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PHARMACOGNOSTIC AND PRILIMINERY PHYTOCHEMICAL INVESTIGATION ON LEAF EXTRACTS OF *Myristica dactyloides* Gaertn.

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ABSTRACT

Myristica dactyloides (Syn: *Myristica laurifolia* Hook.,) belongs to the family myristicaceae is indigenous medicinal plants and used very commonly in the management of various diseases like diabetes, bronchitis, constipation and in various skin ailments. Present work is related to standardization of *Myristica dactyloides* by using pharmacognostic (macroscopy, microscopy and physical constants) and phytochemical investigation on leaf of *Myristica laurifolia*. The leaves of *Myristica dactyloides* were extracted with different organic solvents in increasing order of polarity. The results of the preliminary investigation revealed the presence of alkaloids, steroids, flavonoids, terpenoids, glycosides & carbohydrates. The Methanol, Acetone and chloroform extracts were studied and the phytochemical compounds were isolated by using thin layer and column chromatography. The chemical structures of the isolated compounds were established by spectroscopic techniques such as UV, IR spectroscopy. This was again confirmed by co TLC with standard sample. The aim of the present study was focused on the pharmacognostical, physicochemical and phytochemical properties were carried out, which would like to facilitate quick identification and selection of the drug from various adulterates. All the parameters were studied according to the WHO and pharmacopoeia guidelines to standardize the *Myristica dactyloides*.

Key words: *Myristica dactyloides*, Pharmacognostic evaluation, Physico-chemical characterization, TLC, Spectroscopy.

INTRODUCTION

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularize in developing and developed countries owing to its natural origin and lesser side effects. In olden times, vaidyas used to treat patients on an individual basis, and prepared drugs according to the requirement of the patients. But the scene has been changed now; herbal medicines are being manufactured on a large scale in mechanical units, where manufacturers are facing many problems such as availability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of drugs and formulations, quality control parameters and etc., (Agrawal OP, 2000; Agrawal A, 2005; Ali MS *et al.*, 2005).

Myristica dactyloides belonging to Myristicaceae family is commonly known as “Kattu Jathi". It is indigenous in Africa, Indo Malaysian region and cultivated all over India. Flowers numerous, pendent, 7.5cm long, 3.8cm wide. At first they are white in colour then they become deep red (Kirtikar KR & Basu BD, 2006). The Indian traditional system of medicine, lays emphasis on promotion of health promoting, disease preventive and rejuvenation approach. The Indian

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traditional system of medicine, lays emphasis on promotion of health promotive, disease preventive and rejuvenation approach. Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases [Lien A *et al.,* 2008]. From the above literature, it is clear that no pharmacognostic work is carried out. The present study was therefore undertaken to investigate the pharmacognostic characters and phytochemical analysis of the plant.

MATERIALS AND METHODS

Plant Material Collection

The leaves of *Myristica dactyloides* were collected from Kolli hills in the month of November 2010. The plant material was identified and authenticated by Dr. P. Jayaraman, Plant Anatomy Research Centre, Pharmacognosy Institute, Chennai. The leaves were separated from other parts, washed, cleaned and dried for further use.

Reagents

All reagents and chemicals used for testing were analytical grade obtained from Ranbaxy Fine Chemicals Ltd., New Delhi and Loba Chemie, Mumbai, India.

Pharmacognostic studies

Macroscopy

Morphological studies were done by using simple microscope. The shape, apex, base, margin, taste and odor of leaves and flowers were determined (WHO, 1998). The macroscopical characters are shown in Table.1.

Microscopic Evaluation

The crude bark was subjected to microscopical evaluation. The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the section was 10-12 µm. Dewaxing of the section was done by customary procedure. The sections were stained with toluidine blue as per the method of O'Brien. Since toluidine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary sections were also stained with safranin, fast-green and Iodine (for starch) (Johansen DA, 1940; O'Brien TP *et al.,* 1940).

