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ISOLATION AND PHYSICOCHEMICAL CHARACTERIZATION OF BIOLOGICALLY ACTIVE COMPOUNDS FROM AQQAYIN (BETULA SPP.)

Turayeva Dinora Chori qizi
Student at the Faculty of Natural Sciences,
Chirchik State Pedagogical University
@dinoraturayeva2004gmail.com
Turankulova Janiya Daniyar qizi
Student at the Faculty of Natural Sciences,
Chirchik State Pedagogical University
Yodgorov Baxtiyor Orziqulovich
Senior Lecturer in Chemistry, Faculty of Natural Sciences,
Chirchik State Pedagogical University

Abstract: Growing worldwide demand for plant based therapeutics necessitates increased scientific attention to medicinal taxa rich in biologically active and chemically diverse metabolites. The Aqqayin plant (Betula spp.) is an important example of such taxon since it holds important ethnomedicinal status and an excellent resource of various secondary metabolites. The aim of this study is to report on the isolation and physico-chemical characterization of biologically active components from the formulation of the Aqqayin plant, utilizing an integrated approach, from a review of recent studies on phytochemical studies of this taxon to scientifically documented information. The localization of triterpenoides, flavonoids, phenolic glycosides, diarylheptanoides and its derivatives into bark, leaves and buds, as well as their physical chemical behavior, effectiveness of extraction and the biological relevance are specifically presented. Studies worldwide have shown that there is a prevalence of compounds such as betulin, betulinic acid, ursolic acid, arbutin and phenolic antioxidants in Betula species which shows antimicrobial, anti inflammatory, hepatoprotective and anti-proliferative effects.

Keywords: Biologically active substances, phytochemistry, natural products, triterpenoids, betulin, betulinic acid, flavonoids, phenolic compounds.

From the point of view of scientific research in Uzbekistan, investigation of Aqqayin plant is especially interesting. Although the number of phytochemical surveys published abroad are vast, investigations carried out in Central Asia are still limited. This fact does not allow comparing metabolite composition and variability between populations of one species from different ecological conditions, and consequently identifying geochemotypes of valuable medicinal plant. The separation, purification, and physico-chemical investigation of biologically active substances from Aqqayin should be regarded not only as an academic interest, but as a necessary stage for the development of methods of evidence-based phytotherapy and an issue concerning an effective use of natural resources, with further application of traditional regional knowledge in international medical research.

The study of phytochemical components isolated from the Aqqayin plant (Betula pendula Roth.) is of major scientific interest for the purposes of finding biologically active substances, purifying them and determining physico-chemical properties of the isolated compounds. Modern phytochemical investigations demonstrate that medical and biochemical importance of Betula species is linked to a complex composition of secondary metabolites such as triterpenoids, phenolic substances, flavonoids, tannins, and volatile compounds.

Among the pentacyclic triterpenoids betulin and betulinic acid appear to be the most intensively studied compounds, due to their broad range of biological activities and low toxicity. Investigations have revealed that in particular outer birch bark is a rich source of these triterpenoids, which in birch species may reach as high as 20-30 % and even nearly 45% of the dry weight. Such accumulation in Aqqayin makes it one of the unique medicinally important plants to be included in modern drug discoveries.

The physico-chemical nature of betulin determines its biological activity, as well as industrial and practical usage of this substance. Betulin is a pentacyclic lupane-type triterpenoid with a chemical formula 3,28-dihydroxy-lup-20(29)-ene. It is a white crystal powder that possesses low water solubility, but a good solubility in organic solvents such as ethanol, methanol, chloroform and ethyl acetate. While such properties can be used to facilitate isolation and purification processes, solubility problems may limit pharmaceutical development of these compounds. So physico-chemical limiting factors, and not only lack of biological activity may restrict the therapeutic application of betulin-containing preparations. Pharmaceutical chemistry is now seeking to resolve this problem by preparing derivatives or by encapsulating of those compounds in the form of nanostructures.

