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Full Length Research Paper

Pharmacognostical and phytochemical investigations of *Moringa concanensis* (Moringaceae) an ethno medicine of Nilgiris

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The plant *Moringa concanensis* Nimmo is a tree belongs to family Moringaceae locally known as Kattumurungai by tribal peoples of Nilgiris hill region in Tamilnadu. In view of its medicinal importance and taxonomic confusion, pharmacognostic studies, microscopical structure, morphological characters, chemical analysis and numerical values in epidermal study were carried out to supplement the necessary information for the systematic identification and authentication of this plant, as per WHO guidelines. Pharmacognostical and preliminary phytochemical investigations of this plant were carried out and reported.

Key words: *Moringa concanensis*, moringaceae, microscopical characters pharmacognostical investigations, phytochemical studies.

INTRODUCTION

The plant *Moringa concanensis* Nimmo (Moringaceae), locally known as Kattumurungai by tribal peoples of Nilgiris hill region in Tamilnadu is a tree, glabrous except the young parts and inflorescence. Flowers in lax divarieate thinly pubescent panicles reaching 45 cm long, segent white, oblong reflexed. Petals yellow, veined with red, oblong or oblong spathulate, the lower about 1.5 cm long. Capsules are straight, acutely triquetrous, slightly constricted between the seeds. Seeds white or pale yellow, 3 angled, 3 winged wings very thin, hyaline (Kirtikar and Basu, 1985). The plant *M. concanensis* Nimmo has been widely used as antifertility agent for decades by tribals of Nilgiris hill region. The tribals of Nilgiris, the hill region of the Western Ghats in Tamilnadu, were known to practice traditional medicine and our interaction with these tribals have given us the leads to several research projects with the possible presence of a therapeutic rationale in their claims. Earlier reports showed that the presence of ascorbic acid (Dogra et al., 1975), myristic acid, palmitic acid, oleic acid, stearic acid, arachidic acid and linoleic acid (Verma et al., 1976) from the fruits of *M. concanensis* and seed respectively. The medicinal importance and taxonomic confusion of this plant trigger us to study the pharmacognostical characteristics (microscopical structure and morphological characters) and preliminary phytochemical analysis as per WHO guidelines to supplement the necessary information for the systematic identification and authentication of this plant. The present paper describes pharmacognostic and preliminary phytochemical investigations of the plant *M. concanensis.*

MATERIALS AND METHODS

Pharmacognostical studies

Collection and authentication of plant materials: The plant materials *M. Concanensis* were collected from the hills of Barliar, authenticated at Botanical Survey of India, Coimbatore, India. The

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voucher specimens are preserved in our laboratory for further reference. After authentication, the plant materials were dried under shade. After optimum drying, the materials were coarsely powdered separately and stored in well-closed containers for further use.

Plant anatomical studies: The fresh, healthy samples were cut and removed from the plant and fixed in Formalin, Acetic acid and Ethyl alcohol (FAE) mixture. After 24 h fixing, gradual series of Tertiary - butyl alcohol (TBA) was used to dehydrate the specimens as per the schedule given by Sass (1940). Infiltration of the specimens was carried out by gradual addition of paraffin wax (m. p. 58 - 60°C) until TBA solution attained supersaturation. The specimens were cast into paraffin blocks. The paraffin embedded specimens were sectioned (10 - 12 µm) with the help of Rotary Microtome and dewaxing of the sections was done by the procedure described by Johansen (1940). The sections were stained with Toluidine blue safranin, fast green and Iodine and potassium iodide (O'Brien et al., 1964). The stained specimens were observed and recorded under different magnifications using Nikon, Japan, Labphot 2 Microscopic unit. Normal observations were done using bright field and for the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the Scale-bars (Esau, 1964).

