



The Pharmacognostical Evaluation of the *Marrubium vulgare* Linn Collected from the Pulwama District of Jammu and Kashmir State in India

Vineet Mittal* and Arun Nanda

Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak (124 001), Haryana, India

ABSTRACT

The objective of the present research work was to establish the various pharmacognostical parameters for the *Marrubium vulgare* Linn (Family- Lamiaceae) collected from the Pulwama district of Jammu and Kashmir region of India. The evaluation of organoleptic, microscopical features (root, stem and leaf) and leaf constant such as vein islet number, vein termination number, stomatal index and palisade ratio aids in detection of adulteration and allied species of the plant. The study of various physicochemical parameters helps in determining the quality of the herb. The higher alcohol soluble extractive value ($8.66 \pm 1.2\%$) suggests that most of the constituents of the plant were soluble in alcohol. The total ash acid insoluble ash and water soluble ash was found to be 10.7 ± 0.46 , 1.73 ± 0.61 , $8.9 \pm 0.65\%$ respectively. The moisture content and total fibre content of the drug was $17.2 \pm 0.35\%$ and $9.5 \pm 0.88\%$ respectively. The plant was also extracted by percolation and microwave assisted method of extraction and the yield of the extract was almost double, from 11.27 ± 1.2 in percolation method to $20.23 \pm 1.91\%$ w/w in novel technique. The preliminary phytochemical screening of the extracts confirmed the presence of alkaloids, terpenoids, steroids, flavonoids and carbohydrates in both the extracts. The quantitative estimation of two extracts revealed that the microwave technique significantly increase the total phenolic content from 61.44 ± 2.01 to 93.42 ± 1.04 mg of GAE/gm of extract and total flavonoid content to 37.7 ± 1.66 from 23.25 ± 0.94 mg of RUE/gm of extract as compared to extract obtained by conventional method.

Keywords: Phytochemical screening; Fluorescence; Microscopy; Morphology; Microwave

INTRODUCTION

Since long ages, the herbs have been used by human being to cure vast number of disease. The classical literature mentioned in text books such as Materia medica, Sushruta samhita, Charak samhita purely relied upon the plant based extractives. Also in modern era, a huge proportion of world population used the plant based products for their primary health care needs [1]. The natural products derived from herbs may serve as lead compounds and even the World Health Organization (WHO) has also realized the need of development of novel entities from plant actives to combat with various diseases. The pharmacognostical evaluation helps to ensure the quality, safety and efficacy of raw material of plant origin. It is also the primary step to check the identity of selected medicinal plant before evaluation of therapeutic potential [2].

The *Marrubium vulgare* Linn (Family- Lamiaceae) is a perennial herb belongs to genus *Marrubium* and is geographically distributed to North Africa, Europe and temperate regions of Asia continent. In India, it is generally found in Kashmir region at an altitude of 5000-6000 feet. The herb has been conventionally applied for the treatment of joint pain, bronchitis, inflammation, sore eyes, cough, cold, pulmonary infections and night blindness. The perennial plant also act as purgative, diuretic, bitter tonic, carminative, appetizer and helps in expulsion of foetus [3]. The researchers had also established the cardioprotective, hypotensive, vasorelaxant, antinociceptive, analgesic, antispasmodic, antidiabetic, immunomodulatory, antioedematogenic, gastroprotective and antioxidant potential of the plant [4-15]. The large number of traditional uses and the well proved therapeutic significance of the selected

herb justifies it to be developed as herbal formulation. Therefore the present research has been designed to evaluate and establish the various pharmacognostical parameters which would help in confirmation of identity and quality of the plant before inclusion in a polyherbal formulation.

EXPERIMENTAL SECTION

Chemicals

The various solvents and reagents used in the standardization of selected medicinal plant were of analytical grade. The gallic acid and rutin were procured from Loba Chemie Pvt Ltd., Mumbai, India.

Plant material

The whole selected herb, *Marrubium vulgare* Linn was collected from the Pulwama district of Jammu and Kashmir state in India and was authenticated by a taxonomist (Dr. Sunita Garg) from National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India (NISCAIR/RHMD/consult/2013/2336-116 dated 19/11/2013). A specimen of the herb was also deposited in the Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak for ready reference.

