	Mixer grinder	FecI <sub>3</sub> solution
Dropper	Spatula	Nahco <sub>3</sub>
Beaker	Sleave	Distilled Water
Crucible	Tray dryer	Hydrochloric acid
Reagent bottle	Grinder machine	Feric chloride
Capillary tube	Reprostar	Methanol
China dish	HPTLC	Folin's reagent
Pipettes	Linomat	Acetone

**Sample collection :-**The whole plant of millettia pinnata was collected from Arogyadham Ayurved Sadan chitrakoot, satna [M.P].Taxonomical identification and authentication of millettia pinnata plant were done by Dr.Manoj Tripathi department of Ayurveda Sadan Arogyadham.Plant were washed thoroughly in running water to remove soil and running foreign particles. These were cut into small pieces dried in shade. Shade dried plant sample were powdered using grinder and was stored in air tight container for further chemical analysis. The parameters which were analyzed in the present study are:

### The parameters which were analysed are :-

#### [1] Physicochemical Analysis:-

1. LOD (Lose on drying at 105°C,
- Extractive value:-

2. Water Extractive value
3. Methanol extractive value
4. Ethanol Extractive value
5. Benzene extractive value
6. Acetone extractive value.

**[2]. Ash value**

1. **Total ash**
2. **.Acid insoluble ash**

**[3]. phytochemical analysis:-**

1. Test for alkaloids
2. Test for flavonoids
3. Test for tannins
4. Test for resins
5. .Test for carbohydrate
6. Test for proteins
7. .Test for saponins
8. .Test for cardiac Glycosides

**[4]. High performance Thin Layer Chromatography (HPTLC) Fingerprint profile:-****1. Determination of Moisture Content (Loss on drying at 105°C)**

2gm of sample powder was transferred to weighed thin porcelain dish and kept in oven at 105°C for 5 hours. Dish was cooled in desiccators and weighed. The dish again kept in oven for 30 minute. Dish again cooled in desiccator and take weight. The loss in weight is calculated as percentage.

\*Extractive value

**1 Determination of water soluble extractive**

Weighed accurate 2g powder sample and transferred in 250 ml Iodine flask. Add 100ml water solvent. Provide continuous shaking for 6 hrs and leave for 18 hrs (maceration) After this extract was filtered by using what man filter paper no. 1 and weighted in thin orcelain dish, solvent was evaporated on water bath and residue was weighed. Percentage of extractive value (w/w) was determined

**2.Determination of methanol soluble extractive**

Weighed accurate 2gm powder sample and transfer in 250 ml Iodine flask. Add 100ml alcohol solvent. Provide continuous shaking for 6 hrs and leave for 18 hrs (maceration). After this extract was filtered by using what man filter paper no. 1 and weighted in thin orcelain dish, solvent was evaporated on water bath and residue was weighed. Percentage of extractive value (w/w) was determined.

**3.Determination of ethanol Extractive value :-**

Weighed accurate 2gm powder sample and transfer in 250 ml Iodine flask. Add 100ml ethanol solvent. Provide continuous shaking for 6 hrs and leave for 18 hrs (maceration). After this extract was filtered by using what man filter paper no. 1 and weighted in thin orcelain dish, solvent was evaporated on water bath and residue was weighed. Percentage of extractive value (w/w) was determined.

**4.determination of Benzene extractive value :-**

Weighed accurate 2gm powder sample and transfer in 250 ml Iodine flask. Add 100ml benzene solvent. Provide continuous shaking for 6 hrs and leave for 18 hrs (maceration). After this extract was filtered by using what man filter paper no. 1 and weighted in thin orcelain dish, solvent was evaporated on water bath and residue was weighed. Percentage of extractive value (w/w) was determined.

**5.Determination of Acetone Extractive value:-**

Weighed accurate 2gm powder sample and transfer in 250 ml Iodine flask. Add 100ml acetone solvent. Provide continuous shaking for 6 hrs and leave for 18 hrs (maceration). After this extract was filtered by using what man filter paper no. 1 and weighted in thin orcelain dish, solvent was evaporated on water bath and residue was weighed. Percentage of extractive value (w/w) was determined.