Determination of Physical Constants

(i) Ash Values

Different Ash values like total ash, water soluble ash and acid insoluble ash were evaluated (Indian Pharmacopoeia, 1985).The results are shown in table 2.

(ii) Extractive Values

Different extractive values like water-soluble and alcohol-soluble extractive values were evaluated (Indian Pharmacopoeia, 1985). The results are shown in table 2.

(iii) Foreign Organic Matter and Moisture Content

Foreign organic matter was determined from the weight of the drug taken. Moisture content is determined by Loss on drying method in terms of percent w/w.

(iv) Extraction

The coarse powder of the bark was used for extraction. Continuous hot extraction was carried out by using different solvents of increasing polarity [petroleum ether (60-80^{o} C), ethyl acetate, ethanol and chloroform] in Soxhlet extractor. Finally the marc was allowed for maceration about 24 hours with distilled water to obtain the aqueous extract (Harborne JB, 1998; Mukherjee PK, 2002). The results are shown in Table 3.

(v) Qualitative Phytochemical Screening

Freshly prepared bark extracts were tested for the presence of phytochemical constituents by using reported methods (Khandelwal KR, 2005; Kokate CK, 1994; Farnsworth NR, 1966). The results are shown in Table 4.

(vi) Thin layer chromatography

For the TLC fingerprint the petroleum ether extract and methanolic extract were subjected to thin layer chromatography analysis, to find the presence of a number of Chemical constituents to support the chemical test (Table 6). Analytical TLC plates were prepared by pouring the silica gel G slurry on the glass plates. Drying the thin layer plates, for 30 min in the air and then in an oven at 110°C for another 30 min. For qualitative work, a spot was applied in a row along one side of plate, about 2 cm from the edge, by using capillary tubes. The range of sample volume was controlled, spreading not more than 0.5 cm. The plate was placed in previously saturated TLC chamber with the mobile phase. The Rf values were compared to standard drug and colors were recorded. (Mendham J *et al.,* 2002; Wagner H, 1996).

RESULTS AND DISCUSSION

Table 1. Macroscopy of leaf

Parameters	Features
Color	Outer surface – brown, inner surface– whitish brown to buff
Odor	Characteristic
Taste	Acrid
Size	5 – 12 mm thick
Shape	Quadrangular, fluted

Fig 1. Leaf of *Myristica dactyloides*

Microscopy of leaf

The midrib is 1.33mm thick. The adaxial part is 700µm wide and the abaxial part is 102mm wide. The midribconsist of a ground tissue and two stranded vascular system. Epidermis is narrow with small, thick walled cells, the cuticle is thick and smooth. It is 30µm thick. The ground tissue is parenchymatous, the cells are circular or angular.

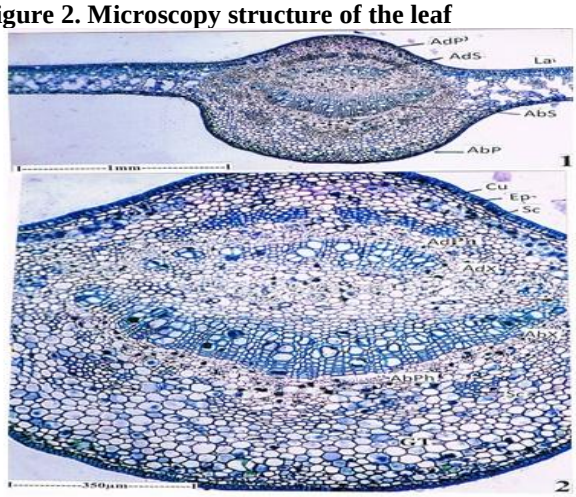
TS of Lamina

The lamina is smooth and even on the adaxial side and rough surface on the abaxial side. The adaxial epidermis is 15µm thick and the abaxial epidermis is more than 25 µm thick. The mesophyll tissue is differentiated into adaxial palisade, which consist of three compact layers of vertical oblong cells, the abaxial portion is the spongy mesophyll, there are 9 or 10 layers of large, loosely arranged cells with wide air chambers. The total thickness of the lamina is 260µm.

