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Phytochemical analysis and pharmacognostic studies of leaves, stem and root of *Misopates orontium* L.

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Misopates orontium L. is a traditionally used medicinal plant belonging to family Scrophulariaceae. The present research work is focused on pharmacognostic standardization including macroscopic, microscopic evaluation and micrometery of leaf, stem and roots powders of *M. orontium*. TS observation of leaf, stem and root showed the shape and arrangements of different cells. In conjunction with these observations palisade ratio, stomatal index, vein termination, vein inlet number of leaf were also calculated. Histochemical features were studied by using Phlorogucinol, Conc. HCl, Iodine solution, Ferric chloride and Sudan III solution. These reagents were used to locate the presence of Ca+2 oxalate crystals, lignin, starch, tannins and oil globules, respectively. Powder study of the leaf showed the presence of epidermal cells, diacytic stomata, glandular trichomes and vessels. In stem powder collenchyma, reticulate vessels, fibers, cortex cells with tracheids were observed. While in root powder boarderd and pitted vessels along with the reticulate vessels, cork cells, parenchyma, oil ducts and oil cells were observed. Different colors were observed under ordinary light, short and long wavelength of UV light influorescence analysis. Phytochemical analysis of the methanolic extract of whole herb confirmed the presence of alkaloids, glycosides, flavonoids, saponins, sterols, triterpenoids, carbohydrates, proteins and tannins. All these results obtained will provide guide lines in establishing the quality control parameters which will help in setting down the pharmacopoeial standards for *M. orontium* L.

Keywords: *Misopates orontium* L, Histochemical, Pharmacognostic, Phytochemical, microscopy

INTRODUCTION

Medicinal plants provide an effective assistance to health care and disease free life. They contained physiologically active principles that have traditional importance for the treatment of various ailments (Qureshi et al. 2009; Rani and Khullar, 2004; Lev, 2006). These medicinal plants are used in the treatment of various diseases either alone or in combination with other plant drugs (Kaur et al. 2011). Plants are a valuable source of a wide range of secondary metabolites, which are used for treatment and prevention of the diseases (Al-Snafi, 2016). *Misopates orontium* L. of family Scrophulariaceae is an annual herbaceous plant found in hilly areas of Pakistan. It has bitter and stimulant properties, the whole plant has been employed for the treatment of tumors and ulcers (Jabeen et al. 2009). The scientific approval and the mechanisms of such claims are lacking in the literature (Lönnig et al.

2007). Traditionally it is used as a diuretic, for treatment of scurvy, in liver disorders, in tumors, as detergent and as astringent. The plant was also used in the treatment of all kinds of inflammation and in haemorrhoids. It contained amino acids, pigments, oils, anthocyanidins, flavonols, flavones, aurones, flavanones, cinnamic acids and many other compounds (Al-Snafi, 2016). Therefore, an extensive anatomical, physicochemical and phytochemical screening is required that will be helpful to avoid any ambiguity (Perveen et al. 2020). Morphological results are helpful in explanation of an exclusive drug with a major focus on quantitative and qualitative microscopy (Demirpolat et al. 2019). Therefore, the present research comprises of anatomical, structural and histochemical evaluations of the leaf, stem and root, along with the whole herb assessment of physicochemical parameters, fluorescence analysis and preliminary phytochemical properties.

MATERIALS AND METHODS

Plant collection

The plant was collected from Bhimber (Bandiala), Kotli, Azad Kashmir and got authenticated by Dr. Uzma Hanif, Department of Botany, Government College University (GCU), Lahore, Pakistan under voucher No: GC. Herb. Bot. 3458.The plant was dried under shade for about 15 days and then pulverized. Powder was stored in amber colored bottles at dry place.

Morpho- anatomical evaluations

Morphological evaluation

Macroscopic evaluations were carried out on 3samples of each part according to Evans (2009). TS of leaf, stem and root were made by commonly used blade and razor method.

Anatomical evaluation

Fresh sections were stained with safranin and fast green dye (Jaiswal et al. 2014).

