

Original Article

STANDARDIZATION OF DRIED FLOWERS OF *MORINGAOLEIFERA* (LAMK.) AND *JASMINUMSAMBAC* (L.) AITACCORDING TO WHO GUIDELINES

MADIHA KHAN, GHAZALA H. RIZWANI, HINA ZAHID*

Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, 75270 Pakistan
Email: zindagi_zh@yahoo.com

Received: 18 Feb 2015 Revised and Accepted: 02 Sep 2015

ABSTRACT

Objective: The aim of the present study is to evaluate various pharmacognostic standards like macroscopy, microscopy, fluorescence analysis, preliminary phytochemical screening and thin layer chromatography of *Moringa oleifera* (Lamk.) and *Jasminum sambac* (L.) Ait.

Methods: Cellular arrangement of the flower of *M. oleifera* and *J. sambac* were examined under electronic microscope. Fine powder of both flowers was used for powder microscopy. The phytochemical screening of the aqueous and ethyl acetate extracts of flowers of *M. oleifera* and *J. sambac* was performed for carbohydrates, terpenoids, alkaloids, flavonoids, saponins, tannins, steroids, lignin and fixed oil. The color intensity or the precipitate formation was used as analytical responses to these tests. The aqueous and ethyl acetate extracts of both plants were gone through TLC monitoring by reconstituting each extracts with 5 ml solvent respectively.

Results: Under microscopic observation the dried powder of *M. oleifera* revealed abundant calcium oxalate crystals, small spherical oil globules, starch grains, irregular fragments and tannin content. While powder microscopy of flower of *J. sambac* showed tannin, oil globules and simple fibers. Preliminary phytochemical analysis of aqueous and ethyl acetate extracts of both flowers revealed that the strength of active agents i. e, carbohydrate, tannins, fixed oil, terpenes in variable percentage.

Conclusion: The findings could be helpful in identification and authentication of *Moringa oleifera* and *Jasminum sambac* in future for further research and utilization.

Keywords: Standardization, Pharmacognostic evaluation, *Moringa oleifera*, *Jasminum sambac*.

INTRODUCTION

Plant material and herbal remedy derived from plants represent a substantial proportion of the global drug market and in this respect internationally recognized guidelines (WHO) are necessary for their quality assessment. Herbal medicines provide widespread aspects for drug discovery in the field of pharmaceutical research. It has been estimated that 80% of people living in developing countries are almost completely dependent on traditional herbal remedies. Hence it becomes extremely important to document the research work carried out on herbal medicines and make an effort towards standardization of plant material to be used as medicine [1]. WHO specification for the evaluation of the safety, efficacy and quality of herbal medicine is an important requirement for attaining uniformity throughout the world. Correct knowledge of crude drugs is very important aspect in preparation, safety and efficacy of the herbal product. The process of standardization can be achieved by stepwise pharmacognostic studies [2]. Standardization is a system to ensure that every packet of medicine that is sold has the correct amount and will induce its therapeutic effect [3].

Moringa oleifera (MO) belonging to Moringaceae family and is commonly known as the suhujana or drumstick tree [4]. It is a fast growing, deciduous, evergreen perennial tree reaching up to about 10-12m. It is one of the important medicinal plants commonly found in Pakistan and most abundantly found in Karachi. While it grows best in dry, sandy soil, it tolerates poor soil, including coastal areas. All parts of the Moringa tree—leaves, flowers, fruits, and roots are edible and have long been consumed as vegetables [5, 6]. Moringa seed oil, also known as Ben oil, has been used in salads, for industrially as fine machine lubrication, and in the manufacture of perfume and hair care products [7]. The flowers of the plant are a rich source of nutrients and mineral contents. They have also been reported to contain flavonoid pigment and nine amino acids as documented in the literature. It has also been employed in the synthesis, characterization and SERS activity of biosynthesized silver nanoparticles [8, 9]. The diverse range of medicinal uses for MO, include its use as an antioxidant, anticarcinogenic, anti-

inflammatory, antispasmodic, diuretic, antiulcer, antibacterial, antifungal and its antinociceptive properties, as well as its wound healing ability has been demonstrated. Additionally, the root bark has been used as an analgesic, alexeteric, antihelminthic, and treatment for heart complaints, as well as for eye diseases, inflammation and dyspepsia [10-14].

