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Comprehensive Evaluation of Analytical Parameter of *Madhushigru Kanda Twaka* (Stem Bark of Moringa Concanensis Nimmo.)

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ABSTRACT:
Background: *Madhushigru* (*Moringa concanensis* Nimmo.) is one of the most important plant for its nutritional as well as medicinal use in Ayurveda. This present study has been carried out to establish the stem bark of plant for its physicochemical characters with different phytochemical qualitative tests as per API and High-Performance Thin Layer Chromatographic analysis. Physicochemical parameters showed pH (6.28), Loss on drying (7.24 %w/w), Total Ash value (3.95 %w/w), Acid insoluble ash (0.57 %w/w), water soluble extractive (15.01 %w/v) and alcohol soluble extractive (23.39 %w/w). Preliminary phytochemical analysis for the presence of various functional groups such as Alkaloids, Flavonoids, Tannin, Saponin, Steroids were also studied. These observations can be helpful for identification and standardization of *Madhushigru* (*Moringa concanensis* Nimmo.).

KEY WORDS: *Madhushigru*, *Moringa concanesis* Nimmo., Stem bark, Physicochemical, Phytochemical, Analytical.

INTRODUCTION

Madhushigru, botanically identified as *Moringa concanesis* Nimmo. is a tree growing all over the tropical places of world.[1] *Moringa concanensis* Nimmo. is a small sized tree resembling *Moringa concanensis* Nimmo. found in Rajasthan, Dry hills of Konkan, Andhra Pradesha and Coimbtore.[2]

It grows about 10 meters in height. The tree produces long, slender pods that resemble drumsticks. Leaves are alternate, 2-3 pinnate, obovate and caduceus. The leaves are somewhat longer than *Moringa oleifera*. Flowers are large, **white**, hermaphrodite, and irregular in axillary panicles. Calyx is thinly tomentose, long segments, white, oblong and reflexed. Petals are **yellow with red veins** and oblong. Stamens are 5 fertile and have 5 staminodes. Capsules are straight, actively triquetrous, slightly constricted between seeds. Seeds are three angled white and pale yellow in colour.[3]

The tribal people use it for eye care, leukorrhea, thyroid problems, menstrual pain, splenomegaly, jaundice, aphrodisiac, tiredness, high blood pressure, constipation, intestinal worms, diabetes, headache and spinal cord pain[4]. General properties of this plant are similar to *Moringa oleifera*.[5]

Many studies have been conducted on different part of this plant such as Pharmacongnostical studies of different part (Flowers, Leaves, Roots, Stem bark etc.), Pharmacological studies and Clinical studies mainly carried out for eye and ENT disorders.

Table No. 1 -Taxonomical classification of *Moringa concensis* Nimmo.[6]

Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Sub class	Magnoliidae
Order	Brassicales
Family	*Moringaceae*
Genus	*Moringa*
Species	*Moringa concanensis*

Table No. 2-Vernacular names of *Madhushigru*[7]

English	Konkan Moringa
Hindi	Jangali Sargua, Senjana
Gujrati	Kadvo saragvo
Marathi	Mashinga, Rava Shevga
Kannada	Nugge, Kaadu Nugge
Tamil	Katu Murangi

AIM:

To perform Physicochemical, Phytochemical and HPTLC study of *Madhushigru* (*Moringa concanensis* Nimmo.) stem bark.

OBJECTIVE:

1. To determine physicochemical parameter of *Madhushigru* (*Moringa concanensis* Nimmo.) stem bark.
2. To determine preliminary phytochemical parameters of *Madhushigru* (*Moringa concanensis* Nimmo.) stem bark.
3. To determine High Performance of Thin Layer Chromatography (HPTLC) of *Madhushigru* (*Moringa concanensis* Nimmo.) stem bark.

