

# Screening of Bioactive Compounds from the Leaves of *Moringa Concanensis* Nimmo

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**ABSTRACT:** During the last decade, the use of Traditional Medicine (TM) has expanded globally and is gaining popularity. It has continued to be used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system. The objective of the present study was to investigate the presence of various phytochemicals from different solvents like ethanolic, aqueous, chloroform, etc extracts of *Moringa Concanensis* Nimmo leaves. The phytochemical analysis revealed the presence of alkaloids, flavonoids, carbohydrates, terpenoids, tannins, reducing sugar and amino acid in the ethanol and aqueous extracts followed by methanol and chloroform.

**KEYWORDS:** *Moringa concanensis*, phytochemicals, ethanol, chloroform, bioactive compounds.

## I. INTRODUCTION

Herbal plants as the major remedy in traditional system of medicine have been used in medical practices since antiquity. The medicinal plant of the study area *Moringa concanensis* Nimmo was mostly indicated to be abundant and rare. *Moringa concanensis* Nimmo (Moringaceae) is one of the important medicinal trees which are restricted in its distribution. *M. concanensis* occurs in tropical dry forest from southeastern Pakistan almost to the southern tip of India. It has recently been found in western Bangladesh. Indigenous knowledge of this plant in that region has not been so far studied.

The family moringaceae has a single genus *Moringa*, with 13 species, of which only 2 species have been recorded in India, *Moringa concanensis* and *Moringa oleifera*. *M. concanensis* Nimmo is a small tree indigenous to Northwest India resembling *M. oleifera* and it is abundant in Rajasthan, the dry hills of Konkan, Andhra Pradesh and is commonly found on recent alluvial land in or near the sandy beds of rivers and streams (1). Perambalur is one of the District of Tamilnadu which is surrounded by south Arcot in the North, Trichy on south, Salem on west and Thanjavur on the east. *Moringa concanensis* is widely distributed on dry lands of Villamuthur, Perambalur, Veppanthattai and Kunnam of Perambalur district.

*Moringa concanensis* Nimmo is a medium sized deciduous tree with thick bark. Bark is fissured to 10cm deep, and is corky grey. The tree is hairless except younger parts and inflorescence. Leaves are bipinnate (very rarely tripinnate), 45 cm long. Primary pairs are 5-6, distant, 10-20 cm long. Leaflets are 4-6 pairs with an odd one, broadly elliptic to round, blunt at both ends, often notched at the tip, 2.5-3.8 cm long, 1.25-2.5 cm broad, pale beneath (Figure-1). Leaf fall is usually found in the month of January. Flowering occurs during February-April. Fruiting is from April onwards (2). The plant has different vernacular names in more than eight vernacular languages. There are twenty four vernacular names in Tamil. *Avacunari*, *Kacappumurunkai*, *Kanalmurunkai*, *Kanamurunkai*, *Karikamuli*, *Karimurungai*, *Katarini*, *Kattamurungai*, etc. The Indian traditional systems of medicine, especially ayurveda, have put forward a number of therapeutic claims for the plant drugs.

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Figure-1 Image showing *Moringa Concanensis* Nimmo(whole plant) with a view of leaves.



## II. BACKGROUND OR RELATED WORK

### Preliminary phytochemical analysis

Plants have an almost limitless activity to synthesize bioactive substances, most of which are secondary metabolites of which at least 12000 have been isolated (3). A *phytopharmaceutical preparation* or *herbal medicine* is any manufactured medicine obtained exclusively from plants (aerial and non-aerial parts, juices, resins and oil), either in the crude state or as a pharmaceutical formulation. The compounds such as muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicines and phorbol esters, all obtained from plants, are important tools used in pharmacological, physiological and biochemical studies (4,5). The plants can be subjected to successive extraction and purification procedures to isolate the compounds of interest, which can themselves, be active and used directly as a drug, examples being quinine, digoxin and ergotamine (6).

## III. SCOPE OF RESEARCH

Ayurveda has a unique way of understanding plants. Starting from the earliest text available, *i.e.*, Charaka samhita (1500 BC - 400 AD), exhaustive descriptions about plants and their medicinal uses are available. All part of the tree *Moringa concanensis* are reported to be used in the treatment of as cites, rheumatism, venomous bites, painful swellings, gastrointestinal motility disorders as cardiac and circulatory stimulant. Along with other therapeutic

# International Journal of Innovative Research in Science, Engineering and Technology

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applications, 'The Ayurvedic Pharmacopoeia of India' indicated the use of dried leaves in goiter, glycosuria, and lipid disorders.