Processing and extraction

The identified medicinal plant was dried in shade and pulverized using a household mixer grinder. The powder was sieved through a mesh size of 60-80 and stored in an air tight container till further use.

Extraction by percolation

The powdered herb (50 gm) was filled in the extraction chamber and percolated with ethanol at $70 \pm 2^\circ\text{C}$ till the extract became transparent in siphon tube of soxhlet apparatus. The extract was filtered and concentrated in the rotary evaporator to recover the solvent and percentage yield (w/w) was reported. The *Marrubium vulgare* extract obtained by percolation (MVP) was stored under sodium sulphate in the dessicator to prevent any moisture gain by powder.

Microwave assisted extraction

The drug powder (5 gm) was also extracted by using microwaves as heating source in Microwave synthesis reactor, U-Wave 1000 (SINEO Microwave Chemistry Technology, China) at Power-time mode. The apparatus was run with a frequency of 2450 MHz at an input power of 2000 W at atmospheric pressure. The extract was obtained at experimental conditions of 500 W (irradiation power) for 5 minutes at $70^\circ \pm 2^\circ\text{C}$. After the procedure the extract was centrifuged, filtered and concentrated to distill off the solvent. The percentage yield (% w/w) of *Marrubium vulgare* Linn extract obtained by microwave heating (MVM) was calculated as per the standard formula and reported [16, 17].

Pharmacognostical evaluation

The evaluation of various pharmacognostical characters was carried out to standardize the selected plant.

Morphological evaluation: The morphological characters such as colour, taste, texture, surface, odour strength and sensation were observed by the use of sensory organs and noted down. The macroscopical parameters like shape, size and fracture of the stem and root were also determined. The various diagnostic characters of leaf like type of venation, margin, lamina, base, margin and apex were evaluated and reported [18].

Microscopical evaluation: The wax blocks of the stem, root and leaf part of the plant were prepared and the transverse section (about 10 μm size) was cut down by using the rotary microtome. The sections were transferred to the glass slide with the help of a brush and dewaxed by treating with the xylene for five minutes. The sections were washed with ethanol for two minutes and mounted in glycerine to describe the various anatomical characters [19]. The free hand sections of (4 mm^2) of the lamina surface were cut and chlorophyll was removed by boiling with isopropanol on a water bath. The various leaf constants like vein islet number, vein termination number, palisade ratio, stomatal index on abaxial and adaxial surface was determined as per the standard methods [20].

Physicochemical evaluation: The various physicochemical parameters such as ash value, extractive value and loss on drying were measured and reported. For Total ash value, the 2 g of the powder drug was taken into a silica crucible and incinerated in muffle furnace at 450°C to make it free from carbon. After this process the powder was

weighed and total ash (% w/w) was determined with reference to air dried sample. The water soluble ash, sulphated ash and acid insoluble ash value was also evaluated as per the procedures described in standard books. The extractive value of the herb in different solvents (alcohol, water and ether) was determined by keeping 5 g of the powder sample with particular solvent and the solution was agitated at regular intervals (for first 6 hours) and filtered after one day. The 25 ml of the filtrate was transferred to a pre weighed china dish and put on a hot plate to evaporate the solvent. Care must be taken to avoid the charring of the extract. The dried residue was measured and extractive value for the respective solvent was calculated. The loss on drying of the powder sample was determined as per standard procedure. Briefly, the accurately weighed drug (10 g) was taken into the previously weighed china dish and kept in hot air oven at 105°C and weighed at regular intervals until the difference between two consecutive readings was not more than 0.25%. The drug was cooled in a dessicator and weighed to determine the moisture content [22, 23]

Total fibre content: To determine the total fibre content of the herb, the powdered drug (2 g) was extracted with xylene. The extract was discarded and marc was dried and treated with dilute sulphuric acid and sodium hydroxide successively. The powder drug was washed with water and alcohol and dried in dessicator. A silica crucible was weighed (X_1) and dried drug was transferred into it. The powder sample was kept at 130°C for two hours in a hot air oven and again weighed after cooling (X_2). Now the residue was burnt at 100°C for half an hour and again cool it room temperature and weighed (X_3) [23]. The total fibre content of drug was calculated using formula:

$$\text{Total Fiber Content} = \frac{(X_2 - X_1)(X_3 - X_1)}{\text{Weight of sample (in gm)}} \times 100$$

Fluorescence analysis: The powder of the drug was also analyzed for fluorescence behaviour by treating it with various acidic and basic solutions and evaluated under visible and UV light at short (254 nm) and long wavelength (366 nm) [24, 25].