**\*Determination of Ash values**

The ash value is useful to determine the quality and purity of the drug. Ash contains Inorganic radicals like phosphate, carbonates and silicates of sodium, potassium, magnesium, Calcium, etc. Different ash values such as total ash value and acid insoluble ash value was Determined.

**1.Determination of total ash:-**

2 gm accurately weighed of the ground drug in a tared platinum or silica dish (previously weighed). Material was incinerated with the help of the muffle furnace at 450°C, until vapors almost cease to evolve. Dish was heated until all carbon was burnt off. Dish was cooled, and calculated the percentage of ash value with reference to the air dried drug.

**2.Determination of Acid-insoluble ash :-**

Ash was boiled with 25 ml 5% hydrochloric acid for 5 minutes. Insoluble matter was collected in the ash less filter paper no. 42. It was washed with the hot water until neutralized and ignited to constant weight, cooled in a desiccator and weighed. Calculate the percentage of acid insoluble ash with reference to the air dried drug.

**\*Phytochemicals analysis:-**

Preliminary phyto-chemical tests were carried out on ethanolic and water extract for the presence/absence of phyto-constituents like alkaloids, flavonoids, tannins, resins, Carbohydrates, proteins and saponins.

**\*Test for Alkaloids**

Mayer's test: Add few drops of Mayer's reagents to 1 ml of the acidic, aqueous extract of the Drug. White or pale yellow colour is formed.

- **Wagner's Test:** Acidify 1 ml of the alcoholic extract with 1.5% v/v of HCl and few drops of Wagner's reagent. A yellow or brown ppt. is formed.
- **Test for Carbohydrate**

Benedict's test: To 0.5 ml of aqueous extract of drug, add 5 ml of Benedict's solution and boil for 5 minutes. Formation of coloured ppt. is due to presence of carbohydrates.

- **Fehling's test:** To 2 ml of aqueous extract of drug, add 1 ml of mix. Of equal parts of Fehling's solution A and Fehling's solution B and boil the content of the test tube for few minutes. A red or brick red ppt. is formed.
- **Test for resins**

Dissolve the 1 ml of extract in 1 ml of acetone and pour the solution into 5 ml distilled water. Turbidity indicates the presence of resins.

- **Test for saponins**

In test tube containing about 5 ml of an aq. Extract of drug, add drops of sodium bicarbonate. Shake it vigorously and left for few minutes. Honey comb – like structure is formed.

- **Test for flavonoids**

In the test tube containing 0.5 ml of alcoholic extract of drug, add 5-10 drops of dil. HCl followed by small piece of 'Mg'. In the presence of flavonoids, pink, reddish pink or brown colour is produced.

- **Test for tannins**

To 1 – 2 ml of extract of drug, add few drops of 5% FeCl<sub>3</sub> solutions. A greenish colour indicates the presence of gallic tannins while brown colour tannins.

**\*High Performance Thin Layer Chromatography (HPTLC) fingerprint profile**

**Development of HPTLC Fingerprint:** High performance thin layer chromatography is automated form of TLC techniques. This method is used for separation of components present in the mixture. Both quantitatively as well as qualitatively. HPTLC of the Test solution of all sample were carried out on Silica Gel 60F254. Pre coated plates (0.2mm thickness, from Merck India Limited Mumbai). A TLC Applicator from Camag Linomat-5 (Camag Switzerland) was used for documentation unit (Camag reprostar -3) was used for documentation of chromatographic fingerprints.

**Preparation of Test solution:** Extract was prepared by taking 5g of sample powder and mixed it with 25 ml methanol and kept on warm water bath for 30 min and filtered and the filtrate was used for HPTLC.

**Procedure:** Applied 7 µL Each of the Test solution at 8 mm band and develop the plate in a solvent system **Toluene: ethylacetate: Formic acid** (7:0:3:0:0.5) to a distance of 9 cm dry the developed plate in air and examine under Ultraviolet light at (254 nm) and at 366 nm before derivatization.

