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Research Article

Encapsulation of edible cuckoopint (*Arum maculatum*) tuber powder

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ABSTRACT

Cuckoopint (*Arum maculatum*), an edible wild plant species, grows in Asia, Europe, and North Africa. The aerial parts of the plant are consumed as food. The plant's tubers are used in traditional folk medicine to treat gastrointestinal disorders. When consumed fresh, the plant's tubers have a toxic effect, and the dried form is safe if it does not exceed a certain amount. Tuber powders can also have a poisonous effect when taken in excessive amounts accidentally and unconsciously. In this study, it was investigated whether it is possible to prepare the powder obtained by drying the tubers of the edible wild plant *Arum maculatum* by encapsulating it with chitosan, an edible, biocompatible, mucoadhesive polysaccharide, in specific doses. *Arum maculatum*-chitosan microcapsules were prepared with *Arum maculatum* tuber powder and chitosan. The effects of medium parameters such as pH, temperature, and ionic strength on the microcapsules' structural integrity and release properties were investigated. Encapsulation of tuber powders prepared in specific formulations into microcapsules can help prevent accidental overdose by the public. Commercial storage, transport, and marketing of cuckoopint tuber powder may be possible through encapsulation.

Araştırma Makalesi

Yenilebilir yılan pancarı (*Arum maculatum*) yumru tozu enkapsülasyonu

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ÖZ

Yenilebilir yabani bir bitki türü olan yılan pancarı (*Arum maculatum*), Asya, Avrupa ve Kuzey Afrika'da yetişir. Bitkinin toprak üstü kısımları besin olarak tüketilir. Bitkinin yumruları, geleneksel halk hekimliğinde mide-bağırsak bozukluklarını tedavi etmek için kullanılır. Taze tüketildiğinde bitkinin yumruları toksik etki gösterir ve kuru hali belli bir miktarı geçmediği takdirde güvenlidir. Yumru tozları da yanlışlıkla ve bilinçsizce aşırı miktarda alındığında toksik etki gösterebilmektedir. Bu çalışmada, yenilebilir yabani bitki *Arum maculatum* 'un yumrularının kurutulmasıyla elde edilen tozun, yenilebilir, biyouyumlu, mukoadhezif bir polisakkarit olan kitosan ile belirli dozlarda kapsüllenerek hazırlanmasının mümkün olup olmadığı araştırılmıştır. *Arum maculatum*-kitosan mikrokapsülleri, *Arum maculatum* yumru tozu ve kitosan ile hazırlandı. pH, sıcaklık ve iyonik kuvvet gibi ortam parametrelerinin mikrokapsüllerin yapısal bütünlüğü ve mikrokapsüllerin salım özellikleri üzerindeki etkileri incelenmiştir. Belirli formülasyonlarda hazırlanan yumru tozlarının mikrokapsüllere kapsüllenmesi, halk tarafından yanlışlıkla aşırı dozun alınmasını önlemeye yardımcı olabilir. Yılan pancarı yumru tozunun ticari olarak depolanması, taşınması ve pazarlanması, kapsülleme yoluyla mümkün olabilir.

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Yazarlar, bu makalede bildirilen çalışmayı etkiliyor gibi görünebilecek bilinen hiçbir rakip mali çıkarları veya kişisel ilişkileri olmadığını beyan ederler. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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1. Introduction

Türkiye has a vibrant medicinal and aromatic plant flora due to its different climatic conditions (Dogan et al., 2004). *A. maculatum* is a wild edible plant that grows naturally in mountainous areas in Türkiye (Ceylan and Akar Sahingoz, 2022).

In addition to being consumed as a local flavor in some regions of Anatolia, *A. maculatum* tubers are widely used in folk medicine to treat colitis, liver disease, or ailments in cases of excessive gastric acid secretion. The plant is also recommended for the treatment of hemorrhoids. The tuber extract of the plant has been clinically tested for its anti-inflammatory activity in the intestinal and respiratory tracts (Kozuharova et al., 2020; Ceylan and Akar Sahingoz, 2022). Some studies report that the plant also has anticholinesterase, antioxidant, and anti-aflatoxigenic activities (Kurt et al., 2018).

The genus Arum (Araceae) consists of 29 tuberous plant species. It is native to Asia, Europe, and North Africa. Herbs from the Arum species have a therapeutic effect on various ailments and are used in folk medicine (Farahmandfar et al., 2019). Archaeological evidence shows that humans have used Arum since ancient times (Azab, 2017).

A. maculatum L., a species of Arum, has been known as a medicinal plant for centuries. It has been used to treat snake bites, wounds, malaria, rheumatism, abdominal pain, hypertension, and diabetes throughout history (Kozuharova et al., 2020). This plant is also called snake pillow, nivik herb, snake ear, bristle, tirşik, Andırın piece, snake tongue, snake blade, or snake beet in Türkiye, depending on the region (Atalay and Yildiz, 2020).