Comparative evaluation of extracts

The whole plant extracts of *Marrubium vulgare* L. (MVP and MVM) were comparatively analyzed for the screening of phytochemical category and quantity of total phenolics and total flavonoids was also estimated.

Preliminary phytochemical screening: The two extracts were subjected to preliminary phytochemical screening to confirm the class of plant actives by the specified procedures mentioned in standard books [26].

Quantitative Estimation of total phenolics content: The quantitative estimation of total phenolic content of the extracts was performed by using UV-Visible spectrophotometer (UV-1800, Shimadzu Scientific Instruments Private Limited) at a wavelength of 760 nm. The test samples were prepared by dissolving the extracts in methanol at a concentration of 10 mg/ml (0.5 ml) and 1.5 ml of aqueous Folin-Ciocalteu reagent (10% v/v) was added. The solution was kept at room temperature for 10 minutes and then 1.5 ml of aqueous Na_2CO_3 (25%) was added. The solution was incubated for 30 minutes at 45°C on a water bath before analysis. The absorbance of the samples was recorded against the blank sample. The standard solution of the gallic acid (50-250 µg/ml) was also prepared in same proportion as of test samples and absorbance was noted. The calibration curve was plotted for absorbance against different concentration of gallic acid (GA). The concentration of phenolics (GA equivalent) in the test samples was determined from straight line equation generated by linear regression of calibration data and expressed as mg of GA equivalent/gm of extract. The analysis was performed in triplicate and values were represented as mean \pm standard deviation (S.D.) [27].

Quantitative estimation of total flavonoids content: The extracts obtained by percolation (MVP) and microwaves (MVM) technique for the selected herb were also evaluated for the total flavonoids content and expressed as mg of rutin equivalent (RUE)/gm of extract. The RUE of the samples (test and standard) was estimated by spectrophotometric method at λ_{max} of 415 nm. The test samples were prepared by taking 1.5 ml of extract solution (10 mg/ml in methanol) followed by the addition of 2% methanolic aluminium chloride solution (1.5 ml). Similarly the standard solutions were formed for rutin (in place of extract) in a concentration range of 50-500 µg/ml. The two solutions were kept at room temperature for one hour and absorbance was measured. The standard graph was constructed for different concentration of rutin against the absorbance and data was analyzed by regression analysis (linear) to develop the equation for straight line. The equation was used to calculate the concentration of rutin

equivalent in test samples. The experiments were carried out in triplicate and data was expressed as mean \pm standard deviation (S.D.) [28].

RESULTS AND DISCUSSION

The evaluation of various pharmacognostical parameters for the *Marrubium vulgare* Linn help in assuring the identity, quality and purity of the selected herb. The evaluation of macroscopical and microscopical features of the herb was useful to distinguish the *Marrubium vulgare* Linn from other species and adulterants. Moreover the shape and size of the leaves also describes the physiological maturity of the plant. The light green, quadrangular shape stem with average size of about 30-46 cm in length and 5-7 mm in width is present (Figure 1).

The fracture of the stem is splintery with smooth texture and surface is densely covered with white fine hairs. The cylindrical shape, creamish brown color roots with length of about 6-12 cm and 3-8 mm width are observed (Figure 2). The hairs are present on the root surface and fracture is woody.



