Current Research in Pharmaceutical Sciences
Available online at www.crpsonline.com

ISSN 2250 – 2688

Received: 08/08/2014
Revised: 15/09/2014
Accepted: 27/09/2014

Priyanka Gupta
Dept. of Pharmacy, AKS University, Sherganj Road, Satna- 485446 (M.P.)

Vikas Jharia
Cipla Pharmaceuticals ltd. Indore- (M.P.)

Correspondence

Priyanka Gupta
Dept. of Pharmacy, AKS University, Sherganj Road, Satna- 485446 (M.P.)

E-mail:
priyankagupta0804@gmail.com

Preliminary Standardization, Extraction & Toxicity study of *Moringa Oleifera* Lam. Stem Bark

Priyanka Gupta and Vikas Jharia

ABSTRACT

Moringa oleifera Lam [Moringaceae] is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. It is known by such regional names as drumstick tree, Sajiwan, kelor, Murungai kaai, marango, mulangay, saijhan, and sajna. Different parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods contain a profile of important minerals, and are a good source of protein, vitamins, β -carotene, aminoacids and various phenolics which act as cardiac and circulatory stimulants, possess anitumor, antipyretic, antiepileptic, antiinflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities, and are being employed for the treatment of different ailments in the indigenous system of medicine. This plant have broad spectrum activities so, further studies on other models and extensive clinical trials are needed to confirm these findings. The present study was undertaken to screen various phytochemicals like flavonoids and terpenoids present in the stem bark and evaluate the potentials for use of this plant, which will help to formulate effective herbal preparation that will be used to combat various disorders.

Keywords: *Moringa oleifera*, β –carotene, antitumor, Drumstick tree, Phytochemical, Nutritional value.

1. INTRODUCTION

Moringa oleifera is a versatile and exceptionally nutritious vegetable tree with a variety of potential uses. It is the most widely cultivated species of Moringaceae family. It can grow well in the humid tropics or hot dry lands, can survive destitute soils, and is little affected by drought. *Moringa oleifera*, native of the western and sub-Himalayan tracts, India, Pakistan, Asia Minor & Africa[1].

Commonly it is known as in English – Moringa or Drumstick tree or Horseradish tree, in Hindi - Sahjan, in Latin – Moringa oleifera, in Sanskrit - Surajana, in Nepali - Sajiwan or Swejan etc. It is useful not only for human beings but also for animals and also in various industrial applications[2]. Different parts of the plant have been used in Indian traditional system of medicine viz. stem bark for the treatment of alzheimers, parkinsons, anti-inflammatitory, anti-asthmatic, anti-anaphylactic, anti-oxidant activity[3].

Moringa oleifera have broad activities like Galactagogue, Rubefacient, Antiscorbutic, Diuretic, Stimulant, Purgative, Antibiotic, Antifungal, Antimicrobial, Antibacterial, Antiinflammatory, Antitumor, Antioxidant, Anti-aging, Estrogenic, Antiprogestational, Hypoglycemic, Antihyperthyroidism, Anti-ulcer, hypocholesterolemic, Antispasmodic, decreasing blood pressure, relieving headaches and migraines etc[4]. The high antioxidant/radical scavenging effects observed for different parts of *M. oleifera* appear to provide justification for their widespread use in traditional medicine in different continents[2].

However detail investigation of stem bark of *Moringa oleifera* had not been carried out so far. Hence this leads us to study the standardization of stem bark of *Moringa oleifera* through various pharmacognostical evaluations on the basis of which provides information for further development.

2. MATERIALS AND METHODS

2.1 Plant material

Stem bark of the plant *Moringa oleifera* has been collected from local garden of AB. Road, Indore (M.P.). Authentification of plant on basis of pharmacognostic study and organoleptic characterstics was done by Botanical survey of India Pune. A voucher specimen no. BSI/WC/Tech./2011/904 of bark of *Moringa oleifera* has been deposited in museum of Dept. of Botany, Botanical survey of India.

