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Original Article

Ethnopharmacological Developments in Traditional Bone Healing Plants: *Ulmus wallichiana, Coelogyne cristata and Pholidota articulata* for Osteoporosis Management

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ABSTRACT

Traditional medicines have afforded a rich source of natural compounds with great success in the field of medicines, pharmacy and biology. Documentation and validation of this indigenous knowledge through scientific investigations has become an emerging trend in the area of natural product research and impetus to prove their therapeutic efficacy and to identify newer, safer and more effective therapeutic agents. This paper provides the latest insights on ethnobotany, ethnopharmacology and phytochemical investigations of *Ulmus wallichiana, Pholidota articulata* and *Coelogyne cristata* for the development of pharmaceutical products for osteoporosis management. Biotechnological development for providing an alternate and renewable source for isolation of bioactive metabolites of *U. wallichiana*, an endangered and folk traditional plant is also discussed in this paper.

Key words: *Ulmus wallichiana*, *Colelgyne cristata, Pholidota articulata,* Bone healing plants, Osteoporosis, Uttarakhand.

1. INTRODUCTION

A systematic search and development of novel or new source of chemical entities, genes, micro and macro-organisms with a potential for value added products or an inventory and assessment of commercially valuable genetic and biological resources with a scientific objective,

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economic goals, conservation and sustainable use with fair & equitable sharing of benefits are the important phenomenon of Convention on Biological Diversity (CBD).These resources offer three fundamental sources of inspiration *i.e.* chemical; gene and design. India is well known for its rich heritage of biological diversity with more than 84.4 million ethnic diversity mainly Gonds, Santhal, Khasis, Angmis, Bhutias and Great Andamanese etc. Indian biodiversity comprises about 45,523 species of plants which is11.80% of the world and nearly 6,500 of them predominantly used in indigenous health care system[1].Among them, the Indian Himalayas alone contributes 18,440 species with more than 40% of plant diversity *i.e.* 8000 species Angiosperms, 44 species Gymnosperm; 600 species Pteridophytes; 1736 species Bryophytes; 1159 species Lichens and 6900 species Fungi and among them more than 45% are known to have medicinal properties[2]. Over the past few decades, medicinal plants used in several traditional remedies have regained wide recognition due to escalating faith on herbal products. There has also been a long standing interest in the identification of new or efficient therapeutic agents with lesser side effects as compared to marketed drugs. Scientific evaluation of traditional medicinal plants, animals, microbes is one of the important phenomena of bio prospecting and discovery of new biological prototypes. Literature reveals that >50000 natural compounds or their derivatives are available for their commercial applications and >120 drugs are in use in different countries[3].

Ulmus wallichiana family Ulmaceae locally known as *Chamormau* is an endangered plant species and endemic to Western Himalayas. The bark of this plant is commonly used for healing fractures in folk tradition of Uttarakhand. Similarly, *Colelgyne cristata* and *Pholidota articulata* (Orchidaceae) are also known to be used for similar purposes (Table1).On the basis of this ethnobotanical information, these plant species were collected and investigated. A number of bioactive molecules including one novel compound for osteogenic activities has been isolated and identified from these plant species (Fig1). Four of them have been licensed to KEMEXTREE, USA for product development. A growing demand from pharmaceutical industries, indiscriminate and distractive collection of raw materials accounts a huge amount of economic loss and rapid depletion of a number of plant species. Plant cell and tissue culture technology offer an alternate and sustainable tool to provide raw materials for their biological products. This paper provides the latest insights on ethnobotanical, phytochemical and pharmacological investigation of these three plant species along with *in-vitro* biosynthesis of bioactive molecules of *U. wallichiana* using plant tissue culture technology.

2. MATERIALS AND METHODS

An extensive ethnobotanical survey of Kumaon Himalaya, Uttarakhand was made during 2008 to 2014 under the routine drug development program of the Institute. The details of survey, a collection of ethno botanical information from the informants and identification of collected plant species have been described earlier [4, 5, 6]. As per the collected ethno botanical information from the informants, the fresh plant samples were collected, dried and processed for chemical and pharmacological investigation[7,8,9,10].Plant species were identified by senior author (KR Arya) and voucher specimen KRA-24443, KRA-24460, KRA-24462were housed in departmental herbarium CSIR-Central Drug Research Institute(CDRI), Lucknow.

