IJBPAS, August, 2024, 13(8): 3742-3761

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

THE FLOWER OF *ROSA ALBA* (L.): PHARMACOGNOSTIC, CYTOMORPHOLOGICAL AND CHROMATOGRAPHIC STUDIES

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Received 15th April 2023; Revised 8th Aug. 2023; Accepted 1st Nov. 2023; Available online 1st Aug. 2024

https://doi.org/10.31032/IJBPAS/2024/13.8.8226

ABSTRACT

The *Rosa alba* L. is a little-large tree or shrub up to 1.8 m in height with wide branches and thorns. It belongs to the family Rosaceae. The purpose of the present study was to ascertain the pharmacognostic, cytomorphological, and chromatographic studies of the flowers of *R. alba*. Microscopic studies of the flower among its different parts like a petal, sepal, pollen grains, anther, ovule, and its powdered drug were performed by routine methods. Results of the microscopic studies reported the occurrence and features of various cellular parts like sclereids, trichomes, crystals, oil globules, vascular bundles, etc. Heavy metals and pesticides in the powdered drug were determined by atomic absorption spectroscopy and gas chromatography equipped with an electron capture detector (GC-ECD) respectively. Nickel, chromium, iron, zinc and cadmium heavy metals were found in the powdered drug but no pesticides were found. A very prompt and precise reversed-phase high-performance liquid chromatography (RP-HPLC) method with diode array detection (DAD) was used for the simultaneous estimation of quercetin, gallic acid, β-sitosterol and vanillin in the hydroalcoholic extract of the flowers. These phytoconstituents were separated from the extract through the C18 column (4.6 mm x 250 mm) using methanol: acetonitrile: water (40: 15: 45, v/v/v) along with 1% acetic acid as mobile phase. Quercetin, Gallic

acid, β-sitosterol, and vanillin were found to be 0.2179, 1.8937, 0.9991, and 0.0065% w/w respectively in the ethanolic (85%, v/v) extract of the flower of *R. alba*.

Keywords: *Rosa alba,* GC-ECD, RP-HPLC, Heavy metal, Pesticide, AAS

INTRODUCTION

Natural products (plants, animals, minerals, micro-organisms, marine, etc.) are a unique source of lead molecules for drug discovery. Therefore, investigators in the pharma field are currently interested in discovering bioactive novel compounds from natural sources. *Rosa alba* L. belongs to the family Rosaceae, commonly known as a white rose. Its white velvety flowers spread a pleasant smell, therefore attracting insect pollination **[1-4]**. The leaves are simple, and palmer in shape with reserved trichomes, reticulated ribs, and small side lances. The central rib is elevated and the fishbone. Three or more flowers collectively form a cluster as a rosette and bloom throughout the year. Its corolla is generally made up of 15-40 pure white-colored petals in an overlapping manner. The stigma of the flower is found unapparent, therefore its carpel is undeveloped, and the androecium has short stamens with poorly formed anthers. Sepals are round and mostly seesaw-teethed **[5]**. There are many groups of phytoconstituents like-flavonoids, anthraquinones, saponins, tannins, monoterpenes, triterpenes, sesquiterpenes, aldehydes, phenolic/alcoholic compounds, and minerals reported in this species **[6-9]**. In the traditional system of medicine, *Rosa alba* L. is used as a rubefacient, lactagogue, insecticidal, antioxidant, anthelmintic, and laxative, for the treatment of piles, diarrhea, cardiovascular diseases, eye troubles, and vaginal candidiasis. Rose oil of the flower is also used as a fragrance or perfuming agent in various topical pharmaceutical preparations. Flower and its oil were found biologically effective as an antioxidant, antimicrobial **[10-13]**, memory enhancing **[14-15]**, antistress **[16]** and antiallergic **[17-18]**. Here, the flowers of *Rosa alba* (*R. alba)* L. were explored based on their traditional values as a therapeutic medicine and selected as a natural source for new drug discovery. Physico-chemical characterization and standardization studies of natural products are one of the main requirements for successful drug development. Therefore, the present study aimed to explore the pharmacognostic, cytomorphological, and chromatographic studies of flowers of *R. alba* before evaluating its biological potential.

MATERIALS AND METHODS

Collection and identification of plant material

Fresh flowers of *Rosa alba* were collected from the garden of Baba Raghav Das Medical College, Gorakhpur. The Plant and its parts were identified and authenticated by Professor (Dr.) V.N. Pandey, Department of Botany, Deen Dayal Upadhyay Gorakhpur University, Gorakhpur (Uttar Pradesh), India. A herbarium specimen of the same has been submitted to the Department vide reference no. "Bot/11017".

All the chemicals and solvents used in the present study were of analytical grade.

Macroscopic characteristic

The flowers of *R. alba* were cleaned properly and the macroscopic characteristics of their different parts such as petal, sepal, androecium, and ovary were evaluated based on their shape, size, colour, odour, taste, and surface characteristics with necked eyes **[19-20]**.

Microscopic characteristic

Transverse sections (T.S.) of the flower's parts of *R. alba* (petal, sepal, pollen grain, anther, and ovule) were prepared by routine methods. Slides of the T.S. and powdered drug were prepared using standard procedures and observed under the microscope. Photomicrography had been done after proper mounting and staining **[21-22]**.

Determination of moisture content

Moisture content was determined by taking 2 g air-dried crude powder of the flowers in a suitable tared weighed Petri plate. Placed the Petri plate at 105°C in the oven until two consecutive weightings did not differ by more than 5 mg. Moisture content or loss on drying (LOD) is the loss of mass expressed as percent w/w **[23]**.

Determination of total ash value

The total ash value was determined by taking 2 g of powdered material of the flowers in a suitable tared-weighed crucible. The material was then incinerated in the muffle furnace by gradually increasing the temperature from 500-600°C until free from carbon, cooled in a desiccator, weighed the crucible, and the percentage ash was calculated as-

$$\%\text{Total ash value} = \frac{(Fw-Pw)\times 100}{W}$$

Fw = Final weight of the crucible with total ash; Pw = Empty weight of the crucible

W = Total weight of powdered plant material

Determination of water-soluble ash

Twenty five milliliters of water was added into the crucible containing the total ash and boiled for 5 minutes. The insoluble matter was collected on an ashless filter paper, then washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C. The weight of this residue was subtracted from the weight of the total ash

and calculated the content of water-soluble ash was in milligrams per gram of air-dried material **[23]**.

