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DETERMINATION OF POLYSACCHARIDES IN THE ROOT NODULES OF ORCHIS MASCULA L. (EARLY SPRING ORCHIS)

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Abstract: Orchis mascula L., commonly known as Early spring orchis, is a perennial medicinal plant of the Orchidaceae family widely distributed in Europe, Asia, and North Africa. The root nodules of this plant have long been used in traditional medicine due to their anti-inflammatory and astringent properties. However, little is known about the chemical constituents, particularly polysaccharides. This study aimed to isolate and quantify water-soluble polysaccharides (WSP) and pectic substances (PS) using two different extraction methods. The results showed that prior removal of lipophilic and phenolic substances significantly influenced the polysaccharide yield, particularly reducing the WSP content. These findings provide preliminary quantitative data that may contribute to future pharmacognostic and phytochemical studies of O. mascula.

Keywords: Early spring orchis, Orchis mascula, root nodules, polysaccharides, pectic substances, phytochemistry

Introduction

Orchis mascula L., or Early spring orchis, is a herbaceous perennial plant of the Orchidaceae family, reaching 20–50 cm in height. It typically grows in calcareous soils and is distributed throughout Europe (including Austria, Denmark, and Greece), Western and Central Asia (notably Turkey, Iran, and Lebanon), and parts of North Africa (including Algeria and Morocco). In traditional medicine, the root nodules—often referred to as salep—are used for their medicinal properties, including astringent, demulcent, anti-inflammatory, and wound-healing effects. They are also employed in folk remedies to treat respiratory and gastrointestinal disorders, such as bronchitis, diarrhea, hemorrhoids, and gout.

Despite its long-standing ethnopharmacological use, the chemical profile of O. mascula remains insufficiently studied. In particular, the role and quantity of its polysaccharide constituents—which may contribute significantly to its therapeutic effects—have not been systematically investigated. Polysaccharides are known to exhibit various biological activities, including immunomodulatory, antioxidant, and wound-healing properties. Therefore, this study aimed to isolate and quantify the primary polysaccharide fractions, specifically water-soluble polysaccharides (WSP) and pectic substances (PS), using two extraction protocols. The findings are expected to provide essential data for future pharmacological evaluation and standardization of this medicinal plant.

Materials and Methods

The root nodules of O. mascula were collected and prepared in Tashkent, Uzbekistan, in 2024. The dried nodules were subjected to two different extraction methods to isolate and quantify WSP and PS.

Method 1:
A total of 20 g of dried root material was ground into a fine powder. To eliminate lipophilic compounds, the powder was extracted three times with chloroform using a 1:3 material-to-solvent ratio. Each extraction lasted 12 hours using the maceration method. After this step, the residue was air-dried at room temperature for 12 hours. Phenolic compounds were then removed by extracting the residue three times with 70% ethanol under similar conditions.

The defatted and dephenolized meal was dried at 30°C for 24 hours and then used for WSP extraction. For this, the meal was transferred to a 250 ml glass beaker, and 200 ml of purified water was added. The mixture was stirred and extracted three times for 4 hours each at room temperature. All aqueous extracts were combined, concentrated to 90 ml, and slowly poured into 200 ml of cold 96% ethanol with continuous stirring. The precipitated WSP was collected by filtration, dried, and weighed.

The residual meal from the WSP extraction was further subjected to pectic substance extraction. The PS extraction involved three successive extractions using a mixture of 0.5% oxalic acid and ammonium oxalate (1:1) in a 1:5 ratio to the raw material. The mixture was heated at 80–85°C for 2 hours. The combined extracts were evaporated to 50 ml and neutralized using dialysis. Cold ethanol (100 ml of 96%) was added to precipitate PS, which was then filtered, dried, and weighed.

Method 2:
In the second method, the same steps were followed but without the preliminary removal of lipophilic and phenolic substances. This was done to evaluate the impact of pre-treatment on the yield of polysaccharides.

Results and Discussion
The results of the quantitative analysis of polysaccharides extracted from the root nodules of O. mascula are as follows:

- **Method 1** (with chloroform and ethanol pretreatment):
  - Water-Soluble Polysaccharides (WSP): **2.51%**
  - Pectic Substances (PS): **0.16%**
- **Method 2** (without pretreatment):
  - Water-Soluble Polysaccharides (WSP): **4.3%**
  - Pectic Substances (PS): **0.16%**

These findings indicate that the removal of lipophilic and phenolic substances before polysaccharide extraction resulted in a significantly lower yield of WSP. This suggests that some WSP may be co-extracted or associated with phenolic compounds in the raw material, and their removal prior to water extraction affects the overall yield. However, the amount of PS remained consistent across both methods, implying that pectic substances are more stably bound within the plant matrix and unaffected by initial solvent treatments.

The relatively higher yield of WSP compared to PS aligns with similar findings in other medicinal plants, where water-soluble polysaccharides often dominate the carbohydrate profile. These polysaccharides are likely to contribute to the traditional uses of O. mascula, especially in soothing and anti-inflammatory applications.

Conclusion
The present study demonstrated that Orchis mascula L. root nodules contain appreciable quantities of polysaccharides, particularly WSP, which may have pharmacological relevance.

The yield of WSP is significantly affected by pretreatment steps, highlighting the importance of extraction protocols in phytochemical analysis. The relatively constant content of PS suggests it is a more stable component of the plant matrix. Future research should focus on the structural characterization and bioactivity of these polysaccharides to better understand their therapeutic potential and validate the traditional uses of O. mascula.

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