**Derivatization:** Possibility of derivatization is a strong point of HPTLC. Chemical reactions are possible on a given plate before or after chromatography. Both possibilities have their advantages. However, the decision depends on the sample matrix, level of detection, and interference present. Post-chromatography derivatization is a more popular technique for which several hundred references in literature are available, as compared to a few pre-chromatographic derivatizations. The results are unique and specific when pre-chromatographic development has been recommended. Derivatize the plate using methanol reagent and heating at 105°C until the bands are clearly visible and examine the plate. The R<sub>f</sub> value and colour of the band resolved were recorded.

#### **\*preparation of HPTLC**

- **Test solution :-** concentrated solution of with methanol.
- **Stationary phase :-** pre-coated plates with silica gel 60F254 of 0.2mm thickness.
- 7. **Mobile phase :-** Toluene, Ethylacetate, Formic acid (7.0:3.0:0.5)
- 8. **Volume of test sample Applied :-** 7 µl
- 9. **Spray reagent :** 5% Methanolic H<sub>2</sub>SO<sub>4</sub>
- 10. **Distance travelled by solvent system :-** 8.9cm.
- 11. **Developed chamber :-** twin through chamber (10×10) cm.
- Results & Discussion

**The parameters which were analysed in the study are :-**

1. **Loss on drying (LOD)**
  - **Extractive value**
2. Water extractive value
3. Ethanol extractive value
4. Methanol extractive value
5. Benzene extractive value
6. extractive value.
- **Ash value**
7. Total ash
8. Acid insoluble
- **Phytochemicals**
9. Alkaloids
10. Flavonoids
11. Saponins
12. Tannins
13. Resin
14. Carbohydrates
15. Phenol
16. Test for cardiac Glycosides
17. Protein test .
- High Performance Thin Layer Chromatography (HPTLC) Fingerprint profile.

And the results are tabulated below from table number 1-8 table.

**1. Table No:- 1 Loss On Drying (LOD value of karanj leaves)**

Sr.No.	Weight of the petridish with sample	Weight of the petridish Sample 1 <sup>st</sup> reading	After ½ hours drying wt. (2 <sup>nd</sup> reading)	Difference
1.	31.8957	31.7942	31.7896	0.1061
2.	39.1819	39.0770	39.0704	0.1115

**1.1<sup>st</sup> Sample weight -2g**

- Average 1<sup>st</sup> weight difference = 0.1061
- LOD % =  $0.1061 \times 100/2$
- LOD% = 5.08%

**2.2<sup>nd</sup> time sample**

- Average 2<sup>nd</sup> weight difference = 0.1115
- LOD% =  $0.1115 \times 100/2$
- LOD% = 5.575%

**2. Water soluble extractive value of millettia pinnata (karanj):-**

Sr. No.	Weight of petridish (A)	Weight of petridish with sample (B)	Difference (A-B)
a)	37.9725	38.0157	0.0432
b)	38.0011	38.0412	0.0401

**Sample (a) Extractive value:-**

- Sample weight = 2 g
- Average weight difference = 0.0432
- Extractive value =  $0.0432 \times 500$
- Extractive value = 21.6%

**Sample (b) Extractive value :-**

- Sample weight = 2g
- Average weight difference = 0.0401
- Extractive value =  $0.0401 \times 500$
- Extractive value = 20.05%

**3:- (b) Ethanol soluble extractive value of Millettia pinnata (karanj)**

Sr. No.	Weight of petridish (A)	Weight of petridish with sample (B)	Difference (A-B)
1.	37.1807	37.1956	0.0149
2.	35.5245	35.5403	0.0158

