



PHARMACOGNOSTIC EVALUATION AND PHYSICO-CHEMICAL ANALYSIS OF *ULMUS WALLICHIANA* PLANCH.

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ABSTRACT

The plant *Ulmus wallichiana* Planch. (Family: Ulmaceae) is found in Himalayan regions from Afghanistan to West Nepal. In India, it is found in Kumaun and Garhwal hills of Uttarakhand (India), locally known as Chamarmou. Bark of *U. wallichiana* is commonly used as Kumauni and Garhwali traditional healers for bone fracture of animals as well as human being. The current work was designed to establish the pharmacognostic parameters like macroscopic & microscopic characters, and physico-chemical analysis for *U. wallichiana*. WHO recommended methods and other standard procedures were employed for the establishment of pharmacognostic and physico-chemical parameters. Transverse section (TS) of the bark under the microscope showed periderm region showing cortex, mucilage canal, cork, phellogen and phelloderm. TS of the bark also showed secondary phloem region, medullary rays, stone cells and fibres. Longitudinal section showed medullary region, epidermal region and mucilage canal. Powder microscopy under the microscope showed calcium oxalate crystals, cork cells, phloem fibre, starch grains, stone cells and lignified phloem fibres. Physico-chemical analysis helps to identify pure plant material and detect adulteration. In the present study, total ash, water soluble ash, acid insoluble ash, sulphated ash and loss on drying were estimated to be 17%, 15%, 3.5%, 5%, 0.15% respectively. The findings of the present study could be useful in authentication of plant material and also in monograph preparation of *U. wallichiana*.

INTRODUCTION

The plant *Ulmus wallichiana* (family: Ulmaceae) found in western Himalayan regions of India, is one of the richest emporiums for medical taxa. Traditionally, *U. wallichiana* has been used in the treatment of digestive tract diseases (1,2). Bark of this tree is commonly used for bone fracture healing of animal as well as human being as folk medicine in Uttarakhand (India) (3). Bark paste of the plant is mentioned in wound healing potential drugs (4). Leaves are used as fodder for sheep and goats in Jammu & Kashmir (India) (5). The current study was designed to establish macroscopic & microscopic characters, and physico-chemical parameters for *U. wallichiana* bark. The biological activities of plants are because of various phytoconstituents present in plants. The therapeutic effects of

herbal medicines may not be consistent because of variation in phytoconstituents and incorrect source of plants. Toxic chemicals are also present in plants therefore, it is necessary to evaluate their efficacy, quality and safety. Correct identification and quality assurance of the starting material is, therefore an essential prerequisite to ensure reproducible quality of herbal products, which contributes to its efficacy and safety. As per the World Health Organization (WHO) report description of macroscopic and microscopic of a plant is the first step towards establishment of its identity and purity therefore, should be carried out before any investigation to be undertaken. However, available literature revealed that there is no pharmacognostic study has been carried out so far on this plant; hence the current study was under taken to establish

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various pharmacognostic and physico-chemical parameters for *U. wallichiana*.

MATERIAL AND METHODS

Plant material

Barks of *U. wallichiana* were collected from near, Budhar, Budhakedar Nath Ghansali, Tehri Garhwal, Uttarakhand (India). The plant was identified and authenticated at the Herbarium of Botanical survey of India (BSI), Dehradun, Uttarakhand (India) vide reference no. BSI/NRC Tec/ Herb (Ident.) / 2016-17/455.

Morphological/organoleptic Evaluation

In this evaluation the plant material was evaluated by investigation studying colour, odour, taste, size, shape, special feature like touch, texture etc.

Microscopical Evaluation

Section cutting of bark

Collect fresh part (Bark) of *U. wallichiana* and perform transactional section and longitudinal section with the help of using different suitable reagents (6).

Powder microscopy

Powder microscopy of bark was done by the Dutch process. 2 g of powder of selected part was taken and add 10% nitric acid solution (50 ml) to it and warm for 2 min. Then filtered the solution and residue obtained, it was wash with hot water and then filter. Then Again residues were taken and add 10% sodium hydroxide solution (50 ml), warm for 2 min then again filtered the solution. Residue washed with hot water and again filtered. Finally, take residue for powder microscopy using a student microscope (Olympus) (6).