The objective of the present study is to investigate the presence of various phytochemicals from different solvents like ethanol, acetone, ethyl acetate, petroleum ether, methanol, water, chloroform and hexane extracts of *Moringa Concanensis* Nimmo leaves. The scope of the research is to make the plant useful for treating different ailments and have a potential of providing useful drugs of human use in future.

## IV. MATERIALS AND METHODOLOGY

### Collection of plant materials

The plant materials used for the study were collected from selected areas of Eastern Ghats, which are an assemblage of discontinuous ranges, hill plateaus and gorges adjoining the plains. The hilly area of Tamil Nadu covers the Javadi Hills, Shervarayans, Kolli Hills, Chinnakalrayan, Chitheris, Bodamalai, Palni Hills, Pacchaimalais and Nilgiris ranges. The Kolli hills has its geographical position between 11°10' to 11°30' N and 78°15' to 78° 30' S, belongs to Namakkal district covering a total area of about 503 sq. km. These come under Perambalur districts of Tamil Nadu respectively. The collected plant materials were air dried and grinded to a fine powder using an electronic blender and was authenticated in the Rapinat Herbarium, St.Joseph's College, Tiruchirappalli, Tamil Nadu,India. Each specimen was labeled, numbered and annotated with the date of collection and the locality.

### Preparation of leaf extracts

Ten grams of the dried powdered plant material (leaves) were soaked separately with 100 ml of each of the solvents viz. ethanol, acetone, ethyl acetate, petroleum ether, methanol, water, chloroform and hexane in a soxhlet apparatus for 48 hrs at 310° C until complete extraction of the materials. At the end of 48 hrs, each extract was filtered through Whatman No.1 filter paper and filtrates were concentrated at room temperature in order to reduce the volume. The sample was concentrated using rotary evaporator and freeze dried to powdered form. In the present study, the extract was prepared according to the polarity and they were concentrated and their value was calculated in reference to air dried drugs. The results are tabulated in (Table 1). The paste like extracts were stored in pre-weighed screw capped bottles and the yield of extracts were weighed. These screw capped bottles were kept in refrigerator. Each of the extract was individually reconstituted using minimal amounts of the extracting solvent prior to use (7).

**Table- 1: Values of leaves extracts of *Moringa concanensis* with different solvents**

Sl.No.	Solvent Extracts	Value%(w/w)
1	Aqueous Extract	1.93
2	Acetone Extract	1.14
3	Chloroform Extract	1.50
4	Ethanol Extract	0.95
5	Ethylacetate Extract	0.90
6	Hexane Extract	0.82
7	Methanol Extract	1.00
8	Petroleum ether Extract	0.86

The most convenient modern technique for purification is chromatography. The purification of the desired compound was done by the precipitation of the impurities by Hydrochloric acid. Chemical tests are conducted on the extracts of each solvent sample and also of the powdered form of the leaves samples using standard methods of (8).

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## Phytochemical analysis of different Crude extracts

The Plant extracts were analyzed for the presence of active principles such as Triterpenoids, Steroids, Glycosides, Saponins, phenolics, Alkaloids, Flavonoids, Tannins, Proteins, Free Amino Acids, Carbohydrate, fatty acids, volatile oils and reducing sugars. Following standard procedures were used.

### Test for Alkaloids:

Hager's Test – Test solution was treated with few drops of Hager's reagent (saturated picric acid solution). Formation of yellow precipitate would show a positive result for the presence of alkaloids.

Mayer's reagent test- About 0.5 ml of test solution was taken with 1 ml of 2N HCl. Aqueous layer formed was decanted to which one or few drops of Mayer's reagent were added. A white precipitation shows the presence of alkaloids.

### Test for Flavonoids:

Magnesium Test- A small piece of magnesium ribbon was added to all extracts of the plant material, and this was followed by the drop wise addition of concentrated hydrochloric acid along the sides of the test tubes. Colours varying from orange to red indicated flavones, red to crimson indicated flavonols, crimson to magenta indicated flavones.

Alkaline reagent Test – Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

### Test for Tannins

Test samples of leaves were boiled with one ml of distilled water in a test tube and then filtered. The filtration method used here is the normal method, which includes a conical flask and filter paper. 0.1% FeCl<sub>3</sub> is added to the filtered samples and observed for brownish green or a blue black coloration, which shows the presence of tannins.