Scientific investigation carried out in the world countries confirmed the presence in birch species not only triterpenoids but also high amount of flavonoids (quercetin and kaempferol derivates), diarylheptanoids, lignanes and phenolic substances (chlorogenic acid, ellagic acid, phenolic glycosides). These substances play important roles in plant defense mechanisms and enzymatic regulation. The inner part of the bark was established to be richer with phenolic substances and flavonoids, whereas outer bark triterpenoids.

Platyphylloside is among other studied phenolic constituents of birch, which can be considered as chemotaxonomic marker in determination of Betula species. Therefore one should keep in mind that in search of biologically active compounds specific extraction methods, particular to certain plant parts, are to be applied to maximize the quantity and purity of targeted chemical.

One of the disputable questions in the science today is the problem of choosing suitable extraction technique to maximize both yield and purity of a biologically active compound. Traditional methods of extraction using organic solvents or ethanol-methanol mixture are widespread; however, they are quite laborious and may not be efficient in the case of labile substances due to need for prolonged period of extraction, large amounts of solvent and heat. Comparisons between extraction of flavonoids from birch leaves by methods with different polarity of the solvents and different duration of extraction revealed that different compounds may be accumulated depending on the choice of conditions. Therefore inconsistent results of the biological activity of a plant may not only be attributed to variation between the plant samples, but also to different methods of extraction.

Consequently, modern extraction technologies have become one of the main innovative approaches within Aqqayin phytochemistry. Ultrasound-assisted extraction, microwave activation, vacuum sublimation and supercritical carbon dioxide extraction will continue to substitute for entirely classical procedures as they offer greater efficiency and are much more environmentally friendly than traditional extraction. Supercritical CO 2 systems offer advantageous prospects because of the ability to isolate lipophilic components selectively and at the same time significantly decrease residues of toxic solvents. According to various comparisons of methods for triterpene extraction from birch bark, the yield may reach close to 40%, but depends on factors like temperature, pressure, solvent composition and pretreatment. Recent research in sustainable extraction methodologies supports the prospect of processing forest by-products and bark waste

into valuable medicinal materials for pharmaceutical purposes; in that way the integration of medicinal chemistry with the concept of the circular bioeconomy.

Isolation itself does not ensure the reliability of scientific results. The study of complex chemical profiles at the highest level require robust physico-chemical characterization to confirm identity and purity of compounds. The contemporary internationally accepted method comprises the application of high performance liquid chromatography, gas chromatography mass spectrometry, Fourier transform infrared spectroscopy, Raman spectroscopy and nuclear magnetic resonance.

Such sophisticated analytical instruments provide possibility not only to identify compound but also to estimate distribution of particular chemical individuals and to describe the exact structure. Investigation on Betula pendula bark extracts by spectroscopic means resulted in the formation of preparations of major betulin fraction by using methanol as solvent and with increased extraction time. Studies carried out by using GC-MS were performed to obtain the identity of pure sample components and betulin was identified as a main one; highest purity 59% was reached using optimal method and major components were betulin accompanied with lupeol and other triterpenoids. Such chemical identification is in our view an important prerequisite of the reliability of medicinal plants research as medicinal applications without confirmation of existence and structure of active principles can't be considered to be proved from scientific view.

Relevance of isolated metabolites in Aqqayin to medicine science, it is another scientific area widely discussed. A link between betulin and betulinic acid to antioxidant, anti-inflammatory, antimicrobial, antiviral, antiparasitic and antiproliferative activities has been established. Some works indicated betulin-betulinic acid effects to control apoptosis, inhibit inflammation-related pathways and prevent activation of tumor-associated signaling pathways. The most recent data deal with the anticancer activity, but still many of biological effects still seem to exist only in vitro.

Many biological effects of betulin, Betulinic Acid on different cell lines remain questionable, a well-founded concern of majority contemporary reports. Lack of knowledge on their pharmacodynamic behavior is considered one of the main problems of birch phytochemistry and large studies on pharmacodynamics and toxicology are necessary for any pharmaceutically oriented development of products.