Physico-chemical Evaluation

Determination of extractive values by cold maceration method

Alcohol soluble extractive

About 4g of drug was taken in a weighing bottle and transferred to a dry 250 ml conical flask and make up the volume 100 ml with 90% alcohol and macerate (cold maceration) and allow for 24hrs. then shaking frequently during the first 6hrs & allowing stand for the 18hrs. Thereafter, filter it rapidly taking precautions against loss of ethanol, evaporate 25ml of the filtrate to dryness in a tarred flat

bottomed shallow dish, dry at 105°C & weigh. Calculate the percentage of ethanol soluble extractive with reference to the air dried drug (6). Percentage of extractive value of air dried material was calculated as:

$$\% \text{Extractive value} = \frac{[\text{Final weight-initial weight}] \times 4}{\text{Weight of drug}} \times 100$$

Water soluble extractive

Water soluble extractive is useful for evaluation of crude drug and gives idea about the nature of the chemical constituents, soluble in that particular solvent. Add 4g bark part to 50ml of chloroform water in a stopper flask. Shake well & allow standing for 10min, cool, add 2g of Kieselguhr and filter. Transfer 5ml of the filtrate to a tarred evaporating disk, 7.5 cm in diameter, evaporate the solvent on a water bath, continue drying for 30min, then dry in a steam oven for 2hrs & weigh the residue. Calculate the percentage of water soluble extractive with reference to the air dried drug. There are chloroform act as a preservative (6).

$$\% \text{Extractive value} = \frac{[\text{Final weight-initial weight}] \times 4}{\text{Weight of drug}} \times 100$$

Petroleum ether soluble extractive

Place about 5gm of coarsely powdered air-dried material, (bark) accurately weighed, in a glass-stopper flask. Macerate with 100 ml of the solvent (petroleum ether) concerned for 6hours; shaking frequently, then allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent, transfer 25ml of the filtrate to a tarred flat-bottom dish and evaporate to dryness on a water-bath. Dry at 105°C for 6 hours, cool in a desiccator for 30 minutes and weigh without delay. Calculate the content of extractable matter in mg per g of air-dried material (6).

$$\% \text{Extractive value} = \frac{[\text{Final weight-initial weight}] \times 4}{\text{Weight of drug}} \times 100$$

Determination of extractive values by hot maceration method

Ethanol soluble extractive

Five gram of coarsely powdered drug separately accurate weighing air-dried material was placed in a glass-stopper conical flask. 100 ml of ethanol was add and weigh to obtain the total weight including the flask. Shake well and allowed to stand for 1 hour. A reflux condenser was attached to the flask and gently boils for 1 hour; cool and weighed. Readjusted to the original total weight with the solvent specified in the test procedure for the plant material concerned. Shake well and filter rapidly through a dry filter. 25 ml of the filtrate was

transferred to a tarred flat-bottomed dish and evaporated to dryness on a water-bath. Dried at 105°C for 6 hours, cooled in a dessicator for 30 minutes, then weigh without delay. The content of extractable matter was calculated in mg per g of air-dried material.

$$\% \text{Extractive value} = \frac{[\text{Final weight-initial weight}] \times 4}{\text{Weight of drug}} \times 100$$

Water soluble extractive

Five gram of coarsely powder (bark) of *U. wallichiana*, accurately weighed air-dried material was placed in a glass-stopper conical flask. 100ml of water was adding and weighs to obtain the total weight including the flask. Shake well and allowed to stand for 1 hour. A reflux condenser was attached to the flask and gently boils for 1 hour; cool and weighed. Readjusted to the original total weight with the solvent specified in the test procedure for the plant material concerned. Shake well and filter rapidly through a dry filter. 25 ml of the filtrate was transferred to a tarred flat-bottom dish and evaporate to dryness on a water-bath. Dried at 105°C for 6 hours, cooling in a dessicator for 30 minutes, then weigh without delay. The content of extractable matter was calculated in mg per g of air-dried material.

$$\% \text{Extractive value} = \frac{[\text{Final weight-initial weight}] \times 4}{\text{Weight of drug}} \times 100$$

Petroleum ether soluble extractive

Five g of coarsely powdered (bark) accurately weigh air-dried material was place in a glass-stopper conical flask. 100ml of petroleum ether will add and weigh to obtain the total weight including the flask. Shake well and allowed to stand for 1 hour. A reflux condenser was attached to the flask and gently boils for 1 hour; cool and weighed. Readjusted to the original total weight with the solvent specified in the test procedure for the plant material concern. Shake well and filter rapidly through a dry filter. 25 ml of the filtrate was transfer to a tarred flat-bottomed dish and evaporated to dryness on a water-bath. Dried at 105°C for 6 hours, cooled in a dessicator for 30 minutes, then weigh without delay. The content of extractable matter was calculated in mg per g of air-dried material.