- **1<sup>st</sup> sample weight = 2g**

Average weight difference - 0.0149

Ethanol soluble extractive value% =  $0.0149 \times 500$ 

Extractive value% = 7.45 %

- **2<sup>nd</sup> sample extractive value**

Sample weight = 2g

Average weight difference = 0.0158

Ethanol soluble extractive value =  $0.0158 \times 500$

Extractive value% = 7.9%

**4:- (c) Methanol soluble extractive value *Millettia pinnata* (karanj) :-**

Sr. No.	Weight of petridish (A)	Weight of petridish with sample (B)	Difference
1.	36.6192	36.6563	0.0371
2.	32.8537	32.8912	0.0375

**a) 1<sup>st</sup> sample of Extractive value of methanol :-**

Average weight difference = 0.0371

Methanol extractive value =  $0.0371 \times 500$

Extractive value = 18.55 %

**b) 2<sup>nd</sup> sample of Extractive value:-**

Average weight difference = 0.0375

Methanol extractive value =  $0.0375 \times 500$

Extractive value = 18.75%

**5:- (d) Benzene soluble extractive value of *millettia pinnata* (karanj) :-**

Sr. No.	Weight of petridish (A)	Weight of petridish with sample	Difference
1.	32.5550	32.5651	0.0101
2.	33.1388	33.1500	0.0112

**1<sup>st</sup> sample of Extractive value :-**

Average weight difference = .0101

Benzene extractive value =  $0.0101 \times 500$

Extractive value = 5.05%

**2<sup>nd</sup> sample of Extractive value :-**

- Average weight difference = 0.0112
- Benzene extractive value =  $0.0112 \times 500$
- Extractive value = 5.6 %

**6 :- (e) acetone soluble Extractive value of *millettia pinnata* (karanj) :-**

1.1<sup>st</sup>sample of Extractive value:-

- Average of Extractive value = 0.0148

Sr. No.	Weight of petridish (A)	Weight of petridish with sample (B)	Difference
1.	27.6522	27.6670	0.0148
2.	31.4078	31.4218	0.014

- Extractive value = 7.4%

**2. 2<sup>nd</sup> sample of extractive value:-**

Average of Extractive value = 0.014

Acetone extractive value =  $0.014 \times 500$

Extractive value = 7.6 %

**7:- (f) chloroform soluble extractive value millettia pinnata (karanj) :-**

Sr. No.	Weight of petridish (A)	Weight of petridish with sample (B)	Difference
1.	37.9760	37.9899	0.0139
2.	29.8952	29.9093	0.0141

- 1.1<sup>st</sup> sample of Extractive value:-

Average of extractive value = 0.0139

Chloroform extractive value =  $0.0139 \times 500$

Extractive value = 6.95%

2<sup>nd</sup> sample extractive value :-

Average of extractive value = .0.0141

Chloroform extractive value

=  $0.0141 \times 500$

Extractive value = 7.05 %

**8.a) Total Ash value of millettia pinnata (karanj) :-**

Sr. No.	Empty weight chinadish (a)	Empty weight chinadish+2g sample (b)	Weight after in			Difference
			1 <sup>st</sup> (c)	2 <sup>nd</sup> (d)	3 <sup>rd</sup> (e)	
1.	17.3142	2.0064	17.5189	17.5110	17.5106	0.1964
2.	18.3866	2.0030	18.5912	18.5036	18.5837	0.1971

- Ash value =  $\text{Difference} \times 100/2$

1<sup>st</sup> time =  $0.1964 \times 100/2$

= 9.82 %

- 2<sup>nd</sup> time Ash value

$0.1971 \times 100/2$

Ash value = 9.85 %

**8:- (b) Acid insoluble ash value of millettia pinnata (karanj)**

Sr. No.	1 <sup>st</sup> weight (A)	2 <sup>nd</sup> weight (B)	3 weight (C)	Difference (C-A)
1.	16.8473	16.8481	16.8485	0.0585
2.	18.4494	18.4486	18.4382	0.0516