Histochemical evaluation

Histopathology of these TS was conducted by using phlorogucinol, conc. HCl, Iodine, Ferric chloride and Sudan III solution to locate the presence of Ca+2 oxalate crystals, lignin, starch, tannins and oil globules, respectively under microscope. (Christodoulakis et al. 2015). Powder microscopy of each part was done according to standard procedure of Evans.

Micrometery of Anatomical sections

Micrometer was used to measure the size of different cellular structures observed in each sample (Kadam et al. 2012). Specific histological features including, stomatal index, vein-islet and vein termination number and palisade ratio were noted (Najafi and Deokule, 2010).

SEM

Scanning electron microscopy was done of each TS by method given by Rashid (2018) from Centre for Advanced Studies in Physics (CASP), Department of Physics, Government College University, Lahore, Pakistan.

Fluorescence analysis

Florescence analysis of each part of plant powder was done according to method of Ishtiaq et al. 2018 by using different reagents.

Preliminary phytochemical analysis

The preliminary phytochemical analysis of whole herb was carried out according to the standard procedures (Ishtiaq et al. 2016).

Physicochemical analysis

Extractive values of different solvent, pH and acid values were calculated according to standard procedure (Ishtiaq et al. 2014).

RESULTS

Morphological evaluation

Misopates/Antirrhinum orontium is commonly called as snapdragon. Flowers are borne in terminal racemes. Literature revealed that both qualitative and quantitative studies are important for identification of raw material during drug manufacturing (Kumar et al. 2011). Herbal drug's purity, safety, identity and quality can be maintained by means of standardization which involve in both the qualitative and quantitative parameters (Ishtiaq et al. 2018).

Anatomical/Microscopic evaluation

Among the qualitative parameters powder microscopy is the simplest and more economical measure. Quantitative parameter included micrometry of cells, stomatal index, vein islet, vein termination number and palisade ratio are specific for certain species and measure. In the findings about *M. orontium* L the TS of leaf showed arrangement of epidermis, collenchyma cells and vascular bundles (Fig.1A, B). The TS of stem vascular bundles arranged in circular form along

with pith cells, cortex and epidermis (Fig. 1C, D). The TS of root also disclosed the presence of cortex, pith, epidermis and pericycle in the middle (Fig. 1E, F).

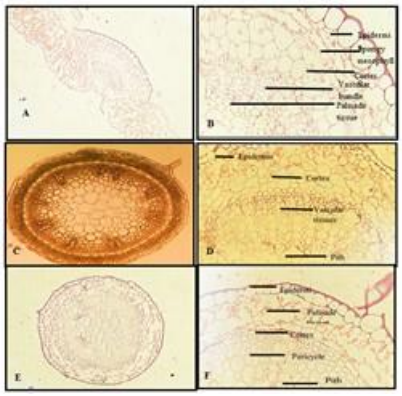

Figure 1: A, B Showing T.S of Leaf, C, D Showing T.S of Stem and E, F Showing T.S of Roots of *M. orontium*.

Histochemical analysis of TS

The histochemistry showed the presence of Ca^{+2} oxalate crystals, tannins and starch granules. However, it didn't show any change when exposed to Sudan III and ferric chloride which confirms the absence of oil globules and lignin.

SEM analysis

Herbal drug technology includes all the necessary steps for the conversion of botanical materials into medicines, where standardization and quality control are essential analytical tools to assure the correct identification of drugs. Advances in microscope technology and improvements in light and scanning electron microscopes have increased the accuracy and capabilities of microscopy as a mean of botanical identification. This approach aims at the establishment of botanical biomarkers based on the major microscopic features observed in the studied drugs. (Fig. 2)

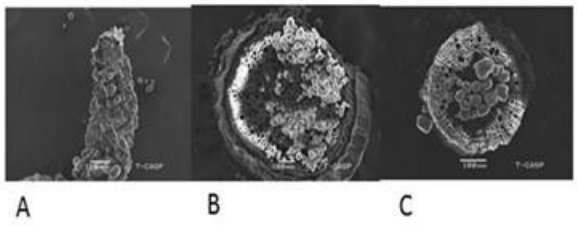

Figure 2: A Showing T.S of Leaf, B Showing T.S of Stem and C Showing T.S of Roots of M. orontium under SEM.

