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Original Article

ISOLATION, IDENTIFICATION, AND QUANTIFICATION OF LECTIN PROTEIN CONTENTS IN *CHAMERION ANGUSTIFOLIUM* L. DRIED RAW MATERIAL AND THE STUDY OF ITS ACTIVITY USING RATUSERYTROAGGLUTINATION

ZEAD HELMI MAHMOUD ABUDAYEH[1*], KHALDUN MOHAMMAD AL AZZAM[2*], ULIANA VLADIMIROVNA KARPIUK[3], LOAY KHALED HASSOUNEH[1], AMJAD ABUIRMEILEH[1]

[1]Faculty of Pharmacy, Isra University, 11622 Amman, Jordan, [2]Pharmaceutical Chemistry Department, Pharmacy Program, Batterjee Medical College for Sciences and Technology (BMC), 21442 Jeddah, Kingdom of Saudi Arabia, [3]O. O. Bogomolets National Medical University, T. Shevchenko Boulevard, 13, Kiev, Ukraine
Email: azzamkha@yahoo.com, zead09@meta.ua

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ABSTRACT

Objective: To isolate, identify and quantify lectin protein contents in *Chamerion angustifolium* L. dried raw material species namely; leaves in the flowering stage, buds, and flowers and the study of its activity.

Methods: Lectin activity has been determined using the biological method called ratuserytroagglutination. This method is based on the formation of aggregates of lectins and rat erythrocytes using the floor amount of lectins that agglutinate erythrocytes as a unit of measurement. Additionally, the protein contents of the extracts have been determined using the Bradford assay method.

Results: lectins activity from *Chamerion angustifolium* L. in leaves in the flowering stage, buds, and flowers were 2.72±0.06, 0.24±0.008, and 0.56±0.014 units/mg protein, respectively. The greatest lectins activity was in the leaves in the flowering stage followed by flowers and then in buds. Protein contents in leaves in the flowering stage, buds, and flowers were 4.71±0.03, 6.77±0.02, and 5.76±0.14 mg/ml, respectively.

Conclusion: All proteins obtained from the *Chamerion angustifolium* L. plant raw material were shown to possess rat erythrocytes agglutinating activity. The crude extract of leaves in the flowering stage exhibited the strongest hemagglutinating activity of about 2.72 units/mg proteins, whereas the buds showed the lowest activity of about 0.24 units/mg proteins. It should be highlighted herein, although many plant lectins mimic the behavior of plant storage proteins, these lectins should not be classified as storage proteins.

Keywords: Lectins, *Chamerion angustifolium* L., leaves, Buds, Flowers, Ratuserytroagglutination.

INTRODUCTION

Chamerion angustifolium L. Holub, also called *Epilobium angustifolium* L., is a perennial medicinal plant of the Onagraceae family [1, 2]. This native plant is considered as a representative of willow herb species which found throughout the lower temperature of Northern region in Ukraine. Willow herb has been used in folk medicine to treat a variety of diseases such as rectal bleeding, benign prostate hyperplasia and related urination problems, relief of menstrual disorders, etc. [2-4]. Nowadays, it is widely used as a domestic herbal for the treatment of various diseases such as inflammation, mouth ulcers, cramps, dysentery, skin sores, burns, etc. Additionally, due to the sweet and pleasant taste, it is commonly consumed in Russia as a tea for the treatment of gastritis, stomach ulceration, and sleeping disorders [2, 5].

In the beginning of this century, the anticancer properties of *Chamerion* angustifolium L. were discovered [4, 6-8]. This is attributed to the presence of oenothein B which belongs to the group of oligomeric ellagitannins [8, 9]. Also antibacterial [10], anti-inflammatory [11], antioxidant [11], and anti-aging [12] properties of *Chamerion angustifolium* L. species have also been reported.

Over the last few decades, lectins have become a hot topic of interest to a large number of researchers and attracted a lot of interest, owing to their potentially exploitable biological properties including immunomodulatory and anti-insect [13], antitumor [14, 15], antibacterial [16], antifungal [17], anti-HIV [14, 17,18], and mitogenic activities [19].

