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Research Article

A comprehensive quality control standardization of *Mirabilis jalapa* L. tuberous root

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ABSTRACT

The aim of the study to established the standard monograph in respect of pharmacognostical, physicochemical and phytochemical characteristics of *Mirabilis jalapa* Linn. tuberous root. The parameters were investigated as per the procedure mentioned in World Health Organisation (WHO) guidelines and Ayurvedic Pharmacopoeia. Morphological characters showed that the roots are conical in shape, fleshy, healthy upper part followed by narrow at the end, branched, presence of buds on the surface, externally brown to dark grey in colour and internally whitish to buffed in colour, taste is slightly sweetest without any odour. Microscopically, the tuberous root showed the presence of Epidermis, cortex, collateral vascular bundle, medullary rays, phloem fibres, acicular crystal of calcium oxalate, starch grain, meta-xylem, proto-xylem etc. In phytochemical analysis the tuberous root extracts shown the presence of carbohydrates, flavonoids, phenolics, alkaloids, steroids and glycosides. Physicochemical parameters like ash value, loss on drying, foreign matter, extractable matter, foaming index, swelling index, volatile oil content, fluorescence analysis, crude fibre content, total number starch grain content and presence of heavy metals were established and reported. The present study will assist the further investigation on this plant.

Keywords: *Mirabilis jalapa;* Tuber; Pharmacognostical; Physicochemical and Phytochemical.

INTRODUCTION

Mirabilis Jalapa L. (Nyctaginaceae) is the herbaceous plant, 0.6 - 1 meter in height. The flowers of this plant open at around 4 o'clock in the afternoon and hence its common name is four o'clock plant. It can be propagated by either from seed or tuber in early spring (Jyotchna et al., 2016). *Mirabilis jalapa* L. is known as a bioactive plant and used in various diseases as folklore medicine. Traditionally, the plant is used to treat inflammatory and painful diseases and is also used as a laxative. Leaf juice is used on boils, to heal wound as external application, bruises and also for allaying itching in urticaria (Muthumani et al., 2009). In traditional medicine *Mirabilis jalapa Linn* is widely used as antidysenteric, antiparasitic, carminative, digestive stimulant, tonic, vermifuge, wound healer seeds are used as cathartic. The root is having aphrodisiac, diuretic and purgative properties, used as dropsy. *Mirabilis jalapa* is also used to cure many infirmities including dysentery, diarrhea, muscular pain and abdominal colic. In ayurveda this plant is used for treating boils, inflammations, constipation, diabetes, urinary disorders. Dried flowers are used as a snuff for headaches, fungal infection and root decoction to wash wounds, treat skin afflictions as leprosy (Kiran et al., 2010; Mahajan et al., 2008). It is reported that the root of *Mirabilis jalapa* contain phytoconstituents such as alkaloids, glycosides and phytosterol (Walker et al., 2008). But the evidences were found in the literature on the root part of this plant which is inadequate to consider as standard. Hence, the present effort has been made to standardize the root scientifically in respect with its pharmacognostical, physicochemical and phytochemical feature which would be supportive for upcoming research work on this plant part.

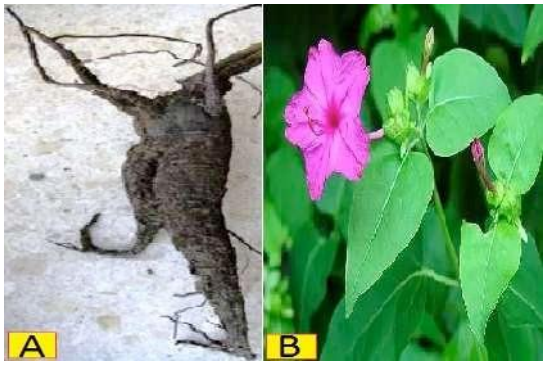

Figure 1: Image of Tuberous root (A) and Leaf, flower (B) of Mirabilis jalapa

MATERIALS AND METHODS

Plant material

Tubers of *Mirabilis jalapa* were collected from local area of Kamrup district, Assam, India and authenticat-

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ed by Dr. P. P. Baruah (HOD, Department of Botany, Gauhati University, India). The voucher specimen of *Mirabilis jalapa* tuberous root (Acc no. 18176) were kept at the Department of Pharmacognosy, Girijananda CHowdhury Institute of Pharmaceutical Sciences, Assam for future reference. The plant material was process and subjected to washing with running water, dried in shade for 10- 15 days and stored in air tight containers until used.

