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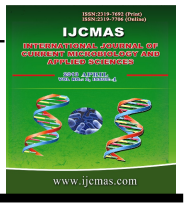

Original Research Article

Evaluation of phytochemical, Pharmacognostical and antimicrobial activity from the bark of *Moringa concanensis* Nimmo.

V.Balamurugan[1,2]* and V.Balakrishnan[3]

[1]Research and Development Centre, Bharathiar University,
Coimbatore-641 046, Tamil Nadu, India.
[2]Department of Biotechnology, Sri Vinayaga College of Arts and Science,
Ulundurpet-606 107, Tamil Nadu, India.
[3]Department of Biotechnology, K.S.Rangasamy College of Technology (Autonomous),
Tiruchengode-637 215, Tamil Nadu, India.
**Corresponding author e-mail: balakal78@gmail.com*

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Antimicrobial activity; phyto-chemical analysis; *Moringa concanensis.*

A B S T R A C T

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. The Indian traditional systems of medicine, especially ayurveda, have put forward a number of therapeutic claims for these plant drugs. The whole plant parts of the tree are used in the treatment of as cites, rheumatism, venomous bites and painful swellings. To the best of our knowledge no scientific report has described traditional claims regarding *M.concanensis.* In the present study the different solvent extracts of *Moringa concanensis* bark were subjected to preliminary phytochemical analysis pharmacognostical and antimicrobial activities against certain bacteria and fungi. The phytochemical analysis revealed the presence of alkaloids, carbohydrates, terpenoids, tannins, reducing sugar and amino acid. The antimicrobial activity was more in chloroform, aqueous extract than the acetone extract.

Introduction

Moringa concanensis Nimmo is a medicinal plant belonging to the family Moringaceae, which is called as Kattumurungai or Peyimurungai in Tamil. It is present in large amount in the district of Perambalur, TamilNadu. Indigenous knowledge of this plant in that region has not been so far studied. Perambalur is one of the District of Tamilnadu is surrounded by South Arcot in the North, Trichirrappalli on south, Salem on west and Thanjavur on the east. *Moringa concanensis* is widely distributed on dry lands.

M. concanensis is a evergreen tree with a spreading crown, up to 8 feet. Leaves alternate, 2-3- pinnate, obovate, caducous. Flowers large, white, hermophrodite, irregular in axillary panicles. Calyx thinly tomentose, long, segments white, oblong, reflexed. Petals yellow, veined with red, oblong. Stamens 5 fertile and 4-5 staminodes. Capsule straight, actively triquetrous, slightly constricted between the seeds. Seeds white or pale yellow 3- angled *Moringa concanensis* is used for treating various human ailments by their own.

The preparation of the drug from this plant is easy and simple. The plant Kattumurungai is entirely different from the Murungai *(Moringa oleifera*). Leaves and flowers are larger in size than *M. oleifera*. The appearance of bark shows distinct feature in both the species of *Moringa*. Bark is very smooth and is very hard in both the plants respectively.

Twenty types of human ailments may cured by using this plant with simple preparations. The therapeutic values of *M. concanensis* are described with disease cured, part used; mode of drug preparation and method of consumption (Anbazhakan *et al.*, 2007*) Moringa concanensis* is a small tree with thick bark, glabrous, except younger parts and inflorescence. Leaves are bipinnate (very rarely tripinnate), ca. 45 cm long. Pods are linear, 30–45 cm long, sharply three-angled. The horseradish odour of *M. concanensis* is more intense than *M. oleifera*. *M. concanensis* has a strong central trunk that is covered with an extremely distinctive layer of very furrowed bark that can be more than 15 cm thick.

The flowers also have distinctive yellow petals, with red or pink veins (Qaiser, 1973; Manzoor, 2007). In addition, *Moringa* is believed to have multiple medicinal qualities. For example, the barks, roots, leaves and flowers of *Moringa* tree are used in traditional medicine and folk remedies in many countries (Anwar *et al.,* 2007; Mormitsu *et al.*, 2000). The stem bark is used to relieve bloating and the gum is used for headache and dental problems. The present study deals to find out the phytochemical characteristics from the bark of *M. concanensis.*

Materials and Methods

Collection of plant materials

The plant materials selected for the present study especially the bark of *Moringa concanensis*. The barks were collected from the Essanai Village of Perambalur District, Tamil Nadu state. After that the plant materials were dried under shade condition. After optimum drying, the bark materials were coarsely powdered separately and stored in well-closed containers for further laboratory analysis.

Preparation of leaf extracts

The dried bark powder material was extracted by using the different solvents such as ethanol, acetone, ethyl acetate, methanol, water, petroleum ether and chloroform, in the increasing order of their polarity (Ozarkar, 2005).The solvent was removed under pressure to obtain a total extracts. Yields were 0.96, 1.94, 0.78, 1.2, 1.7, 1.1, and 1.7% in water, methanol, chloroform, ethanol, petroleum ether, acetone, and ethyl acetate respectively and the extracts were subjected to antibacterial activity assay.