Jasminum sambac (JS) commonly known as jasmine or motia, belongs to family Oleaceae. Motia is a scandent or subrect shrub with young pubescent branches broadly ovate, opposite leaves, white, very fragrant flowers cultivated nearly throughout the tropical and subtropical parts of the world. The plant is much valued for its exquisitely fragrant flower [15].

Plant is widely distributed all over the world, native of South and Central India and Sri Lanka. The plant contain friedelin, lupeol, betulin, α -amyrin, ursolic acid, sambacin, jasmnin, sambacoside A, sambacolignoside, quercitin, rutin, kaempferol, luteolin, phenylmethanol, linalool, ~~terpeneol~~ [16 -18]. Moreover, it is employed for ophthalmic purposes, skin diseases, pyrexia, cephalgia, otopathy, stomatopathy, leprosy, pruritis and ulcers. It also exhibits chemo-preventive effect against DLA-induced lymphoma [19, 20].

However, literature survey revealed that the pharmacognostic studies on dried flowers of MO and JS are lacking hence in the present research investigation was undertaken. The objective of the present study is to evaluate various pharmacognostic standards like microscopy, extractive values, fluorescence analysis and preliminary phytochemical analysis of MO and JS.

MATERIALS AND METHODS

Collection of plant materials

Jasminum sambac flowers were collected in September 2013 at flowering stage. Flowers were shade dried over a period of 24 h inside the room at ambient temperature. While *Moringa oleifera* flowers were collected in January 2014 at flowering stage. The flowers were sun-dried over a period of 48 h.

Histological examination

Fresh flowers of both plants were sliced into thin sections and treated with 10%, 20%, 30%, 40%, 50% alcohol for 2 min each, then add 2 drops of saffranine keep it for 5 min then treat it with 60%, 70% and 80% alcohol followed by the addition of 2 drops of malachite green for 5 min respectively. After treating it with 90% and 100% alcohol pour 1 drop of oil on slide and observe the sections under microscope [21].

Powder microscopy

Dried flowers of MO and JS were crushed in mortar and pestle. Crushing was done with the help of agitation and trituration. A small amount of samples were taken and chloral-hydrate was poured on powder samples respectively. It was allowed to stand for about 3 min and observed under microscope. It was then mounted in glycerin (50%) and observed under microscope. Similarly, the powder was also stained with weak iodine (N/50) solution for the identification of starch grains. Powder was treated with conc. H₂SO₄ for the identification of calcium oxalate crystals [22].

Fluorescent analysis

A pinch of dried and pulverized plant material was taken in clean vials with about 10 ml of solvent like concentrated nitric acid, 50% HCL, 80% sulphuric acid, acetic acid, N/20 iodine solution respectively. All the vials were shaken well and incubated for about 30 min at room temperature. The colors of the drug solutions thus obtained were observed for their characteristic color reaction under the visible light and ultra violet light (UV 254 nm and UV 366 nm) and were recorded [23].

Preliminary phytochemical analysis

The aqueous and ethyl acetate extracts of both plants were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedure [24, 25]. Phytochemical screening of carbohydrates, saponins, tannins, steroids, flavanoids, alkaloids and terpenes was performed by using standard methods.

Thin layer chromatography

The aqueous and ethyl acetate extracts of both plants were gone through TLC monitoring by reconstituting each extracts with 5 ml solvent respectively. After reducing the volume upto 1 ml, each extract (15µl) was applied on TLC plates (20 x 20 cm silica gel 60 precoated plates, fluorescence at 254 nm, Merck, Germany). The plates were developed using solvent systems i. e, Hexane: Chloroform (80:20), Chloroform: Acetone: butanol (70:20:10), Chloroform: Acetic acid (90:10), Ethyl acetate: Methanol: Water (10:65:35), Butanol: Ethanol: Water (50:45:5), Chloroform: Methanol: Water (80:15:5) and Butanol: Acetic acid: Water (40:55:5) respectively. After developing, each TLC plates were dried and visualized under UV lamp (254 and 366 nm) and marked [26].