MATERIAL AND METHODS

Stem Bark of *Madhushigru* (*Moringa concanensis* Nimmo.) was collected from Netranga, located in Rajpipala, Gujarat, is positioned at a latitude of 21.8653° N and an altitude of approximately 73.5001° E meters above sea level, after proper identification from Ayurvedic Pharmacopeia of India. After proper drying stem bark was converted into 80 mesh powder form in the controlled environment at Pharmacognosy Laboratory of Upgraded Department of Dravyaguna, GAC, Vadodara.

1. Physicochemical Parameters

Assessment of the parameter such as pH, loss on drying, ash value, acid insoluble ash, water soluble extractive and alcohol soluble extractive was carried out by following standard procedure recommended by

Ayurvedic Pharmacopoeia of India and other standard texts. Detailed outcome of physicochemical parameters are given in table no. 6.

Determination of Moisture (Loss on drying):

In this method, 5 g powder of stem bark *Madhushigru* was taken in evaporating dish then put into the hot air oven at 105^0 Celsius temperature for 1 hour than evaporating dish was kept in desiccator for cooling and then weight. And continues dry and weighing until the difference between two successive weighing correspond more than 0.5 percent.

The % of loss on drying = $\frac{\text{Difference in weight after heating} * 100}{\text{Weight of sample taken}}$

Determination of total ash:

2 g powder of *Madhushigru* stem bark was taken in crucible than it put in electric muffle furnace at 450 ° Celsius temperature until free from carbon and then kept in desiccator for cooling and weight was taken. Then calculate the percentage of ash with reference to the air-dried drug.

Wt. of Silica crucible = A_1 gm, Wt. of Sample(X) = X gm, Wt. of Silica crucible with Ash = A_2 gm.

Percentage of Total ash = $(A_2 – A_1 / X) *100$

Determination of Acid insoluble ash:

Ash of *Madhushigru* was taken than 25 ml diluted HCL was added and insoluble matter was collected on Ash less filter paper and washed with hot water until the filtrate is neutral. Transfer the filter paper containing insoluble matter to the crucible and dried on hot plate. Ignite to constant weight in muffle furnace. Allow the residue to cool in suitable desiccator for half hour and count the weight. And calculate the content of acid insoluble ash with reference to the air-dried drug.

Acid insoluble Ash value of the sample= $\frac{100*a}{y}$ %

a= weight of the residue, y= weight of the drug taken

Determination Water soluble extractive:

5 g powder of *Madhushigru* stem bark and 100 ml distilled water mixed in glass beaker than shaking frequently during 6 hours and allowing standing for 18 hours. Filter rapidly, taking precaution against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish and was evaporated to dryness on water bath. It was followed by drying at 105^0C for 6 hours. Cooled in a desiccator for 30 minutes and was weighed without delay. Calculate the percentage of Water extractive value with reference to air dried drug.

Determination Alcohol soluble extractive:

This process same like to the Water-soluble extractive but in this procedure methanol solution was used instead of distilled water.

Determination pH value:

Take a two types of Buffer solution no 4 and 7 and digital pH meter then take a buffer solution in beaker and deep the electrode in it. Carry out the same exercise for another buffer solution also, after washing the electrode thoroughly with distilled water, then take a sample and dip the electrode in it and then calculate the value.

Table No. 3: Values of Physicochemical Parameters of Stem Bark of *Madhushigru*

Physicochemical Parameter	Results
Loss on drying(%w/w)	7.04
Total Ash value(%w/w)	3.95
Acid insoluble ash(%w/w)	0.57
Water soluble extractive value(%w/w)	15.01
Alcohol soluble extractive value(%w/w)	23.39
pH (1% Solution)	6.28

2. Preliminary Phytochemical analysis of Stem Bark:

By performing various qualitative tests, one can get idea about the type of Phyto constituents present in the sample. Hence the powder of samples subjected for following tests. Preparation of plant extracts is as per shown in below table no. 7.