3. RESULTS

Ethnobotanical data on the plant species *viz. U. wallichiana, Coelogyne cristata* and *Pholidota articulata* collected from the informants of Almora, Nainital, Bageshwar districts of Kumaon region of Uttarakhand state are described in Table 1. *U. wallichiana* and *P. articulata* are very commonly known for healing the fractured bone in all the three studies districts. However, *C. cristata* is very less known for its fracture healing activities. Among these, the bark of *U. wallichiana* is most popular and frequently used to treat the fractured cases of human as well as domestic animals. Due to very limited abundance in the nature and rapid collection of bark in a non-scientific manner for its different traditional uses, most of the trees died and plant became endangered (IUCN Red List of threatened species, 2006).

Table 1: Ethnobotanical plants used for healing fractures in folk tradition of Kumaon, Uttarakhand

S.No.	Botanical name	Local name	Family	Plant's Part (s)	Mode of administration
1	*Ulmuswallichiana* Planchon	Chamormau	Ulmaceae	Bark	Hot aqueous paste is applied on fractured parts
2.	*Coelogynecristata* Lindley	Hadjojen	Orchidaceae	PX*	Paste is applied on fractured parts and Dry powder is given Orally to the patients
3.	*Pholidotaarticulata* Lindley	Hadjojen	Orchidaceae	PX*	Paste of the fresh plant materials is Applied on fractured parts of the patients

* PX: Whole plant without roots

During chemical linvestigation, four major bioactive metabolites including a novel flavonol rich in C-glycosylated flavonoid and (2S,3S)-aromadendrin-6-Cb-D glucopyranoside (Fig 1) were isolated and identified from the bark of *U. wallichiana*[11]. Pharmacologically these metabolites showed osteogenic properties for prevention and treatment of osteoporotic fractures during menopausal disorders[12]and licensed to KEMESTREE, USA as rapid fracture healing agents for product development. Similarly, 3 compounds Flaccidin, Flavidin and Oxoflavidin (Fig 2) from *P.articulata* and 2 compounds, Coelogin and 3 (3-hydroxy phenethyle) -5 methoxyphenol (Fig. 3) from *C. cristata* were

also isolated and identified and showed strong potential for osteogenic activities during pharmacological investigations[9, 10].

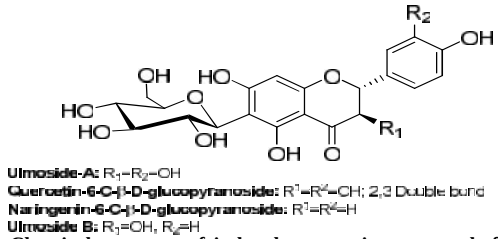

Fig 1: Chemical structure of isolated osteogenic compounds from *U. wallichiana* Planchon

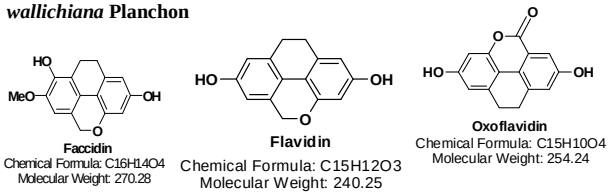

Fig 2: Chemical structures of osteogenic compounds isolated from *P. articulate* Lindley

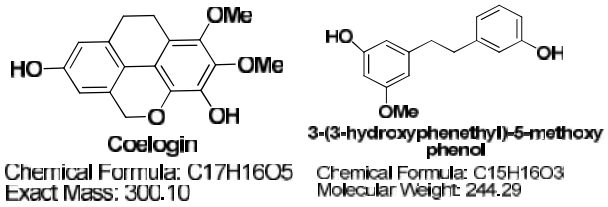

Fig 3: Chemical structures of osteogenic compounds isolated from *C.cristata* Lindley

Fig 4 (A-D): Protocol for establishment of callus and suspension culture for *in vitro* biosynthesis of bioactive metabolites in *Ulmus wallichiana* Planchon. A) Induction of callus from stem explants; B & C) Proliferation and biomass production of callus; D) Cell suspension culture.

