

Original Research Article

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Qualitative and Quantitative Phytochemical analysis of *Moringa concanensis* Nimmo

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ABSTRACT

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Moringa concanensis Nimmo (Moringaceae) is an important medicinal plant. The present study deals with the analysis of Phytochemical constituents by qualitative and quantitative analysis of *Moringa concanensis* leaves, flowers and seeds were done using methanol extract. Alkaloids, flavonoids, terpenoids, carbohydrates, protein and amino acids were analysed. Phenol and saponin were present in only methanol extracts of leaves and flowers. Steroids, anthroquinone, tannin, oils and resins were absent in the extract. Quantitative analysis were also conducted to determine the amount of alkaloids, flavonoids, phenol and carbohydrate.

Introduction

The evaluation of all the drugs is based on phytochemical and pharmacological approaches which leads to the drug discovery referred as natural product screening (Foye *et al.*, 2008). Any part of the plant may contain active components such as bark, leaves, flowers, roots, fruits and seeds (Gordon and David, 2001).

Phytochemicals are the chemicals produced by various parts of the plants. These bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides.

These compounds have various activities such as antimicrobial and antibacterial some have been reported to exhibit hemolytic and foaming activity reported by Feroz *et al.* (1993).

Number of various environmental factors such as climate, altitude, rainfall and other conditions may affect growth of plants which in turn affect the quality of herbal ingredients present in a particular species even when it is produced in the same country. These conditions may produce major variations in the bioactive compounds

present in the plants (Kokate *et al.*, 2004). Qualitative phytochemical screening will help to understand a variety of chemical compounds produced by plants and quantification of those metabolites will help to extract, purify and identify the bioactive compounds for useful aspects to human beings.

Plants have limitless ability to synthesize aromatic substances, mostly phenols or their oxygen-substituted derivatives (Geissman, 1963). Most of the natural products are secondary metabolites and about 12,000 of such products have been isolated so far. These products serve as plant defence mechanisms against predation by microorganisms, insects and herbivores (Fransworth and Morris, 1976). Today there is growing interest in chemical composition of plant based medicines. Several bioactive constituents have been isolated and studied for pharmacological activities. During the last two decades, the pharmaceutical industry has made massive investment in pharmacological and chemical researches all over the world in an effort to discover much more potent drugs, rather, a few new drugs. Plants have successfully passed the tests of commercial screenings.

Furthermore, the use of herbal medicine for the treatment of diseases and infections is as old as mankind. The World Health Organization supports the use traditional medicine provided they are proven to be efficacious and safe (WHO, 1985). In developing countries, a huge number of people lives in extreme poverty and some are suffering and dying for want of safe water and medicine, they have no alternative for primary health care. There is therefore the need to look inwards to search for herbal medicinal plants with the aim of validating the ethno-medicinal use and subsequently the isolation and characterization of

compounds which will be added to the potential list of drugs.

Moringa concanensis is a medicinal plant belonging to the family Moringaceae (Fig.1). It is present in large amount in the Tamil Nadu state, India. *M. concanensis* is widely distributed on dry lands of Tamil Nadu. *M. concanensis* is an evergreen tree with a spreading crown, up to 7-8 feet. Leaves alternate, 2-3- pinnate, obovate, caducous. Flowers large, white, hermaphrodite, irregular in axillary panicles. Calyx thinly tomentose, long, segments white, oblong, reflexed. Petals yellow, veined with red, oblong. Stamens 5fertile and 4-5 staminodes. Capsule straight, actively triquetrous, slightly constricted between the seeds. Seeds white or pale yellow 3- angled. The present investigation to find out the qualitative and quantitative analysis from the leaves, flowers and seeds.

Materials and Methods

Selection of Plant Species

The plant materials (Leaves, flowers and seeds) of *M. concanensis* were collected from the Kunnam of Perambalur District, Tamil Nadu(Fig.2). The plant materials were washed thoroughly 2-3 times with running tap water and once sterile with distilled water. Then the plant parts were shade dried and coarsely powdered separately and stored in well closed bottles for further analysis in laboratory.

Authentication of Plant Materials

The plant was authenticated at The Rapinet Herbarium, St.Joseph's College, Tiruchirappalli, Tamil Nadu and Botanical Survey of India [BSI], Southern Circle, Coimbatore. India. The specimen was labelled, numbered and annotated with the

date of collection and locality.