This wild herb is known to irritate the skin, mouth, tongue, and throat when eaten raw, causing swelling of the throat, difficulty breathing, burning pain, and abdominal pain. However, these effects disappear if the plant is boiled or dried. However, studies have revealed its therapeutic importance as a pain reliever for kidney and liver injuries, hemorrhoids, and many diseases. *A. maculatum* extract has demonstrated antimicrobial and antifungal activities against Gram-positive and Gram-negative bacteria and fungi (Al-Shmgani et al., 2019). In a study, the total phenolic and flavonoid compound contents and fatty acid profiles of the extracts prepared from the leaves and fruits of the plant were determined, and it was reported that the extracts have antibacterial and antifungal effects (Comlekcioglu et al., 2021).

In Türkiye, as in the world, people still resort to traditional medicine to prevent and treat many diseases (Al-Shmgani et al., 2019; Comlekcioglu et al., 2021). Plants with antioxidant properties are now widely used in preventive therapy, functional and therapeutic foods in the food industry, and human nutrition (W[1]sowicz et al., 2004). The intake of such food or food products is generally considered beneficial for preventing and treating cancer and cardiovascular, inflammatory, microbial, and age-related diseases (Kurt et al., 2018).

A. maculatum leaves are widely used as a vegetable by people in some regions. The roots of *A. maculatum* are used in various treatments, such as diaphoretic and expectorant. Terpenes or terpenoids in the structure of the plant are active against bacteria, fungi, viruses, and protozoa. Capsaicin, a terpenoid component, has a wide variety of biological activities that affect the nervous, cardiovascular, and digestive systems in humans and find use as an analgesic (Safari et al., 2014).

As known, *A. maculatum* is a toxic tuberous plant that humans cannot directly consume. In addition to the above-ground parts of *A. maculatum*, its tubers are also consumed by humans as food in some places (Łuczaj and Pieroni, 2016). The lectin content of *A. maculatum* tubers is high, and studies have been conducted on lectin's isolation and biological activity in tubers (Majumder et al., 2005). In some regions of Türkiye, the plant is used in treating hemorrhoids thanks to its antifungal properties. In such treatments, the plant's tuberous roots are crushed, mixed with flour, and swallowed as tablets after processing (Uzun et al., 2004).

The fresh tuber contains volatile, bitter taste components, starch, gum, sugar, lignin, potassium, calcium, oxalate, and saponin salts. The plant's root is edible when well-dried and ground—the saponin oxalates in its fresh form cause wounds and blisters on the skin. It can be highly toxic if prepared incorrectly and should be designed with due care and attention (Usman, 2016). In addition, the binding of calcium with oxalic acids also lowers the available calcium levels in the body (Weaver et al., 1997). Calcium depletion interferes with the electrical activity of the heart, muscles, and nerves. Calcium depletion inhibits the calcium pump's action in the muscles' action potential (Akhan et al., 1995). The calcium salts formed affect the kidneys (Hesse et al., 1993). In fatal cases of ingesting oxalate-containing plants, the pathological manifestations are mainly in the kidneys, digestive tract, and brain. Postmortem findings in the kidneys can lead to small multiple hemorrhages, occlusion, swelling, tubular sclerosis, and hyaline degeneration (Sanz and Reig, 1992; Hesse et al., 1993).

In the literature, it has been reported that the tubers of *A. maculatum* cause poisoning when taken in large amounts, and no specific antidote is used in the treatment (Prakash Raju et al., 2018). The discussion so far shows that the above-ground parts and tubers of *A. maculatum* have high biological activities but are toxic. When prepared and consumed under certain conditions, their toxic effects are eliminated and utilized as a food or therapeutic agent.

Therefore, the encapsulation methods can be used to determine the amount of consumption. Alginate, pectin, carrageenan, gum arabic, chitosan, cellulose derivatives, and cyclodextrin are often used as components of the encapsulating matrix (Augustin and Hemar, 2009). Among them, chitosan (a deacetylated derivative of chitin) is the second most abundant biopolymer in the world after cellulose. It is a polycationic polysaccharide derived from chitin, composed of N-acetyl-d-glucosamine units linked by β-(1,4)-glycosidic bonds, soluble in aqueous solutions such as acetic acid and lactic acid, and its solubility depends on the degree of deacetylation and molecular weight (Chen et al., 2023).

Chitosan is a unique biological material due to its biodegradability. Its non-toxicity, biocompatibility, and good film-forming properties are highly valued by academia and industry in food applications (Gao and Wu, 2022). In addition to these properties, chitosan's excellent antibacterial and antioxidant properties make it widely used in food packaging, food preservation, food additives, and other fields (Sahraee et al., 2019).