Table No.4: Steps for preparation of Plant extracts[8]

Step 1	Samples of powder taken 5 gm and was extracted with methanol (100ml).
Step 2	Kept it for overnight. Initially occasional shaking up to 6 hours was performed and then kept aside for settle down.
Step 3	After 24 hours, it was filtered and alcoholic extract was collected
Step 4	Similarly, water extracts of sample were prepared and collected
Step 5	After that, qualitative tests were done by using appropriate extracts

Phytochemical analysis was performed with following the standard protocol given in API.[9] As like - Alkaloid - Dragendorff's reagent, Saponin - Foam test, Flavonoids, Tannin, Steroids. Observations are mentioned in table no. 8.

Table No. 5: Result of Preliminary Qualitative tests of Stem bark of *Madhushigru* (*Moringa concanensis* Nimmo.)

Plant Metabolites	Results
Alkaloid	+ve
Saponin	+ve
Flavonoids	+ve
Tannin	+ve
Steroids	-ve

3. Sophisticated 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.

Preparation of Test Solution: Weight approximately 1 g of sample in a reflux flask. To it add 20 ml of chloroform and reflux it for 30 min on water bath. On completion of time, remove the flask from the water bath and filter with the help of whatmann No. 1. Use the test solution thus obtained for HPTLC fingerprinting.

Preparation of Spray reagent [Anisaldehyde – Sulphuric acid reagent]: 0.5 ml Anisaldehyde **is** mixed with 10 ml Glacial acetic acid followed by 85 ml methanol and 5 ml sulphuric acid (98 %).

HPTLC of *Madhushigru* was scanned under 254nm, 366 nm and 540nm. Observation of Rf value and number of spots at different visualization tabulated in table no. 9.

Table No. 6: Observation of Rf value and number of spots at different visualization

Visualization	Rf Value	Number of Spots
254 nm	0.55, 0.87	2
366 nm	0.19, 0.84, 0.90	3
540 nm	0.14, 0.25, 0.31, 0.55, 0.68, 0.88, 0.90	7

RESULTS AND DISCUSSION

In the present study, standardization of stem bark of *Moringa concanensis* Nimmo. was done which included physicochemical and phytochemical analysis. This provides the easy, speedy and economical means to establish the identity and purity of drug and it also provides a reliable method for identifying adulteration.

In physicochemical analysis parameters like total ash value, acid insoluble ash and extractive values were estimated which serves reliable aid in identification of adulteration and identification of plant material. Ash value gives an idea about inorganic composition and other impurities present with drug. Extractive values are the values which gives the knowledge of chemical constituents of crude drugs that are soluble in particular solvent, which are helpful to determine exhausted and adulterated drugs. Loss on drying should be at minimum level so the bacteria, fungi etc. will not grow during the time of storage.

The chemical constituents of plants/herbs contribute to their physiological properties and consist of primary metabolites, viz., sugars, amino acids, and proteins, along with secondary metabolites such as alkaloids, flavonoids, tannins, saponin, steroids etc. In the present study, different qualitative tests were carried out with the methanol extracts of the *Moringa concanensis* stem bark powder. The results of the phytochemical screening revealed the presence of alkaloids, saponins, tannins, and flavonoids but absence of steroids in the extracts of this the sample.

In HPTLC profile, each and every metabolite has played specific role and function in harmony with other metabolites within the organization framework of the cells in the defence mechanism of the plants. Here in this HPTLC study different peaks are observed at different Rf. Total 2 peaks are observed at 254nm, and 3 peaks are observed at 366nm and 7 peaks are observed at 540nm of UV light out of which 2 peaks resembling each other at Rf 0.50 and 0.90. The number of observed peaks shows the presence of numerous active constituents in the given sample of the stem bark of *Madhushigru*.

CONCLUSION

Madhushigru (*Moringa concanensis* Nimmo.) is an important plant which is used in many diseases mentioned in Ayurvedic literature. This study shows the Phytochemical analysis and physicochemical parameters that can be used for the identification in further research study.