Extraction of the Plant Materials

The fresh plant materials were washed with running tap water and shade dried. The leaves, flowers and seeds were crushed to coarsely powdered. These coarse powders (25g) were then subjected to successive extraction in 250ml of methanol solvent by using Soxhlet apparatus. The collected extracts were stored and then used for further analysis. The DMSO (Dimethyl sulfoxide) is act as dissolved solvents for these extracts.

Qualitative Phytochemical Analysis

Preliminary phytochemical analysis was carried out for the extract as per standard methods described by Brain and Turner (1975) and Evans (1996).

Detection of Alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

Mayer's test: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Wagner's test: Filtrates were treated with Wagner's reagent. Formation of brown/reddish brown precipitate indicates the presence of alkaloids.

Detection of Flavonoids

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates that the presence of flavonoids.

H₂SO₄ test: Extracts were treated with few drops of H₂SO₄. Formation of orange colour indicates that the presence of flavonoids.

Detection of Steroids

Two ml of acetic anhydride was added to five mg of the extracts, each with two ml of H₂SO₄. The colour was changed from violet to blue or green in some samples indicate that the presence of steroids.

Detection of Terpenoids

Salkowski's Test

Five mg of the extract of the leaves, flowers and seeds was mixed with two ml of chloroform and concentrated H₂SO₄ (3ml) was carefully added to form a layer. An appearance of reddish brown colour in the inner face was indicates that the presence of terpenoids.

Detection of Anthroquinones

Borntrager's Test

About five mg of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of CHCl₃ was added to the filtrate. Few drops of 10% NH₃ were added to the mixture and heated. Formation of pink colour indicates that the presence anthroquinones.

Detection of Phenols

Ferric chloride test: 10mg extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates that the presence of phenol.

Lead acetate test: 10mg extracts was treated with few drops of lead acetate

solution. Formation of yellow colour precipitate indicates that the presence of phenol.

Detection of Saponins

About 0.5mg of the extract was shaken with five ml of distilled water. Formation of frothing (appearance of creamy mass of small bubbles) shows that the presence of saponins.

Detection of Tannins

A small quantity of extract was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green colour was formed. It indicates that the presence of tannins.

Detection of Carbohydrates

0.5mg extracts were dissolved individually in five ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

Detection of Protein & Amino acids

Biuret test: To 0.5 mg of extract equal volume of 40% NaOH solution and two drops of one percent copper sulphate solution was added. The appearance of violet colour indicates that the presence of protein.

Ninhydrin test: About 0.5 mg of extract was taken and two drops of freshly prepared 0.2% Ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates that the presence of proteins, peptides or amino acids.

Detection of Oils and Resins

Test solution was applied on filter paper. It

develops a transparent appearance on the filter paper.

It indicates that the presence of oils and resins.

Quantitative Phytochemical analysis

Estimation of Alkaloids

Alkaloid determination by using Harborne (1973) method. One gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and its covered and allowed to stand for 4 h. It was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated NH₄OH was added by drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH₄OH and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of Flavonoids

One grams of plant sample was repeatedly extracted with 100ml of 80% aqueous methanol at room temperature. The mixture was filtered through a Whatman No1 filter paper into a pre-weighed 250ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. (Krishnaiah *et al.*, 2009).

Estimation of Total Phenols

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. Five ml of the extract was pipetted out into a 50 ml flask, then 10 ml of distilled water was added. Two ml of NH₄OH solution and 5 ml of concentrated amyl alcohol were also added.

The samples were made up to mark and left to react for 30 min for colour development. This was read at 505nm.

Estimation of Carbohydrate

100 mg of sample was hydrolysed in a boiling tube with 5 ml of 2.5 N HCl in a boiling water bath for a period of 3 hours. It was cooled at room temperature and solid sodium carbonate was added until effervescence ceases. The contents were centrifuged and the supernatant was made to 100 ml by using distilled water. From this 0.2 ml of sample was pipetted out and made up the volume to one ml with distilled water. Then one ml of phenol reagent was added and followed by 5.0 ml of sulphuric acid. The tubes were kept at 25-30°C for 20 min. The absorbance was read at 490 nm (Krishnaveni *et al.*, 1984).

Results and Discussion

The present study was carried out on the *M. concanensis* revealed that the presence of active phytochemical constituents. The phytochemical active compounds of *M. concanensis* were qualitatively and quantitatively analysed from leaves, flowers and seeds separately and the results are mentioned in Table 1 and Table 2 respectively.

