Development of quality control markers for *Ulmus wallichiana* Planchon: An Indian traditional plant for osteogenic activity

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*Ulmus wallichiana* Planchon, is an Indian folk traditional plant used for the treatment of fractured bones in folklore tradition of Uttarakhand Himalaya, India. During chemical investigations, three major compounds, Ulmoside A & B, and Naringenin-6-C-β-D glucopyranosideis for osteogenic activity have been isolated from bark of this plant species. This paper presents macro and microscopic study, physicochemical parameters and chemo-profiling of stem bark of *U. wallichiana* through Q TOF HRMS for development of quality control markers and identification of crude samples. Transverse section (TS) stem bark and powder microscopy shows crushed rhytidoma consisting plenty of mucilaginous canals followed by crushed cork, cells of the cork filled with dark brown content. Whereas, authenticity and quality of raw materials can be determinate with exact calculated mass value of marker compounds for osteogenic activity at 467, 451 and 435 using Q TOF HRMS technique. Ursolic acid, β-sitosterol and lupeol at Rf value 0.25, 0.36 and 0.44 has also identified as HPTLC marker for identification and authentication of crude drug samples of stem bark of *U. wallichiana*.

**Keywords:** *Ulmus wallichiana*, Bone healing, Q TOF HRMS, Quality control marker, HPTLC

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Himalayan elm, *Ulmus wallichiana* Planchon, family Ulmaceae, locally known as Chamourmou is commonly used for healing fractured bones in animal as well as human being in folklore tradition of Uttarakhand Himalaya, India. During chemical investigations, three major compounds, Ulmoside A & B, and Naringenin-6-C-β-D glucopyranosideis for osteogenic activity have been isolated from bark of this plant species. This paper presents macro and microscopic study, physicochemical parameters and chemo-profiling of stem bark of *U. wallichiana* through Q TOF HRMS for development of quality control markers and identification of crude samples. Transverse section (TS) stem bark and powder microscopy shows crushed rhytidoma consisting plenty of mucilaginous canals followed by crushed cork, cells of the cork filled with dark brown content. Whereas, authenticity and quality of raw materials can be determinate with exact calculated mass value of marker compounds for osteogenic activity at 467, 451 and 435 using Q TOF HRMS technique. Ursolic acid, β-sitosterol and lupeol at Rf value 0.25, 0.36 and 0.44 has also identified as HPTLC marker for identification and authentication of crude drug samples of stem bark of *U. wallichiana*.

**Methodology**

*Ulmus wallichiana* Planchon, large deciduous tree with rough grey exfoliating bark, up to 30 m high; young branches pubescent to tomentose. Leaves elliptic-acuminate to obovate-cuspidate,7-15 cm long, 4-6 cm broad, base obliquely cuneate to rounded,
sharply biserrate, teeth arching with 2-4 secondary teeth, upper surface pubescent to scabridulous, lower surface densely pubescent to tomentose; petiole 6-10 mm long, pubescent. Inflorescence axis elongated, pedicels more than 5 mm long, articulated; 1/3rd lower portion of pedicel uniformly pilose; flowers in clusters appearing before leaves on branches of previous season, perianth tube narrowed into the pedicel, lobes 5-6, obtuse, pubescent to sub glabrous; stamens 5-6, filaments longer than the perianth, anthers red; ovary slightly pubescent all over; fruit samara, orbicular-obovate, 12-15 mm, narrowed into a short stipe, 2-3 mm long, stipe longer than the perianth, central seed hirsute to sub glabrous, wing membranous, reticulate, margin ciliolate (Fig. 1A).

Stem bark of *Ulmus wallichiana* was collected from three different altitudinal locations of Uttarakhand Himalaya, viz. Nainital (1800 M), Almora (2500 M) and Bageshwar (3000 M) during April, 2009. All the samples were properly identified by one of the senior author (KRA) and compared with Flora of The District Garhwal, North West Himalaya6. Herbarium specimens were deposited in departmental herbarium, CDRI, Lucknow, vide voucher specimen numbers KRA 24432, KRA 24443, and KRA 24444, respectively. Analytical grade chemicals from SD Fine, Mumbai (India) and standards (Lupeol, β-sitosterol and ursolic acid) were procured from Sigma, St. Louis, MO, USA.

Macro and microscopy of bark and powder were studied13-15. Air dried plant material was used for quantitative determination of ash and extractive values10. Swelling Index was calculated by modifying WHO method due to high percentage of mucilage. 100 mg powder was kept in 25 ml of water in a glass-Stoppard measuring cylinder; the material was shaken repeatedly for 2 hrs and then kept for 24 hrs to settle down. Volume of the mixture (ml) was measured as swelling Index. Marker compounds Ulmoside A, B and Naringenin-6-β-D glucopyranoside were identified through Q-TOF HRMS fitted with an electro spray (ESI) interface. Analyses utilized the positive ion mode m/z (M+H)+ for detection of compounds.