Pharmacognostical evaluation

Macroscopic, Microscopic and powder drug evaluation

The morphological evaluation was carried out as per the standard methods available in the literatures to determine the shape, size, taste, colour, odour of drug. Transverse section of the tuberous root specimen was done by using microtome technique. The sections were dehydrated with varying strength of absolute alcohol and placed on glass slide and then stained with the mixture of phloroglucinol and conc. HCl (1:1) to identify different cell under the microscope. For the powder study the dried plant material was subjected for size reduction to fine powder using mechanical grinder. The powder was then passed through sieve no 60 where the fines were collected and subjected for the powdered study. For microscopical observation of various powder characters, different detecting reagents were used such as Phloroglucinol and conc. HCl (1:1) and iodine solution. Photographs of different magnifications (10× and 40×) were taken (Khandelwal, 2012)

Determination of physicochemical parameter

As per method described in WHO guideline all the physicochemical parameters like ash value, loss on drying, foreign matter, extractable matter, foaming index, swelling index, volatile oil content and presence of heavy metal were established for the powdered drug of *M. jalapa* tuberous root (World Health Organisation, 2002).

Fluorescence analysis of powdered drug

Fluorescence of powedered drug was evaluated as per the methods of described literature (Chase and Pratt, 1949; Kokoski et al, 1958). About 1-2 gm of powdered drug was treated with different chemical reagents and examined under ordinary light and UV light with short (λmax 254 nm) and long (λmax 365 nm) wave length.

Quantification of crude fibres and starch grains content

About 2 gm of powdered crude sample of the tuberous root was boiled with 10% nitric acid, filtered, washed the residue with boiling water and the residue was treated with 2.5% NaOH. The crude fibres thus obtained were filtered, weighed and the % w/w of crude fibres present in air dried plant material was calculated (Kokate, 2014). The quantitative determination of starch grains was done by incorporating the Wallis's Lycopodium spore method [Khandelwal, 2012].

Phytochemical evaluation

Preparation of Extract

The dried tuberous roots were subjected to size reduction. About 50 gm of crude powder drug was subjected to successive extraction by cold maceration process with petroleum ether, benzene, diethyl ether, chloroform, acetone, ethyl acetate and methanol for about 48 hrs with frequent shaking up to 6 hrs. Extracts were then filtered, concentrated under reduced pressure in rotary evaporator (Buchi India Pvt Ltd.) at 40°C and stored in a desiccator until further use (The Ayurvedic Pharmacopoeia of India, 2007)

Preliminary phytochemical screening

The concentrated extracts were subjected to preliminary phytochemical screening for the identification of the various phytochemical classes such as alkaloids, carbohydrate, glycosides, saponins, steroids, terpenoids, phenolics, flavonoids and protein (Khandelwal, 2012; Kokate et al., 2014).

Quantitative estimation of polyphenolic components

Standard procedures were followed for the estimation of total polyphenolic components such as total flavonoid content (Kumaran and Karunakaran, 2007), total phenolics and tannins content (Makkar, 2000) incorporating UV spectrophotometer. Rutin is used as reference standard for the estimation of total flavonoid content; whereas, tannic acid is used as reference standard for the estimation of total phenolics and tannins content. Results were calculated and expressed as the Mean±S.E.M using statistical linear regression method.

RESULTS

Pharmacognostical evaluation

Morphological evaluation

Tuberous roots shown in Fig. 1 are conical in shape measuring 5-33 cm long and 3-7 cm width. The roots are fleshy, branched, occasionally presence of bud on the surface, externally brown to dark grey in colour and internally whitish to buffed in colour, taste is slightly sweetest without any odour.

Microscopical evaluation

Transverse section of the *Mirabilis jalapa* tuberous root is represented in Fig. 2. The root is externally characterized by the presence of a well define layer of epidermis (Ep) composed of few layer of narrow, irregular cork cells followed by randomly arranged parenchymatous layer (8-10 layers) composing of the cortex (Cr) region. Collateral closed types of vascular bundle were seen where phloems (Ph) and xylems (MXy and PXy) are arranged in side by side. Xylem are made up of vessels and are lignified in nature where as phloem

were composed of phloem fibres which are clearly stained yellowish in phloroglucinol and HCl. Vascular bundles are embedded in parenchymatous ground tissue. Uniseriate medullary rays (MR) were observed to be originating from the cortex region which extends up to the centre passing through the xylem. Pith was found to be absent.