Extractive value: The extractive value is an approximate measure of a certain constituents or a group of related constituents present in the sample. Water soluble and ethanol soluble extractive values were determined based on the procedure described earlier (Anonymous, 1996) using hydro alcohol, chloroform, petroleum ether, ethanol, and ethyl acetate as solvents.

Preparation of the extracts

The plant materials were shade dried and powdered separately. All plant materials were passed through sieve no. 40 and used for extraction. A weighed quantity of each of the plant powder was extracted with Alchol: Water (1:1) by cold maceration. The extract was evaporated to dryness under reduced pressure and controlled temperature (40 - 50°C).

Fractionation of the extracts

The hydroalcoholic extracts was taken and suspended in water and successively extracted with petroleum ether 40 - 60°C, chloroform, ethyl acetate and ethanol. Petroleum ether, chloroform, ethyl acetate fractions were dried over anhydrous sodium sulphate and concentrated to dryness under reduced pressure and controlled temperature (40 - 50°C) using rotary evaporator. The ethanol fraction directly concentrated to dryness under reduced pressure and controlled temperature.

Preliminary phytochemical studies

Qualitative phytochemical analysis of the extracts and fractions: The hydro alcoholic extract of the plant and their fractions were subjected to the different chemical tests for the identification of various phytoconstituents (Kokate, 1999). The presence of carbohydrates was tested by Molisch test, Fehling's test, Benedict's test and Barfoed's test and the presence of alkaloids were confirmed by Dragendorff's test, Wagner test, Mayer's test and Hager's test. Libermann Burchard test and Salkowasky tests were used to confirm the steroids and sterols and Legal test, Baljet test, Borntrager's test and Keller Killiani test were used to confirm the presence of glycosides. Further, Foam test and Hemoglobin test employed to identify the saponins in the extract and Shinoda test used for the flavonoids. Tannins and triterpenoids were tested by Lead acetate test, Gelatin test and Tin and Thionyl chloride test, respectively. The proteins and amino acids presence in the extract were indentified using Biuret test, Ninhydrin test and xanthoprotein test.

Thin layer chromatography analysis

Thin layer chromatography technique was adopted for the identification of different classes of components present in plant extract and fractions based on the methods described earlier (Wagner and Bladt, 1996; Harborne, 1983). Methanol: chloroform (90:10) was used as the solvent system.

High performance thin layer chromatography (HPTLC) analysis

HPTLC is a sophisticated and automated technique, which is useful in separation of compounds. Precoated plates and auto sampler are used for precision and to achieve significant separation. UV, visible and fluorescence scanner are used for qualitative and quantitative estimation (Stahl, 1969).

The extracts and fractions were dissolved in respective solvents (1% w/v) and were applied with Linomat applicator - IV on the plates. The quantity of samples applied was 10 µl each. The samples were applied as a band of 10 and 8 mm apart from each other; 6 x 10 cm plates were used. The plates were developed in CAMAG twin trough chamber up to a distance of 8 cm using methanol: chloroform (90:10) as the solvent system. After completion of the run, plates were taken out of the chamber and dried with dryer and then scanned under UV light at 366 nm. The scanning data were subjected to integration through the 'Cats software'. The chromatograms and the R_f data were collected.

RESULTS AND DISCUSSION

Anatomy of the bark

The surface of the bark is uneven, shallow fissured, fissures wide and irregular. The outer surface is brownish-grey; the inner surface is flesh colored (Figure 1). The bark has no specific taste or odour. It is light, soft and easily breakable. The cut surface appears granular and a slimy substance oozes out from the cut end.

The bark has single, superficial wide periderm comprising of wide, suberised, wavy zone of phellem and radial bands of distinct and wide phelloderm. Total thickness of the bark is nearly 2 cm. The phellem consists of deeply suberised thin walled tabular cells, the phellem is homogeneous. The phelloderm comprises of a wide zone of brick-shaped cells with wide, simple pits; some of the phelloderm cells have calcium oxalate crystals as shown in Figure 2. Large masses sclereids develop from the old phelloderm cells.