Determination of acid-insoluble ash

25 ml of hydrochloric acid (~70g/l) was added into the crucible containing the total ash, covered with a watch glass, and boiled gently for 5 minutes. The watch glass was rinsed with 5 ml of hot water and added this liquid to the crucible. The insoluble matter was collected on ashless filter paper and then washed with hot water until the filtrate was neutral. Filter paper containing the insoluble matter was transferred into the original crucible. Dried it on a hot plate and ignite to constant weight. The residue was allowed to cool and weighed. The content of acid-insoluble ash was calculated in milligrams per gram of air-dried material.

Determination of water and alcohol soluble extractive values

About 5.0 g of powder of flowers was macerated with 100 ml of solvent (water and ethanol separately) in a conical flask for 24 hours and shaken frequently for 6 hours then allowed to stand for 18 hours. It was then filtered rapidly. 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed dish at 105°C for 6 hours, cooled in a desiccator for 30 minutes, and weighed to a constant weight. The percent of water and alcohol-soluble extractives was calculated concerning the air-dried powder.

Determination of swelling index

About 1.0 g of powder of flowers was accurately weighed and soaked in 25 ml of water in a measuring cylinder. The mixture was shaken thoroughly every 10 minutes for 1 hour, and then it was set aside for 3 hours at room temperature. The volume (in ml) has to be measured which is occupied by the plant material, including any sticky mucilage, considered as the swelling index.

Determination of foaming index

About 1.0g of powder was transferred to a 500 ml conical flask containing 100 ml of boiling water. The mixture was maintained at moderate boiling for 30 minutes. Cooled and filtered into a 100 ml volumetric flask and sufficient water was added up to the mark. The decoction was then poured into 10 stoppered test tubes in successive portions in 1ml, 2ml 3ml, and up to 10ml. The volume of each test tube was made up with water to 10 ml then the tubes were stoppered and shaken in a lengthwise motion for 15 seconds (two shakes per second). All the tubes were allowed to stand for 15 minutes and measured the height of the foam. The results were assessed as follows.

- When the height of foam in each test tube is less than 1 cm, the foaming index is considered to be less than 100.
- If the height of the foam in a test tube is measured as 1 cm, the volume of decoction in this tube is used to determine the index. If this tube is the first or second tube in the series, an intermediate dilution is prepared similarly to obtain more accurate results.
- When a foam height greater than 1 cm is obtained in each tube, the foaming index is considered to be greater than 1000. In this case, the determination is repeated using a new series of dilutions of the decoction to obtain accurate results.

The foaming index was calculated as-

Foaming index=1000/A

Where, A= the volume in a milliliter of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm was observed **[23]**.

Determination of pesticides by gas chromatography

Pesticides in the air-dried powder of the *R. alba* flowers were determined by GC-ECD.

Preparation of standard samples

Standard samples of the pesticides were prepared in acetonitrile at the concentration of100 ng/ml.

Preparation of test sample

A test sample of the powdered drug of *R. alba* was prepared in duplicate by the QuEChERS extraction method for multipesticide residue analysis. According to this method- 10 g of the powdered drug was macerated in 10 ml Milli-Q water and set aside for 20 minutes. The macerated sample was then thoroughly mixed with 10 ml acetonitrile, 4 g magnesium sulfate, and 1 g of sodium chloride for 10 minutes through a Rotospin test tube rotator at 50 rpm. The extract was then centrifuged at 10,000 rpm for 10 minutes. For the sample clean-up step, a fixed amount of PSA (primary secondary amine) along with 150 mg magnesium sulfate was added to 1.0 ml of the prepared extracts, and the mixture was shaken with a vortex shaker for 10 min, followed by centrifugation at 10,000 rpm for 10 minutes. The supernatant was collected after centrifugation and filtered with a Whatman no. 1 filter paper **[24]**.

Chromatographic specifications

Chromatographic specifications were that a 5% diphenyl/95% dimethyl polysiloxane column was used at 170°C as the initial oven temperature for 05 minutes. The final temperature of the oven was maintained at

290°C for 50 minutes. Samples were injected by automatic injection mode at 280°C. The detector was maintained at a temperature of 310 °C. Nitrogen was used as carrier gas with a flow rate of 60 ml/min.

Determination of heavy metals

Heavy metals were analyzed according to the APHA (23rd edition), 3111B method **[25]**. A total of 1.0 g of dried coarse powder drug was placed in a crucible and set in the furnace for 2.0 hours to ash. Then, 5.0 mL of nitric acid was added to the sample and boiled to almost dryness. Next, 10 ml of hydrochloric acid was added and diluted up to 100 ml, which was then taken for analysis by atomic absorption spectrometry (AAS). The quantity of all heavy metals was calculated by extrapolation of their absorbance with the concentrations of a calibration curve by plotting a linear graph of the absorbance of standards versus their concentrations.

Quantitative analysis by RP-HPLC

Preparation of Extract (RA)

About 200 g powder of the flower of *R. alba* was extracted with 85% v/v ethanol (≈300mL) by continuous hot extraction in the soxhlet apparatus. The solvent was filtered and evaporated to dryness (concentrated) using a rotary vacuum evaporator to obtain the dry extract. The dried extract was kept in a desiccator for further use.

Preparation of standard solutions

Stock solutions of each authentic/reference standard Quercetin (QU), gallic acid (GA), β-sitosterol (BS), and vanillin (VA) were prepared in ethanol at a concentration of 0.02% w/v and filtered through a Millipore injection filter (0.45µm).

Preparation of sample solution

The sample solution of RA was prepared in the mobile phase at a concentration of 0.40% w/v and filtered through a Millipore injection filter (0.45µm).

Standard solutions, sample solution, and mobile phase were deaerated/degassed ultrasonically before use.

Chromatographic conditions and descriptions

Reverse phase-high performance liquid chromatography (RP-HPLC) analysis of the test sample and standards were performed by Agilent 1260 infinity II HPLC system equipped with a UV detector. The Agilent C18 reversed-phase column (4.6 mm X 250 mm) packed with 5µm particle diameter was used as the stationary phase. Methanol: acetonitrile: water (40: 15: 45, v/v/v) containing 1% acetic acid filtered through a Millipore injection filter (0.45µm) was used as the mobile phase. QU, GA, BS, and VA were estimated by diode array detection (DAD) at 368, 273, 206, and 231 nm

wavelengths respectively after separation by RP-HPLC. The volume of the injection and flow rate in the analysis were 10µl and 1.0 ml/min respectively **[26]**. Samples were injected by automatic injection mode. Peaks of the sample in chromatograms were identified by comparing their retention time (RT) and pattern of the spectrum with those of authentic standards. Quantitative analysis of the compounds was determined by the integration of their peaks using an external standard method. The total run time of each sample in HPLC was 12 minutes at ambient temperature.