RESULTS

Pharmacognostic studies are a straightforward and reliable tool by means of which inclusive information of the crude plant material can be obtained. Macro morphological studies of *M. oleifera* revealed that the flowers are fragrant; white or creamy white in colour. While *J. sambac* flowers are white with pleasant fragrance. Proper organoleptic examination of both flowers was illustrated in table 1.

Table 1: Morphological depiction of flower of *Moringa oleifera* and *Jasminum sambac*

Source	Plant Pictures	Taste	Odour	Texture	Color
<i>Moringa oleifera</i>		Sweet	Slight fragrance	Rough	Lemon white
<i>Jasminum sambac</i>		Acrid	Pleasant	Rough	White

Histological examination provides a tool for the determination of cellular type and shape of the sample.

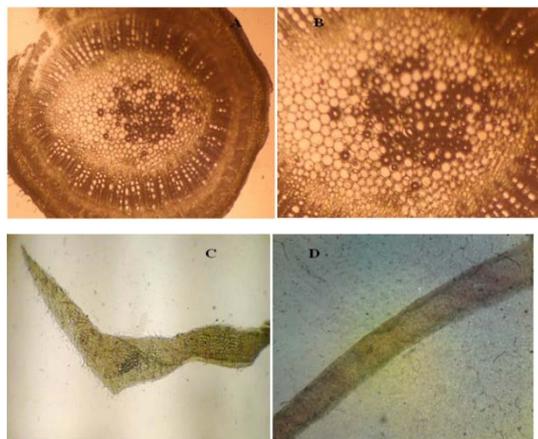


Fig. 1: Histological examination of *M. oleifera* and *J. sambac* flower. A: T. S of pedicle of *M. oleifera*; B: T. S of center part of pedicle of *M. oleifera*; C: T. S of petal of *M. oleifera*; D: T. S of petal *J. sambac*

The transverse section of pedicle of MO showed cork tissues, cambium, parenchymatous cell, collenchyma cells, phloem and xylem vessel. The outer most layer consist of cork cells arranged in

radial rows. The cork layers are followed by cork cambium which are thick layered containing pigments. Cortical region is surrounded by the collenchyma cells. The thin walled parenchymatous cells containing calcium oxalate, starch grains and oil globules. The centre region of pedicel consists of hexagonal cells containing heavy deposition of tannins. Histology of petal showed sclerids, fibrovascular tissue and cortical cell. While the anatomical examination of JS showed fibrous layering over the surface (fig. 1).

Microscopic cellular fragments were visualized in three different detecting reagents (50 % Glycerine solution, 10 % Chloral hydrate solution and 5 % Iodine solution). Under microscopic observation the dried powder of MO revealed abundant calcium oxalate crystals, small spherical oil globules, starch grains, irregular fragments and tannin content (fig. 2-A). While powder microscopy of flower of JS showed tannin, oil globules and simple fiber (fig. 2-B).

Powder samples of both flower were treated with different reagents and was examined under day light and UV light (254 and 366 nm), the results are shown in table 2.

Preliminary phytochemical analysis of aqueous and ethyl acetate extracts of both flowers revealed that the strength of active agents i. e, carbohydrate, tannins, fixed oil, terpenes in variable percentage presented in Table-3. The presence of these phytoconstituents makes the plant useful for treating different ailments and providing useful herbs of human consumption.

TLC finger prints of both aqueous and ethyl acetate extract of MO and JS showed complex mixtures of non polar and polar compounds in different solvent systems (table 4).

