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Spectroscopic analysis, Morpho-anatomical Characterization and Phytotoxic Evaluation of *Rosa multiflora* Thunb

Jehan Zada[1,3*], Sumaira Shah[1], Ali Hazrat[2], Syed Hidayat Yar[3], Zahid Ullah[1] , Ikram Ullah[1]

[1]*Department of Botany, Bacha Khan University Charsadda, KP, Pakistan.* (24420)

[2]*Department of Botany, University of Malakand, Chakdara, KP, Pakistan.* (18800)

[3]*Department of Botany, Govt; Degree College Lal Qilla, Pakistan. (*18800*)*

Correspondence
Jehan Zada,
jehanzada777@gmail.com

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Abstract

This study presents a comprehensive analysis of *Rosa multiflora* leaves focusing on their morphological characters, anatomical feature, phytotoxic potential and spectroscopic analysis. The leaf anatomy exhibited a typical bifacial structure with specific characteristics such as palisade palisade ratio (9.5), vein islets number (16.5 per mm^2), vein termination number (14.5 per mm^2), stomatal number (121.5 per mm^2) and stomatal index (9.5). Various structures like trichomes, mineral crystals, and phloem tissues were observed in powder microscopy of crude drugs. Elemental analysis detected essential minerals like K (4.159 mg/L), Ca (8.030 mg/L), Na (1.787 mg/L) and Fe (1.710 mg/L) in fairly good amounts. These minerals play crucial role in various biological processes highlighting the potential nutritional and medicinal value of the selected plant. Additionally, the study evaluated chlorophyll content in fresh leaves, which varied across different size samples. The existence of bioactive compounds and antioxidant enzymes suggests the potential of *Rosa multiflora* leaves for medicinal applications. These findings can benefits pharmaceutical industry, students and scientific community for further research and development, highlighting the plant's promise for various uses.

KEYWORDS

Anatomical; Phytotoxic; Elemental; Medicinal

1.0 INTRODUCTION

Medicinal plants are a rich source of bioactive compounds that offer effective treatment option for various human diseases. Natural remedies derived from these plants are not only affordable but often considered safer, with few side effects compared to synthetic alternatives. It makes the chief source of new health care materials and pharmaceuticals [1]. *Rosa multiflora* Thunb is deciduous scrambling or arching shrub climbing over other plants up to a height of 7 m. Stem features curved prickles. Leaflets typically 5 but it may be 7-9, measure up-to 5 cm in length, with acuminate tips. The margins are usually simple, but occasionally doubly serrate, and sparsely hairy above and beneath. Stipules bears long prickle along the margin. Flowers are white, occasionally pink, inflorescence panicles and rarely corymb that reflexed after flowering. The fruits are small, globose, and 6-7mm in diameter, with a hairy, elongated column of styles. Fruits are achene and become red on ripening [2]. Aqueous extract of flower and leaves are active against gram-positive