The concentration of the identified phytoconstituents was determined about their peak areas using the formula as-

$$\frac{\text{(Peak area of TS) x (Concentration of SS) x 100}}{\text{(Peak area of TS) x (Concentration of SS) x 100}}$$

$$\text{(Peak area of SS) x (Concentration of TS)}$$

TS= Test sample; SS= Standard sample

RESULTS AND DISCUSSION

Macroscopic analysis of the fresh flower of *R. alba* and its parts was evaluated based on its color, smell, taste, size, shape, and floral arrangements **(Table 1)**. Crude drugs when supplied in intact form can be identified by morphological characteristics. However, in case of doubt, the same can be investigated for histological characteristics to confirm the identity of the supplied drug.

Table 1: Macroscopic/Organoleptic characters of the flowers of *R. alba*

Parameter	Characteristics
Colour	Pure White
Odour	Pleasant fragrance
Taste	Slightly sweet and mucilaginous
Calyx	Sepal 5, green, polysepalous with valvate Aestivation.
Corolla	18-20 polypetalous, white, but fused at the base with twisted aestivation.
Pistil and Stamen	Stigma was not apparent. The androecia have short stamens with anthers. The type of fixation of the anther was dorsifixed.

The microscopic studies often yield information that cannot be obtained by any other method **[23]**. Therefore, a histological examination of the different parts of the flowers was conducted on its transverse section (T.S.) and powdered drug. Cytomorphological characters in the T.S. of the petal, sepal, and powder of the whole flower were differentiated microscopically by staining with suitable reagents. The wavy epidermis, spongy parenchyma with clearly visible vascular bundles, and non-glandular (conical) trichomes were observed in the mesophyll tissues of the petal.

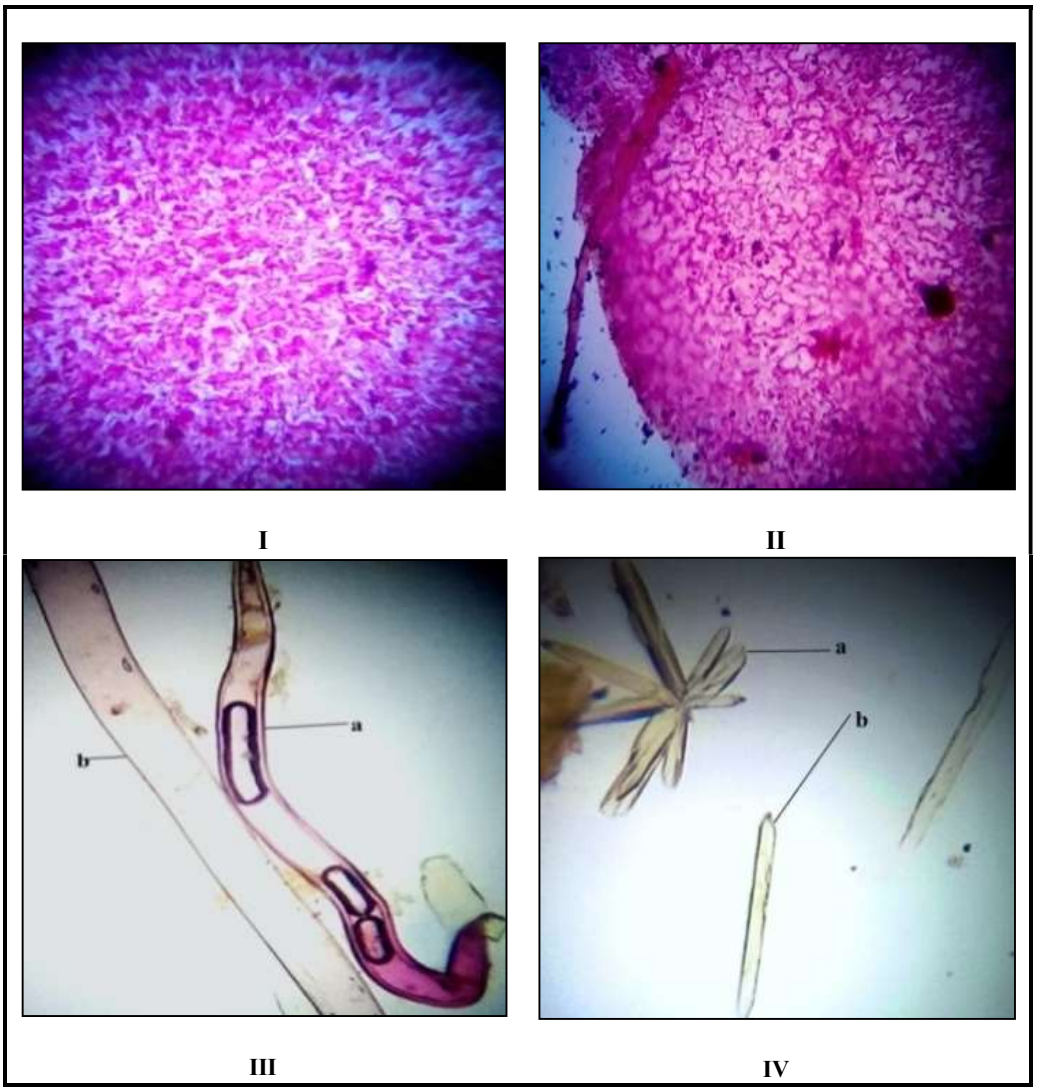

Figure 1 : Microscopic studies of petal
T.S. of petal: (I) Wavy-walled epidermis with spongy parenchyma (II) Epidermis of the petal with non-glandular trichome Powder microscopy of the petal: (III) (a) Uniseriate, multicellular, wavy covering trichomes with a blunt tip (b) Unicellular trichomes (IV) Petal powder: calcium oxalate crystals. (a) Rosette crystal (b) Single acicular crystal

Powder microscopy provides detailed information about the crude drug through its analytical uses like the property to magnify permits the fine structures of the miniature object to be visualized and thus corroborate the structural details. It can also be used in the determination of the optical as well as micro-chemical properties of the plant drugs under examination **[23]**. Consequently, the powder microscopy of the flower was performed to relate its histological characteristics. Powder microscopic studies of the petals showed uniseriate, multicellular (6-10 cells), wavy covering trichomes with a blunt tip. Single acicular and rosette-shaped calcium oxalate crystals and oil globules were also observed **(Figure 1)**. Spongy parenchymatous epidermal cells of the sepals showed capitate glandular

trichome (with unicellular stalk and unicellular head) and non-glandular trichomes (unicellular, uniseriate). Powder of the sepals showed capitate glandular trichomes with unicellular stalk and a unicellular head, capitate sessile trichome with a unicellular head without stalk, non-glandular unicellular-uniseriate trichomes along with acicular calcium oxalate crystals and oil globules **(Figure 2)**. Pollen grains of the flower were found to be dicolpate shape with a visible thick lining of cellulose, intine, exine, and pollen sac **(Figure 3)**. Microscopic evaluation of the anther also showed uniseriate, multicellular, wavy non-glandular trichomes of a blunt tip with acicular-shaped calcium oxalate crystals and oil globules. The type of fixation of the anther was dorsifixed and its fibrous layer contained reticulated cells **(Figure 4)**. Non-glandular multicellular, uniseriate trichomes were observed in the microscopic slides of the mature ovary **(Figure 5)**.