$$\% \text{Extractive value} = \frac{[\text{Final weight-initial weight}] \times 4}{\text{Weight of drug}} \times 100$$

Determination of Moisture (loss on drying)

3g of shade-dried drug (*U. wallichiana*) was taken into a weighed flat and thin porcelain dish. Then dried in the oven at 100° or 105° after that cool in desiccators and calculate the loss

in weight is usually recorded as moisture (6). Calculate the percentage of moisture content of air dried material as:

$$\% \text{moisture content} = \frac{\text{loss in weight of sample}}{\text{total weight of drug}} \times 100$$

Determination of Ash Value

Total ash

Firstly weigh and ignite flat, thin porcelain dish silica crucible and then weigh about 2 g of powdered drug into the dish/ crucible and incinerated in a crucible at a 500°C-600°C in a muffle furnace till carbon free ash was obtained then it is cool, weight and percentage of yield was calculated as per reference (6,7). The %w/w of total ash was calculated as follows:

$$\text{total ash (\%w/w)} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

Determination of acid-insoluble value

After as per the above step ash using 25 ml of dilute-hydrochloric acid wash the ash from the dish using for total ash into a 100ml beaker. Then place wire gauze over a Bunsen burner and boil for five minutes. Filter through an ash-less filter paper, wash residue twice with hot water. Ignite the crucible in the flame cool and weigh, after that put the filter paper and residue together into crucible; heat gently until vapours' cease to be evolved and then more strongly until all carbon was remove. Cool in a dedicator weigh the residue and finally calculate acid insoluble ash of the crude drug with reference to the air dried sample of the crude drug (Khandelwal 2006). The % w/w of acid insoluble ash was calculated as follows-

$$\text{Acid insoluble ash (\%w/w)} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

Determination of water soluble ash

Total ash was boiled for 5 min with 25 ml water and insoluble matter which was collect on an ash-less filter paper was washed with hot water and ignited for 15 min at a temperature not exceeding 450°C in a muffle furnace. Difference in weight of ash and weight of water insoluble matter gave the weight of water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried powered drug (8). The (%w/w) of water soluble ash was calculated as follows:

$$\text{Water soluble ash (\%w/w)} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

Determination of Sulphated Ash

A silica or platinum crucible was heated to redness for 10 minutes, allowed to cool in desiccators and weigh. Unless otherwise specified in the individual monograph, 1g of the

substance was transfer to the crucible under examination and the crucible and the content was weigh accurately. Gently, ignite at first until the substance was thoroughly charred. The residue was cool and moistened with 1ml of sulphuric acid, gently heated until the white fumes was be no longer evolved and ignited at $800^{\circ} \pm 25^{\circ}$ until all black particles was disappear. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool and few drops of sulphuric acid were add and heated. Ignite again as before, allowed to cool and weigh. The operation was repeated until two successive weighing do not differ by more than 0.5mg (9).

The (% w/w) of sulphated ash was calculated as follows:

$$\text{Weight of sulphated ash (\% w/w)} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

RESULTS

Pharmacognostic evaluation of *U. Wallichiana*

Macroscopic/organoleptic characters of *U. wallichiana* bark are shown in table 1. Results of powder microscopy and TS and LS are presented in Fig. 1- 8.

Table 1: Morphological/organoleptic characters of *U. wallichiana* bark

Morphological/ organoleptic characteristics	Inference
Colour	Blackish brown
Odour	Pleasant
Taste	Slightly acrid
Shape	Flakes, slightly curved
Size	1- 1.6 cm in thickness
Habit	Tree

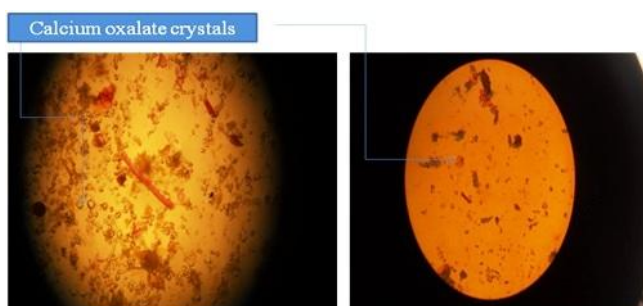


Figure 01. Microscopic characteristics of bark powder of *U. wallichiana* indicate presence of calcium oxalate crystals (10X)