bacteria also used as a laxative in Japan. The fruits are diuretic, anodyne, hypoglycaemic and laxative. It is also used to treat constipation and articular pain and foul ulcers, wound sprains and injuries [3]. Medicinal plants are the reservoir of important bioactive phyto-constituents and serve as a path for modern drug preparation [4]. Pharmacognostic procedures are useful for the identification and authentication of drugs from plant origin. For the standardization of plant material, pharmacognostic procedures such as biochemical, microscopic and macroscopic studies are useful for the identification and standardization of medicinal plants and its materials. Leaf anatomy is useful for the identification of medicinal plants. Some microscopic leaf constant parameters like stomatal density, stomatal index, termination of the vein, vein islets, the ratio of palisade cells etc. are also used to identify and standardized crude [5]. The number of stomata, numbers of vein islets, vein termination numbers, ratio of palisade cells, are the leaf constant, Stomatal numbers, stomatal index, vein islet number, vein termination number and palisade cells ratio are the leaf constant, commonly used to evaluate the leaf drugs on the microscopic basis [6]. The fluorescence study is also useful in the identification and determination of active materials in the crude drugs of plants. Therefore it may be used as the first step for the identification of drugs of plant origin [7, 8]. Various fluorescence analyses on different medicinal plants like *polygonum cuspsidatum* [6, 9] and *Hygrophila auriculata* [10] were worked out. Various micro and macro elements play an important role in contesting against numerous health problems. The decrease in calcium level causes degradation of tooth, hypertension, paralysis and increase in lipids level especially in cholesterol content in a human being [11]. Each medicinal plant has pharmacologically important phytoconstituents and having its own elemental composition [12, 13]. In pharmacy, chlorophyll molecules are used as a photosensitizer for cancer therapy. Chlorophyll is known to have multiple medicinal uses and has its place in modern medicine and therapy. In traditional medicine, the chlorophyll and its derivatives have a long history [14, 15] and also numerous therapeutic uses including the anti-inflammatory agent [16, 17], wound healing [18] and internal deodorant [19]. Weeds compete with cereal crop for available resources and effect crops yield because the growth of weeds is fast as compare to crops [20]. The major problem in Pakistan is the massive loss of crops due to poor or lack of weeds management.

2.0 MATERIALS AND METHODS

2.1. Plant Collection and Preservation

Fresh leaves of *Rosa multiflora* were collected from Maidan Valley, District Lower Dir, Khyber Pakhtunkhwa, Pakistan. Some fresh leaf samples were used for macroscopic and microscopic study. The remaining collected leaves of the selected plant were washed, cleaned with tap water, separated and dried in shade. The dried leaves were grinded to fine powdered by the electric grinder and used for

different analysis i.e., microscopic, spectroscopic and phytotoxic evaluation.

2.2. Morphological Study

The morphological features of the selected plant for plant identification will be studied by following the organoleptic methods of Trease, [5] for the study of following leaf parameters i.e. leaf size, odour, color, taste, phyllotaxis, insertion, leaf base, petiole, leaf lamina and venation.

2.3. Anatomical Study

Transverse sections of the *R. multiflora* leaf were prepared through sharp razor for leaf epidermal study. Thin transverse section was taken and stained with phloroglucinol and hydrochloric acid was placed on a glass slide in glycerine and examined under Labomed microscope with camera fitted [21].

2.4. Leaf Surface Study

The arrangements of most of the cells are destroyed in powder drugs, therefore for the evaluation of drugs, a microscopical study of the leaves was carried out, which include;

2.4.1. Vein Termination Number and Vein Islets

The research plant leaf was cut into various sections from side to a central vein cleaned by boiling in a test tube containing 200% Choral hydrate by keeping in a water bath [22]. The cleared leaf part of 1-mm is cut and mounted on a slide and observed by using a 4mm objective of the compound microscope and stage micrometer. After fixing, the stage micrometre was removed and a cleared leaf specimen mounted on the slide was focused. Light is focused on the 1mm along with stetting of iris diaphragm closer to a certain extent. The entire islet was counted from one side in the square and those which touch the square boundary also included in counting. Veinlet terminations were also counted along with veins-islets inside the square.

2.4.2. Stomatal Density and Stomatal Index

The fresh leaf epidermis from both sides removed and added to dilute solution of glycerine with the help of forceps and studied under compound microscope. The epidermal cells number along with stomatal number was noted through 100x magnification. Additional information such as epidermal cell size stomatal size was measured through ocular micrometer and stage micrometer. The existence and absence of stomata, stomata types etc. were also observed [23, 24].

2.4.3. Ratio of Palisade Cells

Various small thin sections of the leaf cut from leaf lamina and cleaned by boiling in a test tube containing 200% choral hydrate by keeping in the water bath [25]. Under the compound microscope, a very thin section was adjusted as such that palisade cells and the cells of epidermis arranged under them can be observed at the same time with a slight adjustment. By dividing the resulted value by 4, the Palisade ratio was obtained. Different pieces were examined to get correct and standard results [23].