Anatomical analysis of leaf, stem and root

The powder microscopy of leaf showed; epidermal cells with numerous stomata, epidermal tissue contain various layers of palisade tissues, oil cavity, group of vessels, numerous glandular trichomes with base attached to the surface of epidermal cells. Stem powder showed thin walled cork cells, lignified xylem parenchyma cells, widely distributed reticulate vessels, glandular trichomes with covered head and calcium oxalate crystals. While root showed; epidermal cells with few stomata, thick walled scelerenchyma cells, pith cells, part of cork and phelloderm region, thin wall parenchyma cells, phloem cells with few calcium oxalate crystals, reticulate vessels, fibers, oil cavity and oil cells with associated tissues (Fig. 3, 4, 5).

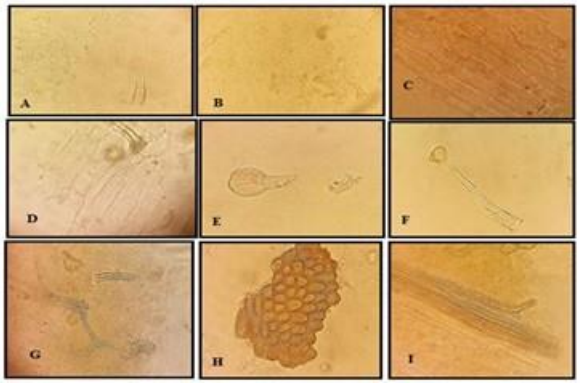

Figure 3: Leaf Powder of M.orontium; A, B,C Indicated Epidermis with Trichome and Stomata; D, E Showed Lamina with Oil Cavity; F Unicellular Glandular Trichome, G, Network of Vessels with Xylem H and I Epidermis of near Margin

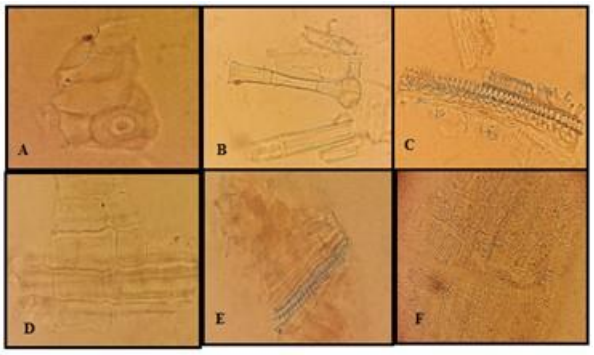

Figure 4: M. orontium Stem Powder; A, B Glandular Trichrome with Multicellular Base, C, Part of Cork and Phelloderm D, Trachedial Vessel E, F Medullary Rays Containing Acicular crystals of Calcium oxalate

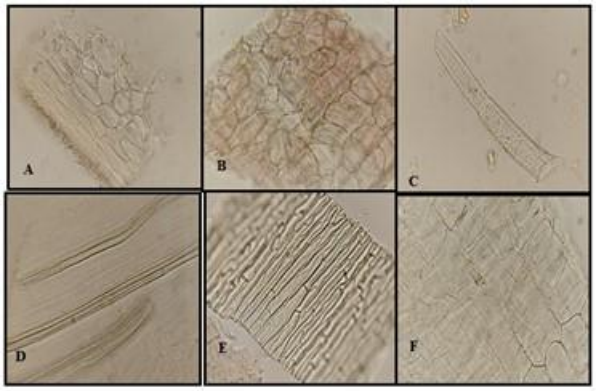

Figure 5: M. orontium Root Powder A. Cork, B, C Fiber with Bordered Pitts, D Fibers Associated with Reticulate Pitted, E and F Parenchyma Cells with Striations.

Quantification of Anatomical analysis

The cellular components are observed in leaf, stem and root of *M. orontium* L and their measurements are taken by using micrometer (Table 1) and quantitative evaluation of leaf constants are also calculated (Table 2). The quantitative evaluation of cellular parts in powder study and TS of leaf helped in the establishment of leaf constants that proved to be a helpful tool to aid in the authentication and confirmation process of this therapeutically important drug (Table 1 & 2).