Secondary phloem constitutes the inner bark. It can be differentiated into outer zone of collapsed phloem and inner zone of non-collapsed phloem as seen from Figure 3. The collapsed phloem constitutes the bulk of the bark and it is the widest part of the secondary

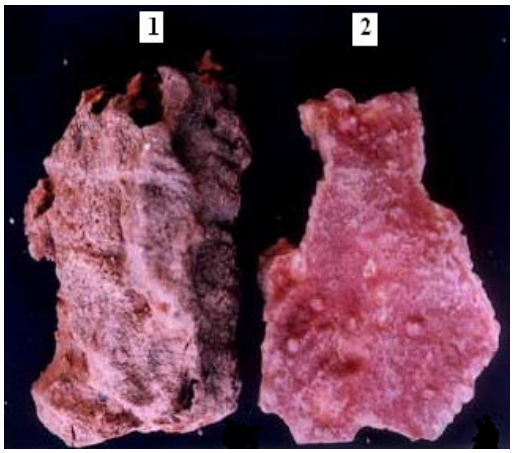

Figure 1. Surface features of the bark of *Moringa Concanensis*.
1. Outer surface. 2. Inner Surface.

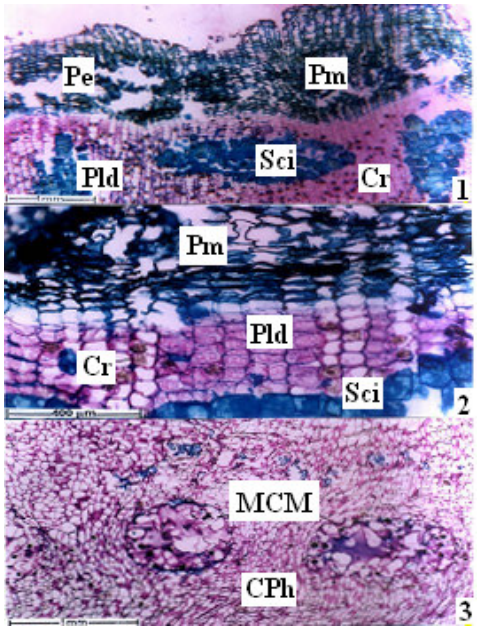

Figure 2. Anatomy of the outer bark of *Moringa Concanensis*
1. Phellem and phelloderm enlarged.
2. Circular cell mass in the outer bark
3. Mucilagenous cell mass in the outer bark. (**CPh**; Collapsed phloem, **Cr**; Crystals, **MCM**; Mucilagenous cell mass; **Pe**; periderm, **Pm**; Phellem, **Pld**; Phelloder, **Sci**; Sclereids).

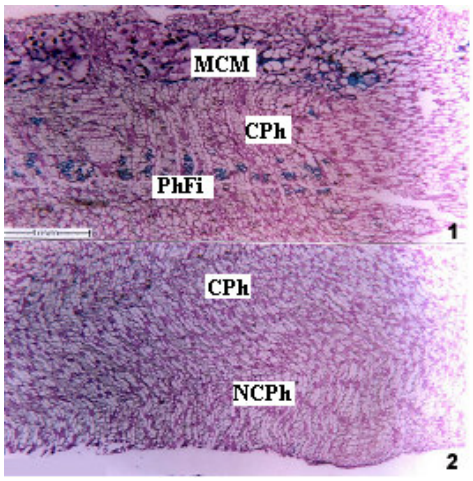

Figure 3. Secondary phloem tissues – Collapsed and non-collapsed, phloem.
1. Col-lapsed phloem with mucilaginous cell mass and scanty sclerenchyma.
2. Non-Collapsed phloem and collapsed phloem. [**CPh**; Collapsed Phloem, **MCM**; Mucilagenous cell mass, **NCPh**; Non-Collapsed phloem; **PhFi**; Phloem fibers].