2.5. Powder Drugs Microscopy

Leaf Powder drug of *R. multiflora* was examined for their microscopical and physical features. Physical features include colour, taste and odour while

microscopical observation was work out for the determination of the specific structure existing in the leaf powder. The proposed study was carried out by adopting the following techniques. A small amount of leaf powder was mounted on slide, various liquids such as iodine solutions, chloral hydrate and water for clear observation. The prepared a slide of powder was examined with the help of microscope using 10X and 45X objective for 45X and 10X objective lenses for better study of various structures and then drafted. Some structures of powder were also examined with the help of a camera fitted Labomed microscope [26, 27].

2.6. Fluorescence Study

Fluorescence study is used as a standard for identification and standardization powder drugs [6]. Fluorescence study of *R. multiflora* leaf powder untreated as well as treated samples with various chemicals (50% H_2SO_4, NH_3 solution, 50% HNO_3, Sodium hydroxide (NaOH) in ethanol, Sodium hydroxide (NaOH) in water, Picric acid, Iodine solution 22 and 10% solution of $FeCl_3$) was performed by examining the sample in visible light and Ultra Violet light of both short wavelengths [23, 28, 29].

2.7. Elemental Analysis

Elemental study of leaf powder was work out with an absorption spectrophotometer for the determination of some macro and microelements. The sample mixture was filtered and the whole filtrate was diluted properly with distilled water up-to-the 100ml solution by following the method of [30]. The prepared samples analysed through Atomic Absorption Spectrophotometer [31]. The elements were detected through the atomic absorption spectrometer and value achieved in ppm (part per million). The curve of the calibration was established using standards 1000 ppm for the individual elements.

2.8. Leaf Chlorophyll Content

The leaf chlorophyll content of the proposed plant was estimated by using the procedure of Arnon [32].

2.9. Phytotoxic Activity

The phytotoxic activity of *Rosa multiflora* leaf extract was evaluated using *Lemna minor* as test species [33].

2.9.1. Preparation of media for Phytotoxic activity

E-medium was prepared from various minerals nutrients with specific concentrations. The salts were first weighed and then dissolved in distilled water and then water is added to make a volume of 1000ml. The pH of E-medium was adjusted from 5.5-6.0 by the addition of potassium hydroxide (KOH).

2.10. Statistical Analysis

The experiments were performed in triplicate and the data obtained was analyzed and values are expressed as the mean ± standard deviation (SD).

3.0. RESULTS AND DISCUSSION

Plants play a crucial role in the treatment and prevention of various diseases and still remain a leading choice for many people worldwide including Pakistan. In most countries of the world natural drugs are either used for the ordinary medical treatment or as a food supplement to prevent diseases. Various medicinal plants are used as an alternative of synthetic drugs for the treatment and

prevention of a variety of diseases. Due to a number of reasons such as availability, preparation, preservation, storage and processing natural's products of medicinal plants are preferred over synthetic drugs [34].

3.1. Leaf Morphology

Morphological and organoleptic characteristics of leaf and flower are significant for identification and authentication as shown in Table (1). The combination of pinnatifid (fringed) stipules and hairless styles are a unique feature to this species of a rose. The organoleptic/ morphological characteristics of *R. multiflora* leaf are listed in Table (1). The present investigation revealed that morphological characteristics of a leaf of *R. multiflora* were beneficial in species identification and separation from closely related species of family rosacea. Leaf Leaves are 8 to 11 cm long, alternate and pinnately compound (Table 1). Each leaflet has 5 to 11 elliptic, ovate or oblong leaflets, each 1 to 5 cm long with serrated edges. The upper surface of leaflets is glabrous dark green and the lower surface is pubescent light green. The combination of pinnatifid (fringed) stipules and hairless styles are unique features to this species of rose. Other researchers conducted the macroscopic, microscopic study of various parts of *Holoptelea integrifolia* in terms of organoleptic, microscopic and physical parameters [35].