The moisture content/LOD is the loss of mass expressed as percent w/w in powdered drugs, determined by the gravimetric method **(Table 2)**. Moisture is one of the major factors responsible for the deterioration of drugs. Low moisture content is always desirable for higher stability of drugs; therefore moisture must be eliminated from crude drugs by drying as far as possible. Insufficient drying supports the spoilage of the drug by the growth of molds and bacteria and makes possible the enzymatic destruction of active principles **[23]**. The ash values (total ash, acid-insoluble, ash, and water-soluble ash values) of the powdered drugs were determined and represented in **Table 2**. The ash residue consists of inorganic materials like metallic salts (carbonates, phosphates, silicates, etc.) and silica. The extractive value of any crude drug in a particular solvent is the amount of phytoconstituents soluble in that solvent. In the present study, the water and ethanol-soluble extractive values of the powder of *R. alba* were determined and found 3.81% and 3.90% respectively.

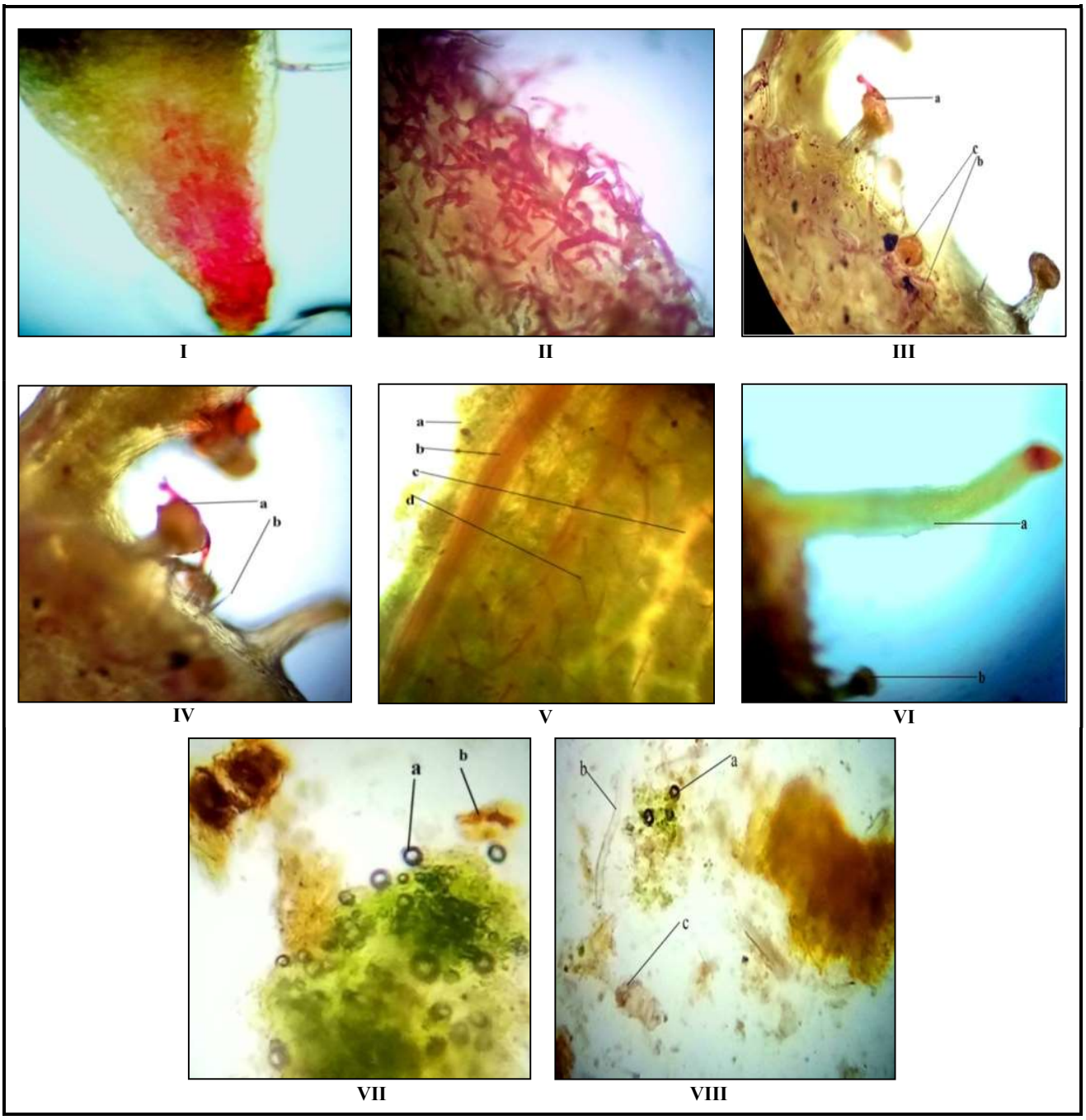

Figure 2: Microscopic studies of sepal
T.S. of sepal: (I) Vascularization in the T.S. of sepal (II) Non-secretory hairs in a sepal (III) (a) Capitate glandular trichome with unicellular stalk and unicellular head sepal (b) Papillae (c) Capitate sessile trichome; (IV) (a) Secretrory trichome (Capitate trichome) (b) Non-secretory (Non-glandular) trichome (V) (a) Epidermis (b) Vascular bundle (c) Spongy parenchyma (d) Trichome; (VI) (a) Glandular trichome(pillate) (b) Glandular trichome (capitate) Powder microscopy of sepal: (VII) Sepal powder (a) Oil globules (b) Calcium oxalate crystals; (VIII) Sepal powder (a) Oil globules (b) Fragments of trichomes