Stomata

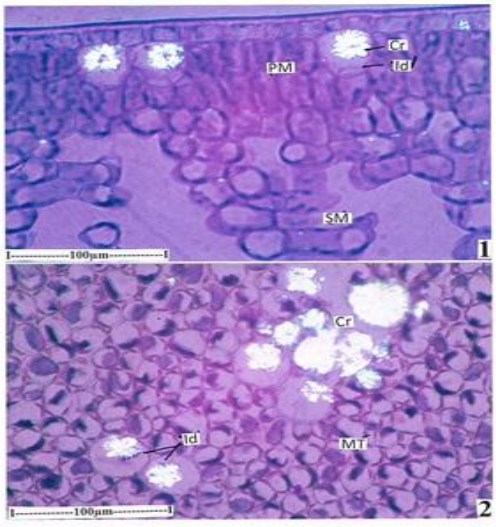
Stomata occasionally on the abaxial epidermis. The stomata are cyctocytic type having five or more subsidiary cells encircling the cells the epidermal cells are fairly large.

Figure 2. Microscopy structure of the leaf



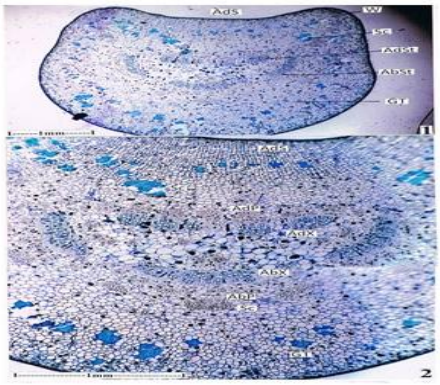
AbS	-	Abaxial strand
Abph	-	Abaxial Phloem
Abx	-	Abaxial Xylem
Adp	-	Adaxial part
Adph	-	Adaxial Phloem
Ads	-	Adaxial strand
Adx	-	Adaxial Xylem
Cu	-	Cuticle
Ep	-	Epeidermis
GT	-	Grant Issue
La	-	Lamina
Sc	-	Sclerenchyma

Figure 3. TS of Lamina



Cr	-	Crystal
Id	-	Idioblast
Mt	-	Mesophyll tissue
Sm	-	Spongy mesophyll
Pm	-	Palisade mesophyll

Figure 4. Abaxial surface



Abt	-	Abaxial phloem
Ab st	-	Abaxial strand
Abx	-	Abaxial xylem
Adp	-	Adaxial phloem
Ads	-	Adaxial side
Ad st	-	Adaxial strand
Adp	-	Adaxial phloem
Adx	-	Adaxial Xylem
Gt	-	Guard tissue
Sc	-	Sclerenchyma

Table 2. Data showing the Physico Chemical Standards of *Myristica dactyloides*

S.No	Total Ash % w/w	Water Soluble Ash % w/w	Acid Insoluble Ash % w/w	Sulphated Ash % w/w	Loss on Drying % w/w	Water soluble Extractive % w/w	Alcohol Soluble Extractive % w/w
1	8.82	5.12	3.52	5.12	8.02	18.92	13.59
2	8.92	4.81	2.51	5.56	7.56	17.56	15.63
3	8.51	4.88	3.32	4.96	8.36	18.27	14.58
4	9.22	5.16	2.95	5.32	7.23	16.98	15.42
5	8.58	5.19	2.29	5.25	7.92	18.59	14.92
Minimum	8.51	4.81	2.29	4.96	7.23	16.98	14.58
Average	9.00	4.89	2.946	4.588	7.14	16.68	14.74
Maximum	9.22	5.19	3.32	5.56	8.36	18.92	15.63

Table 3. Successive Solvent Extraction of leaves of *Myristica dactyloides*

S No	Solvents used	Colour & Consistency	Extractive values w/w
1	Petroleum Ether	Green Colour	0.925%
2	n – hexane	Green &sticky with oily mass	0.761%
3	Chloroform	Dark green	1. 539%
4	Acetone	Dark green sticky mass	0. 654%
5	Alcohol	Brownish green colour	0.829%
6	Aqueous	Brown colour	1.52%

