International Journal of Pharmacy and Pharmaceutical Sciences
ISSN- 0975-1491 Vol 2, Issue 4, 2010

Research Article

PRELIMINARY PHYSICOCHEMICAL AND PHYTOCHEMICAL EVALUATION OF *MORINDA CITRIFOLIA* FRUIT EXTRACTIVES

SMITA NAYAK* AND SUSHMA MENGI**

***Gahlot Institute of Pharmacy, Plot no. 59, Sector 14, Koparkhairane, Navi Mumbai – 400709, Maharashtra, India, **C.U.Shah College of Pharmacy, S.N.D.T. Women's University, Juhu-Tara Road, Santacruz (West), Mumbai – 400 049. Maharashtra, India,**
E-mail: smitanayak12@yahoo.co.in

Received: 18 May 2010, Revised and Accepted: 15 Jun 2010

ABSTRACT

Morinda citrifolia Linn (Rubiaceae) (noni) is a small evergreen tree growing in coastal and forest regions. In traditional plant based medicine, the fruits have been used for diverse medicinal purposes. Detailed physicochemical evaluation of the plant was carried out to observe its microscopic characteristics and to determine ash values and extractive values. Phytochemical screening by qualitative chemical methods was also carried out. Additionally, fractionation methodologies were developed and standardized for isolation of components rich in polysaccharides, anthraquinones and alkaloids. Our TLC studies confirmed the presence of scopoletin, an important marker in the identification of Morinda fruits. This study can serve as a guideline for laying down specifications for dried Morinda fruits as well as fruit powder so as to prevent the adulteration of the raw material.

Keywords: *Morinda citrifolia* Linn, polysaccharides, anthraquinones, alkaloids, scopoletin.

INTRODUCTION

The plant *Morinda citrifolia* Linn commonly known as noni belongs to the Rubiaceae family. Reports of the medicinal properties of this plant have been found in ancient literature as well as in traditional folklore[1,2,3]. The flowers, leaves, fruits, barks and roots of the plant have been reported to have antibacterial, anti-viral, anti-fungal, anti-tumor, anthelmintic, analgesic, hypotensive, anti-inflammatory and immune-enhancing effects[4,5]. An exhaustive survey of the reported uses of *Morinda citrifolia* revealed that the fruit is most valuable in numerous applications such as poultice on wounds, boils, pimples; as a purgative; as a blood purifier; in the treatment of tuberculosis, diabetes, heart ailments and high blood pressure. In addition, its immunostimulant potential has led to its use in the prevention of AIDS, prevention of Epstein-Barr virus activation and as an anti-cancer agent[6]. The present investigation was carried out to study the physicochemical and phytochemical characteristics of *M. citrifolia* fruits. Additionally, chromatographic fingerprints of the extracts were also obtained using suitable solvent systems.

MATERIALS AND METHODS

Plant Material

The fruits of *Morinda citrifolia* were obtained from M/s Anju Phytochemicals Pvt. Limited (Bangalore, India) and authenticated at Nicholas Piramal Research Center, Mumbai. A voucher specimen (No. 4944) was deposited in the herbarium of the institute. The crude drug was evaluated for physicochemical characteristics as per Indian Herbal Pharmacopoeia[7]. Macroscopic characteristics of the fresh fruits were noted (Figure 1). Powder (60 #) of the dried fruits of *Morinda citrifolia* was treated with phloroglucinol-HCl solution, glycerin and iodine solution to determine the presence of lignified cells, calcium oxalate crystals and starch grains. Total ash, water-soluble ash and acid-insoluble ash were determined. Alcohol and water-soluble extractive values were determined to estimate the amount of water and alcohol soluble components[8].

Extraction methodologies

Aqueous extract

The dried powder (50 g) was refluxed with 200 ml of distilled water for 12 hr. The extract was filtered, cooled and evaporated to dryness under reduced pressure in a rotary evaporator. The yield of the aqueous extract was 23.7% w/w of the dried powder.

Hydroalcoholic extract

Morinda citrifolia fruit powder was continuously extracted in a Soxhlet apparatus with 300 ml of 50% v/v ethanol till extraction was complete. The extract was filtered, cooled and evaporated to dryness under reduced pressure in a rotary evaporator. The yield of the hydroalcoholic extract was 25.4% w/w of the dried powder.