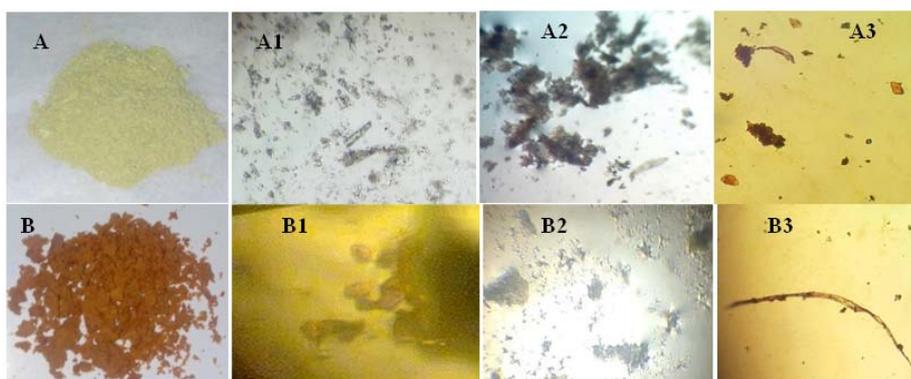


Fig. 2: Powder microscopy of *M. oleifera* and *J. sambac* dried flower. A: Pulverized sample of *M. oleifera*; A1: Abundant Calcium oxalate crystals with small spherical oil globules and starch grains; A2: irregular fragments; A3: tannin content; B: Pulverized sample of *J. sambac*; B1:Tannin; B2: oil globules; B3: simple fiber

Table 2: Fluorescence analysis of powdered flower of *M. oleifera* and *J. sambac*

Particular of Treatments	Ordinary light		U-V light (254 nm)		U-V light (366 nm)	
	<i>J. sambac</i>	<i>M. oleifera</i>	<i>J. sambac</i>	<i>M. oleifera</i>	<i>J. sambac</i>	<i>M. oleifera</i>
Powder as such	Brown	Yellowish green	Brown	Black	Blackish brown	Black
Powder+distill water	Orange	Creamy white	Brown	Yellow	Yellow	Yellow
Powder+HCl	Yellow	Yellowish orange	Creamy white	White	Cream colour	Creamy White
Powder+H ₂ SO ₄	White	Creamy white	Orange	Dark brown	Yellow	Orangish brown
Powder+iodine solution	Brick red	Brick red	Black	Black	Black	Black
Powder+acetic acid	orange	Creamy white	Brown	yellow	White	Yellow

Table 3: Preliminary phytochemical screening of extractsof *M. oleifera* and *J. sambac*

Phytochemicals	Test reagents	<i>Jasminum sambac</i>		<i>Moringa oleifera</i>	
		Aqueous extract	Ethyl acetate extract	Aqueous extract	Ethyl acetate extract
Carbohydrates	Molish's Test	++	+++	+	+++
Saponins	Frothing test	++	-	-	-
Lignin	Phloroglucinol Test	+	-	+	-
Tannins	Lead acetate Test	+++	-	+++	-
Steroids	Salkowski Test	++	-	+	-
Flavonoids	Alkaline reagent Test	-	+	-	+
Terpenes	Liebermann Burchard's Test	+	+	+	+
Alkaloids	Dragendroff Test	++	-	-	-
Fixed oil	Spot Test	+++	-	+	-

DISCUSSION

Standardization of medicinal plants involve complete data on herbs such as taste, smell, appearance, microscopic characterization and finger print profile of the active constituent present in the plant [27]. Standardization of herbal medicines involve several steps, however, the source and quality of raw materials play a pivotal role in

guaranteeing the quality and stability of herbal preparations. Other factors such as the use of fresh plants, temperature, light exposure, water availability, nutrients, period and time of collection, method of collecting, drying, packing, storage and transportation of raw material, age and part of the plant collected, etc., can greatly affect the quality and consequently the therapeutic value of herbal medicines [28].