Plate No: 2

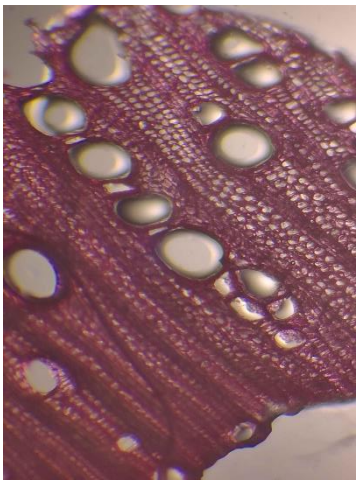


Fig. 3c (Xylem, Phloem)

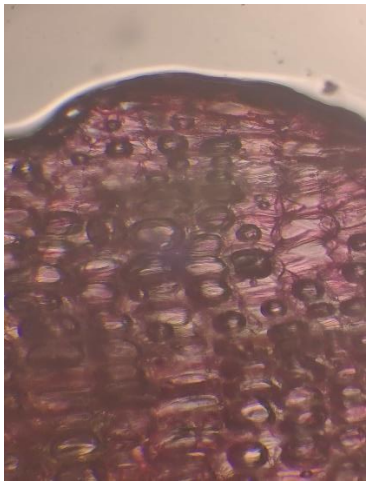


Fig. 3d (Crystals)

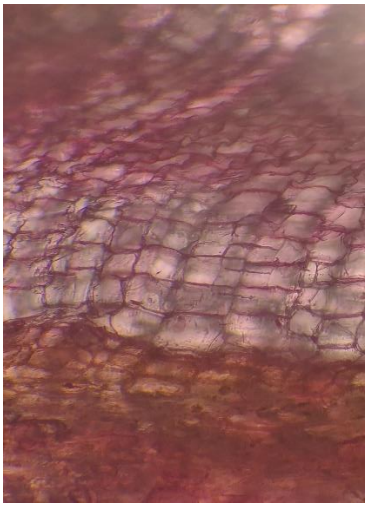


Fig.3b (Cork cambium, Sclereid)