Polysaccharide enriched fraction (F I)

Morinda citrifolia fruit powder (50 g) was defatted using methanol (300 ml) and refluxed with distilled water (300 ml) for 12 hr. The aqueous extract was filtered and concentrated to 100 ml. The polysaccharides were precipitated by pouring the concentrate into 500 ml of acetone. The crude polysaccharide (1 g) was then dissolved in 50 ml of water. To this solution 25 ml of 12% w/v aqueous trichloroacetic acid was added. The protein impurities which precipitated out were filtered off. The residue was poured into 500 ml acetone to precipitate pure polysaccharides. The precipitate was filtered off under vacuum and air-dried[9]. From the dried powder, 6.24 % w/w of F I was obtained.

Anthraquinone enriched fraction (F II)

The powdered dried fruits (50 g) were refluxed with a mixture of 100 ml methanol and 150 ml of water for 3 hr. This extract was then acidified with 2 ml of concentrated hydrochloric acid and 5 ml of 5% methanolic solution of ferric chloride and refluxed for 6 hr. The anthraquinones were extracted in chloroform using a separating funnel. The chloroform extract was filtered and evaporated to dryness[10]. The yield of F II was 0.47% w/w of the fruit powder.

Alkaloid enriched fraction (F III)

The powdered dried material (25 g) was refluxed with 100 ml of a mixture of ethanol-chloroform (1:3) containing 2 % v/v of strong solution of ammonia for 6 hr. The extract was further extracted with three 20 ml portions of 2 N HCl. The combined acid extracts were made alkaline with strong ammonia solution and extracted with two 50 ml portions of chloroform. Each chloroform portion was washed with 20 ml of water. The combined chloroform extract was then evaporated to dryness[11]. The yield of F III was 0.12% w/w of the fruit powder.

Preliminary Phytochemical Studies

The dried powdered material was subjected to phytochemical evaluation for identification of various phytoconstituents[12].

HPTLC fingerprinting

Suitable solvent systems were developed for both the aqueous and the hydroalcoholic extract. The extracts were spotted on HPTLC silica precoated plates 60F (E Merck) and the plates were developed using suitable solvent systems. The developed plates were dried and

scanned on a Camag HPTLC scanner at 254 nm and 366 nm. These plates were sprayed with suitable spray reagents for detection of other functional groups. A suitable solvent system was developed for the identification of scopoletin, a reported marker in the fruits of *Morinda citrifolia*[13].

RESULTS AND DISCUSSION

Microscopic evaluation of the powder showed the presence of single acicular calcium oxalate crystals (average length = 0.04 mm), lignified cells, starch cells and oil globules. The ethanol soluble and water-soluble extractive values were found to be 13.4 % and 19.0 % w/w respectively. The total ash, water soluble and acid insoluble ash values were observed to be 8.5 %, 4.89 % and 0.019 % w/w respectively. The moisture content of the powder estimated as percentage loss on drying (LOD) was found to be 5.9 % w/w.

Both the aqueous and the hydroalcoholic extracts were dark brown in color and semi-solid in consistency.

Phytochemical investigations revealed the presence of citric acid in both the extracts. This citric acid was responsible for the acidic nature of the extracts. The presence of polysaccharides, anthraquinone glycosides, amino acids, coumarin glycosides, essential oils, tannins, phenolic compounds, alkaloids and citric acid as phytoconstituents in the aqueous extract and the presence of phytoconstituents such as polysaccharides, amino acids, proteins, fats and oils, anthraquinone glycosides, flavanoids, coumarin glycosides, tannins, alkaloids, phenolic compounds and citric acid in the hydroalcoholic extract was confirmed by employing phytochemical tests.

Morinda citrifolia **branch bearing a fruit and a flowering body**

Morinda citrifolia **fruits (whole and section)**

Fig. 1: It shows macroscopic characteristics of *Morinda citrifolia* fruits

The transverse section of fresh *Morinda citrifolia* fruits showed a single layered epidermis and mucilaginous hypodermis region containing oil glands (Figure 2). The mesocarp was identified by the presence of vascular bundles.