Microscopical evaluation of powder drug

The powdered drug was mounted in the mixture of phluroglucinol with HCL (1:1) for the detection of lignification and also with diluted iodine solution for examining the persence of starch grains. Various isolated cells like Phloem fibre (PhF), Starch grain (SC), Acicular crystal of calcium oxalate (ACC), Parenchylatous cell (PC), Xylem vessel (XyV) were observed with different magnification (10× and 40×) (Fig. 3)under the compound microscope.

Physicochemical characteristics

Physicochemical parameters were evaluated and the results were shown in the table 1.

Fluorescence powder drug analysis

The fluorescence analysis of powdered tuberous root of *M. jalapa* were performed and the colour of fluoresence were noted down which is indentified by comparing with the standard colour index chart. The result is represented in Table 2.

Quantification of crude fibre and starch grains content

Crude fibre content was found to be 0.30% w/w per gram of the plant material. The number of starch grain present was found to be 75,200 in 1 mg of powdered drug of tuberous root of *M. jalapa*.

Phytochemical evaluation

Quantification of extractive value of different extracts

The results for the cold macerated extractive value of the individual solvent extract were shown in the Table 3. It was observed that methanol extract produces the maximum percentage yield of the extract followed by ethyl acetate and chloroform as solvents.

Preliminary phytochemical screening

The results from the preliminary phytochemical screening of different extract of *Mirabilis jalapa* tuberous root showed the presence of phytocostituents like alkaloids, carbohydrate, glycosides, steroids, saponins, phenolics, flavonoids. (Table 4).

Quantitative estimation of polyphenolic components

Quantitative estimation of total phenolic, total tannin and flavonoid were determined from the crude drug of *M. jalapa* by UV spectrophotometer. Total phenols, tannin and flavonoid content were found to be 12.39±0.023 mg/gm equivalent to tannic acid, 11.72±0.15 mg/gm equivalent to tannic acid (TAE) and 126.23±0.41 mg/gm equivalent to rutin (RE) respectively.

DISCUSSION

As per the WHO guideline, standardization is the initial steps required to diagnose the organized or unorganized crude drugs with its actual quality and property and also to identify the adulterant (Damiki et al., 2014). In recent years herbal formulations are occupying global economy. People are showing maximum interest on it rather than the modern synthetic medicines. WHO stated that about 70-80 percent of world population have faiths on herbal medicines because of less side effects and are significantly effective in treating disease ailments (Muniappan and Savarimuthu, 2011) 25% of modern medicines are directly or indirectly derived from plants sources [Mohd et al., 2011]. Hence, proper identification is required in order to maintain the quality, safety and efficacy of unexploited medicinal herbs [Majumder and Paridhavi, 2013]. The foremost aim of this study to the set up a standard monograph profile of the tuberous root of *M. jalapa* by pharmacognostically, physicochemically and phytochemically standardize methods. As the plant is ethnomedicinally documented in many literatures and is also utilized by the villagers of North East as well as eastern part of India; therefore, this effort will be helpful for further investigation on this plant. Pharmacognostical evaluation is considered as preliminary characterization of medicinal plant where the crude drugs are authenticated macroscopically and microscopically (Sahu et al., 2014). Macroscopically external features like size, shape, colour, taste, odour were revealed. Microscopically, the tuberous root has shown the important diagnostic characters of Nyctaginaceae family [Metcalfe and Chalk, 1950] such as the presence and arrangement of collateral closed vascular bundles embedded in parenchymatous ground tissue, lignified xylem elements with spiral thickenings, uniseriate medullary rays, acicular calcium oxalate crystals and many others.