Lectins, derived from Latin—'*Legree*' = to pick or select [20], are carbohydrate binding proteins [20-24] which present in most of plants and in some animals [20]. They are stable, most can be freeze-dried, are generally functional at 5<pH>10, and are commercially available [25]. They are highly variable in their amino acid sequences and widely distributed in microorganisms, viruses, animals and higher plants [23]. Plants are the richest and most convenient source of lectins. Thus, it has been attracting much attention because of their ease of isolation and usefulness as an adaptogen. Lectins were identified by their agglutination ability of human and animal red blood cells [22]. Additionally, specific amino acids residues are considered essential for maintaining the carbohydrate binding as well as hemagglutinating activity of lectins [26].

A few studies have been reported in the analysis of *Chamerion angustifolium* L. studying the composition of fresh and dried material using chromatography, spectrophotometric and chemometric techniques have been reported [8]. Moreover, the determination of the highest amount of flavonoids and the highest radical scavenging activity of *Chamerion angustifolium* L., which was collected in different vegetation phases (intensive growing, bud, massive blooming, ripening of fruits (seeds) and the end of vegetation) and in different parts of the plant (blooms, leaves and stems) has also been conducted [2].

Additionally, it has been reported that the physiological activity of some kinds of lectins involves the use of plant extracts to control biochemical processes in the human body and, on the other hand, to increase its immunity and capacity to fight cancerous tumors [27]. Therefore, lectins and the study of their properties are still an urgent task not only in modern medicine but also in pharmacology.

Information regarding the lectin protein contents and its activity in the plant of the *Chamerion angustifolium* L. is almost completely deficient. To our knowledge, this is the first comparative study of the lectin protein contents and the study of its activity from *Chamerion*

angustifolium L. raw material in different parts of the plant (leaves in the flowering stage, buds, and flowers), growing in Ukraine.

MATERIALS AND METHODS

Chemicals and reagents

Sodium chloride solution (0.9%), trisodium citrate, potassium chloride, disodium phosphate, and Coomassie Brilliant Blue G-250 were purchased from Sigma-Aldrich (St Louis, MO, USA). All other reagents used were of analytical grade.

Plant material

Chamerion angustifolium L. medicinal plant raw material was collected from the Zhitomir oblast region, located in the Northern of Ukraine during July to August in 2013–2014. The temperature at harvesting time ranged between 30–35 °C. It was collected at three different stages of vegetation (leaves in the flowering stage, buds, and flowers). The raw material was allowed to dry at room temperature in a well-ventilated shadow place. Dried material samples were kept in a dry and dark place in a multilayer paper bags. Dried raw material samples were ground with the aid of a food processor Power Plus 1300 (Braun, Germany). The obtained particle fractions were 0.1–0.3 cm. The obtained powder of the plant raw material was stored in a dry and dark place at room temperature in the polyethylene bags till used.

Macroscopic and microscopic techniques for identification/ authentication of *Chamerion angustifolium* L. plant

As known, authentication/identification of raw material is the essential starting point in developing a botanical product. Nevertheless, inherent chemical variability will certainly be observed with any botanical. Additionally, each step of harvest, storage, processing, and formulation may significantly change the quality and uniformity of the final product, whether by preserving the desired marker components or by eliminating unwanted contaminants [28]. Therefore, methods to assure quality control in manufacturing and storage are required tools not only to ensure optimal efficacy but also the safety of these products. Moreover, such controls are important for the evaluation of toxicological, pharmacological, or clinical studies involving botanical dietary supplements.

Macroscopic study

The herb of *Chamerion angustifolium* L. is a well-known folk medicinal plant commonly used in Russia as a tea to treat a variety of diseases. The macroscopic study of the morphological description of the plant parts was conducted by our naked eye and with the aid of a magnifying lens.