Sr. No.	Photochemical	Test	Observation	Dist. Water	Methanol	Benzene	Acetone	Chloroform
1.	Carbohydrates	Fehling's test	First yellow then brick red precipitate occurs	+	+	+	+	+
		Benedict test	Appears green, yellow Or red.	+	+	+	+	+
2.	Alkaloids	Wagner's test	Yellow brown	+	+	+	+	+
		Dragendroff's test	Orange or reddish brown precipitate	+	+	+	+	+
		Hager test	Reddish brown precipitate indicates	+	+	+	+	+
3	Flavonoids	Shinoda test	Yellow coloration Occurs	+	+	+	+	+
4.	Saponins	Froth test	Resistance froth of 1-1.5 CM indicate of saponins	+	-	-	-	-
5	Tannins		Greenish colour indicate the presence of white brown colour for tannins.	+	+	+	+	+
6.	Resins	FeCl <sub>3</sub>	Blue colour	+	+	+	+	+
		Cons. HCl	Reddish pink	+	+	+	+	+
7.	Phenol	Folins	Brownish green indicates of phenolic compound.	+	Not detected	+	+	+
8.	Test for cardiac Glycosides	Killer killani test	Reddish brown colour at the two liquid layer and upper layer appear.	Not done	Not done	+	+	+
9.	Protein	Buirate test	Formation of red or violet colour indicates the presence of protein.	+	Not done	not done	not done	Not done

- **1st time**

- Sample weight = 2g

$$= \text{difference} \times 100/2$$

$$= 0.05850a \times 100/2$$

$$= 2.92 \%$$

- **2<sup>nd</sup> time**

$$= \text{Sample weight} = 2g$$

$$= \text{Diff.} \times 100/2$$

$$= 0.0516 \times 100/2$$

$$= 2.58 \%$$

## 9. Preliminary phytochemicals investigation :-

### Table :- above show the presence of:-

- Carbohydrates in all the sample extract of water, methanol, benzene, acetone, chloroform.
- Alkaloids in all the sample extract of water methanol, benzene, acetone, chloroform.
- Flavonoids were present in all five extract.
- Saponins were found present in water extract only.
- Tannins present were present in all five extract.
- Resins were found present in water, methanol, benzene, acetone.
- Phenolic compounds were present in the four extracts except in methanol extract.

- h) Cardiac Glycosides were present in three extract except in water and methanol.  
i) Protein were found present in only water.

## 10.FINGERPRINTING PROFILE OF MILLETIAPINNATA PLANT :

Table -10: Rf value of HPTLC fingerprinting profile on milletia pinnata,

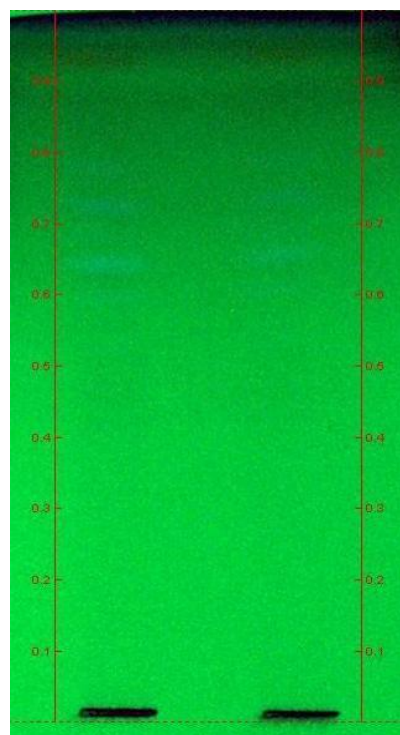
Sr. No.	Rf values	254 nm Before Derivatization	366 nm before Derivatization	366nm after Derivatization
1.	Rf1	0.09(blue)	0.2 (green)	0.2(black)
2.	Rf2	0.18(purple)	0.31(Sky blue)	0.42(green)
3.	Rf3	0.94(green)	0.42(Sky blue)	0.45(sky blue)
4.	Rf4		0.45(Sky blue)	0.53(green)
5.	Rf5		0.52(Sky blue)	0.62(green)
			0.6(Sky blue)	0.64(green)
			0.64(Sky blue)	0.73( green)
			0.7(Blue)	0.81(green)
			0.72(Sky blue)	0.93(red)
			0.79(Blue)	
			0.94(Red)	

Table -show spots at :-

(a):- At 254 nm ( before derivatization ) : fine spots were observed at 0.09(blue), 0.18(purple), 0.94(green).