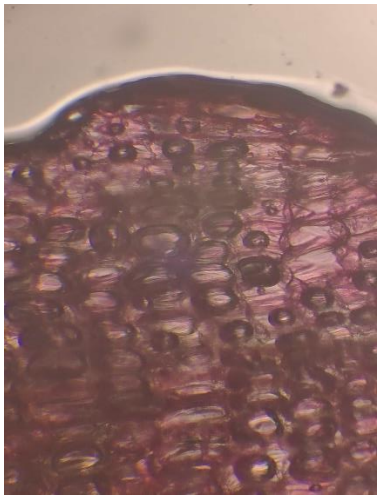
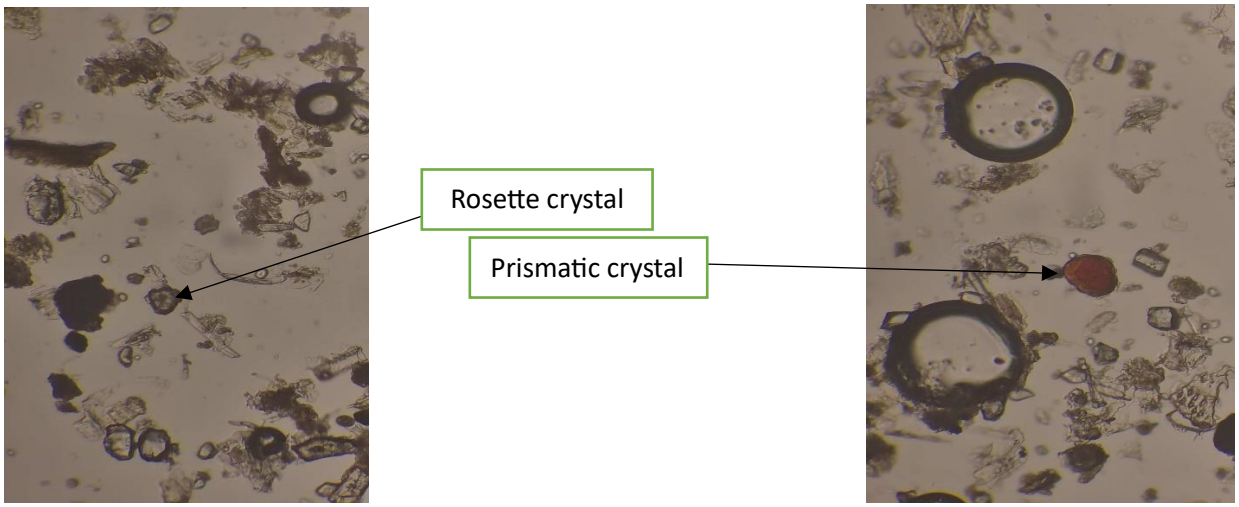
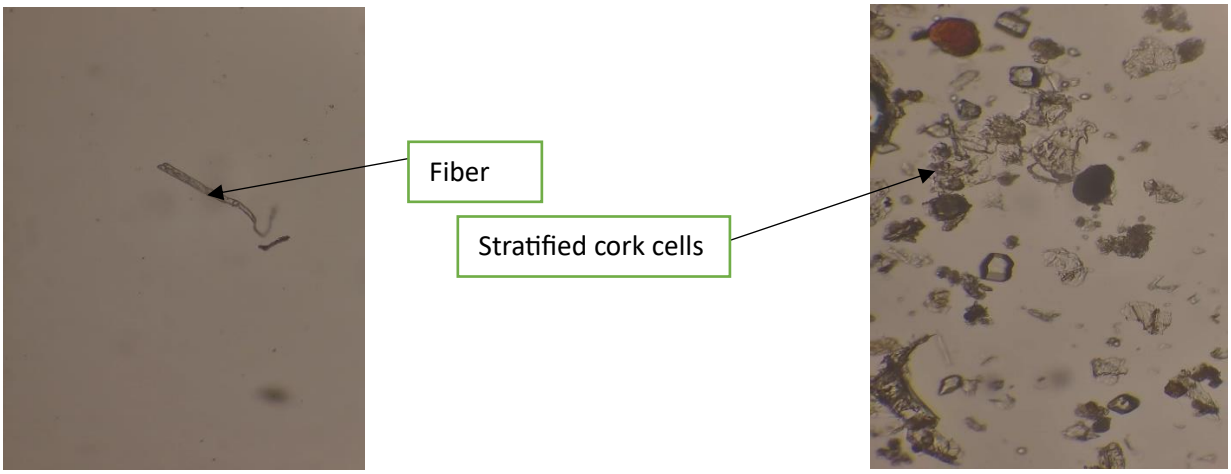
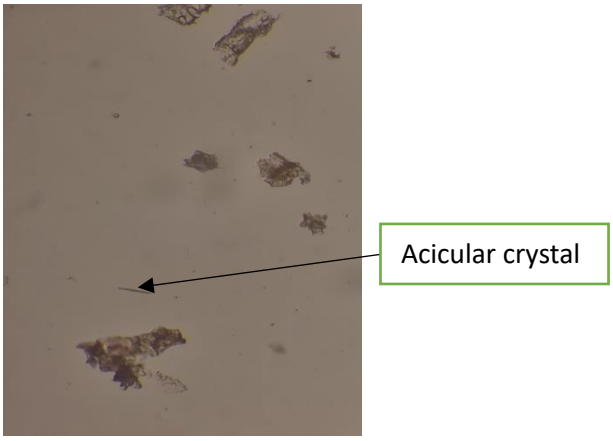


Fig. 3a (Periderm)

Plate no: 3







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