Table 4. Data Showing the Preliminary Phytochemical Screening of *Myristica Dactyloides*

Constituents	Petroleum ether extract	n-hexane extract	Chloroform extract	Acetone extract	Alcohol extract	Aqueous extract
Alkaloids	+	-	+	+	+	+
Carbohydrates	-	+	-	+	-	-
Glycosides	+	+	+	+	+	+
Phytosterols	+	+	+	+	+	+
Volatile oils	+	+	+	+	+	+
Saponins	+	-	-	-	-	-
Phenolic Compounds & Tannins	-	-	+	+	-	+
Lignin	+	+	+	+	+	+
Protein & free Amino acid	-	-	-	-	-	-
Gums & Mucilage	+	-	+	-	-	-
Flavonoids	+	-	+	+	+	-

(+) - Presence (-) – absence

Table 5. Fluorescence Analysis of Leaves of *Myristica Dactyloides*

Reagents	Alcohol extract		Acetone extract		Aqueous extract	
	Day Light	UV Light	Day Light	UV Light	Day Light	UV Light
1N NaOH (Aqueous)	Green	Light green	Brown	Light green	Green	Fluorescent green
1 N HCl	Green	Fluorescent green	Green	Pale green	Green	Yellowish green
50% H_2SO_4	Green	Fluorescent green	Green	Fluorescent green	Green	Light green
50% HNO_3	Brown	Light green	Green	Light green	Brown	Fluorescent green
Methanol	Green	Yellowish green	Green	Fluorescent green	Green	Pale green
Sample + NH_3 Solution	Green	Light green	Brown	Pale green	Green	Fluorescent green
Sample + Iodine	Brown	Light green	Green	Fluorescent green	Yellowish green	Fluorescent green
Sample + $FeCl_3$	Green	Fluorescent green	Brown	Pale green	Light green	Yellowish Green

Table 6. Data Showing the Thin Layer Chromatography of Chloroform Extracts of *Myristica dactyloides*

Parameters	Stationary phase	Mobile phase	No of Spot	R_f value
Alkaloids	Silica gel G	Benzene: ethanol (9:1)	5	0.4, 0.66, 0.73, 0.83, 0.86
Glycosides		Ethyl acetate: n-butanol: water (4:4:3)	5	0.26, 0.46, 0.6, 0.73, 0.86
Flavonoids		Petroleum ether: ethyl Acetate (2:1)	4	0.13, 0.2, 0.33, 0.26
Steroids		Chloroform: ethanol (96:4)	7	0.13, 0.2, 0.33, 0.4, 0.46, 0.53, 0.6
Essential oils		Pure chloroform	2	0.66, 0.4

Qualitative Phytochemical Screening

The qualitative chemical tests of the petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts revealed the presence of alkaloids, tannins, saponin, flavonoid, amino acid, flavonoids, steroids, glycosides and carbohydrates.

Fluorescence analysis of extracts

All the leaf extracts are examined in daylight, short and long UV to detect the fluorescent compounds by the reported method.

Figure 5. Thin Layer Chromatography of Chloroform Extracts of *Myristica dactyloides*