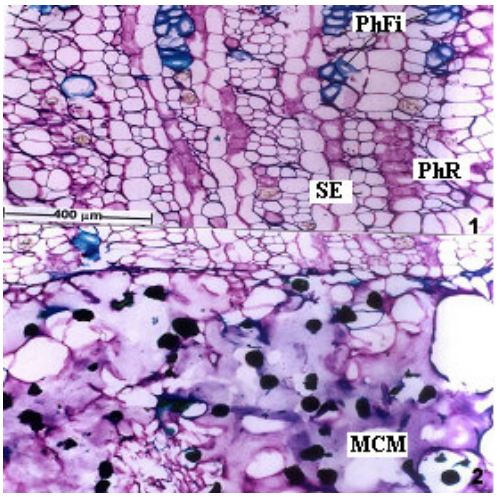

Figure 4. Elements of secondary phloem and mucilaginous cell mass.
1. Secondary phloem showing phloem fiber bands and collapsed sieve elements.
2. Mucilagenous cell mass enlarged. [**MCM;** Mucilagenous cell mass, **PhFi**; Phloem fibers, **PhR**; Phloem ray, **SE**; Sieve elements].

phloem (Figure 4). Mucilaginous cell masses are seen in a tangential band outer both sclerenchyma band. The masses are either discrete circular masses or in continuous wide tangential band (Figure 4). The parenchyma cells disintegrate losing their contents into the cavity. Large, spherical bodies of unknown chemical composition are seen within the mucilaginous cell masses.

The collapsed phloem comprises of crushed sieve elements, highly dilated phloem rays, dilated parenchyma cells and scanty phloem sclerenchyma. The sieve elements are crushed into thin, dark lines due to excessive dilation of the axial parenchyma and phloem rays; the crushed sieve elements are seen in radially oblique lines and in parallel bands in the midst of dilated cells

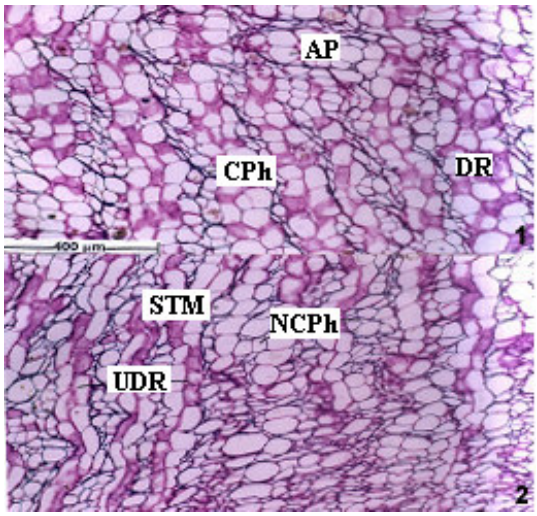

Figure 5. Collapsed phloem-Structure and organization
1. Radial oblique lines of crushed sieve elements and dilated rays.
2. Undilated rays and non-collapsed phloem. [**AP**; Axial parenchyma, **CPh**; Collapsed phloem, **DR**; Dilated ray, **NCPh-Non**; Collapsed phloem, **STM**; Sieve Tube Member, **UDR**;Undilated Ray].

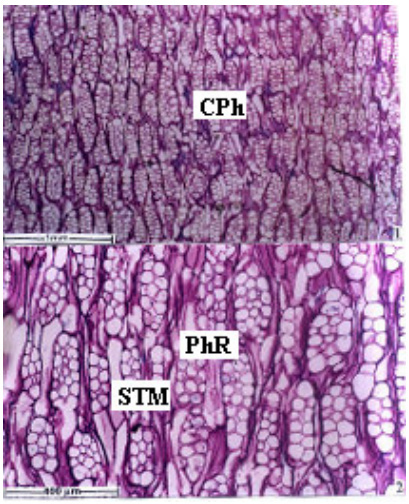

Figure 6. Tangential longitudinal section Profile of secondary phloem.
1. Storied arrangement of phloem rays.
2. Phloem ray seviation and sieve tube members. [**CPh**; Collapsed phloem, **PhR**; Phloem ray, **STM**; Sieve tube member].