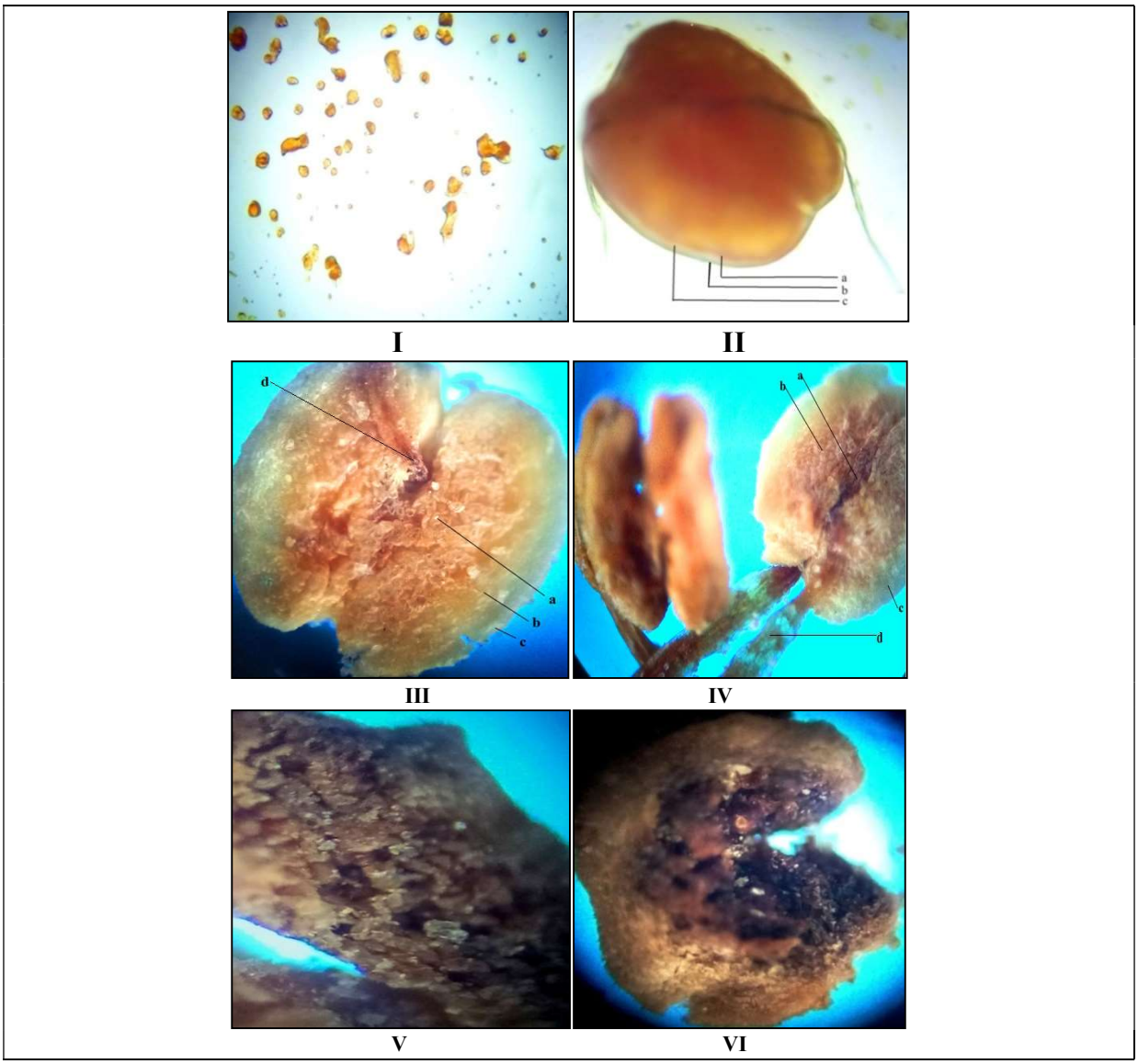

Figure 3: Microscopic studies of pollen grains
(I) Pollen grains; (II) Pollen (dicolpate shape) - (a) Intine (b) Exine (c) Thick lining of cellulose; (III) Cross section of the anther (a) Pollen sac (b) Endothelium (c) Epidermis (d) Pollen tube; (IV) Cross section of anther- (a) Pollen sac (b) Endothelium (c) Epidermis (d) Filament; (V) Pollen sac of the anther; (VI) Pollen aperture of *Rosa alba*

It was observed that the percent ethanol-soluble extractive value is slightly higher than the water-soluble, indicating that the ethanol-soluble phytoconstituents in the plant are slightly higher than the water-soluble one. It also indicates whether the crude drug is exhausted or not **[27]**. The swelling index is defined as the volume in milliliters, which is produced by the swelling of one gram of plant material under particular sets of conditions.