Table. 1 Morphological/ Organoleptic characters of *Rosa multiflora* leaf.

S. No	Characteristics	Finding
1	Leaf Size	5cm long, 1.5-2.5 cm wide
2	Ordour	Not specific
3	Phyllotaxis	Alternate
4	Venation	Reticulate
5	Shape	Ovate
6	Taste	Slightly bitter
7	Color	Upper is glabrous dark green and lower surface is pubescent light green
8	Lamina	Narrow broad
9	Leaf base	Symmetric
10	Petiole	Present
11	Stipule	Fringed
12	Margin	Serrated

3.2. Leaf Epidermal Study

The foliar epidermal anatomical parameters of leaf such as the shape of the epidermal cell, stomata type were studied. These studies indicated that these parameters showed variation in different species (Fig.1--a, b, c). The recorded observations were listed in Table (2). The upper surface of leaf consist densely arrange palisade cells containing the greater amount of chlorophyll.

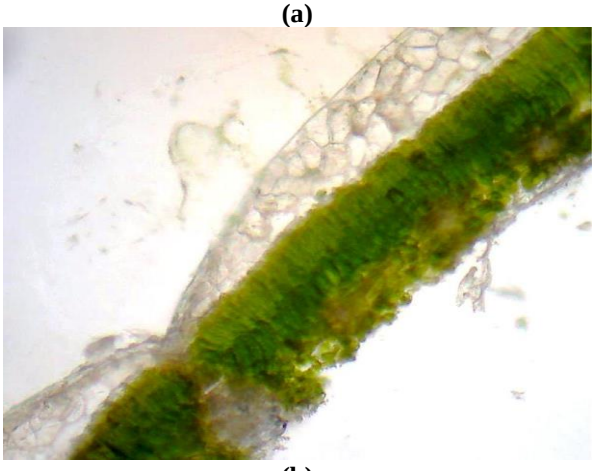
The epidermal study of *R. multiflora* leaf was carried out under a compound microscope. The microscopic study shows that stomata are present only on the lower surface of the leaf (Fig.1--d). The recorded observations are listed in Table (1). Thin section of leaf through the midrib region under the compound microscope showed usual bifacial structure other leaf diagnostic features of identification such as palisade ratio (9.5), vein islets number (16.5 per mm^2), vein termination number (14.5 per mm^2), stomatal number (121.5 per mm^2) and stomatal index (9.5). The number of stomata, numbers of vein islets, vein termination numbers, ratio of palisade cells, are the leaf constant, Stomatal numbers, stomatal index, vein islet number, vein termination number and palisade cells ratio are the leaf constant, commonly used to evaluate the leaf drugs on the microscopic basis [6]. Foliar epidermal parameters of leaf such as the shape of the epidermal cell, stomata type were observed. The recorded observations were listed in Table (2). The upper surface of the leaf consists densely arrange palisade cells containing a sufficient quantity of chlorophyll. Stomata

were confined to the lower surface of the leaf and such leaf is known as Hypostomatic leaf (Fig. d). Stomata on the lower surfaces were arranged on angles to each other. Capellades *et al.*, [36] reported the same results for *Rosa multiflora* from the stomatal anatomy of cultured *R. multiflora* under the influences of the environment.

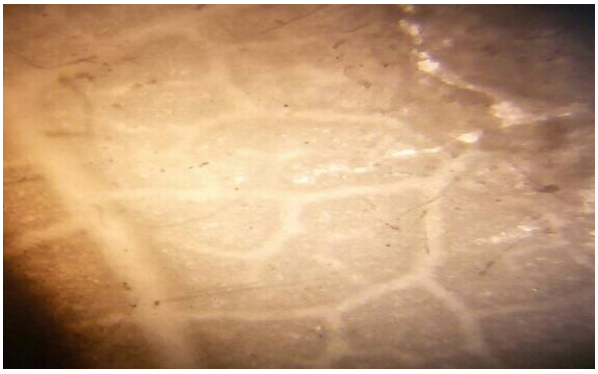
Table 2. **E**pidermal features of *R. multiflora* leaf.

