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VALIDATION OF TRIBAL CLAIMS ON *MORINGA OLEIFERA* LAM. USING PHYTOCHEMICAL ANALYSIS

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ABSTRACT

Moringa is a medicinally important genus that has long history of traditional use as a remedy to cure wounds and various ailments such as colds, diabetes, digestive problems etc. In addition, the species is consumed as a source of nutritive food and used as vegetables worldwide. The genus consists of 13 species that have been cultivated throughout Asia and Africa for their multiple purpose use value. The current study is aimed to validate the traditional medicinal uses of *Moringa oleifera*, provide scientific insights on the phytochemistry, biological activities and thereby correlating its therapeutic potential for future prospects. Analysis of phytochemical profile showed the presence of the major important bioactive compounds (saponin, tannin, flavonoids, phenolics, and reducing sugar) that were assessed in aqueous, methanolic aqueous and acetone-aqueous extracts following standard procedures. This study provides the foundation to explore the tribal medicinal use complemented with the scientific evaluation. Our study reinforce further phytochemical study with advanced technologies for future research opportunities of this species as it is an interesting plant containing commercially important active compounds that enable to determine pharmacological significance, and socio-economic potential.

Keywords: *Moringa oleifera*, Phytochemical analysis, Medicinal uses

INTRODUCTION

Moringa oleifera Lam. (Moringaceae) is a small, graceful, deciduous tree with sparse foliage, often resembling a leguminous species at a distance, especially when in flower, but it shows difference in its fruit. The tree grows to 8 m high and 60 cm. Bole crooked, often forked from near the base. Bark smooth, dark grey; slash thin, yellowish. Twigs and shoots short but densely hairy. Crown wide, open, typically umbrella shaped and usually a single stem; often deep rooted. The wood is soft that produces resin exudates (Gopalakrishnan *et al.*, 2016).

Anatomical structures of *Moringa oleifera*

a) Stem: In young stem 16 - 18 collateral vascular bundles are present in a ring. Pith is large and parenchymatous. Pericycle is composed of alternate groups of fibers and parenchyma cells in young stem but it turns into a complete circular band of fibers in old stem. In old stem vascular cambium produces small amount of secondary phloem and large amount of secondary xylem, which is composed of uniseriate xylem rays, lignified thick-walled xylem fibers and roundish vessel elements (Haines, 1924; Saxena and Brahmam, 1995).

b) Root: Young root has tetrarch xylem. In old root

vascular cambium is 6 - 8 layered which produces many roundish vessel elements embedded in large number of xylem parenchyma cells. Starch grains are observed in xylem parenchyma. Phellogen is 3 - 4 layered, which forms phellem of rectangular or squarish cells. Phellogen is large and consisted of thin walled parenchymatous cells. Many of them contain tannin. Scattered groups of fibers are embedded in this region. Wall of phellem cells is suberized (Haines, 1924; Saxena and Brahmam, 1995).

c) Leaf: Lamina epidermal cells of adaxial surface are larger than cells of abaxial epidermis. Palisade cells are elongated, present in a single row and have dense tanniferous content. Stellate crystals of oxalate are observed in cells of lamina, midrib and petiole. Cell wall of epidermal cells is sinuous on abaxial side and non-sinuous on adaxial side. Leaf is hypo-stomatic with anomocytic stomata. Contiguous stomata at right angle to each other are also observed (Haines, 1924; Saxena and Brahmam, 1995).

d) Midrib: The main parts of midrib have hypodermis, cortex and a large semi - triangular collateral vascular bundle. Hypodermis is made of collenchyma and present only on abaxial side. Parenchymatous cells are observed below abaxial epidermis. Cortex is parenchymatous (Haines, 1924; Saxena and Brahmam, 1995).

e) Petiole: Cells of epidermal and sub epidermal layers of petiole are radially elongated. Some of them have tanniferous content. The rest of the part of petiole is made up of central vascular cylinder and collenchyma cells containing large number of oxalate crystals (Haines, 1924; Saxena and Brahmam, 1995).

f) Rachis: Epidermis has thin and smooth cuticle. Tanniferous contents are observed in cortical cells. Pericycle is composed of fibers and parenchyma groups present external to the ring of 10 - 12 collateral vascular bundles. Glandular unicellular filiform trichomes are present on the epidermis of stem, lamina, midrib, petiole and rachis. They have wafted cell wall (Haines, 1924; Saxena and Brahmam, 1995).