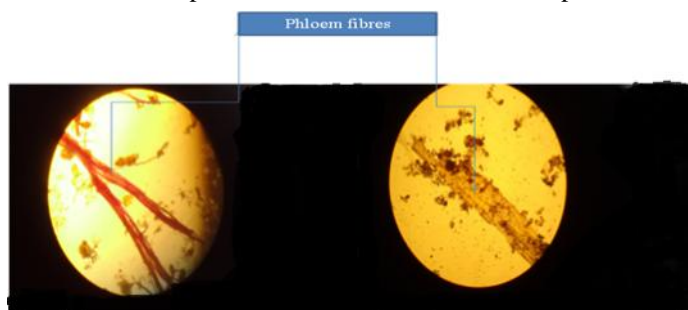


Figure 02 Microscopic characteristics' bark powder of *U. wallichiana* indicate presence of Lignified phloem fibre (10X)

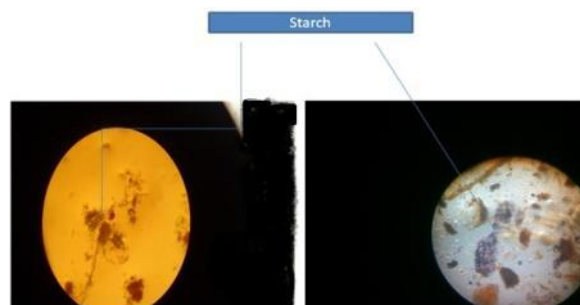


Figure 03. Microscopic characteristics of bark powder of *U. wallichiana* indicate presence of Starch grains (10X)

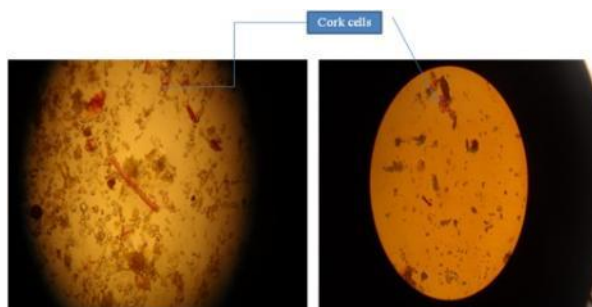


Figure 04. Microscopic characteristics of bark powder of *U. wallichiana* indicate presence of Cork cells (10X)
Transverse section

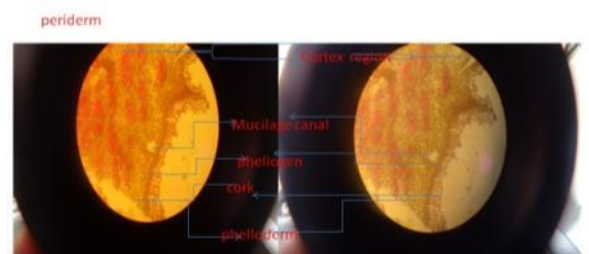


Figure 05. Transverse section of *U. wallichiana* bark indicate presence of periderm region (10X)

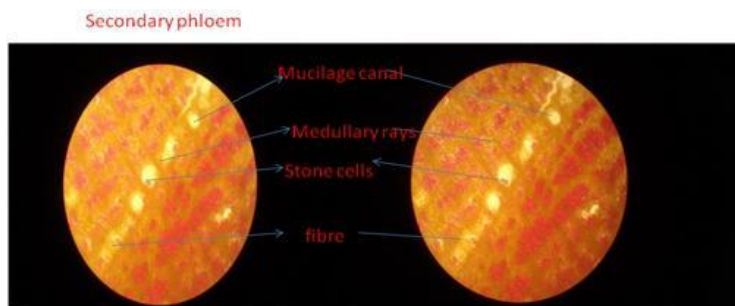


Figure 06. Transverse section of *U. wallichiana* bark indicate presence of secondary phloem region (10X)

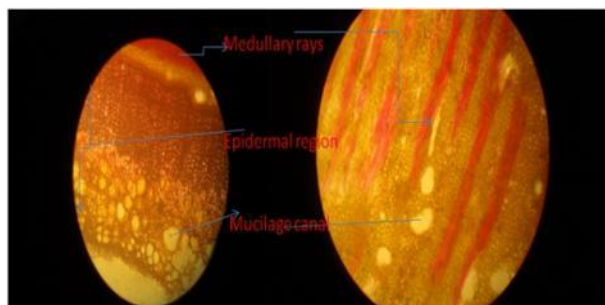


Figure 07 Longitudinal section of *U. wallichiana* bark

Physico-chemical Parameters

Results of total ash and loss on drying of *U. wallichiana* bark are shown in table 2.

Plant Extractive Values

Yield of various extractive values (hot and cold extraction) of *U. wallichiana* bark are reported in table 3.