Microscopic study

The microscopic study of the current work, the anatomical study, was done using the procedure prescribed in our previous work [29]. In brief, the structure of epidermis of the leaf, petals and sepals of *Chamerion angustifolium* L. was studied using the scanning electron microscopy. Their main diagnostic characters (from the type of surface, the presence of cuticle, epidermal cells form, stomatal, trichomes and wax types) were identified. Moreover, several numeric indices of the studied materials (moisture, total ash content and ash insoluble in of hydrochloric acid) were determined accordingly.

Preparation of lectin-containing extracts

Lectin-containing extracts (LCE) was prepared by weighing 1 g sample of the ground plant material which was transferred to a porcelain mortar. 10 ml of physiological sodium chloride solution (0.9%) was added and ground together for 5 min to attain a homogeneous state, filtered through a double layer gauze filter and centrifuged at 3000 rpm for 15 min. Lectin activity was then tested using the supernatant containing lectin extracts.

Preparation of erythrocyte suspension

Red blood cells (RBC's) were obtained from citrate solution of rat blood (one part blood was taken in nine parts chilled 3.8% solution of trisodium citrate). The mixture was centrifuged at 1500 rpm for 5 min. Citrate supernatant was removed and this step was repeated four times under the same conditions. Then, the procedure of washing of RBC's was conducted to remove plasma proteins; which may inhibit cell-lectin interactions. After a third washing, the cells were re-suspended in 25 ml of 0.15 M NaCl to make a 2% suspension. The cell suspension obtained was stable and refrigerated at 4 °C till used.

The reaction of hemagglutination to free erythrocyte sedimentation rate

The commonly accepted method for the determination of lectins is the reaction of hemagglutination erythrocytes. The presence of lectin in raw material characterizes agglutination activity. Lectin activity was determined in raw material by biological testing–ratuserytro agglutination according to the method of Pogorila *et al.*, [30]. In brief, this method is based on the principle of agglutination mediated by lectins, namely the formation of aggregates by non-covalent binding of the inverse of the active center of the protein subunits of the lectin with complementary receptor on rat erythrocyte membranes.

A 0.1 ml erythrocyte suspension of packed RBC's was added into a vial dispenser with the aid of 5 ml of saline solution and shaken gently. The reaction of hemagglutination to free erythrocyte sedimentation rate (RHG) was performed at the immunological plate with U-shaped apertures. In each of the 8 holes of the vertical row, 0.05 ml of the buffered saline solution was added (contains 1 l of water, 8 g sodium chloride, 0.2 g potassium chloride and 1.0 g of disodium phosphate). The pH of the solution was adjusted to pH 7.4 using 1N HCl solution.

To avoid the unacceptable artifacts related to both inadequate removal of citrate blood plasma and RBC's from hemolysis, the sample on the spontaneous deposition of washed RBC's, i.e., in the test-system is not injected LCE, but only twice the amount of saline solution 0.1 ml and 0.05 ml of 2% suspension of rat erythrocytes was placed (single vertical row of holes). To prepare a series of successive twofold dilutions of LCE, 0.05 ml of LCE was added in the first hole of the above-mentioned vertical row, stirred, and 0.05 ml was collected, which was then transferred to the lower hole number 2, stirred and 0.05 ml was collected, and then transferred to the following lower hole number and son on till reach hole number 8, where 0.05 ml portion was removed. Then, 0.05 ml 2% of erythrocyte suspension was added to each hole and then left for 60–90 min at 25 °C. Testing lectin substances of plant extract was carried out in the threefold analytical repetition.

The intensity of the reaction of agglutination of RBC's (IRAE) was determined by the titer for individual holes. Hemagglutination titer was taken as the maximum dilution at which erythrocytes were completely agglutinated, (reflects the lectin activity and allows agglutination), and recorded as one hemagglutination unit as prescribed by Pogorila *et al.*, [30]. The higher the titer value, the higher is the lectin activity. Moreover, lectin specific activity (LSA) was expressed as a titer over mg of protein.