Physicochemical parameters were evaluated to determine the presence of adulteration as well as to measure the purity of medicinal plant in powder form. Ash value was performed to measure the presence of inorganic matters in the drug (Manish et al., 2014). The result of total ash indicate the presence of salt of carbonate, phosphate, silicates of sodium, calcium and magnesium as impurities, while acid insoluble ash was performed only to detect the presence of silica. Water soluble ash was estimated to detect the amount of inorganic substances exhausted by water, whereas sulphated ash represents the amount of salt present in powder drug. Extractive values were estimated to examine the solubility of drug in different solvents, quantity of active ingredients present in the drug and the nature of bioactive compound presence in the drug ((WHO, 2002). Loss on drying was estimated to determine the presence of moisture content present in the

Table 1: Physicochemical parameters of tuberous root of Mirabilis jalapa

SN	Physicochemical parameters	Result
1	Ash value *Total ash* *Acid insoluble ash* *Water soluble ash* *Sulphated ash*	 12.2% w/w 4.3% w/w 8.4% w/w 6.7% w/w
2	Loss on drying	6.66 % w/w
3	Foreign matter	0.85 % w/w
4	Extractive value *Alcohol soluble extractive* *Water soluble extractive* *Ether soluble extractive*	 7.2% w/w 10.6% w/w 2.1% w/w
5	Swelling factor	Below 1
6	Foaming index	Below 100
7	Volatile oil content	Absent
8	Heavy metals Lead (Pb) Cadmium (Cd) Zinc (Zn) Mercury (Hg)	 Not more than 0.005 PPM Not more than 0.0001 PPM Not more than 0.118 PPM Not more than 0.111 PPM

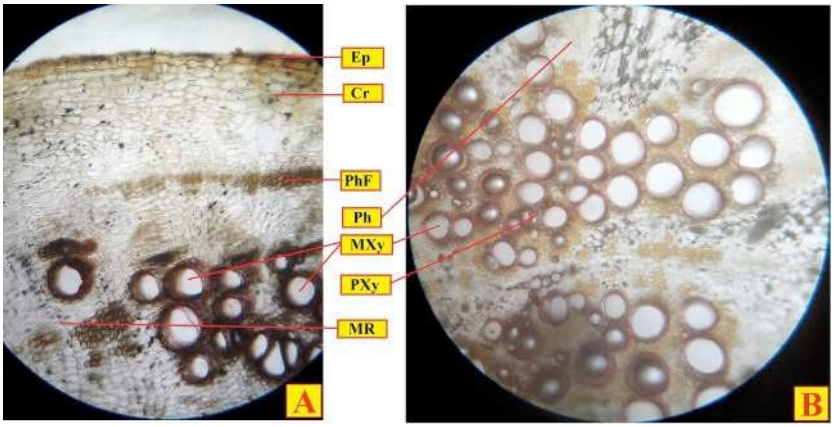

Figure 2: Transverse section (10× magnification) of the tuber of Mirabilis jalapa through the periphery (A), and centre region (B) [*Abbreviations*- Ep: Epidermis; Cr: Cortex; PhF: Phloem fibre; Ph: Phloem; MXy: Meta xylem; PXy: Proto xylem; MR: Medullary ray]