Biotechnological developments for *in vitro* biosynthesis of bioactive metabolites in *U. wallichiana* Planchon

It is important to establish the optimal cultural conditions for the establishment of *in vitro* cultures for biosynthesis and enhancement of bioactive metabolites from medicinal plants of pharmaceutical application. *Ulmus wallichiana*, is an endangered plant species and all the bioactive metabolites for osteogenic activity were isolated from the bark of this plant species. Considering the medicinal properties of its bark as the main source of its bioactive metabolites, development of an alternate and renewable pathway was essential and logical. Plant tissue culture technology has been investigated for industrial production of several secondarymetabolites[13, 14]andprove to be an effective tool for *in vitro* synthesis and enhancing the quality and quantity of secondary metabolites[15, 16, 17]. Plant growth regulators are one of the most important factors to influence cell growth, differentiation and metabolite formation[18]. The appropriate concentration and combination of auxins and cytokinins in the culture media are one of the critical determinants in controlling callus growth, regulation of cells and biosynthesis of secondary metabolites. Murashige and Skoogs, 1962 (MS) media fortified with different auxins(2,4-D; NAA; IBA; IAA) and cytokinins (Kn, BAP; 2-iP) in various concentration and combinations were applied for induction and establishment of callus and cell suspension cultures[19]. MS medium containing 0.25 mg/L 2, 4-dichlorophenoxy acetic acid (2, 4-D) and 0.1 mg/L Kn showed the optimal growth condition for establishment of callus and cell suspension culture (Fig 4). Q-TOF LC-MS analysis of these cultures showed the presence of similar nature of osteogenic compounds as they were isolated from the parent plants[19]. The developed *in vitro* technology for isolation of these metabolites under controlled environmental conditions may ensure the production and enhancement of adequate quantity of materials during product development process.

4. DISCUSSION

The major objective of this work was to explore the traditional use of these three plant species used for the treatment of fractures in folk tradition of Uttarakhand Himalaya and to identify their bioactive compounds for modern therapeutics. The relevance of these ethno botanical uses for the study in osteoporosis management was the hypothesis of the scientific mechanism involved in healing fractures. Bone is a dynamic organ and is constantly being remodeled in order to facilitate growth and repair. This process requires the involvement of bone forming osteoblast and bone resorbing osteoclast cells, which function in generating and mineralizing bone, giving strength and rigidity to the skeletal system. Phytoestrogens are a group of natural compounds that exert estrogenic activity and are used for the treatment of menopausal disorders and have a protective effect on osteoporosis and cardiovascular system. These well-known facts brought our attention that these traditionally bone healing plants may also have the bioactive compounds which may exert same properties of osteoblast differentiation, bone mineralization, improvement of bone biochemical properties as these are the prerequisite phenomenon of healing fractures. Hence, keeping in view to these mechanistic facts, these well-known traditional plants used for healing fractures in folk tradition of Uttarakhand Himalaya were investigated as a core idea for identification and isolation of bioactive molecules for osteoporotic fractures which may help in the management of osteoporosis.

Worldwide, it is estimated that one osteoporotic fracture occurs in every 3 seconds and annually it accounts 8.9 million[20]. India is more alarming country as approximately

300 million people are suffering from osteoporosis[21]. In India, the ratio of osteoporosis patient in male and female is 1:8 and 1:3 respectively. These data depict India as one of the largest affected countries in the world and being repeatedly increasing. It is estimated that about 51% women are suffering from osteoporotic problems in India; however, the actual numbers accounts more than 200 million which is comparatively higher than the men at any elderly age group[22]. In most western countries, the maximum osteoporosis incidence occurs at the age of 70-80 years. Meanwhile in India, it occurs 10-20 years earlier *i.e.*at the age of 50-60 years[23]. In USA, more than 1.5 million osteoporosis-related fractures were reported annually, which includes around 46% vertebral fractures, 20% hip fractures, 16% wrist fractures and 20% other fractures[24]. According to an estimate, osteoporosis hip fractures in India may increase to 600,000 per year by 2020 and upto more than 1 million by 2050[25]. Pharmacological agents which are approved by FDA (Food and Drug Administration) for prevention or treatments of osteoporosis are classified as anti-resorptive and anabolic agents. On the other hand, anabolic agents appear to promote osteoblast functions which ultimately enhance bone formation. Anabolic therapeutic options of osteoporosis are limited to only synthetic analogs of parathyroid hormone (PTH) like teriparatide. It is extremely costly and has side effects. It can cause low blood pressure, orthostatic hypotension and increase in blood calcium level and rarely osteosarcoma which is a serious form of cancer[26].

5. CONCLUSION

Traditional medicines have considered to providing affordable and safest treatment with very fewer side effects as compared to allopathic medicines. To till date, a number of traditional medicinal plants, their formulations and other natural products had been scientifically investigated for isolation of bone anabolic agents. Scientific investigations on *U. wallichiana, Coelogyne cristata* and *Pholidota articulate* reported in this paper validates the traditional claims of bone healing properties. The identified compounds have a great potential to provide a better option and resources for the development of effective, safer and affordable therapeutical agents for the management of osteoporosis.

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