2.2 Chemicals and Reagents

All the chemicals and reagents used were purchased from Kasliwal Brothers and S.K. Traders Indore.

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2.3 Preparation of the Plant Extracts

Dosage form of the extract was prepared with distled water. The extract was provided by oral administration. The volume of administration of drug was calculated based upon the body weight of animal. Volume of drug administration was kept constant with respect to their body weight (10 mL/kg). The extract was kept in dessicator and after that in air tight container. The dosage form was prepared freshly at the time of administration.

2.4 Experimental animals and protocol

Albino wistar rats of both sex weighing between 150-250 g were used. The experimental protocol was approved from Institutional Animal Ethics Committee. Animals were housed under standard conditions of temperature (24 ± 2°C) and relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were kept for 1 week to acclimatize to laboratory conditions before starting the experiment; they were given free access to water and standard rat feed except during experimentation. All experimental procedures and protocols used in this study were revised and approved by the Institutional animal ethical committee (IAEC) of college of pharmacy IPS Academy, Indore, constituted under Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). Ethical guidelines were strictly followed during all the experiments (Protocol no. CPCSEA/46/2011)[5].

2.5 Determination of Physical Constants

2.5.1 Macroscopy

The plant stem bark has been observed for its appearance, size, shape, colour, odour, taste and smell.the plant has also been authenticated by Botanical survey of India, Pune.

2.5.2 Microscopy

Stem bark was dried under shade and powdered manually. Boiled with water in the ratio of 1:16 (decoction) untill it remains one eighth part. Extract obtained, concentrated and Store in air tight container. The stem bark has been allowed to soak in the microscopic slide.

2.5.3 Ash values

Ash values are helpful in determining the quality and purity of a crude drug, especially in the powdered form. The objective of igniting vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a crude drug reflects the care taken in its preparation. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high[6].

2.5.4 Total ash value

Weighed accurately about 2 to 4 g of the powdered drug in a tared silica crucible. Incinerated at a temperature not exceeding 450 – 550º C for 4 h, until free from carbon, cooled and weighed. Calculated the percentage of ash with reference to air-dried drug using following formula[6].

% Total ash value = Wt. of total ash x100 /Wt. of crude drug taken

2.5.5 Water soluble ash value

Boiled the ash with 25 ml of water. Filtered and collected the insoluble matter on an ashless filter paper, washed with hot water and ignited in a tared crucible at a temperature not exceeding 450ºC for 4 h. Cooled in a desiccator and weighed. Subtracted the weight of insoluble matter from the total weight of ash.

The difference in weight represented weight of water soluble ash. Calculated the percentage of water soluble ash with reference to the air-dried drug using the following formula[6].

$$\% \text{ Water soluble ash value} = \frac{\text{Wt. of total ash - Wt. of water insoluble ash X 100}}{\text{Wt. of crude drug taken}}$$

2.5.6 Acid insoluble ash value

Boiled the ash for 5 min with 25 ml HCl. Filtered and collected the insoluble matter on an ashless filter paper, washed with hot water and ignited in a tared crucible at a temperature not exceeding 450ºC for 4 h. Cooled in a desiccator and weighed. Calculated the percentage of acid insoluble ash with reference to the air-dried drug using following formula,

$$\% \text{ Acid insoluble ash value} = \frac{\text{Wt. of acid insoluble ash x 100}}{\text{Wt. of crude drug taken}}$$

2.6 Plant extraction

The ayurvedic literature reveals the traditional claim for the use of fresh bark of *Moringa oleifera* for the treatment of ulcer (vrad dosh nasak). The bark is powdered and lepa is applied externally and given orally. The decoction of the plant is prepared as shobbhajanakwatha for the treatment of various ailments as in spleen enlargement, since the traditional claim involves its use by administrating the plant as decoction[7].