**Chromatographic analysis and HPTLC profile**

Dried stem bark of *U. wallichiana* were powdered and sieved through 44 meshes. Powdered sample (5 gm) was exhaustively extracted with methanol (4 × 25 mL, each time for 15 min) under reflux on a water bath at 100°C. Extracts were filtered through Whatman No. 1 filter paper (separately for each sample), concentrated under reduced pressure, and lyophilized17-18. A solution (10 mg mL⁻¹) of these extracts and standard solution (0.1 mg mL⁻¹) of β-sitosterol, ursolic acid and lupeol were also prepared in methanol. Known quantity of methanolic extracts along with chemical markers (β-sitosterol, ursolic acid and lupeol) were applied on to Higlachrosep nano silica UV 254 HPTLC plates of 10x10 cm with 0.2 mm nano silica with fluorescent indicator (S. D. Fine-Chem. Ltd. India) with the help of CAMAG Linomat Applicator 5, positioned 15 mm from side and 10 mm from bottom of the plate. Plate was eluted to a distance of 8.0 cm at room temperature (24ºC) in a solvent system – toluene: ethyl acetate : formic acid (9 : 1 : 0.1) in previously saturated twin trough chamber (CAMAG). Plate was derivatized by spraying with anisaldehyde sulphuric acid reagent. After heating at 110°C for 10 min plate was documented under visible light and scanned at wavelength 600 nm using CAMAG TLC Scanner 3 with software Win Cats 3.2.1.

**Results and discussion**

**Macroscopic studies**

Bark fibrous, rough grey exfoliating in diamond shaped flakes. Dried mature bark slightly curved, 1 to 1.5 cm in thickness, outer surface blackish brown in colour, rough due to the presence of irregular cracks and rhytidoma which peeled off at some places leaving rough, protuberated brown coloured surface; internal surface brown in colour, smooth or with very fine, wavy, transverse and longitudinal striations; fracture hard and splintery; mucilaginous and slightly acrid in taste with pleasant odour (Figs.1 B & C).

**Microscopic studies**

TS bark shows crushed rhytidoma consisting of plenty mucilaginous canals followed by crushed cork, cells of the cork filled with dark brown content. Cork cambium is not distinct. Phelloderm is very broad traversed with plenty schizogenous mucilage canals, some stone cells, fibres and prismatic crystals of calcium oxalate. Parenchymatous cells also filled with mucilage. Secondary phloem is very narrow as compared to phelloderm. Phloem consists of sieve tubes, companion cells, phloem
parenchyma, abundant mucilage canals and tangential bands of crystalloid fibres interrupted by medullary rays. Mucilage canals are arranged in tangential and longitudinal rows, forming a checker board appearance. Frequency of prismatic crystals is more in phloem region. Medullary rays, heterogenous, multiseriate, upto 20 cells high, cells circular to polygonal in shape, prismatic crystals of calcium oxalate abundant in rows (Fig. 2).

Powder

It is reddish brown in colour, mucilaginous and acrid taste without any odour. Under the microscope it shows plenty mucilaginous canals, numerous prismatic crystals and some cluster crystal of calcium oxalate, groups of parenchymatous cells, crystalloid, septate and non-septate fibres, cork cells in transverse and surface view and stone cells (Fig. 3).

Physico-chemical analysis

Ash values (total, acid insoluble, water-soluble), alcohol soluble, and water-soluble extractives are presented in Table 1. Slight variation was observed in Physico-chemical parameters of all the samples. For example, ash values and extractive values were found maximum in Bageshwar sample while mucilage content was found more in Almora sample. Q-TOF mass spectrum (Fig. 4) indicated that peak at m/z 467 and 435 could be due to bioactive markers ulmoside A and peak at 451 could be due to ulmoside B or eriodictyol-6-C-β-D glucopyranoside. HPTLC fingerprint profiles of all the three samples collected from Nainital, Almora and Bageshwar districts along with chemical markers have been developed (Fig. 5). Three HPTLC marker compounds, viz. ursolic acid, β-sitosterol and lupeol at Rf value 0.25, 0.36 and 0.44, respectively were observed in all three samples.
The wealth of traditional knowledge system related to the use of plants is not important to record this knowledge just to store, but it is important to keep it alive and make available for future as a unique resource for identification of novel pharmacological agents. In recent years, various factors like gradual depletion of natural pockets of indigenous flora and fauna, decreasing interest shown by traditional
healers in this profession due to less economic gain, natural calamities etc., led to the loss of huge amount of indigenous knowledge. Due to gradual loss of this generational traditional knowledge, these herbal texts become increasingly valuable and need rapid interaction between scientist and traditional communities. Sharing of benefits arises from the product development will further encourage their confidence and may leads more potential pharmacological agents for therapeutic values.

Conclusion

*U. wallichiana* is popular for its medicinal property of healing fractured bones in folk tradition of Uttarakhand Himalaya and some of its traditional claims have been scientifically validated. However, a well established quality control and identification parameters are highly essential for crude drug identification and authentication of this plant species. It is notable that the osteoprotective compounds ulmoside A, B and naringenin-6-C-β-D glucopyranoside is isolated from bark of this plant species are very complex in nature and have been licensed to Kemextree, USA for product development. From ongoing discussions, it was revealed that the molecular mass spectrum, exact calculated mass value and molecular ion peaks at 467, 451 and 435, identified as Ulmoside A, B and Naringenin-6-C-β-D glucopyranoside through Q-TOF HRMS and macro and microscopic studies, physico-chemical parameters, high mucilaginous substances on soaking in water and HPTLC marker compounds (ursolic acid, β-sitosterol and lupeol) identified in the stem bark may be used as standard parameters for authentication and quality evaluation of commercial samples of *U. wallichiana* during product development stage.

Fig. 5—HPTLC fingerprint profiles of methanolic extract of all the three samples of stem bark of *U. wallichiana* along with chemical markers. 1- Almora; 2- Bageshwar; 3- Nainital; R1- β-Sitosterol; R2- Ursolic acid; R3- Lupeol
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