Gelatin Test – Test solution when treated with gelatin solution would give white precipitate indicating the presence of tannins.

### Test for Steroids

Few ml of all extracts of the powdered leaves were evaporated to a dry mass and the mass was dissolved in 0.5 ml of chloroform. Acetic anhydride (0.5 ml) and 2 ml of concentrated sulphuric acid were added. A blue or green colour or a mixture of these two shades was regarded as positive for the presence of steroidal compounds.

### Test for Terpenoids

3 ml of all extracts of sample is mixed with 0.5ml of glacial acetic acid followed by CHCl<sub>3</sub> in a test tube. 1.5 ml of concentrated H<sub>2</sub>SO<sub>4</sub> is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

### Test for Saponins

Froth Test- One gram of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5 ml of the filtrate was added to 5 ml of sterile distilled water in a test tube. The test tube was shaken vigorously for about one minute to obtain a stable persistent froth. The frothing is then mixed with 3 drops of olive oil and it was then allowed to stand for 15 minutes. Honeycomb emulsion indicates the presence of Saponins.

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## Test for Glycosides

Bromine water test - Test solutions were dissolved in bromine water and observed for the formation of yellow precipitate to show a positive result for the presence of glycosides.

Fehling's Test- Coarsely powdered samples were added into two separate beakers. To one of the beakers was added 5 ml of dilute sulphuric acid while 5 ml of water was added to the other beaker. The two beakers were heated for 3-5 min and the contents were filtered into labeled test tubes. The filtrate was made alkaline with 5% sodium hydroxide and heated with Fehling's solution for 3 min. The presence of reddish precipitate in the acid filtrate was regarded as positive for glycosides.

## Test for Proteins

Biuret Test – Test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink color.

## Test for Gums and Mucilage

About 5 ml of various extracts were added separately to 15 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

## Test for Carbohydrate

Benedict's test – Test solutions of various extracts were mixed with 5-8 drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath. The formation of reddish brown precipitate shows the presence of carbohydrate.

## Test for Amino Acids

Ninhydrin Test – Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple color suggesting the presence of free amino acids.

## Test for Phenols

To 2ml of test solution, added equal amount of alcohol and then a few drops of neutral ferric chloride solution was added. The test result was observed for the appearance of blue colour which indicates the presence of phenols.

## V. RESULTS AND DISCUSSION

The present investigation of preliminary phytochemical results showed the presence and absence of certain phytochemicals in different extracts. The tests were performed using different organic solvents; Ethanol, Aqueous, Acetone, Chloroform, Hexane, Methanol, Petroleum ether, Ethylacetate, extracts respectively. In the present investigation, the aqueous extracts of leaves showed the presence of flavonoids, alkaloids, saponins, gums and mucilage. Further, the aqueous extracts of the leaves showed the absence of steroids, terpenoids, tannins, lignin and glycosides. Hexane and ethyl acetate showed the least performance, since the phytochemicals present were in trace or negligible amount. The ethanolic leaves extract revealed the presence of almost all the bioactive compounds except amino acids which were present in traces (Table - 2).

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**Table- 2: Preliminary Phytochemical analysis of *Moringa concanensis* leaves extracted with different solvents**

Sl.No:	Chemical Tests	Solvent Extracts							
		Aqueous	Acetone	Chloroform	Ethanol	Ethylacetate	Hexane	Methanol	Petroleum Ether
1	Alkaloids	+	-	-	+	-	-	+	-
2	Flavonoids	+	+	-	+	-	-	+	-
3	Tanins	-	+	-	+	-	-	-	+
4	Steroids	-	-	-	+	-	-	-	-
5	Terpenoids	-	-	-	+	-	-	+	-
6	Saponins	+	-	+	+	-	-	+	-
7	Glycosides	-	-	-	+	-	-	-	+
8	Proteins	-	-	+	-	+	-	+	-
9	Gums & Mucilage	+	+	-	+	-	-	+	-
10	Carbohydrates	-	-	+	+	+	-	+	-
11	Amino acids	-	+	-	-	-	+	-	+
12	Phenols	-	+	+	+	-	-	+	+

(+) - Present, (-)- Absent

Tannins, phenolics, saponins, alkaloids and flavonoids have been linked or suggested to be involved with antibacterial and anti-viral activity while tannins and flavonoids are thought to be responsible for antidiarrheal activity (9). Herbs that have tannins as their component are astringent in nature and are used for the treatment of intestinal

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disorders such as diarrhoea and dysentery, this finding support the reasons why *Moringa concanensis* Nimmo has position among medicinal plants used for the treatment of microbial infection. Tannins have been observed to have remarkable activity in cancer prevention and anticancer (10).

Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these compounds were found to be present in the aqueous and ethanolic leaves extracts, it accounts for their medicinal value and future application of this in drug manufacturing.

Investigations of the mode of action indicate that tannins and flavonoids increase colonic water and electrolyte reabsorption and other phytochemicals act by inhibiting intestinal mobility, while some components have been shown to inhibit particular enteropathogens (9). Flavonoids also exhibit anti-inflammatory, antiangiogenic, anti-allergic effects, analgesic and antioxidant properties (11). Flavonoids have the ability of scavenging hydroxyl radical, superoxide anion radicals and lipid peroxyradicals which highlight many of the flavonoids health- promoting functions in organism, and are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA (12). Flavonoids in human diet may reduce the risk of various cancers, as well as preventing menopausal symptoms (11). In addition to the antimicrobial activities exhibited by flavonoids, it also exhibits antitrypanosomal and antileishmanial activities (13). Some flavonoids have also been reported to behave like some coumarins in the inhibition of giant cell formation in HIV-infected cell cultures (14).

The petroleum ether extract of leaves showed moderate amount and ethanolic extract showed appreciable amount of glycosides. Glycosides are known to work by inhibiting the  $\text{Na}^+/\text{K}^+$  pump. This caused an increase in the level of sodium ions in the myocytes and then led to a rise in the level of  $\text{Ca}^{2+}$ . This inhibition increases the amounts of  $\text{Ca}^{2+}$  ions available for contractions of the heart muscles which improves cardiac output and reduces distention of heart and thus are useful in the treatment of congestive heart failure and cardiac arrhythmia (15). This compound has been reported to be a novel cancer therapeutic agent (16).

Saponins from plants have long been employed for their detergent properties. They are used as mild detergents and in intracellular histochemistry staining to allow antibody access to intracellular proteins. The ethanolic and aqueous leaves extract showed precipitation followed by emulsion formation. In medicine, they are used in hypercholesterolaemia, hyperglycaemia, antioxidant, anti-cancer, anti-inflammatory and weight loss etc. (17,1). Sandeep et al.,(2013) reported that saponins has anti-carcinogenic properties, immune modulatory activity and cholesterol lowering activity. It is also reported to have anti-fungal properties (18).

Terpenes are very important group of organic compounds that have been reported as potent drugs used in treatment of a wide range of ailments. They can range from simple essential oils to the more complex triterpenes and teraterpenes. The most rapidly acting anti-malarial, artemisinin and its derivatives are terpenes (14). The presence of terpenes in ethanolic leaves extract encourages further research possibilities for new drugs leads. The presence of terpenes were in trace amount in petroleum ether and chloroform extracts.

Steroids in modern clinical studies have supported their role as anti-inflammatory and analgesic agents. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones (19).The ethanolic and methanolic leaves extract revealed appreciable and moderate amounts of steroids respectively.

## VI. CONCLUSION

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. Phytochemical test performed in *Moringa concanensis* leaves revealed the presence of Alkaloids, tannin, carbohydrate, saponins, flavonoids, steroids etc in ethanolic extracts. Since the yield of bioactive metabolites in a plant extract also varies considerably with the (20,21) method/solvent of extraction it is plausible that the ethanolic extracts of *Moringa concanensis* Nimmo were generally more potent than the aqueous extracts probably because the active principles in the plant dissolved more readily in and were better extracted by a less polar solvent (ethanol) than water. A plethora of traditional medicine references attest to its curative power, and scientific validation of these popular uses in developing to support to at least some of the claims *Moringa concanensis* preparation known to have antibiotic menstrual pain, constipation, jaundice, diabetes and skin tumours and to reduce cholesterol levels and blood pressure. The results form a good preliminary basis for the

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prioritization of candidate plant species for further in-depth phytochemical and pharmacological investigations toward our quest to unearth lead antiparasitic, anticancer and antimicrobial compounds.

## ACKNOWLEDGEMENT

I am greatly thankful to the management, and lab technicians for providing necessary laboratory facilities to carry out the work.

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