The quantitative estimation of primary metabolites revealed that the various phytochemical constituents present in the plant extract (Table-2). In seed sample of *M. concanensis*, the alkaloid content was 2.15(W/w), flavonoids content was 10.08(W/w), phenol content was not detected and the carbohydrate content was about 5.86(W/w). But in the case of flower sample the alkaloids content found to be 4.37(W/w), flavonoids content was 15.12(W/w), phenol content was

30.18(W/w) and carbohydrate about 7.49(W/w). Finally the leaf sample contains the alkaloid content about 5.92(W/w), the flavonoid content was 15.74(W/w), phenol content 37.52(W/w) was very higher than the other two sample and finally the carbohydrate contents was found to be 9.15(W/w).

Fig.1 *Moringa concanensis* Nimmo- Habit



Fig.2 Perambalur District-Map

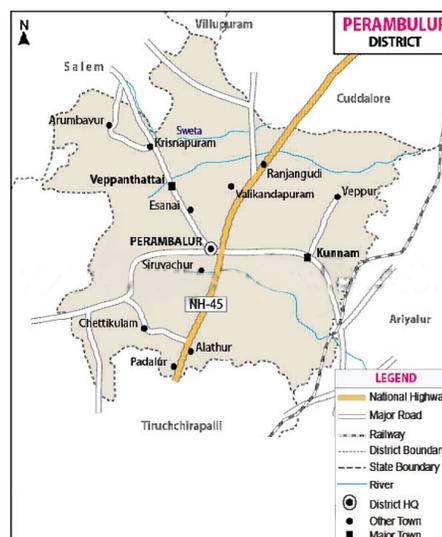


Table.1 Qualitative Phytochemical Analysis of *M. concanensis* Leaves, Flowers and Seeds Extracted with Methanol Solvent

Phytochemicals	Methanol Extracts		
	Leaves	Flowers	Seeds
Alkaloids			
Mayer's test	+	+	+
Wagner's test	+	+	+
Flavonoids			
Lead acetate test	+	+	+
H ₂ SO ₄ test	+	+	+
Steroids			
Liebermann-Burchard test	-	-	-
Terpenoids			
Salkowski test	+	+	+
Arthroquinone			
Borntrager's test	-	-	-
Phenols			
Ferric chloride test	+	+	-
Lead acetate test	+	+	-
Saponin	+	+	-
Tannin	-	-	-
Carbohydrates	+	+	+
Proteins & Amino acids			
Biuret test	+	+	+
Ninhydrin test	+	+	+
Oils & Resins	-	-	-

(+) Present (-)Not detected

Table.2 Quantitative Phytochemical Analysis of *M. Concanensis* Leaves, Flowers and Seeds Extracted with Methanol Solvent

S.No	Phytochemicals	Leaves (W/w)	Flowers (W/w)	Seeds (W/w)
1	Alkaloids	5.92	4.37	2.15
2	Flavonoids	15.74	15.12	10.08
3	Phenol	37.52	30.18	-
4	Carbohydrates	9.15	7.49	5.86

The present investigation shows that significant variation in the contents like alkaloids, flavonoids, phenol and carbohydrate when compared to above mentioned results. These variations are due to number of environmental factors such as climate, altitude, rainfall etc. as mentioned (Kokate et al., 2004). Saponins act as

antimicrobial activity and extremely to cold-blooded animals, but toxicity to mammals is low (Sneh verma et al., 2013). Saponins are a mild detergent used in intracellular histochemistry staining to allow antibody access to intracellular proteins. The saponins are used in hypercholesterolaemia, hyperglycemia, antioxidant, anticancer, anti

inflammatory activity and weight loss (Manickam murugan *et al.*, 2014).

The phytochemical screening of flowers and flower buds are not been reported earlier although flower and flower buds of *M.concanensis* also help in abortion and leucorrhea (Anbazhakan *et al.*, 2007). Alkaloids have been used as both antibacterial and antidiabetic properties and useful for such activities. Phenols and phenolic compounds have been extensively used in disinfections and remain the standard with which other bactericides are compared (Akinyeye *et al.*, 2014).

In the present study conclude that the *M. concanensis* leaves have the potential to act as a source of useful drugs because of presence of various phytochemical constituents such as alkaloids, flavonoids, phenol, terpenoids, saponin and carbohydrates. These phyto constituents seemed to be the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital role for good health.

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