Table 02: Total ash and loss on drying

Parameters	% (w/w)
Ash value:	
✓ Total ash	17%
✓ Water soluble ash	15%
✓ Acid insoluble ash	3.5%
✓ Sulphated ash	5%
Loss on drying	0.15%

Table 03. Extractive values of different extracts of *U. Wallichiana*.

Extract	Yield (%w/w)	Yield (%w/w)
	Cold extraction	Hot extraction
Petroleum ether	0.16	0.24
Chloroform	0.18	0.20
Ethyl acetate	0.24	0.32
Ethanol	0.36	0.70
Water	0.60	0.76

DISCUSSION

The present study was emphasized on macroscopic and microscopic studies, and physico-chemical analysis of *U. wallichiana* bark. The plant material was evaluated morphologically/ organoleptically by studying colour, odour, taste, size, shape, special feature like touch, texture etc. In this study, Transverse sections (TS) and powdered plant material was evaluated for internal structure and cells of plant like type of vascular bundles, epidermis, calcium oxalate crystals, trichomes etc. Such descriptions form the basis for the identification of drugs. In the present study, outer surface of bark of *U. wallichiana* observed blackish brown in colour. Odour and taste was pleasant and slightly acrid respectively. Shape appeared like flakes slightly curved and thickness measured about 1-1.6 cm. TS of bark under the microscope showed periderm region showing cortex, mucilage canal, cork, phellogen and phelloderm. TS of bark also showed secondary phloem region, medullary rays, stone cells and fibres. Longitudinal section showed medullary region, epidermal region and mucilage canal. Powder microscopy under the microscope showed calcium oxalate crystals, cork cells, phloem fibre, starch grains, stone cells and lignified phloem fibres.

In the present study, various physico-chemical parameters were also determined. physico-chemical parameters give an idea to identify genuine plant material and help to identify adulteration in the plant material. The extracts obtained by exhausting drugs are indicative of approximate measures of the chemical constituents. Taking into consideration the diversity in chemical nature and properties content of drugs, various solvent are used for determination of extractives. In the present study, physico-chemical parameters like total ash, acid insoluble ash, water soluble ash, sulphated ash and loss on

drying were estimated to be 17%, 3.5%, 15%, 5% and 0.15% respectively. Extractive value determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. In the present study, ethanol, chloroform, acetone, petroleum ether and water were used to evaluate the extractable constituents by cold maceration and hot extraction method. Petroleum soluble, chloroform soluble, ethyl acetate, ethanol and water soluble hot and cold extractive values are following. 0.24, 0.20, 0.32, 0.70, 0.76 and 0.16, 0.18, 0.24, 0.36, 0.60 respectively

CONCLUSION

In the current investigation, pharmacognostic standards and physico-chemical analysis may be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs.

Additionally, the present findings could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant *U. Wallichiana*.

REFERENCES

1. Khan MP, Mishra JS, Sharan K, Yadaw M, Singh A.K, Kumar SA. Novel flavonoid C-glucoside from *Ulmus wallichiana* preserves bonemineral density, microarchitecture and biomechanical properties in the presence of glucocorticoid by promoting osteoblast survival: A comparative study with human parathyroid hormone. *Phytomed.* 2013; 20(14): 1256-1266.
2. Ajaib M, Khan ZUD. Ethnobotanical Studies of Useful Trees of District Kotli. *Biologia (Pakistan)*, 2014; 60(1): 63-71.
3. Sharma C, Rajendar K, Kumari T, Arya KR. Indian traditional therapies and bio-prospecting: their role in drug development research. *Int J Pharm Sci Res.* 2014; 5 (3): 730-741.
4. Reddy G, Avinash K, Priyanka B, Saranya CS, Kumar CK. Ashok, Wound healing potential of Indian medicinal plants. *Intl J Phar Rev Res.* 2012; 2 (2): 75-87.
5. Rashid A, Sharma A. Exploration of economically important fodder plants of district Rajouri - Jammu And Kashmir State. *Int J life Sci pharm Res.* 2012; 2(4): 148.

6. Khandelwal KR. Practical pharmacognosy. 19th edition, 2006. P. 149- 156, 158.
7. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues, World Health Organization; 2007.
8. Sharma J, Varma R. A review on endangered plant of *Mallotus philippensis* (Lam.) M. Arg. Pharmacologyonline, 2011; 3: 1258.
9. Indian Pharmacopoeia. I.P, Vol I. Ministry of health and family welfare, government of India. Indian pharmacopoeia commission: Ghaziabad, India; 2010. p. 82-83 & 139 & 201.