Figure 1: Whole plant of *Marrubium vulgare* Linn



Figure 2: Roots of *Marrubium vulgare* Linn

The dark green color (adaxial surface) leaves are of 1.5 to 5 cm in length and with 2 to 6.5 cm of width. The abaxial side (dull green) with multicostate divergent type reticulate venation is observed (Figure 3). The leaves with tomentose type of petiole (4-6 cm) surface and cauline type of insertion are present on the stem. The pinnatifid simple leaves with superposed or verticillate phyllotaxy are present on the stem. The lamina with canescent surface (with white fine short hairs) and almost rotund/orbiculate shape is present. The leaves with crenate margin, subacute apex and symmetric base are present. The taste of the powder of all the parts (stem, roots and leaves) was found to be bitter in nature.

The transverse section (T.S.) of the stem indicates the conjoint and collateral vascular bundles. The pith consist of parenchyma cells are present with reserve food particles (Figure 4).

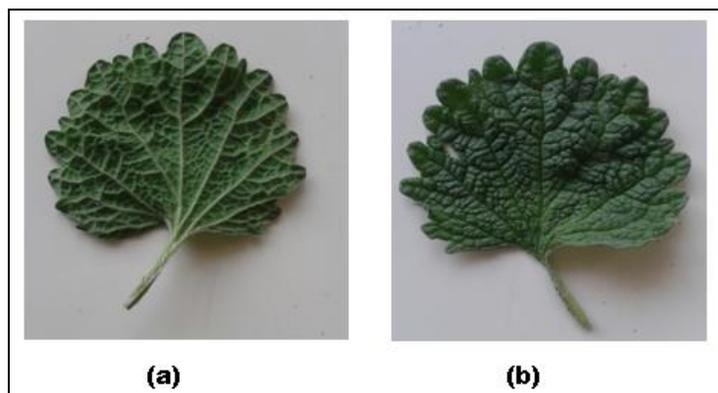


Figure 3: *Marrubium vulgare* Leaf (a) abaxial (b) adaxial surface

The transverse section of the root showed the presence of epidermis, cortex, endodermis and pith composed of parenchyma cells. In this section, the radial vascular bundles with large number of xylem vessels were also observed (Figure 5).

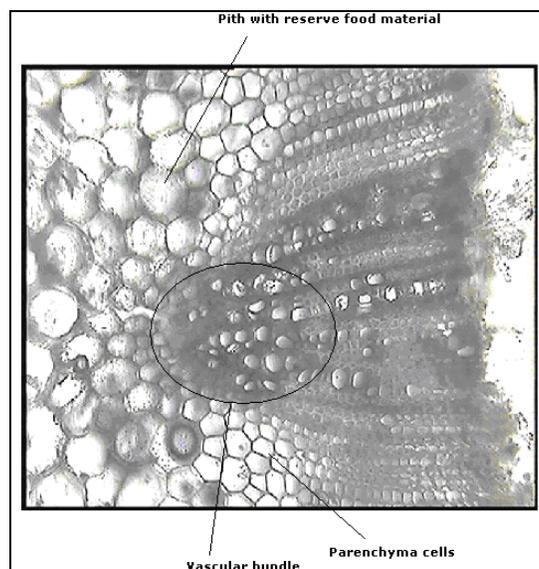


Figure 4: Transverse section through stem

The transverse section of the leaf through the vein confirmed the presence of vascular bundles and epidermal and palisade cells (Figure 6).

The evaluation of the microscopical section of the different parts (stem, root and leaf) confirmed that the *Marrubium vulgare* Linn is a dicot plant. The establishment of various leaf constant such as vein islet number, vein termination number, palisade ratio, stomatal index by the microscopical evaluation of the leaf surface aids in confirmation of genuineness of plant (Figure 7). The results of the quantitative microscopy of the leaf were presented in table 1.

Moreover the determination of physicochemical parameters serves as to ensure the quality of medicinal plant (Table 2). The extractive value is the measure of constituent's nature present in the extracts. The petroleum ether, alcohol and water soluble extractive value of the whole herb was found to be 2.77 ± 0.3 , $8.66 \pm 1.2\%$ and $5.90 \pm 0.8 \%$ w/w respectively. The ash value ($10.7 \pm 0.46\%$) and water soluble ash value ($8.9 \pm 0.65\%$) is the indicative of salts and minerals present in the plant.

