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Research Article

PHARMACOGNOSTICAL STUDIES ON *MORINDA TINCTORIA.* ROXB.

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ABSTRACT

The present study deals with the pharmacognostical studies on *Morinda tinctoria*.(Roxb). The leaves of *M.tinctoria* were shade dried and mechanically powdered after keeping them in an oven at 35ºC for 24 hours. These powdered materials were used for further physiochemical, phytochemical and fluorescent analysis. The leaves contains total ash value is 31%, acid insoluble ash and acid soluble ash values are 29% and 71% respectively. Solubility percentages of alcohol were higher than water. Preliminary Phytochemical tests in the leaves of *M.tinctoria* reveals the presence of alkaloids, tannin and phenol, and flavonoids in aqueous and methanolic extracts. The phytochemical studies namely quantitative analysis proved to be worthy, due to the fact that it showed the presence of primary and secondary metabolites like alkaloid, carbohydrate, protein, phenol, tannin, flavonoids, gums and mucilages. Tannin content of leaves of *M. tinctoria* in our study is 6%. Fresh leaves containing amino acids were screened through the paper chromatography, which consists of Glycine, Proline, Valine and Phenylalanine. These pharmacognostic characters can be used as a diagnostic tool for the authentic identification of *Morinda tinctoria,* which will be very useful in designing the monograph on the drug in the Indian Pharmacopoeia.

Keywords: Pharmacognostical, Anatomical, Phytochemical studies, *Morinda tinctoria*.

INTRODUCTION

The Indian region is well known for its native medicine. More than 80% of the developing world continues to rely on traditional medicines predominantly plants, for its primary health care (Brindha and Saraswathy, 2000). The market for Ayurvedic medicines is estimated to be expanding at 20% annually in India. The compound of herbal medicine component possess some 1200 plant species of therapeutic value used in the traditional medicine by native population and constitute an important resource with tremendous future prospects. Traditionally, plants have served as most important weapon against diseases or ailments of human beings. All sections of the society whether medicine or as folk remedies, widely use medicinal plants. Indian subcontinent is endowed with high floral diversity, of which majority of the plant species have medicinal properties. Man has searched out in past many plant species useful as drugs for combating various diseases and still engaged heavily because of newer and newer diseases and epidermis. Changing patterns of human –life style are also largely responsible for this scenario. In recent years, there has been an increasing awareness about medicinal plants. This is because they are easily available. Less expensive and have least side effects. Thus correct identification and authentication based on accepted scientific criteria are of almost importance in any study on medicinal plants (Chopra *et al.,* 1956). Pharmacognosy is one method for correctly determining the botanical identity of the sample oven in dried or in powdered condition (Karthikeyan, 2011 and Surendrakumar, 2011).

Morinda tinctoria commonly known as Aal or Indian Mulberry is a species of flowering plant in the family Rubiaceae native to Southern Asia commonly found in Srilanka and India. It is an evergreen shrub or small tree growing to 5-10 m tall. The leaves are 15-25 cm long, oblong to lanceolate. Leaves 8-15 [20]×3-5 [8]cm. All parts of *M. tinctoria* has medicinal properties. Leaves are useful as tonic, febrifuge, deostruent and emmenagogue. It is also used for curing dyspepsia, diarrhoea, ulceration, stomatitis, digestion, wound and fever. The leaf juice is useful as a local application. Root is used to cure inflammation and boils. Unripe fruit is used to cure rheumatism. Ash of the fruit prevents dysentery, vomiting, diarrhoea and cholera. Kanchanapoom (2001). Thus the present investigation was carried out to study the Macroscopical, Microscopical, Physicochemical characters, Qualitative and quantitative studies on the phytochemicals of *Morinda tinctoria*. These Pharmacognostical parameters will enable the future investigators for identification of the plant.

MATERIALS AND METHODS

Collection of plant materials

The plant parts of *Morinda tinctoria* were collected from Thanjavur, Tamilnadu.

Anatomical studies

Free hand sections were used for the anatomical studies. These section were mounted in glycerin stained with safranin. Drawing were made with the use of prism type camera lucida and microphotographs were also taken. For quantitative microscopical characters, stomatal index of upper and lower epidermis, Vein islet number, Vein-termination number and palisade ratio were calculated according to the procedures described by Trease and Evans (1983).