Table 1: Micrometry of Some Cells of *M. orontium* L.

Type of cells	Length(µm)	Width (µm)
Epidermal cell	27.66 ± 0.544	20 ± 0.942
Glandular trichome	45.66 ± 7.635	17.66 ± 2.125
Reticulate vessels	199.33 ± 9.90	13.66 ± 1.784
Spongy mesophyll	26.33 ± 2.76	19.33 ± 1.08
Stomatal cell	17 ± 2.624	15.66 ± 1.186
Parenchyma cell	34.66 ± 3.538	15.33 ± 1.186
Cork cells	24 ± 0.942	8 ± 0.471
Head of trichome	25.66 ± 3.538	24.66±1.440
Fibers	163.33 ± 28.414	9 ± 0.942
Oil duct	30.66±0.981	15.33±1.186

Table 2: Leaf constants of *M. orontium* L

Type of cells	Length(µm)	Width (µm)
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Fibers	163.33 ± 28.414	9 ± 0.942
Oil duct	30.66±0.981	15.33±1.186

Fluorescence analysis

The fluorescence analysis is an appreciated, easy and direct method for the identification of fluorescent compounds (Joshi et al. 2012). The powder of whole herb gave various colors when observed under ordinary daylight, in short and long wavelength of UV light (Pekgöz and Çinbilgel 2019) (Table 3).

Table 3: Fluorescence analysis of *M. orontium L*

Leaf constants	Range	Mean ± SEM
Palisade ratio	4.8-5.3	5.05 ± 0.144
Stomatal index of lower epidermis	3.1-6.9	5.0 ± 1.096
Vein-islet number	6.2-7.5	6.85 ± 0.375
Vein termination number	21.5-24.8	23.15 ± 0.952

Physicochemical constants

The preliminary phytochemical screening of methanolic extract of *M. orontium* has been done (Table 4). Extractive values of whole herb powder in methanol, aqueous, chloroform, *n*-hexane, butanol, dilute alcohol and petroleum ether the results are tabulated in (Table 5). The phytochemical screening of whole herb mainly revealed the presence of terpenoids, tannins, glycosides, flavonoids, alkaloids, proteins, carbohydrates, saponins, fats and fixed oils (Table 4). The extractive value of whole herb in methanol was high, followed by dilute alcohol.

Table 4: Preliminary phytochemical analysis of *M. orontium* L.

Protocol	Ordinary light	Short wavelength (254nm)	Long wavelength (365nm)
Powder	Green	Grey	Light pink
$FeCl_3$	Orange	Orange	Light yellow
Conc. HCl	Dark brown	Milky brown	Yellowish brown
1M NaOH	Lemon yellow	Colorless	Pink
$AgNO_3$	Milky yellow	Milky white	Milky white
Water	Light green	Colorless	Pink
CCl_4	Green	Light orange	Yellow
Methanol	Apple green	Dark pink	Golden yellow
CH_3COOH	Light yellow	Orange	Golden yellow
Xylene	Grass green	Light orange	Light green
NH_3	Lemon green	Milky green	Light orange
I_2	Chocolate brown	Black	Orange
Br-Water	Light brown	Light orange	Light orange
Aniline	Dark brown	Dark green	Yellowish orange
Pet-ether	Light brown	Light pink	Purple pink
Ethyl Alcohol	Light green	Orange	Apple green
Picric acid	Yellow	Dark yellow	Green
KOH	Light brown	Light brown	Orange brown

Table 5: Extractive values *M. orontium* L.

Phytochemical group	Test	Methanolic extract
Terpenoids	Salkowaski test Liebermann's test	+ + + +
Tannins	Ferric Chloride test Bromine water test	+ + +
Glycosides	Keller killani test Legal 's test	+ + + +
Flavonoids	Alkaline reagent test Lead acetate test	+ + +
Alkaloids	Mayer 'test Wagner 'test Hager 's test Dragendroff 's test	+ ++ + ++ + ++ + ++
Proteins	Millon 's test Ninhydrin test	+ + +
Carbohydrates	Molisch 's test Benedicts 's test	+ + + ++
Saponins	Foam test	+
Fats and Fixed oil	Spot test	+

The n-hexane extractive value was the lowest as compare to other solvents (Table 5). Fluorescence analysis of all plant samples treated with different reagent showed different color under UV light and day light (Table 3).