The decoction is prepared where the bark is powdered in coarse particles then according to the part of plant it is boiled in water and decoction is prepared, if hard part like bark then 16 times water is taken, if medium hard then 8 times and if soft part then 4 times water is added to make decoction and it is boiled till the extract remains one eighth of the prior addition of water. The decoction is claimed traditionally to use it as according the type of disease. The shelf life of the decoction is 48 hours[8].

The percentage yield of extract was calculated by using following formula

$$\text{Percentage yield} = \frac{\text{Weight of powdered extract x 100}}{\text{Weight of powdered drug taken}}$$

2.6 Phytochemical screening

For the qualitative phytochemical screening, 1 % aqueous solution of the extract in distilled water was used[9] (Table 1).

Table 1. Methods used for the qualitative identification of major group of compounds

Phytochemicals tested	Test method
Alkaloids[10]	Dragendorff's, Hager's, Mayer's and Wagner's reagent[10]
Flavonoids[11]	Shinoda test[11]
Glycosides[11]	Fehlings test[11]
Tannins & Phenols[11]	Ferric chloride test[11]
Amino acids[9]	Ninhydrine test, Millon test[9]

2.7 Qualitative Phytochemical Test

2.7.1 Alkaloids

Preparation of test solution: the test solution was prepared by dissolving extract in the dilute hydrochloric acid solution.
Mayer's test: The acidic test solution with 2ml Mayer's reagent (Pot. Mercuric iodide) gave cream coloured precipitate[10].
Hager's test: The acidic test solution with 2ml Hager's reagent (Saturated picric acid solution) gave yellow precipitate[10].
Dragendorff's test: The acidic solution with 2ml Dragendorff's reagent (Potassium bismuth iodide) showed reddish brown precipitate[10].
Wagner's test: The acidic test solution treated with 2ml Wagner's reagent (Iodine in Potassium iodide) gave brown precipitate[10].

2.7.2 Amino acids

Preparation of test solution: Prepared test solution by dissolving the extract in water (1%).
Ninhydrin test: Test solution treated with 2ml Ninhydrin reagent gave blue colour[9].
Millons test: To the test solution add about 2 ml of Millons reagents, a white precipitate indicate presence of amino acids[9].

2.7.3 Tannins and phenolic compounds

Small quantity of extract was mixed with water in the proportion of 1%, and heated on water bath. The mixture was filtered and 2ml ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins[11].

2.7.4 Saponins

About 0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins[11].

2.7.5 Flavonoids

Extract of about 0.2 g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless indicate presence of flavonoids[11].

2.7.6 Glycosides

The extract about 0.2 g was hydrolysed with HCl solution and neutralized with NaOH solution. A few drops of fehling solution A and B were added. Red precipitate indicates the presence of glycosides[11].

2.7.7 Proteins

Preparation of Test Solution: The test solution was prepared by dissolving the extract in water.
Biuret test: Test solution was treated with 40% sodium hydroxide and addition of 2 ml dilute copper sulphate solution gave blue colour[9].
Xanthoproteic test: Test solution was treated with conc. HNO_3 and boiled which gave yellow precipitate [9].

2.7.8 Terpenoids (Salkowaski test)

0.2 g of the extract of the whole plant sample was mixed with 2 ml of Chloroform and concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interfaces was formed to indicate positive results for the presence of terpenoids[11].

2.8 Quantitative phytochemical test

2.8.1 Alkaloids content

5 g of sample in 250 ml beaker was taken to this 40 ml of 10% acetic acid in ethanol was added then it was covered with aluminum foil and allowed to stand for 4 h. Filtered and filtrate was concentrated on water bath to one-quarter of the original volume. Conc. ammonium hydroxide was added dropwise to the extract until precipitate was completed and precipitate was allowed to settle down and collected and washed with ammonium hydroxide and then filtered. The alkaloids residue dried and then weighed. Value was expressed on g % of dry weight[12]. The value is expressed in table 3.