Maceration Techniques

Jaffrey's maceration method was adopted small pieces of specimens (1mm thickness) were kept in a solution of equal part of 10% chromic acid and nitric acid for over night or until the materials become "mushy" in texture. After maceration the tissues were washed in water and stained with safranin. The macerated and stained tissues were spread on slides and mounted in glycerine for observation.

Analysis of powder of *M. tinctoria*

The leaves of *M.tinctoria* were shade dried and mechanically powdered after keeping them in an oven at 35ºc for 24 hours.These powdered materials were used for further physiochemical, phytochemical and fluorescent analysis. The analytical procedures recommended in Indian Pharmacopoeia (Anonymous, 1965) were followed for calculating the total ash, acid-in soluble ash and loss on drying at 110ºc. Fluorescent analysis was carried out by using the method of Chase and Pratt(1949). Behaviour of powdered treated with different chemical reagents was treated with carried out as mentioned by Kokoshi *et al*.,(1958)

Qualitative phytochemical studies

Alkaloids (Roberts and James 1947), carbohydrates, tannins and phenols (Aparna Bazarbaruna, 2000), flavonoids, gums and mucilages, phytosterol, proteins and amino acids, fixed oils and fats, volatile oil and saponins were qualitatively analysed. Qualitative phytochemical analysis was done by using the procedures of Kokate (1994). In quantitative analysis, Alkaloids, Tannins and Phenols, Free amino acids Flavonoids were determined. Determination of

Amino acids by Chromatograpic method using a method of Jayaraman,(1981). The biochemical parameters like Total phenols (Bray and Thorpe, 1954), Total alkaloids, Kokate (1994), β-sitosterol and flavanoids (Aravindakshan nair and Ramiah.,1982), were quantitatively estimated.

RESULTS AND DISCUSSION

Organoleptic characters of root

The roots are slender and cylindrical in shape 2 to 6mm in diameter. Fresh roots are yellowish brown in colour and become brown when dry. Outer surface of the root has transverse cracks and longitudinal wrinkles. Inner region of the bark is whitish in colour. Powdered roots are brown in colour. Taste is sweets, odour is strong, distinct and pleasant.

Quantitatively microscopical studies on the leaves of *M. tinctoria*

Surface view of the epidermal opening of the adaxial surface reveals epidermal cells with wavy margins devoid of stoma. In the abaxial surface, the epidermal cells are wavy in margin, paracytic type of stomata is present. Stomatal index number is 18-20-22 [Fig-10]. Stomatal frequency is 23-24/mm2.Vein islet number and Vein termination numbers are 1-2 and 2-3 respectively. The Palisade ratio of the leaves are 12-14/mm^2.[Fig-11].

Phytochemical analysis of *M. tinctoria*

Table -1 shows the quantitative values of the leaves of *M.tinctoria are* presented in Total ash value is 31%, acid insoluble ash and acid soluble ash values are 29% and 71% respectively. The solubility percentages of alcohol is higher than water. Table -2 shows the quantitative analysis, Table -3 shows the fluorescent characters and behaviour of the powdered leaves treated with different chemicals. Table- 4 shows Preliminary Phytochemical tests of leaves of *M.tinctoria.* Presence of alkaloids, tannin and phenol, and flavonoids in aqueous and methanolic extracts of *M. tinctoria*. Study of aqueous extracts shows the presence of saponins, gums and mucilages. Total tannin and phenol content of the leaves of *M.tinctoria* is 0.9mg and 6mg respectively. The amount of alkaloid,Flavanoid and β-sitosterols are 11mg,1.3mg and 0.5mg respectively[Table-5]

Composition of Amino acid

Fresh leaves containing aminoacids were screened through the paper chromatography, which consists of Glycine, Proline, Valine and Phynylalanine.

Table 1: Quantitative ash values of *Morinda tinctoria.*

Sl. No	Parameters	Percentage values
		Plant
1.	Total ash value	31%
2.	Acid insoluble ash value	29%
3.	Acid soluble value	71%
4.	Solubility % in alcohol	47%
5.	Solubility % in water	41%

All the values are the averages of five observatios.