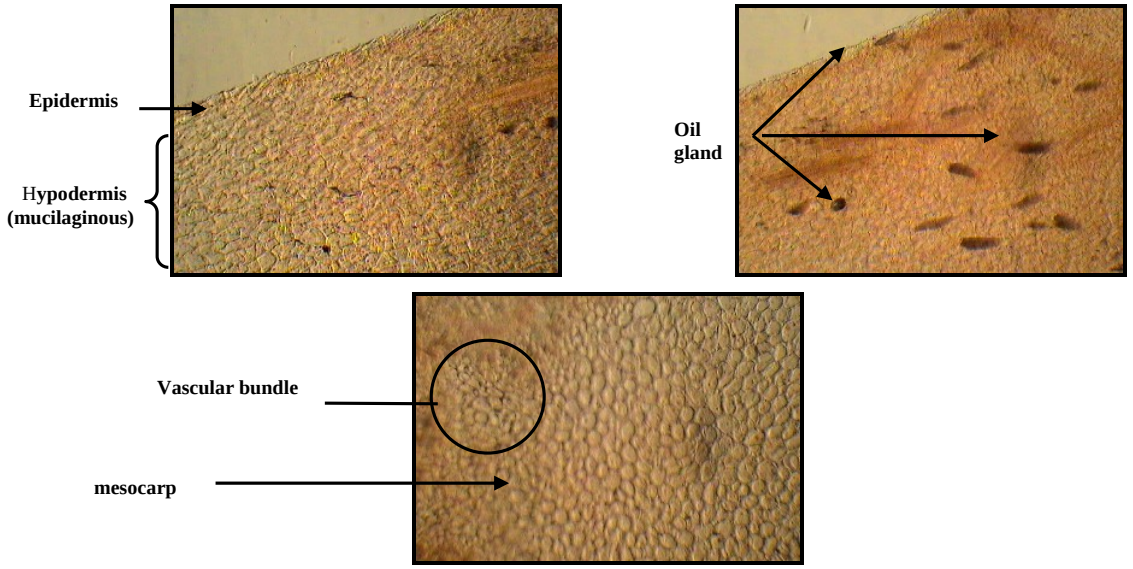

Fig. 2: It shows Transverse sections of fresh *Morinda citrifolia* fruits

TLC profile and HPTLC fingerprint of the aqueous extract developed using propanol: water (5.5:0.5) as a solvent system revealed the presence of nine compounds (Figure 3 and 4). At UV 254 nm, the aqueous extract depicted quenching zones, which may be due to the presence of anthraquinones, alkaloids and polysaccharides.

A blue, green, red and brown coloration obtained on spraying the plate with Aniline Sulphuric Acid reagent (A.S.R.) indicated the presence of essential oils. The plates sprayed with 10% ethanolic KOH showed blue fluorescence at 366 nm indicating the presence of coumarins.

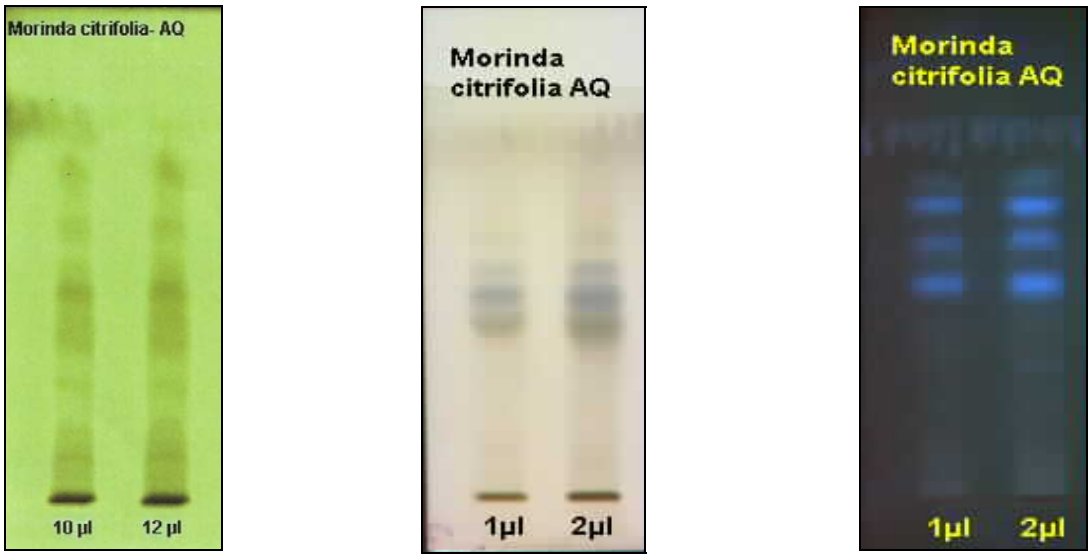

IMAGE 254nm **IMAGE VIS. (A.S.R)** **IMAGE 366nm (10 %KOH)**

Fig. 3: It shows TLC profile of aqueous (AQ) extract of dried fruits of *Morinda citrifolia*