(b):- At 366nm (before Derivatization) : 11 spot were observed at 0.2(green) , 0.31(sky blue), 0.42(sky blue), 0.45(sky blue), 0.52(sky blue), 0.6(sky blue), 0.64(sky blue), 0.7(blue), 0.42(sky blue), 0.79(blue), 0.94(red).

(c):- At 366nm (after Derivatization) : 9 spot were observed at 0.2(black), 0.42(green), 0.45(sky blue), 0.53(green), 0.62(green), 0.64(green), 0.73(green), 0.81(green), 0.93(red).

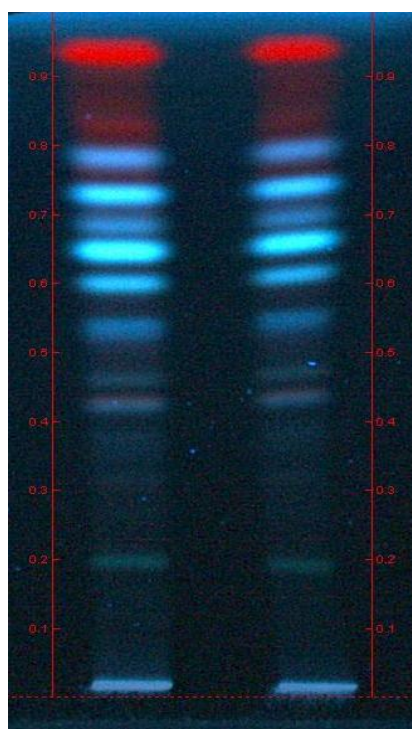


A

B

254nm

Fig-1



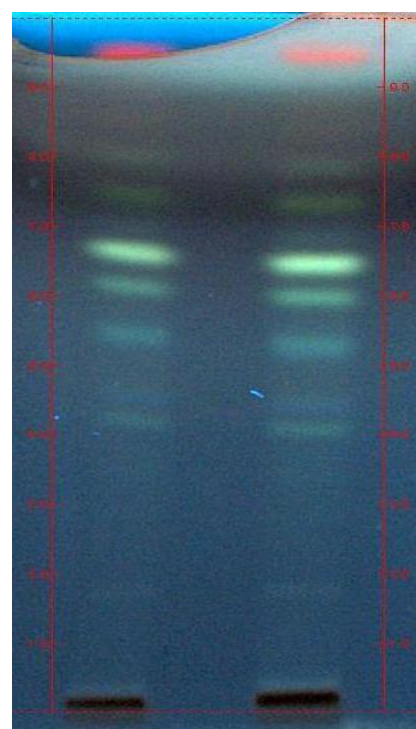
A

B

366 nm

Derivatization

Fig-2



366nm after

Fig -3

Where :-

Track; A:-Test solution of millettia pinnata.

Track;-B:- Test solution of millettia pinnata.

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## Conclusion

From the time immemorial, plants have been widely used as curative agents for variety of ailments. the leaves of *Millettia pinnata* were dried and Paralyzed in Pyrolysis reactor. The This analysis creates a platform to screen many bio active chemical constituents present in *Millettia pinnata* to treat various diseases . In the traditional systems of medicines, such as Ayurveda . the *millettia pinnata* plant is used for anti-inflammatory, anti-plasmodial, anti-nonceptive, anti-hyperglycaemic, anti-lipidperoxidative, anti-diarrhoeal, anti-ulcer, anti- hyperammonic and antioxidant activity. The Present Study reveals the presence of various Phytochemical. HPTLC fingerprint can be used as quality evaluation and Standardization of plant powered of *millettia pinnata* .it can be concluded that plant is a good source of antioxidants due to presence of phenolic and Flavonoid and their derivative. Phytochemical techniques and chemical profile, biological activity characterization may be helpful in development of specific drug of *millettia pinnata*.

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