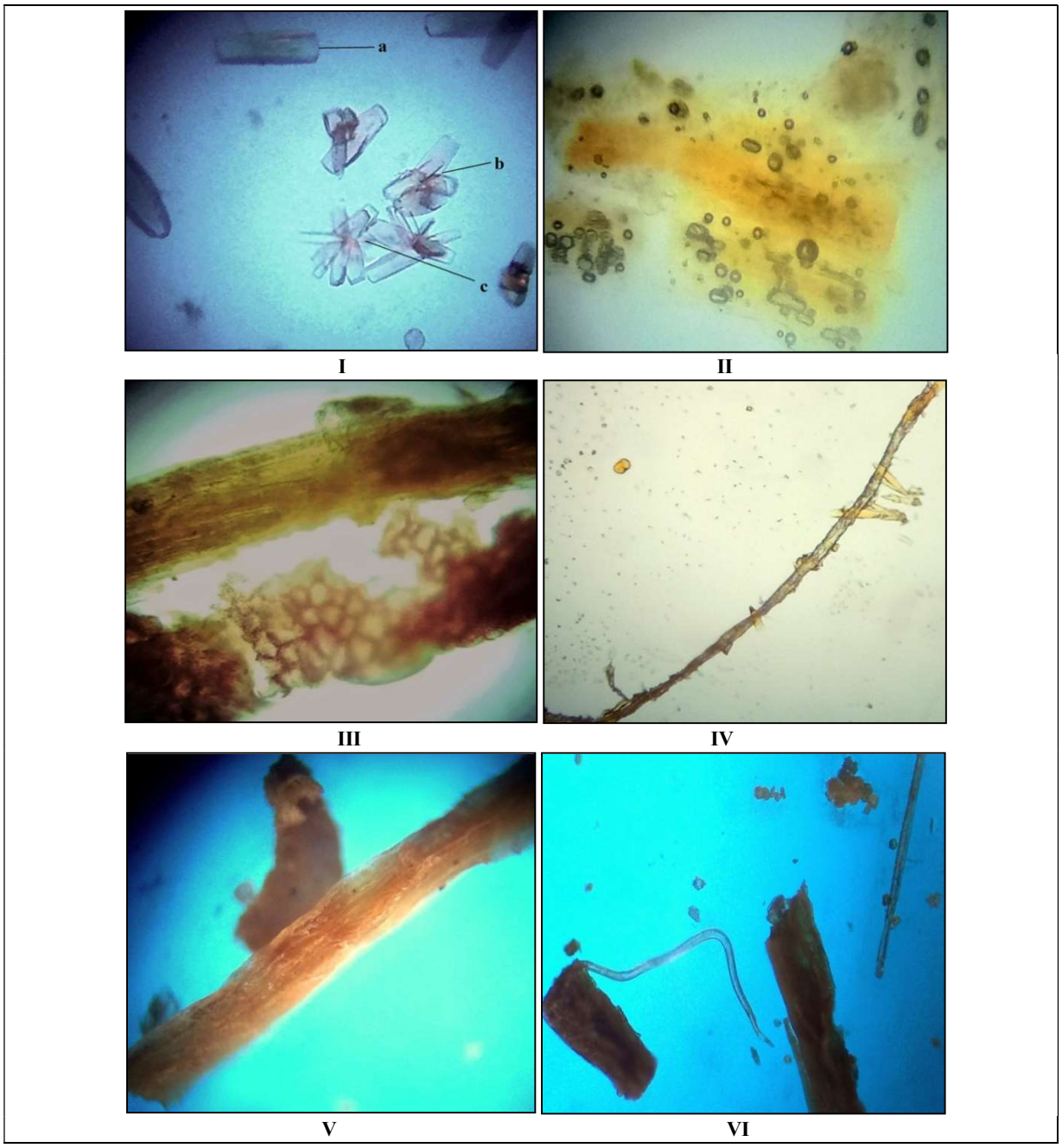

Figure 4: Microscopic studies of anther
(I) Powder of anther - (a) Single acicular (b) Bundle of acicular (c) Rosette crystal (II) Oil globules (III) Pollen tube and a fibrous layer of anther with reticulated cells (IV) Pollen tube (V) Base of the stamen filament (VI) Trichome (uniseriate, multicellular, wavy non-glandular trichomes with a blunt tip.)

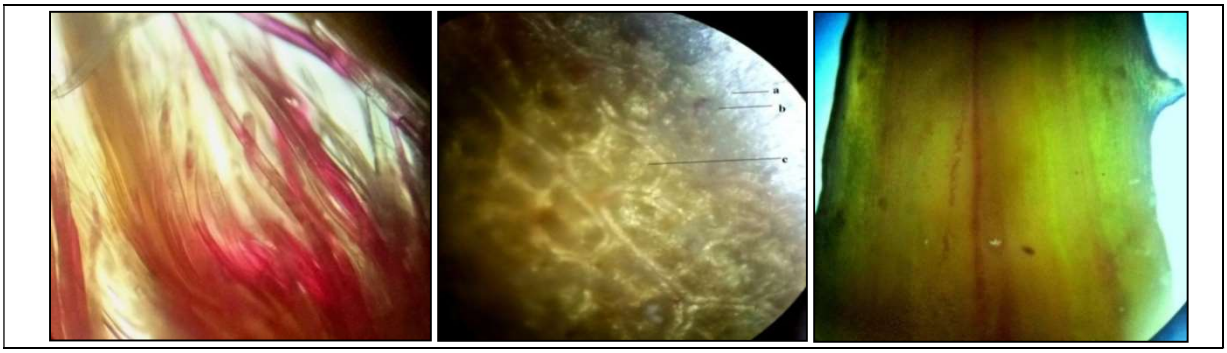

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Figure 5: Microscopic studies of ovule
(I) Mature ovary with non-glandular multicellular, uniseriate trichomes (II) Pedicel (a) Non-glandular trichome (b) Cuticle (c) Collenchyma (III) T. S. of pedicel with trichome

It gives an idea about the mucilage content of the drug **[28]**. The foaming ability of an aqueous decoction of the powdered drug and its extracts is measured in terms of the foaming index. *R. alba* showed low values of swelling and foaming index **(Table 2)**, which indicate that the sticky mucilaginous material and saponin are present in the drug in fewer amounts respectively.

Table 2: Physicochemical parameters of the flowers of *R. alba*

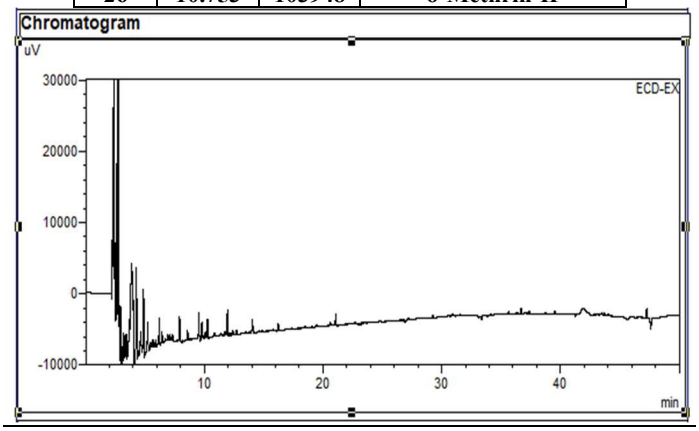
Parameter	Result
Loss on drying (LOD)	12 % w/w
Total ash value	15.235 % w/w
Water soluble ash	0.575%w/w
Extractive value (water)	3.81%w/w
Extractive value (alcohol)	3.90%w/w
Swelling index	12 ml.
Foaming index	Over 1000

Since plants are an essential part of the human diet and therapy, the quality of these products is extremely important and often determined whether they will be used as foods or medicines. Hence analysis of pesticides in the flower of *R. alba* was conducted by GC-ECD and the chromatograms obtained from this analysis showed no peaks of pesticides when compared with the chromatograms of the standard sample of pesticides **(Figure 6)**. There were nickel, chromium, iron, zinc, and cadmium heavy metals are detected in the powdered drug while mercury, lead, and arsenic were not detected.