(Figure 5). Collapsing of the sieve elements occurs gradually, proceeding from the outer part to the inner part. The phloem rays undergo extensive dilation as well as cell divisions; they become 2 - 4 layered with circular of rectangular thin walled cells. Towards the periphery, they are still wider. Axial parenchyma cells appear quite prominent because of their large size as well as their abundance. They are in wavy radial bands along with the crushed lines of sieve elements (Figure 5).

Sclerenchyma elements are scanty only on their tangential band of fibres occurs in the center border of the collapsed phloem (Figure 5). The fibers are in short radial blocks separated by rays. The fibers are wide, thick walled and lignified. Non-Collapsed the phloem occurs inner to the cambium. The sieve tube members are intact; the phloem rays are narrow, undilated and slightly wavy. The sieve tube members are thin walled and angular in outline. The companion cells are small and less prominent (Figure 5). The sieve tubes are 30 - 40 µm in diameter.

Tangential longitudinal section (TLS) of the bark shows the rays are in more or less regular horizontal tiers. The rays are 2 - 4 seviate, short and wide. They consist of angular or circular, compact, thin walled cells, the rays are homocellular (Comprising of only one type of cells) and are 150 - 280 µm in height and 70 - 120 µm wide. Ray frequency is 8 mm. The sieve tube member in short and wide; they have simple, oblique sieve plates (Figure 6).

Calcium oxalate crystals are abundant in the outer bark, especially the periderm and dilated phloem rays. The crystals are rosette type. The rosette has a central dark organic spot surrounded by petal-like crystals appearing as rose flower (Figures 7 and 2). The rosettes are up to 50 µm in diameter. The rosette crystals are also seen in the lumer of the sclereids (Figure 7) and occasionally prismatic crystals are also seen mixed with rosette containing cells (Figures 7 and 2).

Extractive value

Extractive values of Hydroalcoholic and its various fractions of *M. concanensis* were determined. Extractive values for hydro alcohol, chloroform, petroleum ether, ethanol and ethyl acetate is 12.49, 2.05, 1.12, 3.08 and 5.27 respectively.

Preliminary phytochemical studies

A systematic and complete study of crude drugs should include a complete investigation of both primary and secondary metabolites derived from plant metabolism. The different qualitative chemical tests are needed to be performed for establishing profiles of given extracts and fractions for their nature of chemical composition. The qualitative phytochemical analysis on the various extracts and fractions of the *M. concanensis* were carried out. The results of the studies indicated the presence of carbohydrates, alkaloids, glycosides, tannins, saponins, terpenoids, flavonoids and proteins in hydroalcoholic extract. Alkaloids, tannins and Terpenoids were present in chloroform fraction where as ethyl