Table 4: TLC fingerprinting analysis of floral extractsof *M. oleifera* and *J. sambac*

Solvent systems	<i>Jasminum sambac</i>		<i>Moringa oleifera</i>	
	Aqueous extract	Ethyl acetate extract	Aqueous extract	Ethyl acetate extract
Hexane: Chloroform (80:20)	0.29	0.875	0.88	0.9, 0.4
Chloroform: Acetone: Butanol (70:20:10)	0.77	0.89	0.66	0.71, 0.94
Chloroform: Acetic acid (90:10)	0.91, 0.92, 0.93, 0.94	0.9, 0.45, 0.25, 0.07	0.07, 0.91, 0.96, 0.98, 0.17, 0.92	0.88, 0.08
Ethyl acetate: Methanol: Water (10:65:35)	0.76	0.92	0.8	0.84
Butanol: Ethanol: Water(50:45:5)	0.62	0.8	0.78	0.76
Chloroform: Methanol: Water(80:15:5)	0.59	0.57, 0.63, 0.68, 0.78, 0.9	0.12	0.12, 0.44, 0.63, 0.67, 0.95
Butanol: Acetic acid: Water (40:55:5)	0.39	0.76	0.8, 0.47	0.68

Fluorescence analysis is used for the characterization of the crude drug. Fluorescence is the phenomenon exhibited by the presence of chemical constituents present in the plant material. Some chemical constituents show fluorescence in the visible range (daylight) while some are not visibly fluoresce in daylight. These types of compound produce fluorescence in ultra violet light. Substances that are not fluorescent, they may often be converted into fluorescent products by applying different reagents.

Knowledge of phytochemicals of botanicals are important not for the discovery of therapeutic agents but also for disclosing new sources of economic materials. As commercialization of the natural medicine has occurred, it becomes an important issue to assurance the safety, quality and efficacy of medicinal plants [29]. The extractive values are valuable to estimate the chemical constituents present in the crude drug and furthermore assist in evaluation of definite constituents soluble in a particular solvent [30]. Thin layer chromatography (TLC) is the common, simple and reliable method for analysis and is widely used in natural product extract analysis, stability tests of extracts and finished product and in sample quality control. TLC fingerprint of plant extracts can be used for identification of components which can be achieved on the basis of retention factor (R_f) value and colour spots [31]. TLC finger print profile of both extracts of flower respectively considered as good standardizing tool.

CONCLUSION

The pharmacognostic parameters of flower of *M. oleifera* and *J. sambac* are laid down for the first time and these findings could be helpful in identification and authentication of these plant materials in future for further research and utilization.