MATERIALS AND METHODS

Phyto-chemical experiments were carried out on aqueous, methanolic aqueous and acetone-aqueous extracts of the plant using standard procedures to identify the constituents as described by different workers (Harborne, 1973; Tiwari *et al.*, 2011; Mishra *et al.*, 2012). Each plant extract was tested through different solvent media to detect the presence of different bio-active constituents such as saponin, flavonoid, alkaloid, steroid, phlobatannin, terpenoid, tannin, reducing sugar, carbonyl and phenolic compounds. The leaves of *Moringa oleifera* were collected and washed thoroughly to remove unwanted dust and soil particles and were separately air dried stored in airtight container for phyto-chemical analysis.

Phytochemical assays

Phytochemical analysis was carried out on leaves of the plant using standard procedure to identify the bioactive compounds.

Test for Saponin

1ml of the extract was boiled in 10ml of distilled water and filtered with Whatman filter paper. 5ml of filtrate was mixed with 2 ml of normal distilled water and shaken vigorously. Occurrence of stable persistent froth indicated the presence of saponin.

Test for Tannin

5ml of plant extract was added with 5 drops of 10% lead acetate. Formation of a light-yellow precipitate indicated the presence of tannin.

Test for Flavonoids

To 1 ml of the extract, few drops of dilute sodium hydroxide were added. Presence of flavonoids is indicated upon production of an intense yellow color in the plant extract which became colourless on addition of 2-3 drops

of 50% dilute acid.

Test for Terpenoid

0.5 gm of plant extract was mixed with 2 ml of chloroform and equal volume of concentrated Sulphuric acid was added. Terpenoids is not present due to absence of a reddish-brown colouration of interface.

Test for Phenolic compounds

2 ml of plant extract was added with 5 drops of 1% ferric chloride and 1 ml of potassium ferro cyanide, a bluish-green solution showed the presence of phenolic compound.

Test for reducing sugar

0.5 g of plant extract was dissolved with distilled water and filtered. The filtrate was boiled with 2 drops of Fehling's solution A and B for 5 minutes. An orange-red precipitate obtained indicated the presence of reducing sugar.

Test for Steroid

2 ml of plant extract was dissolved in 5 ml chloroform and then 5 ml of concentrated sulphuric acid was added. Absence of formation of 2 phases (upper red and lower yellow with green fluorescence) indicated the absence of steroid.

Test for Alkaloids

5 ml of plant extract was mixed with 3 ml of aqueous HCL on water bath and then filtered. 1 ml of Dragendorff's reagent was added in the filtrate. The absence of orange-red precipitate indicated the absence of alkaloids in the sample extract.

Test for Carbonyl

2 ml of plant extract was added with 2 drops of 2, 4-dinitrophenyl hydrazine solution and thoroughly shaken, absence yellow crystal formation indicated absence of carbonyl.

RESULTS AND DISCUSSION

The species of *M. oleifera* is considered as an important commodity which has recent global attention as the 'natural nutrition' source as a functional food. In this study, the extracts of leaves of *M. oleifera* were analyzed by qualitative tests to reveal the presence of unique metabolites such as saponin, tannin, flavonoids, phenolic compound, etc. using aqueous, methanol and acetone-aqueous solvents. Different solvent extracts of plant showed both positive and negative results for each compounds as detected in the analyses (Table 2) (Figure

Table 1: Medicinal values of *Moringa oleifera* leaf

State	Tribal uses
Odisha	Leaves are edible
Odisha	Leaves are used as tonic
Odisha	Leaves are used against diabetes
Odisha	Leaves are cooked with other vegetables to reduce the eye problems and blood pressure
Odisha	Leaves are used against poor appetite

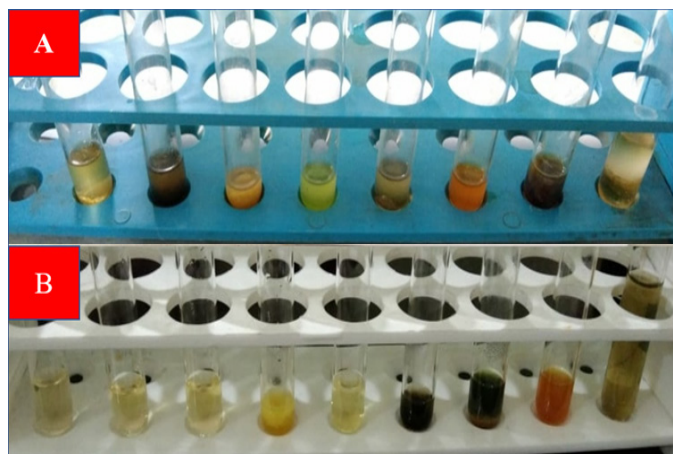
Table 2: Phytochemical testing of crude extracts of *Moringa oleifera*