The acid insoluble ash (1.73 ± 0.61) tells us about the percentage of adulteration with siliaceous material which arise due to the improper collection of the herb [29]. The total fibre content is also considered as a measure to evaluate the

quality of a drug. For the selected herb it was found to be $9.5 \pm 0.88\%$. The high content ($17.2 \pm 0.35\%$) of moisture in the sample ($>10\%$) suggests that the proper storage of the drug is required to avoid the microbial contamination.

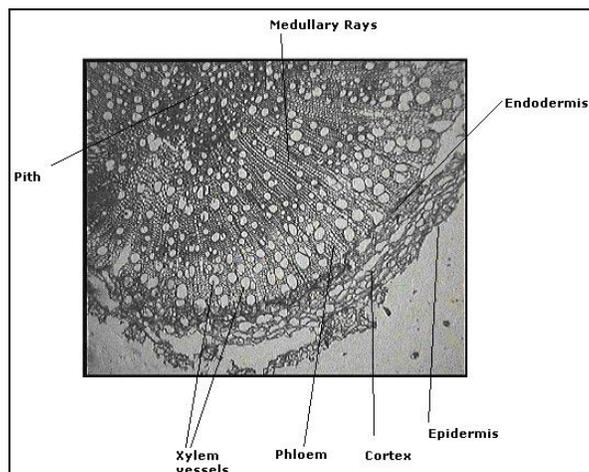


Figure 5: Transverse section through root

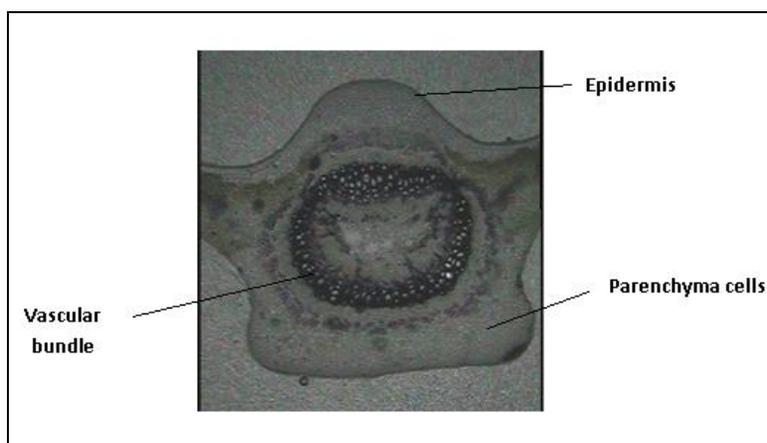


Figure 6: Transverse section of the leaf through vein

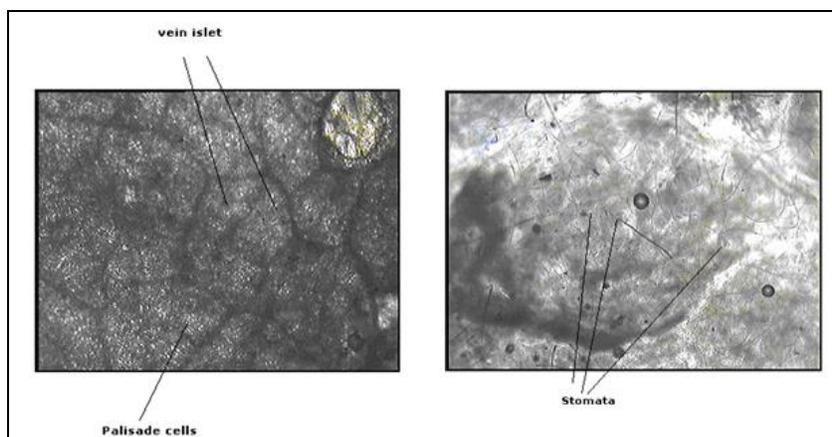


Figure 7: Photomicrographs of leaf surface showing vein islet, palisade cells and stomata

The results of the quantitative microscopy of the leaf are presented in table 1.