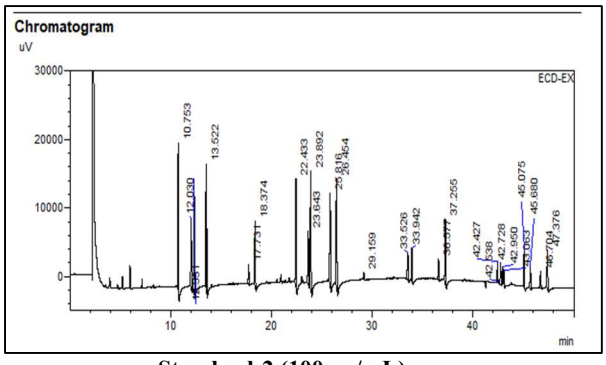
Sample- *R. alba*

Standard-1 (100 ng/mL)

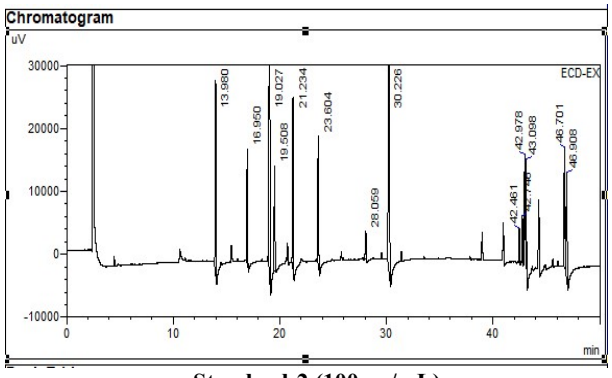
S. No.	RT	Area	Pesticide Name
1	10.753	103948	α-HCH
2	12.030	46273	β-HCH
3	12.331	76768	λ-HCH
4	13.522	86896	δ-HCH
5	17.731	12448	Malathion
6	18.374	49337	Chlorpyrifos
7	22.433	85615	Quinolphos
8	23.643	38093	α-Endosulfan
9	23.892	92241	Profenofos
10	25.816	75681	p p-DDE
11	26.454	93495	β-Endosulfan
12	29.159	6422	p p-DDD
13	33.526	23573	o p-DDT
14	33.942	29005	p p-DDT
15	36.577	15819	Bifenthrin
16	37.255	52740	Fenpropathrin
17	42.427	14048	λ-Cyhalothrin-I
18	42.538	631	λ-Cyhalothrin-II
19	42.728	14407	α-Cypermethrin-I
20	42.950	9654	Fenvalerate-I
21	43.063	7023	α-Cypermethrin-II
22	45.075	26933	α-Cypermethrin-III
23	45.680	15167	Fenvalerate-II
24	46.704	12611	Fenvalerate-III
25	47.376	31344	δ-Methrin-I
26	10.753	103948	δ-Methrin-II



Standard-1 (100 ng/mL)



Standard-2 (100 ng/mL)



Standard-2 (100 ng/mL)

Figure 6: Gas chromatograms of the flowers of *R.alba* and standard samples of pesticides

S. No.	RT	Area	Pesticide Name
1	13.980	167837	Fluchloralin
2	16.950	95339	Alachlor
3	19.027	303570	Aldrin
4	19.508	92404	Dicofol
5	21.234	156036	Pendimethalin
6	23.604	115970	Butachlor
7	28.059	26265	Endosulfan-sulfate-I
8	30.226	282643	Endosulfan-sulfate-II
9	42.461	25751	β-Cyfluthrin-I
10	42.746	35461	β-Cyfluthrin-II
11	42.978	58081	β-Cyfluthrin-III
12	43.098	64785	β-Cyfluthrin-IV
13	46.701	117526	Fluvalinate-I
14	46.908	104459	Fluvalinate-II

Results of the heavy metal determination **(Table 3)** revealed that the flower of *R. alba* may have been growing in clean areas, so the powder of its flower were found to have low concentrations of the tested heavy metals.

Table 3: Results of heavy metals test in the flowers of *R. alba*

Test performed	Test method used	Result (mg/kg)
Nickel as Ni	APHA (23rd Edition) 3111B	0.645
Chromium as Cr		1.115
Iron as Fe		21.94
Zinc as Zn		5.14
Mercury as Hg		Not detected
Lead as Pb		Not detected
Cadmium as Cd		0.1
Arsenic as As		Not detected

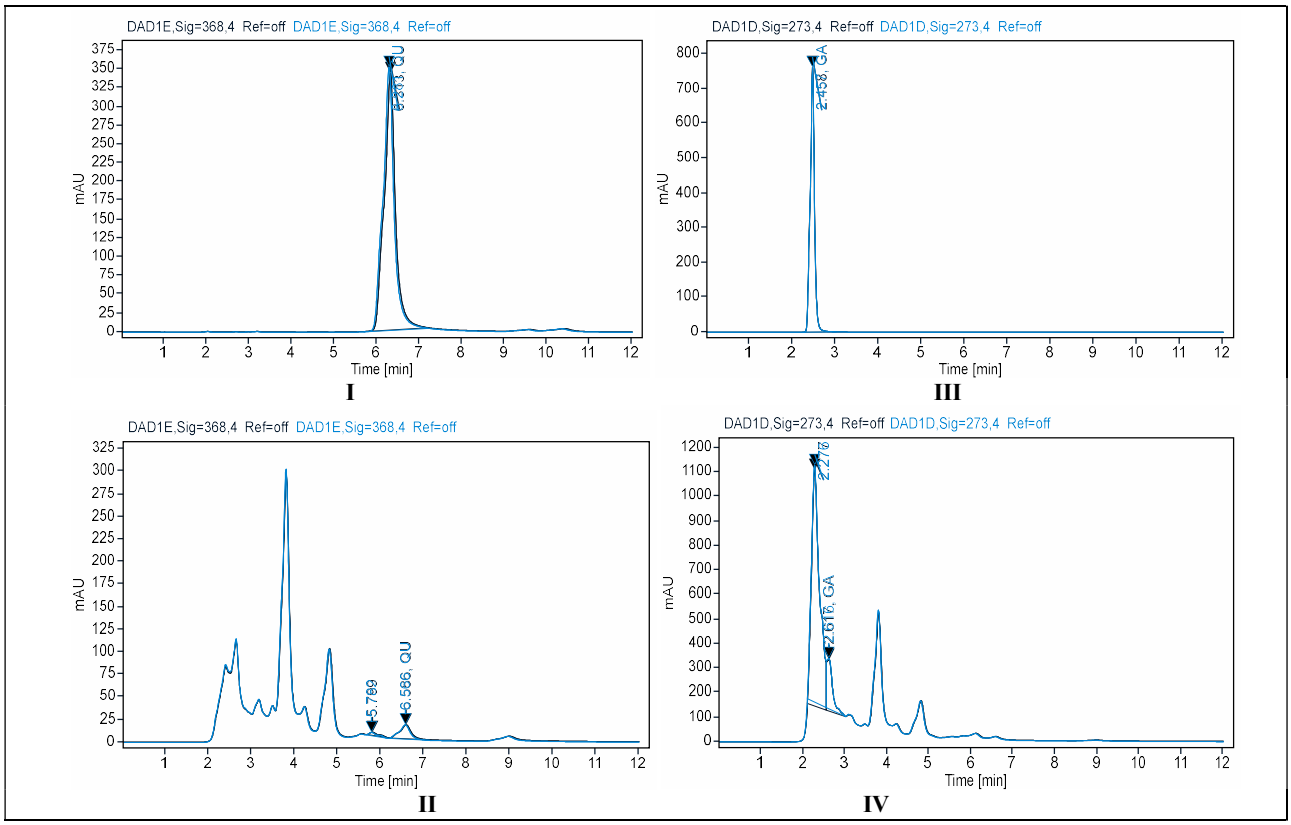


Figure 7: HPLC chromatograms: [I] Standard quercetin; [II] quercetin in RA sample; [III] Standard gallic acid; [IV] Gallic acid in RA sample