Phytochemical analysis

The plant extracts were analyzed by using the following procedures to test for the presence of the alkaloids, fatty acids, emodins, flavonoids, steroids terpenoids, anthracen glycosides, phenolics, saponins, tannins, xanthoprotein, carbohydrate, cardiac glycosides, amino acids, volatile oils and reducing sugars.

Alkaloids

About 0.2g of the extracts was wormed with 2% H2 SO4 for two minutes. It was filtered and a few drops of Dragondorff reagent were added. Orange red precipitate indicated the presence of alkaloids.

Volatile oils:

Two ml of extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl. A white precipitate is formed if volatile oils are present.

Fatty acids

Two ml of solution was evaporated on a filter paper. A translucent spot indicated the presence of fatty acid.

Emodins

To the few ml of extracts 25% (W/V) ammonium hydroxide solution was added the appearance of red color. Indicated the presence of emodins.

Flavonoids

Four ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour was appeared and its indicate that flavones.

Steroids Triterpenoids

Few ml of the extracts was evaporated and the residues were dissolved in 0.5ml glacial acetic acid followed by the addition 0.5ml chloroform and few drops of concentration H2So4. The appearance of green, red and violet colour indicated the presence of steroids triterpenoids respectively.

Anthracen glycosides

The appearance of red colour on the addition of 25 (W/V) ammonium hydroxide to the extracts indicates the presence of Anthracen glycosides.

Phenols

Few ml of extracts were treated with 2ml of water with four drops of fec13 regent was added. The appearance of blue colour indicates that the presence of phenols.

Saponins

Saponins were detected by using the 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 10ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of Saponins.

Tannins

A small quantity of each extracts were mixed with water and heated on water bath and filtered. A few drops of ferric chloride were added to the filtrate. A dark green solution indicates that the presence of tannins.

Xanthoprotein

Few ml of the extracts were treated with HNo3. A few drops liquid ammonia was

added. Formation of reddish orange or reddish ping colour indicates the presence of Xanthoprotein.

Carbohydrate

A few ml of the extracts 10% (W/V) of NaOH solution was added and heated. Reddish brown precipitated formed presence of reducing sugar.

Amino acids

To two ml of extract few drops of amino acid reagents added too formed yellow or purple colour presence of amino acid.

Reducing Sugars

0.5ml of plant extracts 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath. Brick red precipitate indicates the presence of reducing sugars.

Cardiac glycosides

25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, then five ml of Fehling solution added. Glycosides are indicated by a brick red precipitate.

Fluorescence analysis of the extract

The extract were prepare as per their polarity in hot successive extraction technique. Further it was treated with reagent and the colour changes were observed under U.V light.

Antibacterial Activity Assay

Antibacterial activity of aqueous extracts and solvent extracts was determined by cup diffusion method on nutrient agar medium (Satish *et al.*,1999) . Cups were made in nutrient agar plate using sterile cork borer (5 mm) and inoculum containing bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50 µl each of all aqueous and solvent extracts were placed in the cups made in inoculated plates. The plates were incubated for 24 hours at 37°C and zone of inhibition if any around the wells was measured in mm.

Antifungal Activity Assay

Antifungal activity of aqueous extracts and solvent extracts was determined by disc diffusion method on Sabouraud Dextrose agar medium (Satish *et al.*,1999). Discs were made in Sabouraud dextrose agar plate by using sterile cork borer (5 mm) and inoculum containing fungi were spread on the solid plates with a sterile swab moistened with the fungi suspension. Then 50µl each of all aqueous and solvent extracts were placed in the disc made in inoculated plates. The plates were incubated for five days at 25°C and zone of inhibition if any around the Disc was measured in mm.

Results and Discussion

Extractive value

The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent (Ozarkar, 2005). In the present study, the extract was prepared by 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).

Table.1 Extract value of bark extracts of *Moringa concanensis* with different solvents

S.No	Extract	Value % (w/w)
1	Ethanol Extract	0.96
2	Acetone Extract	1.94
3	Ethyl acetate Extract	0.78
4	Methanol Extract	1.2
5	Aqueous Extract	1.7
6	Petroleum Ether Extract	1.1
7	Chloroform Extract	1.7

The extracts were prepared as per their polarity in hot successive extraction technique. Further they were treated with reagents and the colour changes were observed under ultra violet light. All the results are tabulated in (Table 2).