DISCUSSION

Literature revealed that both qualitative and quantitative studies are important for identification of raw material during drug manufacturing (Kumar et al. 2011; Singh et al. 2010). Herbal drug's purity, safety, identity and quality can be maintained by means of standardization which involve in both the qualitative and quantitative parameters (Ishtiaq et al. 2018). Among the qualitative parameters powder microscopy is the simplest and more economical measure to identify and standardize the plant material. Quantitative parameter included determination of the size of different cells by using micrometer, stomatal index, vein islet, vein termination number and palisade ratio are specific for certain species and measure. In the findings about *M. orontium* L the transverse section of leaf showed arrangement of

epidermis, collenchyma cells and vascular bundles. The abundant stomata were also observed in the upper epidermis (Fig.1A, B). The TS of stem vascular bundles arranged in circular form along with pith cells, cortex and epidermis (Fig. 1C, D). The TS of root also disclosed the presence of cortex, pith, epidermis and pericycle in the middle (Fig. 1E, F).

Histochemical assessment of transverse sections were carried out at cellular level which demonstrated the existence of tannins, lignin, Ca+2 oxalate crystals and starch granules in the leaf, stem and root. Effervescent response of all parts indicated the presence of Ca+2 oxalate crystals. Blackish coloration in all parts except leaf indicated the existence of tannins. Megnata coloration in all parts indicated the presence of lignin. Similarly blue color indicated the presence of starch granules in all the parts. No pinkish red coloration showed the negative results of oil cells.

Scanning electron microscopy (SEM) is a modern technique to study the plant powder and their TS for authentication of genuine herbal plant material. It is cheap and easy technique for standardization the herbal drug (Fig 2).

The powder microscopy of leaf of the herb showed the epidermal cells with numerous stomata, epidermal tissue contain various layers of palisade tissues, oil cavity, group of vessels, numerous glandular trichomes with base attached to the surface of epidermal cells. The powder microscopy of the stem showed the thin walled cork cells, lignified xylem parenchyma cells, widely distribution of reticulate vessels through the region, glandular trichomes with covered head and calcium oxalate crystals. The powder study of root revealed the epidermal cells with few stomata, thick walled scelerenchyma cells, pith cells, part of cork and phelloderm region, thin wall parenchyma cells, phloem cells with few calcium oxalate crystals, reticulate vessels ,fibers, oil cavity and oil cells with associated tissues (Fig. 3, 4, 5).

The quantitative evaluation of cellular parts in powder study and TS of leaf helped in the establishment of leaf constants that proved to be a helpful tool to aid in the authentication and confirmation process of this therapeutically important drug (Table 1 & 2). The fluorescence analysis is an appreciated, easy and direct method for the identification of fluorescent compounds. Different compounds give fluorescence when they are exposed to short and long wavelength UV light (Joshi, 2012). The powder of whole herb gave various colors when observed under ordinary daylight, in short and long wavelength of UV light (Table 3). The preliminary phytochemical screening of methanolic extract of *M. orontium* has been done (Table 4). Extractive values of whole herb powder in methanol, aqueous, chloroform, *n*-hexane, butanol, dilute alcohol and petroleum ether the results are tabulated in (Table 5).

CONCLUSION

The study of phytochemical and pharma cognostical features of *M. orontium* had shown the standards, which will be effective parameters in the identification and recognition of its purity and genuineness. Physicochemical parameters such as pH, acid values, extractive values and fluorescence analysis are indicators of the quality of material. These pharma cognostical evaluations and physicochemical characterization all are anatomical features helpful for a researcher in their research work for authenticity of this plant material.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

SA wrote the manuscript and performed the experimental work, SI rechecked the manuscript and designed the experimental work. NA helped in anatomical study and performance of experimental work, SR assisted throughout the experimental as well as theoretical work. SR assisted SI throughout the manuscript writing.

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