Parameters	***R. multiflora* leaf**	
	Range	**Mean**
Stomatal frequency (Lower Epidermis)	120-123	121.5
Stomatal index (Lower Epidermis)	8-11	9.5
Vein Islet Numbers	15-18	16.5
Vein Termination Numbers	13-16	14.5
Palisade Ratio	7-12	9.5

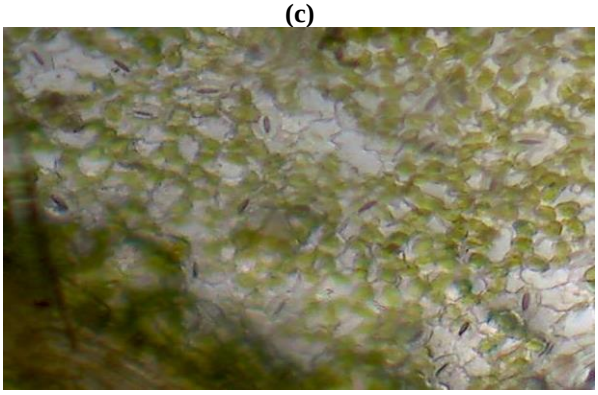
(a)



(b)



(c)



(d)

Fig.1. Leaf anatomy and venation study
(a) Leaf upper epidermis of *R. multiflora* leaf.
(b) Transverse section of *R. multiflora* leaf.
(c) Vein islets and vein termination *R. multiflora* leaf.
(d) Stomata on lower side leaf of *R. multiflora*.

3.3. Leaf powder microscopy

Leaf powder of *Rosa multiflora* looked bright green in colour with an unpleasant odour and slightly bitter and contemplate taste. The structures observed under the microscope are following (Table 3). The presence of trichomes in leaf powder is one of the characteristic features of this plant. The current microscopy of *Rosa multiflora* leaf also indicated the presence of the following characteristic structures (Fig.2--e, f and g).

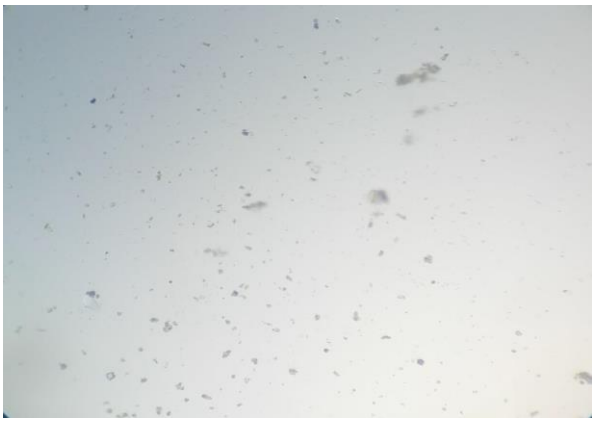
Leaf powder of *Rosa multiflora* looked bright green in colour with an unpleasant odour and slightly bitter and contemplate taste. The structure observed under the microscope are listed in Table (3) shows various structures such as xylem tissues, starch grains,

trichomes and phloem with attached companion cells under microscopy.