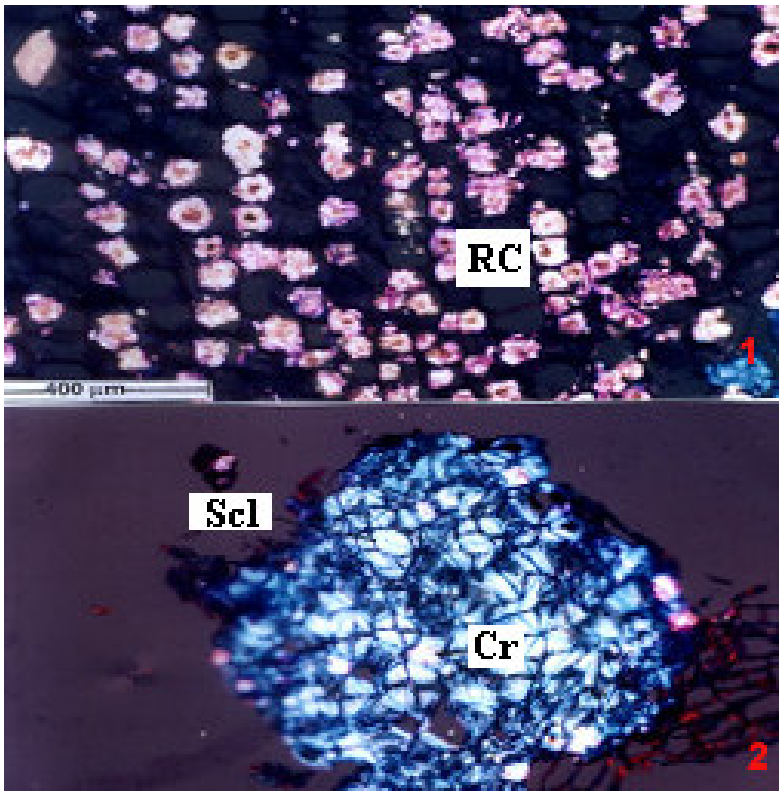

Figure 7. Crystal distributions in the bark.
1. Radial rows of rosette crystals in the phelloderm cells
2. A mass of sclereids with rosette crystal in the cell lumen. [**Cr**; Crystal, **RC**; Rosette Crystal, **Scl**; Sclereids].

as ethyl acetate fraction contains carbohydrate, glycosides, tannins and flavonoids. Petroleum ether fraction contains steroids and terpenoids where as ethanol fraction contains carbohydrates, glycosides, tannins, saponins, flavonoids and proteins.

Thin layer chromatography

The hydro alcoholic extract of *M. concanensis* gave 12 spots when chromatogrammed on silica gel GF_{254} in solvent system chloroform: methanol (90:10). Further the fractions were chromatogrammed on same conditions. Petroleum ether fraction gave 2 spots; chloroform fraction gave 4 spots, Ethyl acetate fraction gave 9 spots and ethanol fraction gave 12 spots, respectively. In all chromatograms two compounds were found to be common having R_f 0.06 and 0.96 respectively and were common in all the fractions. Where as in chloroform, ethyl acetate, ethanol and hydro alcoholic extracts and another spot having R_f 0.71 was found to be common.

HPTLC

The HPTLC finger print analysis of crude drug(s) is a useful tool in the qualitative analysis that gives an idea about the major components present. Hence we subjected various fractions and the total extracts of *Moringa concanensis* for finger printing analysis using HPTLC (CAMAG V 4.06 with Linomat IV applicator). The HPTLC plates were scanned at 366 nm; the chromatogram patterns were documented.

The hydro alcoholic extract of *M. concanensis* showed the presence of 12 spots and 2 of them with R_f values 0.06 and 0.95, were found to be the major one's with about 67.85 and 19.16% peak areas, respectively. Similarly the petroleum ether fraction, showed the presence of 2 spots, and 1 of them with R_f value 0.83, was found to be the major one's with about 88.26% peak area, respectively. The chloroform fraction showed the presence of 4 spots, and 2 of them with R_f values 0.06 and 0.96, were found to be the major one's with about 22.48 and 73.25% peak areas, respectively. The ethyl acetate fraction showed the presence of 9 spots, and 2 of them with R_f values 0.06 and 0.97, were found to be the major one's with about 52.20 and 25.73% peak areas, respectively. The ethanol fraction showed the presence of 12 spots, and 2 of them with R_f values 0.06 and 0.96, were found to be the major one's with about 67.26 and 18.83% peak areas respectively (Figure 8).