Table 2: Qualitative analysis of *Morinda tinctoria*

Treatment	Visible light		UV light	
	Water extract	Alcohol extract	Water extract	Alcohol extract
Powder+1N Hcl	Brownish yellow	Black	Red	Green
Powder + as such	Brown	Green	Brown	Black
Powder + conHNO3	Black	Yellowish brown	Green	Black
Powder+conc.H2SO4	Brownish yellow	Black	Brown	Black
Powder+ Acetic acid	Brown	Yellowish green	Green	Black
Powder+ 10% NAOH	Brown	Yellowish green	Red	Light green
Powder+ 1N HCL	Brownish yellow	Black	Red	Green
Powder + Iodine solution	Yellowish brown	Brownish black	Brown	Black
Powder+ Ferric chloride solution	Black	Black	Brown	Green

Table 3: **Fluorescent analysis of powdered leaves of *Morinda tinctoria***

Treatment	Visible light		UV light	
	Water extract	Alcohol extract	Water extract	Alcohol extract
Powder+1N Hcl	Brownish yellow	Greenish black	Red	Green
Powder +as such	Brown	Green	Brown	Black
Powder+1N HNO3	Black	Yellowish brown	Green	Black
Powder+1N NAOH in water	Brown	Yellowish brown	Dark brown	Black
Powder + 1N NAOH in alcohol	Yellowish brown	Brown	Red	Dark black

Table 4: Qualitative analysis of *Morinda tinctoria* in leaf materials + -Present

S. No	Name of the compounds	Name of the test	Status of the substance	
			Aqueous	methanol
1.		a. Fehling's	-	-
	Carbohydrates	b. Molisch's	-	+
2.	Alkaloids	a. Mayer's	-	-
		b. Hager's	-	-
		c. Wagner's	+	+
		d. Dragen Dorfff's	-	-
3.	Steroids	Chloroform + acetic acid +. H_2SO_4	-	-
4.		a. 10% Lead acetate	+	-
	Tannin &Phenols	b. 5% Ferric Chloride	-	+
		c. 1% gelatin	+	+
		d. 10% Sodium chloride	+	-
5.	Saponins	Foam test	+	-
6.	Fixed oils & Fats	Spot test	-	-
7.	Gums & Mucillage	Alcoholic precipitation	+	-
8.	Proteins	Biuret test	+	+
9.	Flavonoids	OH / HCL	+	+
10.	Volatile oils	Hydro distillation method	-	-

Table 5: Quantitative phytochemical analysis of leaves in Morinda tinctoria

Sl. No	Parameters	Percentage values mg/g
		Plant
1.	phenol	0.9±0.012
2.	Alkaloid	11±0.034
3.	Tannin	6±0.014
4.	B- sitosterols	0.5±0.098
5.	Flavonoids	1.3±0.23.

All the values are the averages of five observations. (Mean ± SD)

DISCUSSION

The anatomical features of interest is that needle shaped crystals are seen in the cells of petiole, leaf and stem. This can be used as a diagnostic characters and this observation is identical to that of earlier reports of Venkataraman and Gopalakrishnan (1996), in *Borreria articularis*. Metcalf and Chalk(1957) observed raphides in Morinda, we also observed distribution group of needle shaped calcium oxalate crystals in the stem, leaf, root and petiole of *M.tinctoria.*

Present work as well as earlier reports of Dhanalakshmi and Roseline (2000) reveals the lower epidermis of leaves contains paracytic stomata. Generally most of the members of Rubiaceae has paracytic stomata only restricted on the lower epidermis. Sclerids are absent in powdered leaves of the *M. tinctoria* (Dhanalakshmi and Roselin 2002).This character is also observed in the present study. Presence of accessory vascular bundle in the petiole is a distinct character of rubiaceae, but there is no accessory vascular bundle was observed in the sample collected. These differences might be due to the observation on different specimens collected from different geographical location.

There is no mechanical tissue (sclerids) was observed in the root, stem and leaves of the materials studies. This observation proves that the plant may be mesophytic in nature but due to the presence of 2 to 3 layered palisade cells in the leaves indicates that this plant is semi-xerophytic in nature. Leaves of *Asclepias syriaca* when grown in xerophytic condition develop a second layer of palisade cells (Metcalfe and Chalk,1957).Anatomically, presence of unicellular, unbranched, trichomes on leaves and petioles, collenchymatous hypodermis. Needle shaped crystals in the petiole and leaves are the distinguish characters of *M. tinctoria*. The phytochemical studies namely quantitative analysis proved to be worthy, due to the fact that it showed the presence of primary and secondary metabolities like alkaloid, carbohydrate, protein, phenol, tannin, flavonoids, gums and mucilages.

These pharmacognostic characters can be used as a diagnostic tool for the correct identification of *Morinda tinctoria*, which will be very useful in designing the monograph on the drug in the Indian Pharmacopoeia.

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