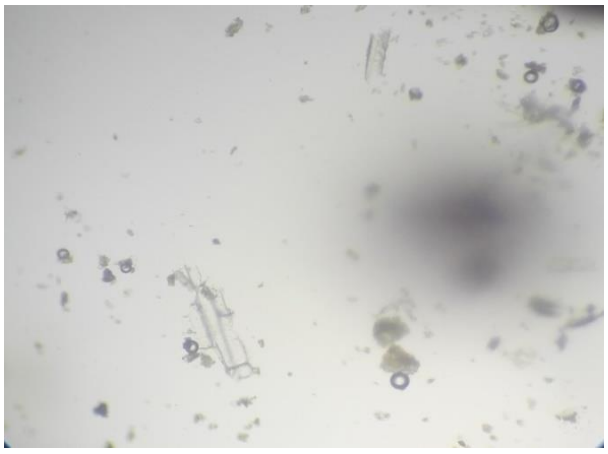
The powder microscopy of *Mollugo oppositifolia* L. shows the presence of vessel elements, phloem fibers and tannin cells [37]. The powdered microscopy of *Calotropis procera* conducted [38] and leaf powder of *Tridax procumbens* was observed and recorded the characteristic feature of the powders [39]. The presence of trichomes in leaf powder is one of the characteristic features of this plant.

Table 3. Structure observed under the microscope in leaf powder of *R. multiflora*.

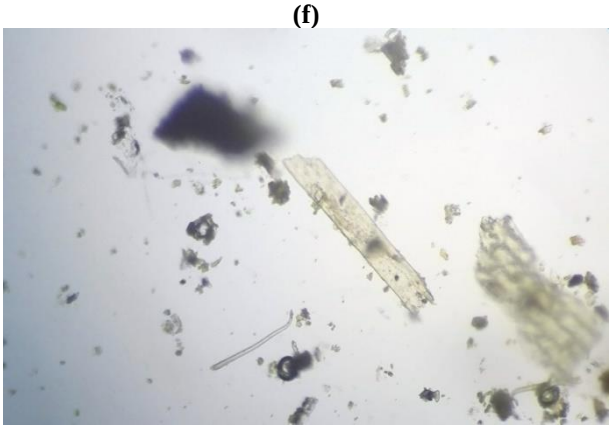
S. No	Structures observed
1	Isolated xylem tissues were observed.
2	Phloem tissues with attached cells.
3	Various size powder and isolated prismatic crystal of calcium oxalate.
4	Starch grains, trichome and leaf hairs of different sizes.



(e)



(f)



(g)

Figure.2. Powder microscopy of *Rosa multiflora*
(e) Microscopy with low power. **(f)** Microscopy indicating tissues. **(g)** Microscopy reflecting trichome, phloem tissues

3.4. Fluorescence study

The fluorescence study of leaf powder of *R. multiflora* was carried out with different reagents under ultraviolet and visible lights. The UV light (254nm) was used for fluorescence analysis leaf powder drugs. The observation has shown marked variation in coloration based on the nature of reagents and wavelength of UV light and the results are listed in table (4). The results are listed in the Table (5) shown marked variation in coloration based on the nature of reagents and wavelength of UV light (254nm). Our observations are in accordance with fluorescence study of leaves of *Catunaregum spinosa* [40], root and stem of *Ichnocarpus*

frutescens [41], *Hygrophila auriculata* [10] and *Crocus sativus* [42].

Table 4. Fluorescence analysis of leaf powder of *R. multiflora* with different reagents.

Reagent	Leaf Powder	
	Visible light	**UV light**
Untreated	Grey Brown	Greenish
50%HNO3	Reddish brown	Light green
50%H2SO4	Dark green	Dark blue
NaOH in water	Brown	Dark brown
Picric acid	Light yellow	Green yellow
NaOH Ethanol	Light grey	Light green
50% HCl	Dark brown	Dark green
Water	Brown	Dark green
Ethanol	Dark green	Blue

3.5. Elemental analysis

The fine leaf powder of *R. multiflora* was analysed for the determination of various elements. The results are given in Table (5) indicated the presence and concentration of various minerals in different concentration in a given sample (Fig.3-- h). The examined minerals and trace elements play both therapeutic and preventive role in combating various diseases of humans. The elemental analysis revealed the existence and different concentrations of various minerals as indicated (Table 6). The reasonable amount of Na, Fe, Ca, K, Mg, Mn and Cu were present in different concentrations in a given sample of leaf powder (Fig.3). The examined minerals and trace elements play both therapeutic and preventive role in combating various diseases [43] and there is an extensive scope to exploit the protective medicinal aspects of various minerals and trace elements [44].