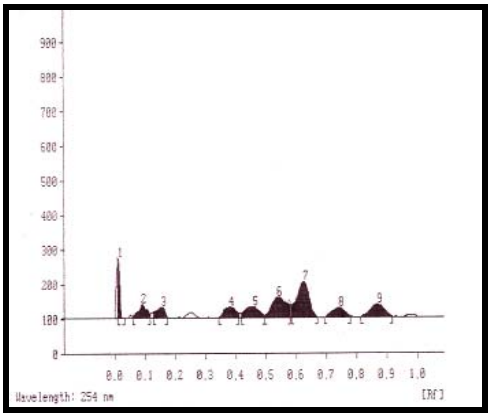

Fig. 4: It shows HPTLC fingerprint of aqueous extract of dried fruits of *Morinda citrifolia*

Table 1: It shows Rf values of the different components of aqueous extract of dried *Morinda citrifolia* fruits

Rf	Peak area
0.01	631.0
0.09	693.4
0.16	553.7
0.38	733.4
0.46	943.2
0.54	1802.0
0.63	2636.6
0.74	748.9
0.87	1106.6

HPTLC fingerprint of the hydroalcoholic extract developed using n-butanol: glacial acetic acid: water (5:1:2) as a solvent system revealed the presence of five compounds (Figure 5 and 6). When scanned at UV 254 nm, the hydroalcoholic extract depicted quenching zones, which may be due to the presence of anthraquinones, alkaloids and polysaccharides. A blue, green, red and brown coloration obtained on spraying the plate with Aniline Sulphuric Acid reagent (A.S.R.) indicated the presence of essential oils. The plates sprayed with 10% ethanolic KOH showed blue fluorescence at 366 nm indicating the presence of coumarins.

Table 2: It shows the Rf values of the different components of hydroalcoholic extract of dried *Morinda citrifolia* fruits

Rf	Peak area
0.01	2457.7
0.14	459.8
0.19	870.8
0.51	2392.5
0.66	1533.1

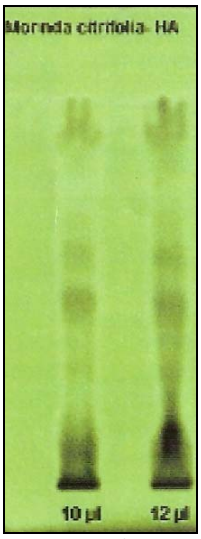

IMAGE 254nm

Fig. 5: It shows TLC profile of hydroalcoholic (HA) extract of dried fruits of *Morinda citrifolia.*

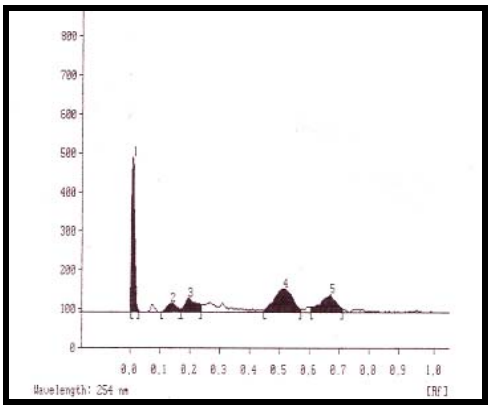

Fig. 6: It shows HPTLC fingerprint of hydroalcoholic extract of dried fruits of *Morinda citrifolia.*

Both the hydroalcoholic and aqueous extracts were extracted with a mixture of water: butanol: methanol: dichloromethane (1:3:5:5). The fingerprint was developed using ethyl acetate: methanol: water (5:0.3:0.2) as a solvent system and scanned at 366 nm. Blue fluorescent band having Rf value of 0.86 and 0.87 was seen in the chromatograms of the two extracts revealing the presence of scopoletin in both the extracts (Figure 7).

