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PRELIMINARY PHYTOCHEMICAL, PHARMACOGNOSTIC EVALUATION AND ANTIMICROBIAL ACTIVITY OF *MORINGA CONCANENSIS* NIMMO LEAF

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

KEY WORDS: *Moringa concanensis, Antibacterial Activity, Phytochemical Analysis, Fluroscence Analysis.*

INTRODUCTION

The plant *Moringa concanensis* Nimmo (Moringaceae), locally known as Kattumurungai by tribal peoples of The Nilgiris in the region of Tamil Nadu state. The plant is a tree, glabrous except the young parts and inflorescence. Flowers in lax divaricated thinly pubescent panicles reaching 45 cm long, segent white, oblong reflexed. Petals are 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[(1)].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 Tamil Nadu, 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. Most of the reports showed that the presence of ascorbic acid[(2)], myristic acid, palmitic acid, oleic acid, stearic acid, arachidic acid and linoleic acid [(3)] 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 *Moringa concanensis* Nimmo.

MATERIALS AND METHODS

Collection of plant materials

The plant materials selected for the present study especially the leaves of *Moringa concanensis*. The leaves 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 leaf materials were coarsely powdered separately and stored in well-closed containers for further laboratory analysis.

Preparation of leaf extracts

The dried leaf 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[(4).] The solvent was removed under pressure to obtain a total extracts. Yields were 2.5, 3.45, 3.65, 3.66, 3.85, 4.25 and 4.45% 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 glycosider, 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% H_2SO_4 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 color was appeared and its indicates 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 color indicated the presence of steroids triterpenoids respectively.

Anthracen glycosides
The appearance of red color on the addition of 25 (W/V) ammonium hydrooxide 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 color 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 color indicates the presence of Xanthoprotein.

Carbohydrate
To 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 color 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 [5]. 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 [5]. 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 50 µl 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

Extractive value
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.

Phytochemical Evaluation
Phytochemical analysis of all the extracts revealed that alkaloids, tannin, amino acids reducing sugar and cardiac glycosides are generally present in the all extracts. Volatile oils, fatty acids and emodins are absent in all the extracts. The results are shown in table 2.

Fluorescence analysis of the extracts
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 3.

Antibacterial Activity
The aqueous, acetone and chloroform extracts were tested at 50 µl concentrations against various microorganisms are presented in Table 1. Antibacterial activity of aqueous extracts recorded as significant antibacterial activity against all the test bacteria. *Lactobacillus bulgaricus* found to highly susceptible to aqueous extract, where as *E.coli* was less susceptible to aqueous extracts. 9, 8, 8, 7 and 5 mm inhibition zone was observed against *Lactobacillus bulgaricus, Pseudomonas sp, Vibrio cholera, Bacillus sp, Micrococcus luteus and E.coli.* Zone of inhibition for aqueous extract against test bacteria varied significantly. Highest antibacterial activity was observed *Lactobacillus bulgaricus* against followed by *Pseudomonas sp, Vibrio cholera, Bacillus sp, Micrococcus luteus and E.*

coli. Eventhough antibacterial activity was observed against other bacteria also it was not detected significant level.

Antibacterial activity of acetone extracts recorded as significant antibacterial activity against all the test bacteria. *Vibrio cholera* found to highly susceptible to acetone extract, where as *Lactobacillus bulgaricusa* and *Micrococcus luteus* were less susceptible to acetone extracts. 9, 9, 6, 5 and 5 mm inhibition zone was observed against *Vibrio cholera, Bacillus sp, Pseudomonas sp, Lactobacillus bulgaricus, and Micrococcus luteus.* Zone of inhibition for acetone extract against test bacteria varied significantly. Highest antibacterial activity was observed *Vibrio cholera* against followed by *Bacillus sp, Pseudomonas sp, Lactobacillus bulgaricus, and Micrococcus luteus* even though antibacterial activity was observed against other bacteria also it was not detected significant level. In chloroform extract *Vibrio cholera* and *E. coli* only having antibacterial activity others not detected. The result was shown in (Table 4).

Antifungal Activity

The chloroform and aqueous extract of *Aspergillus flavus, Aspergillus niger and Aspergillus oryzae* having higher antifungal activity and zone of inhibition. The results were tabulated in (Table 5).

DISCUSSION

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 [6]. In the present investigation, the preliminary phytochemical analysis indicated presence of alkaloids, tannin, amino acids, reducing sugar and cardiac glycosides. It is due to their specific healing properties, healthy action and non-toxic effects [7]. It is interesting to note that antimicrobial activity was highly pronounced in aqueous extract compared to solvent extract. It is also important to note that susceptibility of the bacteria was varied to solvent extract and aqueous extract. This is indicates the presence of more than one active principle in *Moringa concanensis.* Plants are rich reservoir of antimicrobials [8] it is observed that a single plant is known to contain several active principles of biological significance [9]. The present finding is hence highly encouraging in recognizing a plant of interesting antimicrobial activity.

TABLE 1. Extract value of leaf Extracts of *Moringa concanensis* with different solvents

S.No.	Extract	Value % (w/w)
1.	Ethanol Extract	3.66
2.	Acetone Extract	4.25
3.	Ethyl acetate Extract	4.45
4.	Methanol Extract	3.45
5.	Aqueous Extract	2.5
6.	Petroleum Ether Extract	3.85
7.	Chloroform Extract	3.65

TABLE 2. Preliminary Phytochemical analysis of *Moringa concanensis* leaf extracted with different solvents

S.No	Tests	Aqueous extract	Acetone extract	Chloroform extract	Methanol extract	Petroleum ether extract	Ethyl acetate extract
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	+	+	+	+	+	+

TABLE 3. Fluorescent analysis of leaf extract of *Moringa concanensis* with different solvent

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+Aq.NaoH	Yellow	Dark Green	Light Green	Dark Green	Green	Dark Green
2	Extract + NaoH	Yellow	Light Green	Green	Light Green	Yellow	Green
3	Extract + alc-NaoH	Yellowish Green	Dark Green	Dark Green	Light Green	Pale Yellow	Dark Green
4	Extract + HCL	Light Yellow	Green	Greenish Brown	Brownish Green	Dark Green	Brown
5	Extract + 50% HNO_3	Dark Green	Greenish Brown	Yellowish Green	Light Green	Green	Yellow
6	Extract + 50% H_2SO_4	Light Green	Dark Green	Yellow	Dark Green	Dark Green	Yellowish Green
7	Extract + Methanol	Dark Green	Yellowish Green	Green	Dark Brown	Brown	Dark Brown
8	Extract + ammonia	Greenish Brown	Light Green	Brownish Green	Light Green	Green	Brown
9	Extract + $I_{2\ Solution}$	Brownish Green	Dark Green	Light Green	Light Green	Brown	Yellowish Brown
10	Extract + $Fecl_3$	Dark Green	Greenish Brown	Dark Green	Dark Green	Dark Green	Yellowish Green

TABLE 4. Antibacterial Activityof different extract of *Moringa concanensis* leaf

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

TABLE 5. Antifungal Activity of different extract of *Moringa concanensis* leaf

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

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