Table 5. Elemental analysis of *R. multiflora* leaf.

	Element	Mean (mg/L)	S.D
Macroelements	Sodium (Na)	1.787	0.0080
	Potassium (K)	4.159	0.0290
	Calcium (Ca)	8.030	0.0764
	Magnesium (Mg)	1.879	0.0041
Microelements	Manganese (Mn)	0.312	0.2692
	Iron (Fe)	1.710	0.2910
	Copper (Cu)	0.005	0.0017

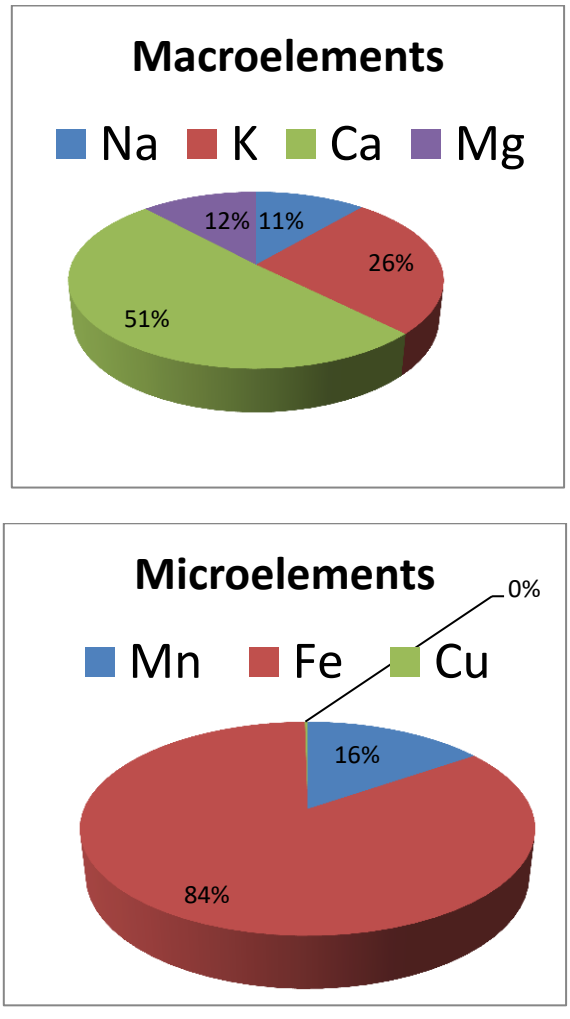


Fig.3. Macro and microelements analyzed in leaf powder.

3.6. Chlorophyll Contents (mg/g) and its Medicinal Application

The chlorophyll content (Chl. a & b) in the different leaf of *R. multiflora* is determined quantitatively at 645and 663nm. The results of chlorophyll content are given in Table (7). The chlorophyll determinations were made on base leaf sizes i.e. large size, medium size, small size and extra small size leaf. Maximum chlorophyll "a & b" content was recorded in large leaf followed by medium size leaf as shown in the Fig. (4). The chlorophyll content (Chl. a & b) determinations were made on bases of leaf sizes. The results given in table (7) reflect that maximum chlorophyll "a" content was recorded in the large leaf (0.062) followed by medium size leaf (0.056) as shown in the (Fig. j). The maximum chlorophyll "b" content is determined in the large size leaf (0.095) followed by medium size leaf (0.079) as shown in (Fig. j). The present finding shows that leaves of *R. multiflora* has a sufficient amount of chlorophyll content and can be used medicinal purposes. Chlorophyll is known to have multiple medicinal uses and has its place in modern medicine and therapy [45]. The structure of chlorophyll and its derivatives, bioavailability, stability, and their cancer preventing activity were studied [46].

Table 7. Chlorophyll "a" and "b" content of *Rosa multiflora* leaves (mg/g).