Preliminary phytochemical analysis

The presence of phytochemicals in *Moringa concanensis* bark extract revealed that, tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer; similar reports were also made by previous Researchers (Ruch *et al.*,1989 and Motar *et al.*,1985). Flavonoids serve as health promoting compound as a results of its anion radicals (Hausteen,1983). These observations support the usefulness of this plant in folklore remedies in the treatment of tress related ailments and as dressings for wounds normally encountered in circumcision rites, bruises, cuts and sores (Mathekga,2001; Lourens,2004; Fergusion,2001 ; Grierson, 1999).Saponins, which are present in plants, have been suggested as possible anticarcinogens. They possess surface-active characteristics those are due to the amphiphilic nature of their chemical structure. The proposed mechanisms of anticarcinogenic properties of Saponins include direct cytotoxicity, immune-modulatory effects, bile acid binding and normalization of carcinogen-induced cell proliferation.

However, the anticarcinogenic effects of Saponins from commonly consumed plant foods have not been studied. (Rao *et al.,* 1995). In the present investigation of Preliminary phytochemical results showed the presence and absence of certain Phytochemical in the extract. The tests were performed using different organic solvents; Aqueous, Acetone, Chloroform, Methanol, Petroleum ether, Ethylacetate, extracts respectively. Phytochemical test revealed the presence of Alkaloids, tannin, carbohydrate Reducing Sugar are generally present in some of the extracts Volatile oils, fatty acids and Anthracene glycosides, Phenoils, Xantho Protein, Cardiac Glycosides are absent in all the extracts. The result was shown in table 3.

Antimicrobial activity

The ethanol, chloroform and aqueous extract showed considerable activity against *Salmonella typhi.* The ethanol extract was more active than the standard against *Salmonella typhii*. Previous study conducted by (Ben Gueddeur, 2002) suggests that the essential oil of *O. majorana* posses antibacterial activity. The work conducted by (Farooqi and Sreeramu, 2004) reveals that the leaves of marjoram have antimicrobial activity against *Escherichia Coli, Pseudomonas aeroginosa, Staphylococcus aureus* and *Salmonella typhi*. Similarly antimicrobial

activity of ethanol, chloroform and water extract of *Marrubium vulgare*, was further assessed against, *Salmonella typhi, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*, were recorded (Al Bakri and Afifi, 2007). In the present study the selected bacteria such *as Pseudomonas* sp., *Staphylococcus* sp., *Bacillus* sp., *Vibrio cholera*, *E. coli, Lactobacillus brevis, Lactobacillus bulgaricus, Micrococcus luteus, Proteus vulgaris*. All the examined extract showed varying degrees of antibacterial activities against the pathogens (Table 4).The antibacterial activity of aqueous extract of *Moringa concanensis* showed maximum zone of inhibition (9 mm) against *E.coli* showed the minimum inhibitory zone (4 mm) against *Pseudomona* sp. 8, 8, 7, 5, 5 and 4 mm inhibition zone was observed.

Table.2 Fluorescent analysis of *Moringa concanensis* bark

S.No	Chemical Test	Ethyl Acetone Extract		Acetone Extract		Ethanolic Extract	
		Day Light	UV Light	Day Light	UV Light	Day Light	UV Light
1	Extract+Aqueous NaoH	Brown	Dark Green	Dark Brown	Light Brown	Dark Green	Light Brown
2	Extract + NaoH	Yellowish Green	Dark Brown	Brownish Yellow	Brown	Brownish Yellow	Yellowish Brown
3	Extract + alc-NaoH	Brown	Yellowish Brown	Dark Yellow	Yellowish Brown	Yellow	Brown
4	Extract + HCL	Greenish Brown	Brownish Green	Brown	Light Yellow	Dark Brown	Light Brown
5	Extract + 50% HNO_3	Dark Green	Greenish Brown	Dark Yellow	Yellowish Brown	Dark Green	Light Green
6	Extract + 50% H_2SO_4	Dark Brown	Dark Brown	Dark Brown	Yellowish Brown	Dark Yellow	Yellowish Green
7	Extract + Methanol	Greenish Yellow	Brownish Yellow	Light Brown	Dark Brown	Brownish Yellow	Yellowish Brown
8	Extract + ammonia	Dark Brown	Brown	Dark Green	Light Brown	Dark Green	Brownish Yellow
9	Extract + I_2 Solution	Green Brown	Yellow	Light Green	Greenish Brown	Dark Green	Light Green
10	Extract + $Fecl_3$	Light Green	Dark Green	Light Brown	Dark Brown	Yellowish Brown	Brownish Yellow

Table.3 Preliminary Phytochemical analysis of *Moringa concanensis* bark extracted with different solvents