To prove the protein nature of agglutinating substance, the extracts were tested for the presence of protein [31, 32]. This method is based on the reaction of the dye Coomassie (Coomassie Brilliant Blue G-250) with protein to form complex blue color. The unit of lectin activity (LA), the minimum amount of lectin that causes agglutination of RBC's. The calculations were performed using the following equation [3].

$$LA = \frac{T}{C} \times V$$

Where: LA-a unit of lectin activity (U/mg protein);

T-titer;

C-protein concentration (mg/ml);

V-sample volume (0.05 ml).

Protein content determination

The protein contents of crude extract samples were determined using the Bradford assay method [31].

RESULTS AND DISCUSSION

Lectin activity from *Chamerion angustifolium* L. leaves in the flowering stage, buds, and flowers were investigated using agglutination assay. In the presence of lectin, sugars on the surface of RBC's from an interaction with the lectin resulting in agglutination.

Hemagglutination activity

It is well known that lectins are sugar-specific binding proteins and hemagglutination activity is considered as one of their key properties [22]. In the current study, lectins were isolated, identified, and quantified from *Chamerion angustifolium* L. species. To investigate hemagglutination activity, freshly collected rat RBC's were employed. Agglutination assay test was carried out using erythrocytes of 2% suspension in saline buffer, pH 7.4. The agglutination strength was estimated as the titer strength of lectin protein (table 1).

The results obtained upon analysis are summarized in table 1 showing that the estimated values of the hemagglutinative activity of the studied *Chamerion angustifolium* L. species which differ significantly among them.

Table 1: **Protein contents and lectins activity of raw materials of *Chamerion angustifolium* L. in different plant species**

Plant species	Titer[a]	Protein content (mg/ml)[b]	HA[c]	LSA (titer/mg)[d]	Lectin activity(units/mg protein)
Leaves in the flowering stage	32	4.71±0.03	1600	135.9	2.72±0.06
Buds	256	6.77±0.02	12800	756.3	0.24±0.008
Flowers	64	5.76±0.14	3200	222.2	0.56±0.014

[a]Titer is the reciprocal of the lowest dilution that was positive for lectin activity., [b]Protein content was determined using Bradford assay method., [c]Hemagglutination Activity (HA), is titer multiplied with sample volume (0.05 ml)., [d]LSA, Specific Activity is HA divided by the protein content.

The IRAE in the studied extracts from *Chamerion angustifolium* L. in different species via ratuserytroagglutination was found to correspond titers of 32, 256 and 64, for the leaves in the flowering stage, buds, and flowers, respectively. Additionally, protein contents of the extracts of *Chamerion angustifolium* L. in leaves at flowering stage, buds, and flowers were 4.71±0.03, 6.77±0.02, and 5.76±0.14 mg/ml, respectively.

There was a significant difference in lectins activity in species of the *Chamerion angustifolium* L. mainly in the leaves in the flowering stage (2.72±0.06 units/mg protein) compared to the buds and flowers where the activities were 0.24±0.008 and 0.56±0.014 units/mg protein, respectively. We conclude that lectin protein contents in dried crushed raw materials can save time and could be used over a longer period not only during the plants' growing season. The results of the current study could be taken into consideration in further studies dealing with the possibilities of using the investigated plant as sources of raw material for pharmaceutical substances.

CONCLUSION

All proteins obtained from the *Chamerion angustifolium* L. plant raw material were shown to possess rat erythrocytes agglutinating activity (table 1). The crude extract of leaves in the flowering stage exhibited the strongest hemagglutinating activity of about 2.72 units/mg proteins, whereas buds showed the lowest activity of about 0.24 units/mg proteins.

In this study, *Chamerion angustifolium* L. samples were collected during 2013-2014 and lectins activity were compared after analysis accordingly. The mean values of LSA (lectins specific activity) were 135.9, 756.3 and 222.2 (titer/mg) for leaves in the flowering stage, buds and flowers, respectively (table 1). Compared to the previous studies, this study has shown that *Chamerion angustifolium* L. lectin activity was not affected by storage.

AUTHOR'S CONTRIBUTION

Dr. Zead Helmi Mahmoud Abudayeh and Dr. Khaldun Mohammad Al Azzam contributed equally to this work.

CONFLICT OF INTERESTS

Declared none

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