CONFLICT OF INTERESTS

Declared None

REFERENCES

- Kunle OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines-A review. *Int J Biodiversity Conservation* 2012;4:101-12.
- Nivedithadevi D, Somasundaram R. Pharmacognostical and qualitative phytochemical studies on the aerial parts of *Tephrosia purpurea* (L.). *Int J Curr Res Biosci* 2012;2:48-53.
- Nasreen S, Radha R. Assessment of quality of *Withaniasomnifera* Dunal (Solanaceae) Pharmacognostical and physicochemical profile. *Int J Pharm Pharm Sci* 2011;3:152-5.
- Morton J. The horseradish tree, *Moringa pterygosperma* (Moringaceae)-A boon to Arid Lands? *Econ Bot* 1991;45:318-33.
- Anwar F, Bhangar MI. Analytical characterization of *Moringa oleifera* seed oil grown in temperate regions of Pakistan. *J Agric Food Chem* 2003;51:6558-63.
- Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J Agric Food Chem* 2003;51:2144-55.
- Tsaknis J, Lalas S, Gergis V, Dourtoglou V, Spiliotis V. Characterization of *Moringa oleifera* variety Mbololo seed oil of Kenya. *J Agric Food Chem* 1999;47:4495-9.
- Nadkarni KM. *Indian Materia Media*. Vol. I. Bombay Popular Prakashan Mumbai; 2005. p. 811-6.
- Prasad TNVKV, Elumalai EK. Biofabrication of Ag nanoparticles using *Moringa oleifera* leaf extract and their antimicrobial activity. *Asian Pac J Trop Biomed* 2011;1:439-42.
- Verma AR, Vijaya kumar M, Mathela CS, Rao CV. *In vitro* and *in vivo* antioxidant properties of different fractions of *Moringa oleifera* leaves. *Food Chem Toxicol* 2009;47:2196-201.
- Bharali R, Tabassum J, Azad MR. Chemomodulatory effect of *Moringa oleifera*, Lam on hepatic carcinogen metabolizing enzymes, antioxidant parameters and skin papillomagenesis in mice. *Asian Pacific J Cancer Prevention* 2003;4:131-9.
- Cáceres A, Saravia A, Rizzo S, Zabala L, De Leon E, Nave F. Pharmacologic properties of *Moringa oleifera*. Screening for antispasmodic, anti-inflammatory and diuretic activity. *J Ethnopharmacol* 1992;36:233-7.
- Caceres A, Cabrerre O, Morales O, Mollinedo P, Mendia P. Pharmacological properties of *Moringa oleifera*.1: Preliminary screening for antimicrobial activity. *J Ethnopharmacol* 1991;33:213-6.
- Sulaiman MR, Zakaria ZA, Bujarimin AS, Somcent MN, Israf DA, Moin S. Evaluation of *Moringa oleifera* aqueous extract for antinociceptive and anti-inflammatory activities in animal models. *Pharm Biol* 2008;46:838-45.
- The wealth of India, Raw Material. Vol 5th. H-K; 2012. p. 289-90.
- Rastogi RP, Mehrotra BN. *Compendium of Indian Medicinal Plants*. Vol. 4. 2002. p. 407-8.
- Rastogi RP, Mehrotra BN. *Compendium of Indian Medicinal Plants*. Vol. 2.; 2000. p. 395-6.
- Tanahashi T, Nagakura N, Nishi T. Sambacosides A, E and F novel tetrameric iridoid glycosides from *Jasminum sambac*. *Tetrahedron Lett* 1998;29:1793-6.
- Kiritikar KR, Basu BD. *Indian medicinal plants with Illustrations*. 2nd Edition. Vol. 7. 2003. p. 2093-6.
- Kalaiselvi M, Narmadha R, Ragavendran P, Vidya B, Gomathi D, Raj CA, et al. Chemopreventive effect and HPTLC fingerprinting analysis of *Jasminum sambac* (L.) Ait. Extract against DLA-induced lymphoma in experimental animals. *Appl Biochem Biotechnol* 2013;169:1098-108.
- Wallis TE. *Text book of Pharmacognosy*. 5th Edition. T. A. Churchill, London; 1985. p. 561.
- Johansen DA. *Plant Microtechnique*, McGraw Hill, New York; 1940. p. 183-203.
- Pratt RT, Chase ER. Fluorescence powder vegetable drugs in particular to development system of identification. *J Am Pharm Assoc* 1949;38:324-31.
- Trease GE, Evans WC. *Pharmacognosy*, East Bourne: Bailliere Tindal, London; 1986. p. 171-2.
- Harborne JB. *Phytochemicals Methods*. Chapman and Hall Ltd, London; 1973. p. 49-188.
- Sherma J, Fried B. *Handbook of thin layer chromatography*. 3rd Edition, CRC Press; 2003. p. 62-129.
- Page LR. Whole herb or standardized plant constituents. *Total Health* 2001;23:20.
- Koo BC, Nottenberg, Pardey PG. Plants and intellectual property: an international appraisal. *Science* 2004;306:1295-7.
- Rice-Evans CA, Miller NJ, Paganga G. Structure antioxidant activity relationship of flavonoids and phenolic acids. *Free Radicals Biol Med* 1996;20:933-56.
- Shwetajain, Chanderachud Sharma, Pankaj Khatri, Atul Jain, Ankur Vaidya. Pharmacognostic and Phytochemical investigations of the Leaves of *Zizyphusxylopyrus* (Retz) Willd. *Int J Pharm Pharm Sci* 2011;3:122-5.
- Cimpoiou C. Analysis of some natural antioxidants by thin layer chromatography and high performance thin layer chromatography. *J Liq Chromatogr Relat Technol* 2006;29:1125-42.