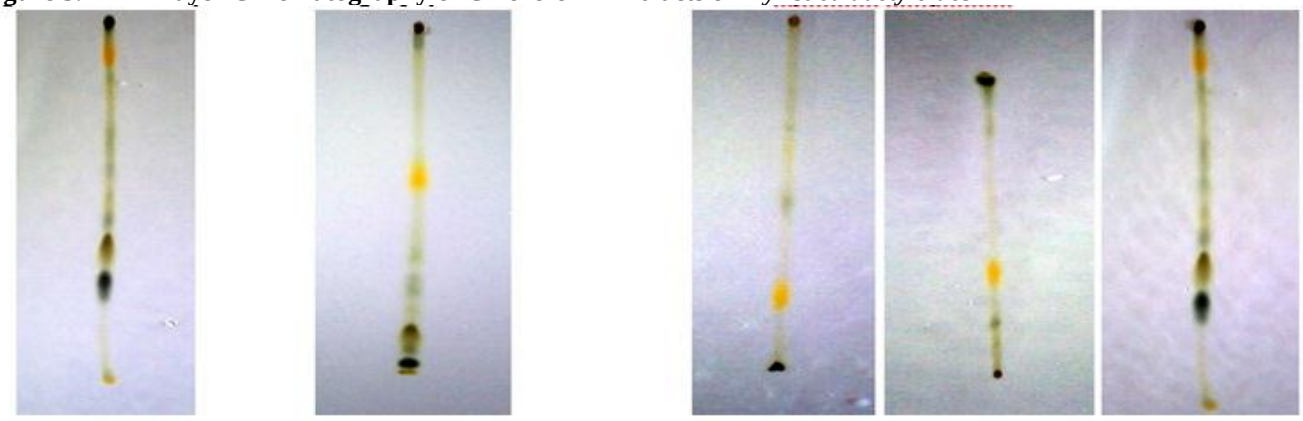


Figure 6. UV Spectrum of MDC1

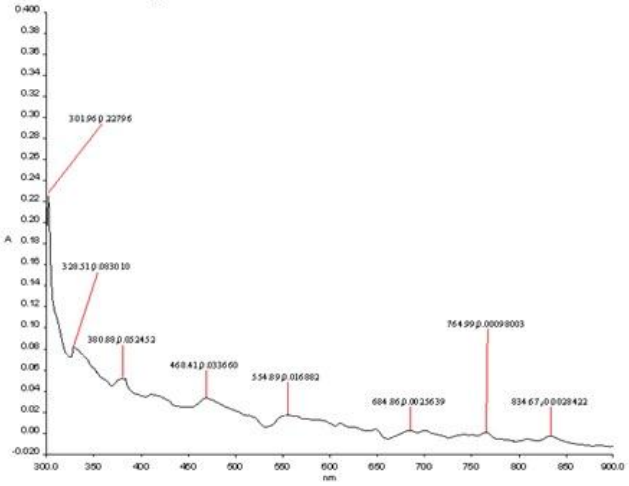
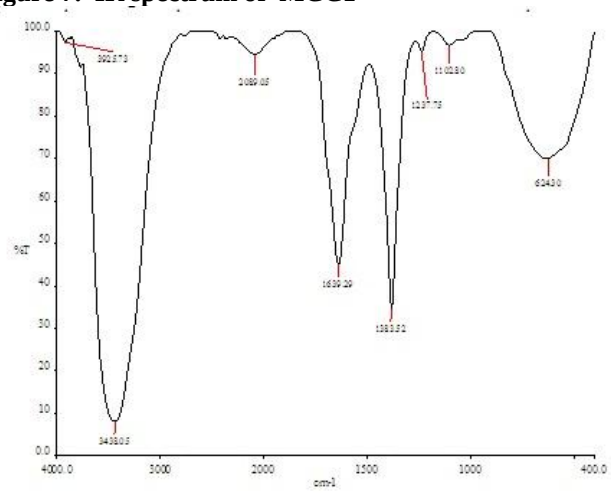


Figure 7. IR spectrum of MCC1



CONCLUSION

Since the plan *Myristica dactyloides* which is also used for the treatment of various diseases and disorders, it is important to standardize it for use as a drug. The pharmacognostic constants for the leaves of this plant, the diagnostic microscopic features and the numerical standards reported in this work could be useful for the compilation of a suitable monograph for its proper identification. The chemical basis for the wide use of this plant as therapeutic agents for treating various ailments. However, there is need to carry out further advanced spectroscopic studies in order to elucidate the structure of these compounds. Further work therefore needs to be carried out on the fractions in order to isolate, purify and characterize the active chemical compounds which could be subjected to further toxicological analysis.

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