Bioactive compounds	Solvent		
	Aqueous	Methanolic aqueous	Ace-tone-aqueous
Saponin	+	+	-
Tannin	+	+	+
Flavonoids	+	+	+
Terpenoids	-	-	-
Phenolic compound	+	-	+
Reducing sugar	+	+	+
Steroids	-	-	-
Alkaloids	-	-	-
Carbonyl	-	-	-

Table 3: Correlation of bioactive compounds by validation of traditional medicine

Medicinal use	Correlation with bioactive compounds	Literature
Against diabetics	Presence of reducing sugar indicates that it might be used against diabetics.	Gothai <i>et al.</i> , (2016).
Tonic	Presence of flavonoids and tannin indicates that it might be used as tonic.	Rao <i>et al.</i> , (2011).
Eye sight	Presence of flavonoids indicates that it might be used for good eyesight.	Mishra <i>et al.</i> , (2018). Ibanez-Calero <i>et al.</i> , (2009).

1). The five out of nine different secondary metabolites examined for the study were found to be present in the extracts of leaves of *M. oleifera*. The leaf exhibited the presence of five diverse bioactive constituents as identified from this study and the plant has been variously used to treat a number of ailments; thus, the species is of high pharmacological significance. It is interesting to note that the flavonoids are found using either of the solvents. The leaf extract showed the presence of flavonoids in all three solvents with tannin & reducing sugar. Flavonoids,

**Figure 1:** A) Acetone extract of *Moringa oleifera*; B) Methanolic extract of *Moringa oleifera*

tannins, phenolic compounds and plant phenols are major groups of compounds that act as primary antioxidant or free radical scavengers. The medicinal plant parts are commonly rich in phenolic compounds such as flavonoids and tannins. The biological function of flavonoids includes protection against allergies, inflammation, free radicals scavenging platelets aggregation, microbes. Reduction of coronary heart disease has been reported to be associated with consumption of flavonoid (Shanmugavel, 2018). Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes (Patel *et al.*, 2014). Saponins have beneficial pharmacological effects. They are anti cholesterolmic due to the formation of a complex with cholesterol in gastrointestinal tract thus preventing absorption. Other activities include anti-inflammation, anti-parasite and anti-virus. Numerous lines of evidence now indicate that saponins can kill tumor cells by triggering tumor cell death via different signaling pathways, by activating death receptors, targeting mitochondria, and inducing oxidative stress. Saponins, by virtue of their multiple apoptotic actions on cancer cells, may provide a new line of anticancer agents. They are also effective against drug-resistant cancer cells (Sharma & Paliwal, 2013). Tannins have revealed potential antiviral, antibacterial and antiparasitic while saponins cause haemolysis of red blood cells (Aliyu *et al.*, 2017). Sugars with reducing property (arising out of the presence of a potential aldehyde or keto group) are called reducing sugars. Some of the reducing sugars are glucose, galactose, lactose and maltose (Nawaz *et al.*, 2009). Reducing our sugar intake can create a stable mood and energy levels. On 9th January 2021, our APRF team interviewed a tribe named Mr. Ajay Kumar from Odisha with semi-constructed questionnaire. We documented the medicinal uses of the '*Sajana sag*' (*M. oleifera*) is being used to improve bad eyesight and to reduce high blood pressure as a part of belief system of the community as explained by the resource person. Besides, the species are also consumed as a routine vegetable that has potential be utilized to improve socio-economical status of the community.

CONCLUSION

The present study revealed that the leaves of *Moringa oleifera* possess diverse bioactive constituents as the plant is variously used by different tribes to cure various diseases or disorders and thus the plant can be a source of high pharmacological importance. The results of the study also showed that the identified bioactive components in leaves act as curative agents that scientifically substantiated the rationale behind the traditional use of a part by different tribes to treat various ailments. However, the study conducted here is preliminary in nature and there are plenty of scopes for further quantitative phytochemical investigation which will unravel the potentiality of traditional medicines.

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CONFLICT STATEMENT

The authors declare that they have no conflict of interest.

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