2.8.2 Flavonoid content

10 g of sample was extracted repeatedly with 100 ml of 80% methanol at room temperature. Filtered through whattman filter paper no. 42 (125 mm), filtrate was transferred into crucible then evaporated to dryness over water bath. Value was expressed on g % of dry weight[12]. The value is expressed in table 3.

2.9 Acute toxicity study[5]

Five groups (n=5) of female albino wistar rats were used in the acute toxicity study of aqueous extract of *Moringa Oleifera*. Animals of all groups were fasted overnight and administered (p.o.) with single dose of 175, 550, 2000, 2000 and 2000 mg/kg of the aqueous extract of the stem bark. Changes in the behavior of animals were observed for 24 h after extract administration. For any sign of toxicity and mortality, animals were observed for 14 days.

The dosing schedule as per the OECD guideline (425) was followed. One rat received a dose at a particular time. First rat received a dose 175 mg/kg p.o. the rat was observed for 3 h, after drug administration, for any toxicity sign, survival or death. If the first rat were died or appear moribund, the second rat received a lower dose (55 mg/kg). The dose progression and reduction factor was 3.2 times of the previous dose. If no mortality was observed in the first rat then the second rat received a higher dose (550 mg/kg). Dosing of next rat was continued depending on outcome previous dosed rat for a fixed time interval (3h).

3. RESULT & DISCUSSION

3.1 Macroscopy

Mature bark, rough, deeply cracked, grey or dark green, 1-3 cm thick depending upon the age of plant; taste bitter and pungent. When wounded, the bark exudes a gum which is initially white in color but changes to reddish brown or brownish black on exposure. The wood is soft and light.

3.2 Microscopy

Cork region very wide, composed of 15-20 layers, thin walled radially arranged, rectangular cells with coloured contents; cork cambium consists of a single thin walled, rectangular elongated cells; secondary cortex very wide composed of cubical to rectangular parenchymatous cells with few crystals of calcium oxalate; several groups of thick walled olygonal stone cells and round to oval starch grains and few oil globules; secondary phloem with many mucilage cavities (fig 1).

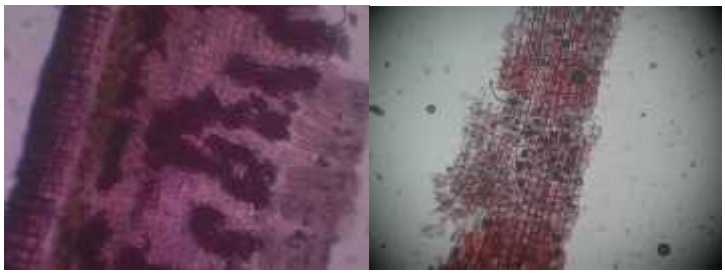

Fig. 1: Microscopy of stem bark of *Moringa oleifera*

3.3 Percentage Yield of Extract

Percentage yield = wt. of extract x 100 / wt. of powdered drug extract

The percentage yield calculated was 12.5%

3.4 Ash Values

Ash value of the crude bark is expressed in table 2.

Table 2. Ash Values

Ash Values	
Total ash	8 % w/w
Acid-insoluble ash	0.5 % w/w
Water soluble ash	8.5 % w/w

3.5 Phytochemical Screening

Preliminary phytochemical analysis of all extracts revealed prescence of alkaloids, carbohydrates, proteins. The other secondary metabolites like flavonoids, terpenoids, etc are also present. The phytochemicals present in various extract are presented in table 3 and table 4.

3.6 Acute toxicity study

The acute toxicity study was carried out to select the dose, by using up & down method. The rats showed no death upto 14 days study period. Even at the high dose no physical signs of toxicity were observed as evidenced by normal breathing and absence of tremors, convulsions, diarrhoeas, salivation, and paralysis, only sedation was observed during first three hours in the treated animals as represented in Table 5.

These observation revealed that the oral LD_{50} of the extract is greater than 2000mg/kg in rat as represented in table 6. Observation of animals over the next 14 days showed no adverse effect of treatment.