Pesticides can be absorbed and stored by plants, and their metabolites can be accumulated in the same way. Therefore, it can be said that the flower of *R. alba* used as herbal medicine is completely safe and standard. Standardization of herbal drugs is a very challenging task in analytical chemistry. Still, in the present research, four different types of phytoconstituents were simultaneously identified, and quantified in the hydroalcoholic extract of *R. alba* by RP-HPLC. Peaks of the QU, GA, BS, and VA of authentic standards were observed at RTs 6.30, 2.46, 2.61 and 3.58 minutes respectively in their respective chromatograms. RTs of the peaks of test samples were compared with the RTs of the standard sample and identified. The concentrations of QU, GA, BS, and VA in the hydroalcoholic extract of the flower were determined by the external standard method using their peak area and found to be 0.2179, 1.8937, 0.9991, and 0.0065% w/w respectively **(Figure 7 and 8)**.

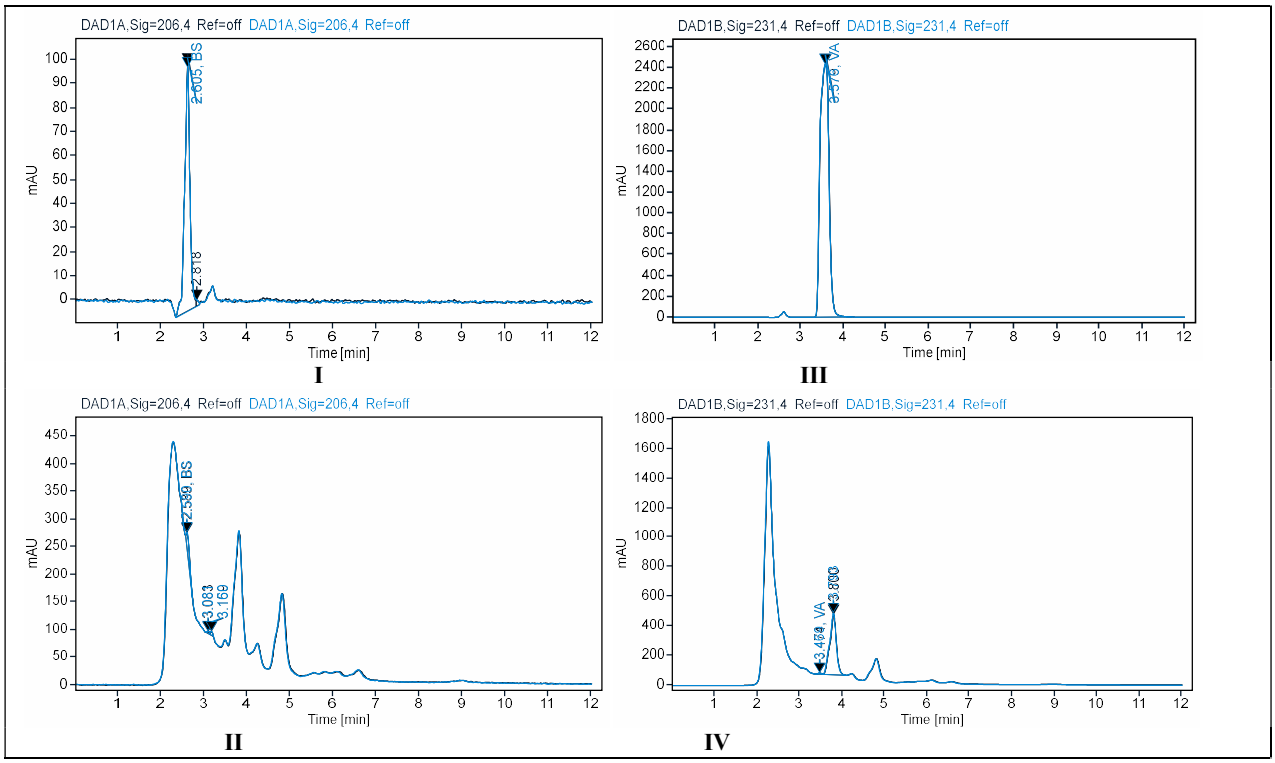


Figure 8: HPLC chromatograms: [I] Standard β-sitosterol; [II] β-sitosterol in RA sample; [III] Standard vanillin; [IV] Vanillin in RA sample

CONCLUSION

In conclusion, the physicochemical and pharmacognostic characteristics of *R. alba* flowers are reported for the first time in the present research. This study reveals the diagnostic characteristics like morphology,

phytochemistry, and anatomy of petals, sepals, anther, and an ovule of the flower of *Rosa alba.* This research may prove to be an important tool for future scientists to identify, authenticate and standardize the raw materials of *Rosa alba* and its formulations. Nickel, chromium, iron, zinc, and cadmium heavy metals are identified in the flowers within acceptable limits. Not a single pesticide is identified in GC-ECD analysis of the powdered drug of *R. alba.* Four compounds with different chemistry were identified and quantified by a single mobile phase using the rapid and advanced method of RP-HPLC. It can be concluded that the outcomes of the current research may provide reference material in the preparation of the monograph on this plant.

ABBREVIATIONS: LOD-Loss on drying; GC-ECD-Gas chromatography with electron capture detector; APHA-American public health association; AAS-Atomic absorption spectroscopy; QU-Quercetin; GA-Gallic acid; BS-Beta setosterol; VA-Vanillin; RA-*Rosa alba*; RP-HPLC- Reverse phase-high performance liquid chromatography; DAD-Diode array detection; RT-Retention time; T.S.-Transverse section.

ETHICAL ISSUES

Not applicable

CONFLICT OF INTEREST

The authors do not report any financial or other conflicts of interest.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. Shashank Mishra, Scientist-C, Biotech Park, Lucknow for providing the results of the heavy metal analysis timely.

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