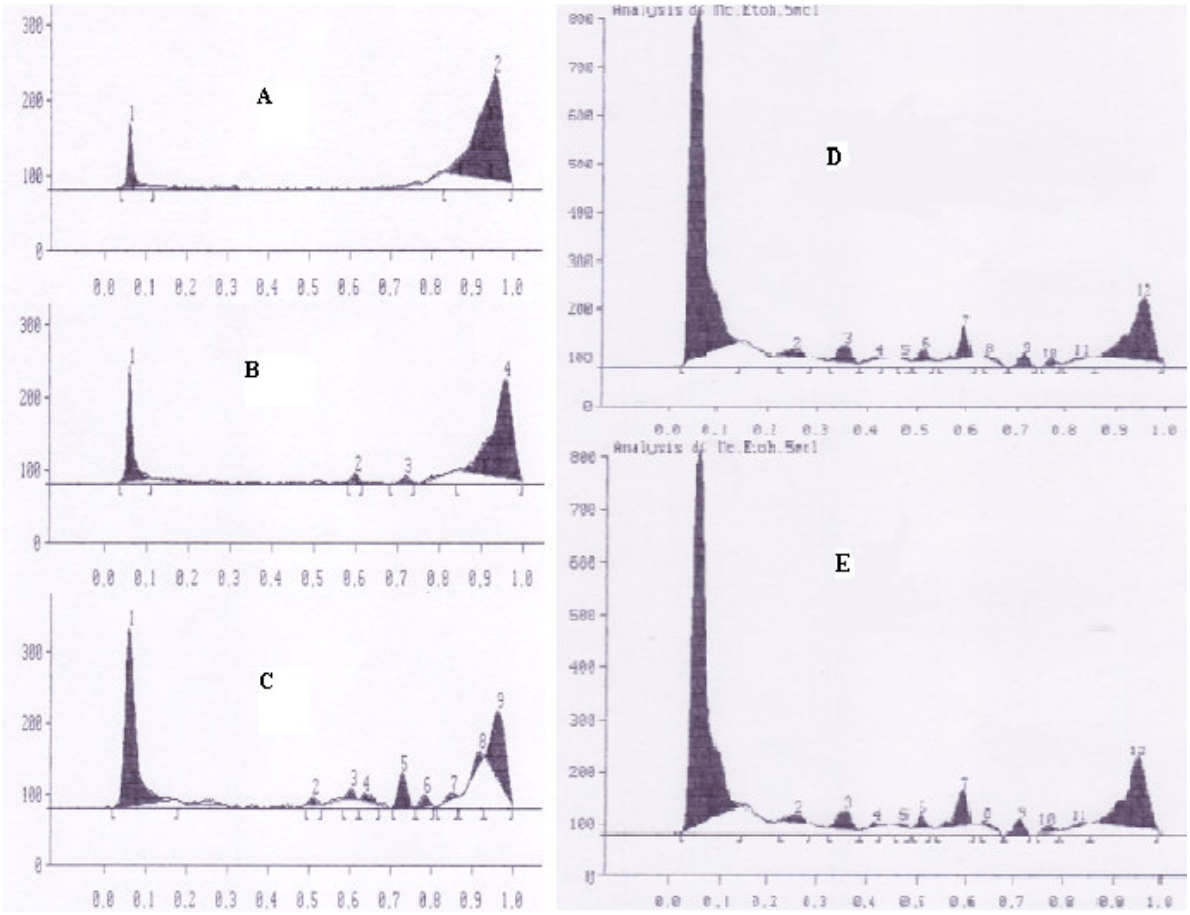

Figure 8. HPTLC Chromatograms of *Moringa concanensis* hydro alcoholic extract and its fractions petroleum ether fraction, B- chloroform fraction, C- ethyl acetate fraction, D- ethanol fraction, E- hydro alcoholic extract.

Conclusion

In the present study we documented the pharmacognostical characters and the preliminary phyto-constituents profile of Moringa concanensis. This information may be useful to future workers to identify and to differentiate from the closely related other species of Moringa.

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