Table 3. Qualitative Chemical Analysis of Aqueous Extract of *Moringa oleifera* Bark

Test	Aqueous extract	
	Observation	Inference
Tannic acid	Coloured precipitate	Presence of Alkaloids (+)
Froth formation	Froth Formation	Presence of Saponins
Acidic $FeCl_3$	Blue-black precipitate	Absence of Tannins
NAOH test	Flavonoids dissolved giving yellow colour	Presence of Flavonoids
HCI test	Solution turns colorless with HCI	
$FeCl_3$	Dark green	Absence of Phenols
Libermann Buchard's	Green- blue colour	Presence of Phytosterols
Salkowski	Reddish brown	Presence of Terpenoids
Fehling's solution	blue colour of Fehling's solutions turned brick red ppt.	Presence of Reducing sugar(Carbohydrates) (++)
Benedict's solution	green colour	Presence of Reducing sugar (++)
Biuret's	violet colour	Presence of Proteins (+)
Heller's	Ppt. and junction formed at two fluid	Presence of Proteins (+)

+indicates presence, ++ more clarity

Table 4. Quantitative Chemical Analysis of Aqueous Extract of *Moringa oleifera* Bark

Test	Percentage
Alkaloids	4.2%
Flavonoids	12%

No mortality was observed after treatment with the highest tested dose (2000 mg/kg *p.o.*) of the aqueous extract. The extract was found to be safe upto the dose of 2 g/kg *p.o.*

The LD_{50} was greater than 2000 mg/kg for the aqueous extract of the stem bark

Table 5. Changes in the animal behavior after administration of *Moringa Oleifera* Extract 2000mg/kg dose

Parameter	Time after administration (h)					
Gross activity	2	3	5	7	12	24
Respiration	-	-	-	-	-	-
Writhing	-	-	-	-	-	-
Convulsion	-	-	-	-	-	-
Salivation	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-
Mortality	-	-	-	-	-	-
Sedation	+	+	-	-	-	-
Skin irritation	-	-	-	-	-	-

+ indicates that change was observed;
– indicates that there was no change

Table 6. Determination of acute oral toxicity and LD_{50} of aqueous extract of *Moringa oleifera*

Animal ID	*Moringa oleifera* Dose(mg/kg)	Result (0/X)
I	175	0
II	550	0
III	2000	0
IV	2000	0
V	2000	0

0 indicate the animal was alive
X indicate the animal was died

4. CONCLUSION

Inspite of use of various alternate medicine therapies, use of herbal therapy is advancing day by day needs as they constitutes a better and important source of potential therapeutic agents. This plant has been observed as a very nutritional evergreen remedy to rejuvenate and for various ailments during the ancient peroid of time. Hence the present study was to investigate the potentials of *Moringa oleifera* stem bark.

Moringa oleifera stem bark was assessed for active principles. The result showed that the plant possessed the active principles like alkaloid, flavonoids, proteins, terpenoids, saponins and glycosides. These metabolites are usually responsible for the pharmacological activities of medicinal plants.

Moringa oleifera contains a number of flavonoids, triterpines, steroids, alkaloids, and many other chemical constituents[13]. The high antioxidant/radical scavenging effects observed for different parts of *M. oleifera* appear to provide justification for their widespread use in traditional medicine in different continents[2]. It is a versatile and exceptionally nutritious vegetable tree with a variety of potential uses. It is useful not only for human beings but also for animals and also in various industrial applications.

The extract is non toxic even at relatively high concentrations. The acute toxicity studies revealed it non-lethal up to the dose of 2000 mg/kg body weight of the animals, and hence these preliminary studies regarding various qualitative and quantitative assessment and phytochemicals, present in the stem bark. Further studies are being carried out to characterize and explore the biological activities that may be present in the extract that will help help to formulate an effective herbal preparation that will be used to combat various disorders.

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