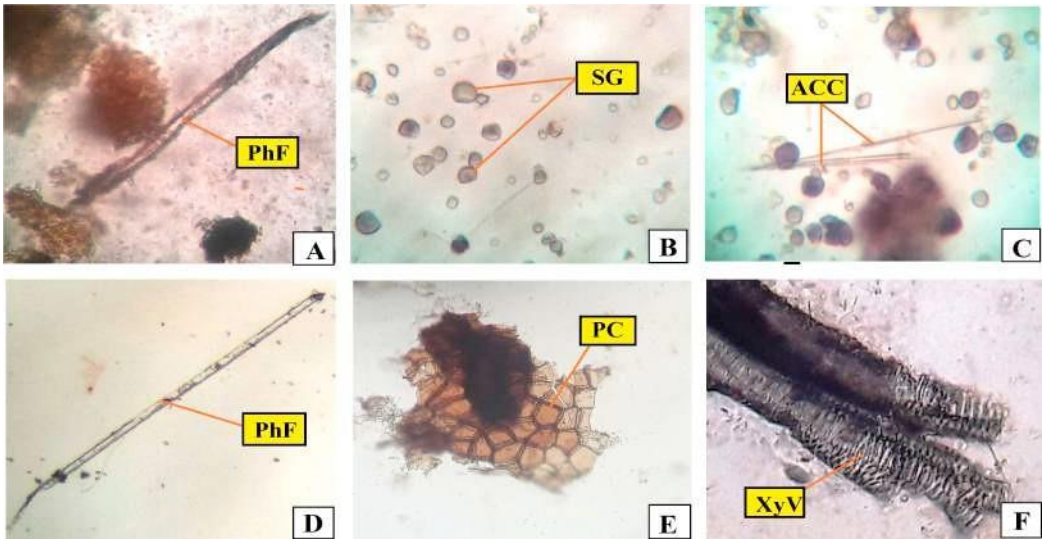

Figure 3: Powder study of the tuber of Mirabilis jalapa [10× and 40× magnification] [Abbreviations- PhF: Phloem fibre (A & D); SC: Starch grain (B); ACC: Acicular crystal of calcium oxalate (C); PC: Parenchymatous cell (E); XyV: Xylem vessel (F)]

Table 2: Fluorescence analysis of powder drug of tuberous root of Mirabilis jalapa

Powder + Reagent	Fluorescence in day light	Fluorescence in (254 nm)	Fluorescence in (365 nm)
Powder drug	NF	NF	NF
Powder + Methanol	NF	Yellowish green	Yellowish brown
Powder + 1 N NaOH in methanol	Light brown	Yellowish green	Greenish blue
Powder + 1 N HCl in methanol	NF	Redish brown	Violet
Powder + 1 N HNO_3 in methanol	NF	Light green	Dark blue
Powder + Picric acid (5%)	Yellow	Pale green	Dark green
Powder + $FeCl_3$ (5%)	Light brown	NF	Dark brown
Powder + KOH (5%)	NF	Pale yellow	Dark green

NF: No fluorescence

Table 3: Percentage yield of crude tuberous root of Mirabilis jalapa extract from different solvents

Sl no	Solvent used	Percentage of yield
1	Petroleum Ether	2.1 % w/w
2	Benzene	1.8 % w/w
3	Diethyl ether	3.9 % w/w
4	Chloroform	9.2% w/w
5	Acetone	8.1 % w/w
6	Ethyl acetate	12 % w/w
7	Methanol	15 % w/w

Table 4: Phytochemical screening of various extracts of tuberous root of Mirabilis jalapa

Bioactive Constituents	Pet. Ether	Benzene	Diethyl ether	Chloroform	Acetone	Eth. Acetate	Methanol
Alkaloids	–	–	–	–	–	–	+
Flavonoids	–	–	–	+	+	+	+
Saponins	–	–	–	–	+	+	–
Carbohydrate	–	–	–	+	+	+	+
Glycosides	–	–	–	+	+	+	+
Steroids	–	–	–	+	+	+	+
Phenolics	–	–	–	+	+	+	+
Terpenoids	–	–	–	–	–	–	–
Proteins	–	–	–	–	–	–	–

drugs, as presence of moisture may lead to the microbial growth as well as deterioration of the nature of active constituent in crude drug (Manish et al., 2014). The result shown the lower level of loss on drying reduced the chance of microbial contamination. Swelling factor indicated the presence of mucilages, gums, pectin, hemicelluloses in the plant sample (Satyendra et al., 2014). So experimental analysis proved that the present in low quantity of above mentioned ingredients in the tuberous roots. Foaming index was determined to have a clear cut understanding on the presence of a remarkable quantity of saponins in the crude drugs (Satyendra et al., 2014). WHO recommended limitation of presence of heavy metal in crude drugs are as follows: Lead (10 ppm), Cadmium (0.3 ppm), Zinc (10 ppm), Mercury (1 ppm) (Sudha et al., 2014) so presence of heavy metals (Pb, Ca, Hg and Zn) in powder drug of tuber was found to be within the prescribed limit, signifying that the plant is safe for consumption and devoid of harmful metals. Fluorescence analysis with powdered drug in presence of different reagents was performed to establish a qualitative identification of crude drug in which few compound present in the drug sample have shown special character in terms of exhibiting fluorescence in day light and under UV lights (Laloo et al. 2012). Phytochemical investigation revealed the presence of phenolics, flavonoids, alkaloids, carbohydrates, steroids as chief constituents. Quantitatively, the tuber also showed the presence of a significant amount of the polyphenolic classes.

CONCLUSION

The overall investigation on different standardization features prvide significant tool for the identification and purity of tuber part of *Mirabilis jalapa* plant and also give support in maintaining the original character of the plant and also prevent the possible steps of adulteration/substitution with other species.

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