Influence of ecological factors to the chemical profile of Aqqayin species, another highly argued scientific approach. Seasonal changes of white birch metabolite profiles and relationship between the phenolics biosynthesis and climatic and soil-based microbial community characteristics have been studied recently. Activity of antioxidants increased significantly with increased content of total phenolics and seasonal and environmental stress on vegetation were confirmed to modify accumulation patterns of metabolites. From a medicinal aspect such finding provides evidence that the harvesting time and habitat significantly affect the chemical composition of a drug and thus also its biological effect. Raw material collection cannot be arbitrary in any sense and it should be governed by ecologically reasoned criteria when developing a scientifically solid product, a concept applied in medicinal plant science for the achievement of reproducible results.

Based on all these finding one would say, the biggest scientific problem about Aqqayin plant is not to demonstrate its possession of biologically active agents which are well known. The real scientific challenge is the development of internationally comparable, standardized extraction processes combined with efficient physico-chemical characterization, ecology-guided standardization of raw material and application studies so that to turn the existing phytochemical potential into real medicinal and industrial products. Such study is also considered important for

Uzbekistan from a strategic viewpoint since its existing biodiversity and increasing medical and industrial capacities are favorable for the exploration of its medicinal plants. Hence, we consider Aqqayin not just a traditional medicinal plant but a promising biochemical resource whose importance is reflected by scientific validation.

The present study focused on comparative phytochemical investigation as well as literature-based analytical review of isolation and physico-chemical characterization of bioactive agents present in Aqqayin plants (Betula spp.). All relevant scientific literature related to Betula phytochemical and pharmacological studies published till the date and indexed on major scientific databases were analyzed comparatively.

A methodology was also used to review physico-chemical characterization data of extraction efficiency, solvent selectivity and compound characterization. We paid special attention to bioactive metabolites identified via using the chromatographical and spectroscopical techniques, namely the HPLC, GC-MS and LC-MS, and only experimental data was used to establish relationship between physico-chemical behavior of majority compounds (betulin, Betulinic Acid, and phenolics) and extraction conditions. This methodological design led us to develop the scientifically well-grounded interpretation on evidence available to date and aimed at establishing analytical reproducibility.

The analysis of literature suggests that Aqqayin plants are chemically rich with biologically active agents, namely triterpenoids, flavonoids and phenolics. Among these betulin, Betulinic Acid were found predominant in bark, compared to greater amounts of flavonoids and antioxidant phenolics in leaf and bud tissues. Experiments revealed that the extraction and yields of these compounds depend heavily on the solvent polarity and conditions and higher recovery was often achieved in alcoholic and hydroalcoholic solutions.

Physico-chemical evaluation demonstrated that the isolated compounds share different solubility, thermal stability and chromatographic characteristics which consequently play role in the purification and in the development of drugs. Methods like chromatography and mass spectroscopy enable successful characterization of compounds and analysis of them. Meanwhile, lack of comprehensive reports on the ecological variations of compounds, limited by small attention of some publications devoted to biological activity on chemical standardization make it hard to transform its pharmaceutical use in to industry level and for medicine purposes.

Based on reviewed data one would state, Aqqayin possess antioxidants and anti-inflammatory as well as antimicrobial activity. Its broader application still depends on adequate extraction procedures and detailed physico-chemical characterization. From this point of view Aqqayin is a good research object not only as an ethnopharmacologically proved medicinal plant but also as a tool for further medicinal studies with regard to its valuable chemical properties.

Reviewed studies indicate that Aqqayin plants are rich in chemically active substances with significant pharmacological properties, predominantly triterpenoids, flavonoids and phenolic compounds, betulin, betulinic acid that are associated with the reported antioxidant, antimicrobial and anti-inflammatory activities in scientific literature, but due to variations in compounds distribution and limited information concerning physico-chemical standardization significant limitations remainto establish industrial and medicinal application. The data confirm need for reliable and efficient extraction methods and advanced analytical methods which contribute to the validation of its medicinal value in a broader spectrum. Aqqayin, as medicinal plant, must be seen not only as an traditional resource but as potential scientific object for evidence-based pharmacotherapeutic applications.

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