Table 1: Various leaf constants of *Marrubium vulgare* Linn

Leaf constant	Range
Stomatal index (adaxial surface)	9.1 - 10.4 - 11.5
Stomatal index (abaxial surface)	18.8 - 20.02 - 21.1
Vein islet number	3 - 3.9 - 4.75
Vein termination number	6.75 - 7.9 - 9
Palisade ratio	2.25 - 2.56 - 3.26

Table2: Various physicochemical parameters of whole plant of *Marrubium vulgare* Linn

Physicochemical parameters	Value
Petroleum ether soluble extractive value	2.77 ± 0.3
Ethanol soluble extractive value	8.66 ± 1.2
Water soluble extractive value	5.90 ± 0.8
Total ash	10.7 ± 0.46
Acid insoluble ash	1.73 ± 0.61
Water soluble ash	8.9 ± 0.65
Total fibre content (%)	9.5 ± 0.88
Loss on drying (%)	17.2 ± 0.35

Values are expressed as mean ± standard deviation (n=3)

The natural products from the plants may produce the fluorescence in visible/UV light as such or on addition of various chemical reagents and thus it constitutes an important parameter for the evaluation of plant based drugs. The results of the fluorescence analysis of the powder of *Marrubium vulgare* Linn are depicted in table 3.

Table 3: Fluorescence analysis of powder of whole plant of *Marrubium vulgare* Linn

Chemical/Reagent	Fluorescence Analysis		
	Visible light	Short UV (λ -254 nm)	Long UV (λ -366 nm)
Drug Powder	Light green	Dark brown	Whitish
Powder + dilute HNO ₃	Light greenish	Dark brown	Light green
Powder + methanolic NaOH	Dark green	Dark black	Light brown
Powder + aqueous NaOH	Dark green	Dark black	Light brown
Powder + CH ₃ COOH	Light green	Dark black	Greenish white
Powder + FeCl ₃	Yellowish	Dark black	Light brown
Powder + HCl	Light brown	Dark black	Light green
Powder + Picric acid	Yellowish brown	Dark black	Light yellow
Powder + H ₂ SO ₄	Yellowish brown	Dark black	Light yellow
Powder + I ₂ solution	Light black	Dark black	Creamish white

The comparative evaluation of the two techniques revealed that the microwave assisted extraction of *Marrubium vulgare* Linn enhance the extract yield from 11.27 ± 1.2 to 20.23 ± 1.91% w/w. Moreover the preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolic compounds, steroids and diterpenes in both the extracts. The quantitative estimation of MVP and MVM indicated that the novel method of extraction significantly increase the total phenolic content (61.44 ± 2.01 to 93.42 ± 1.04 mg of GAE/gm of extract) whereas the total flavonoid content was also enhanced to 37.7 ± 1.66 mg of RUE/gm of MVM from 23.25 ± 0.94 mg of RUE/gm of MVP (Table 4). The high content of flavonoids and phenolic compound justified the use of *Marrubium vulgare* Linn in the diseases (hypertension, diabetes, cardioprotective etc) caused by oxidative stress.

Table 4: Comparative evaluation of two extracts

Parameter	Extract	
	MVP	MVM
% Yield (w/w)*	11.27 ± 1.20	20.23 ± 1.91
Preliminary Phytochemical screening	Presence of alkaloids, steroids, terpenoids (diterpene), flavonoid and phenolic compounds	Presence of alkaloids, steroids, terpenoids (diterpene), flavonoid and phenolic compounds
Total phenolic content (mg of GAE/g of extract)*	61.44 ± 2.01	93.42 ± 1.04
Total flavonoid content (mg of GAE/g of extract)*	23.25 ± 0.94	37.7 ± 1.66

*Values are expressed as mean ± standard deviation (n=3)

CONCLUSION

The pharmacognostical evaluation is an economic and easiest way to confirm the quality of a plant. This could be assumed as reliable method to find out the adulteration and moreover the source and species of selected plant can also be assured. In nutshell, we can conclude that the establishment of range/value of various diagnostic parameters would help in standardization of *Marrubium vulgare* Linn for development as herbal formulation and inclusion in and various pharmacopoeias.

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