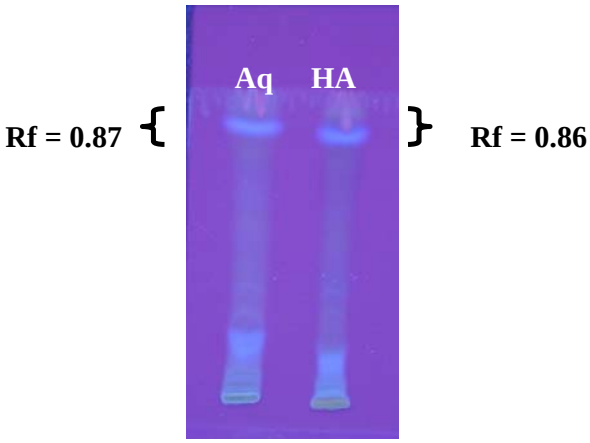

Fig. 7: It shows Scopoletin bands in the aqueous (Aq) and hydroalcoholic (HA) extracts of *Morinda citrifolia* fruits

CONCLUSION

Evaluation of macroscopic, microscopic and physicochemical characteristics of *Morinda citrifolia* fruits was carried out. The results and observations serve as a parameter for establishing the identity of the fruits and can serve as routine quality control tests for monitoring the quality of the fruits as well as fruit powder.

ACKNOWLEDGEMENT

The authors are thankful to the All India Institute of Technical Education (AICTE), India for funding this project.

REFERENCES

1. DerMarderosian A. Guide to Popular Natural Products: Facts and Comparisons, 1st ed. West Port Plaza, A Wolters Kluwer Company; 1999. p. 160.
2. Dittmar A. *Morinda citrifolia* L: Use in Indigenous Samoan Medicine. J Herbs Med Plants 1993; 1(3):77-92.
3. Anonymous. Medicinal plants of India vol.2. New Delhi, Indian Council of Medical Research; 1987. p. 270-72.
4. Wang M, West B, Jensen C, Nowicki, D, Su C, Palu A, Anderson G. *Morinda citrifolia* (Noni): A literature review and recent advances in Noni Research. Acta Pharmacol Sin 2002; 23(12): 1127-41.
5. McClatchey W. From Polynesian Healers to Health Food Stores: Changing Perspectives of *Morinda citrifolia* (Rubiaceae). Integrative Cancer Ther 2002; (2): 110-20.
6. Hirazumi A, Furusawa E. An immunomodulatory polysaccharide-rich substance from the fruit juice of *Morinda citrifolia* (noni) with antitumor activity. Phytother Res 1999; 13(5): 380-87.
7. Indian Herbal Pharmacopoeia, vol I, Regional Research laboratory, Jammu-Tawi and Indian Drug Manufactures association (Mumbai). Published by Regional Research Laboratory. Jammu-Tawi and Indian Drug Manufacturers Association; 1998. p. 179-81.
8. Kokate CK. Practical Pharmacognosy. 4th ed. Vallabh Prakashan, Delhi; 1994. p. 115-17, 123, 124 & 127.
9. Chintalwar G, Jain A, Sipahimalani A, Banerji A, Sumariwala P, Ramakrishnan R, Sainis K. Chintalwar G, Jain A, Sipahimalani A, Banerji A, Sumariwala P, Ramakrishnan R, Sainis K. An immunologically active arabinogalactan from *Tinospora cordifolia*. Phytochemistry 1999; 52: 1089-93.
10. Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. 1st ed. Wright Scientechnica, Bristol; 1975. p.106.
11. Vishin ML, Gupta D. Estimation of alkaloids of Kurchi by non-aqueous titration. Ind J Pharm 1967; 29(1): 3.
12. Khandelwal K. Practical Pharmacognosy 9th ed. Nirali Prakashan, Pune; 2002. p.149-56.
13. Nandhasri P, Pawa K, Kaewtubtim J, Jaemchanya C, Jansom C, Sattaponpun C. Songklanakarin Neutraceutical properties of Thai Yor, *Morinda citrifolia* and Noni juice extract. J Sci Technol 2005; 27(2): 579-586.