S.No	Tests	Aqueous Extract	Acetone Extract	Chloroform Extract	Methanol Extract	Petrolium ether Extract	Ethyl acetate
1	Alkaloids	+	+	+	+	-	-
2	Volatile oils	-	-	-	-	-	-
3	Fatty acids	-	-	-	-	-	-
4	Emodins	-	-	-	+	-	-
5	Flavonoids	-	-	-	+	-	-
6	Steroid Terpenoids	-	+	+	+	-	-
7	Anthracene glycosides	-	-	-	-	-	-
8	Phenoils	-	-	-	-	-	-
9	Saponins	+	+	-	-	-	-
10	Tannins	+	+	+	+	-	-
11	Xantho Protein	+	-	-	-	-	-
12	Carbohydrates	+	+	+	+	-	-
13	Aminoacides	-	-	-	+	+	-
14	Reducing Sugar	-	+	-	+	-	+
15	Cardiac Glycosides	-	-	-	-	-	-

against *Lactobacillus brevis, Micrococcus luteus, Lactobacillus bulgaricus, Staphylococcus* sp. , *Lactobacillus brevis, Vibrio cholera Pseudomons* sp, zone of inhibition for aqueous extract against test bacteria varied significantly. The antibacterial activity of aqueous extract of *Moringa concanensis* showed no zone of inhibition against *Bacillus* sp. and *Proteus vulgaris,* The antibacterial activity of, acetone extract of maximum inhibitory zone (8 mm) against *Proteus vulgaris* 7,7,6,6,6,and 5 mm inhibition zone was observed against *Pseudomons* sp., *Lactobacillus brevis, Staphylococcus* sp. ,*Bacillus* sp., *Lactobacillus bulgaricus* , *E.coli* zone of inhibition for aqueous extract against test bacteria varied significantly. The antibacterial activity of acetone aqueous extract of no inhibitory zone against *Vibrio cholera, Micrococcus luteus.* The antibacterial activity of chloroform extract of *Moringa concanensis* showed maximum zone of inhibition (9 mm) against *Pseudomona* sp. 7,6,6,5and 5 mm inhibition zone was observed against *Micrococcus luteus, Staphylococcus* sp. , *Lactobacillus brevis Bacillus* sp., *Proteus vulgaris*. Zone of inhibition for chloroform extract against test bacteria varied significantly .The antibacterial activity of chloroform extract of *Moringa concanensis* showed no zone of inhibition against *Vibrio cholera* and *E. coli.*

Antifungal activity of the *Moringa concanensis*

Anti fungi activity of the *Moringa concanensis* plant bark extracts was determined against selected fungi showing activities (Table.5.) The Anti fungi activity of aqueous extract of *Moringa concanensis* showed maximum zone of inhibition (6 mm) against *A.oryzae* showed the minimum inhibitory zone (4 mm) against *A. flavus* ,*C. albicans,* 4 mm inhibition zone was observed against *A.sojae* The antifungal activity of aqueous extract of *Moringa concanensis* showed No zone of

inhibition against *A. niger* The Anti fungal activity of Acetone extract of *Moringa concanensis* showed maximum zone of inhibition (6 mm) against *A. niger* showed the minimum inhibitory zone (4 mm) against *A.oryzae,* 5 mm inhibition zone was observed against *C. albicans* The antifungal activity acetone extract of *Moringa concanensis* showed No zone of inhibition against *A. flavus* and *A.sojae.* The anti fungal activity of chloroform extract of *Moringa concanensis* showed maximum zone of inhibition (7 mm) against *A.sojae* showed the minimum inhibitory zone (4 mm) against *A. niger ,A.oryzae* 5 mm inhibition zone was observed against *A. flavus* The antifungi activity chloroform extract of *Moringa concanensis* showed no zone of inhibition against *C. albicans.*

In the present study of emergence of multi drug resistance to human pathogenic infection it has become very necessary to search for new antimicrobial substances from other sources such as plants. In *Moringa concanensis* highly valued plant, with impressive range of medicinal uses and high nutritional value. 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.

Table.4 Antibacterial Activity of *Moringa concanensis* bark

S.No	Organisms	Zone of inhibition (mm)		
		Aqueous Extract	**Acetone Extract**	**Chloroform Extract**
1	*Pseudomona sp*	4	7	9
2	*Staphylococcus sp*	5	6	6
3	*Bacillus sp*	-	6	5
4	*Vibrio cholera*	5	-	-
5	*E.coli*	9	5	-
6	*Lactobacillus brevis*	8	7	6
7	*Lactobacillus bulgaricus*	7	6	-
8	*Micrococcus luteus*	8	-	**7**
9	*Proteus vulgaris*	-	8	5

Table. 5 Antifungal Activity of *Moringa concanensis* bark

S.No	Organisms	Zone of inhibition (mm)		
		Aqueous Extract	**Acetone Extract**	**Chloroform Extract**
1	*A. flavus*	4	-	5
2	*A. niger*	-	6	4
3	*C. albicans*	4	5	-
4	*A.oryzae*	6	4	4
5	*A.sojae*	5	-	7

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