Leaf size	Chlorophyll "a" content (mg/g)	Chlorophyll "b" content (mg/g)
Large leaf	0.062	0.095
Medium leaf	0.056	0.079
Small leaf	0.054	0.065
Extra small leaf	0.052	0.046

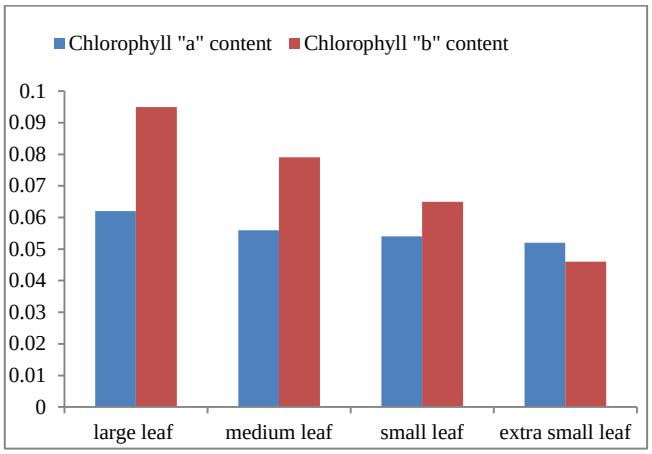


Fig.4. Chlorophyll "a" and "b" content of *R. multiflora* leaves (mg/g).

3.7. Phytotoxic activity

Phytotoxic screening of crude ethanolic extract of *R. multiflora* leaf was carried out using *Lemna minor* as test species. A significant plant inhibition was detected for all the tested samples at various concentrations of the crude drug (Table 8). *R. multiflora* ethanolic extract showed 100% inhibition at 75 mg/ml followed by 50 mg/ml (41.13), and less inhibition shown by 25 mg/ml (18.9±2.77) respectively. The ethanolic extract of *R. multiflora* leaf show inhibitory effect against *Lemna minor* test species. A significant plant inhibition was detected for all the tested samples at various concentrations of the crude drug (Table 8). 100% inhibition was recorded at 75 mg/ml followed by 50 mg/ml (41.13), and less inhibition shown by 25 mg/ml (18.9±2.77) respectively. The results show that the phytotoxic effect of research plant is dose dependant and has great potentials as weedicides. Phytotoxic activities were evaluated on various plants and its parts including *Phyllanthus muellerianus* [47], *Zizyphus jujube* [48] against *L. minor* plant and similar

findings were recorded which support the present study. To discover its phytotoxic mechanism and also to detect and quantify the phytotoxic constituents of *R. multiflora*, additional studies are necessary. It may be helpful to explore its efficacy in detail as pesticides, weedicides and disease control agents.

Table 8. Phytotoxic activity of ethanolic leaf extract of *Rosa multiflora*.

Tested Plant	Concentration of extract (mg/ml)	No. of Frond		%Growth Regulation
		Sample	Control	
Lemna minor	25	24.33	30	18.9%
	50	17.66	30	41.13%
	75	0	30	100%

CONCLUSION

The morphological evaluations of the *R. multiflora* leaf were beneficial in species identification and separation from closely related species of family Rosaceae. Leaf epidermal parameters like vein islets number, vein termination number, palisade ratio, stomatal number and stomatal index were determined. These parameters are considered as an essential tool for microscopical identification and standardization of natural drugs. Microscopy of leaf powder showed different structure including trichomes and vascular tissues and fragments which will help in the identification. Fluorescence study of leaf powder treated with various chemical reagents under visible light and UV light showed different fluorescence which provides a basis for standardization of drug. Elemental analysis revealed the existence of various macro and microelements in the leaf of the selected plant. Chlorophyll analysis shows the existence of a sufficient amount of chlorophylls and can be used as antioxidants due to the presence of antioxidant enzymes. *Lemna minor* phytotoxic bioassay of leaf ethanolic extract showed 100% inhibition at 75 mg/ml.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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