In this study, it was investigated whether it is possible to encapsulate the powder obtained from the tubers of the edible wild plant *A. maculatum* with an edible, biocompatible, mucoadhesive polysaccharide chitosan (a cationic chitin-derived biopolymer) in specific formulations. In this study, the tuber powder of *A. maculatum* was encapsulated with chitosan as microcapsules. In the preparation of the microcapsules, the ratio of tuber powder

to chitosan, the effects of environmental parameters such as pH, temperature, and ionic strength on the structural integrity of the microcapsules, and the release properties from the microcapsules were investigated. No toxic chemicals were used in the preparation of the microspheres; chitosan, a biocompatible edible polysaccharide, was used as the encapsulating agent, acetic acid as a solvent for chitosan, and sodium hydroxide solution was used as the gelation medium to ensure the mechanical stability of the microcapsules.

2. Material and method

2.1. Materials

A. maculatum plant samples were collected in May (2023) in Hadim (Konya, Türkiye). The plant species were defined as *Arum maculatum* by the Department of Biology, Faculty of Science, Selcuk University (Konya, Türkiye). The plant tuber samples were washed, freed from the soil, peeled, cut into small pieces, and dried by laying on blotting papers at room temperature. The dried tuber samples were ground and stored in closed glass bottles at 4°C. Low molecular weight chitosan was used as the encapsulation matrix (Low molecular weight chitosan, 448869-250G, Sigma-Aldrich). Acetic acid solution (Merck) was used to dissolve chitosan, NaOH (Merck) and HCl (Merck) were used for pH adjustments, and NaCl (Merck) was used for ionic strength studies.

2.2. Method

2.2.1. Preparation of A. maculatum-chitosan microcapsules

Chitosan solutions were prepared by mixing 4.0 g of chitosan in 200 mL of acetic acid solution (2% by volume) under continuous stirring for 24 hours. 2.0 g of tuber powder was added to the chitosan solution and stirred for 2 hours to ensure homogeneity. Certain concentrations of sodium hydroxide solution (0.5, 1.0, and 1.5 M) were prepared. The mechanical stability of the microspheres was best observed in a 1.5 M NaOH solution. 1.5 M NaOH (200 mL) was prepared, and 32.0 mL of ethyl alcohol was added. The prepared chitosan-tuber powder mixture was transferred to the burette and dropped into the NaOH-ethanol solution. *A. maculatum*-chitosan microcapsules in the gelation solution were collected by filtration. The resulting microspheres were washed with distilled water until neutralization. After absorbing the water of the microspheres on the filter paper, they were taken to the glass surface and left at room temperature to dry.

Scanning electron microscope (SEM) images of the *A. maculatum*-chitosan microcapsules in the structurally stable formulation were taken, and their surface properties, dimensions, and size distributions were studied.

2.2.2. Determination of the effect of ambient conditions on microcapsules

The stability of the microcapsules at different pH (1.0-9.0), temperature (4, 20, 37°C), and ionic strength (1.0-0.5-0.1 M NaCl) were tested. pH adjustments were made with dilute NaOH and HCl solutions. Temperature studies were performed in a temperature-regulated incubator shaker (50 rpm). The effect of ionic strength was carried out in 1.0-0.5-0.1 M NaCl solutions. After each study, the microcapsules were recovered from the environment by filtration and were examined under the light microscope for possible deformations. The light microscopy images were recorded but not presented here.

2.2.3. Content release from microspheres

Dry tuber powders (200 mg) were placed in a dialysis bag (Thermo Scientific SnakeSkin Dialysis Tubing, 3,500 MWCO). Then, the release (at 50 rpm at 37°C) into an aqueous medium (500 mL of distilled water) at different pH values (pH 1.0-9.0) was followed by a UV-vis spectrophotometer. Release from dry *A. maculatum*-chitosan microcapsules (200 mg in a dialysis bag) was also carried out under the same conditions and followed.

3. Results and Discussion

3.1. Morphological features of chitosan-A. maculatum microcapsules

Scanning Electron Microscopy (SEM) images of chitosan-*A. maculatum* microcapsules are presented in Fig. 1. It was observed that the microspheres, which were close to spherical in the solution medium, retained their sphericity to some extent during the drying phase. Flattening was observed mainly in the parts where the microspheres were in contact with the ground due to their weight. In the surface images, it was determined that the tuber powders were covered with chitosan. After dissolving chitosan in a dilute acetic acid solution, its gelation in alkaline solutions makes it possible to use chitosan as an encapsulating matrix (Fu et al., 2018). Therefore, chitosan is a highly preferred biopolymer, especially for coating food products (Qu and Luo, 2020). It has been observed that chitosan is a suitable matrix for encapsulating *A. maculatum* tuber powder. The ratio of chitosan/*A. maculatum* tuber powder by mass was found to be 2/1.

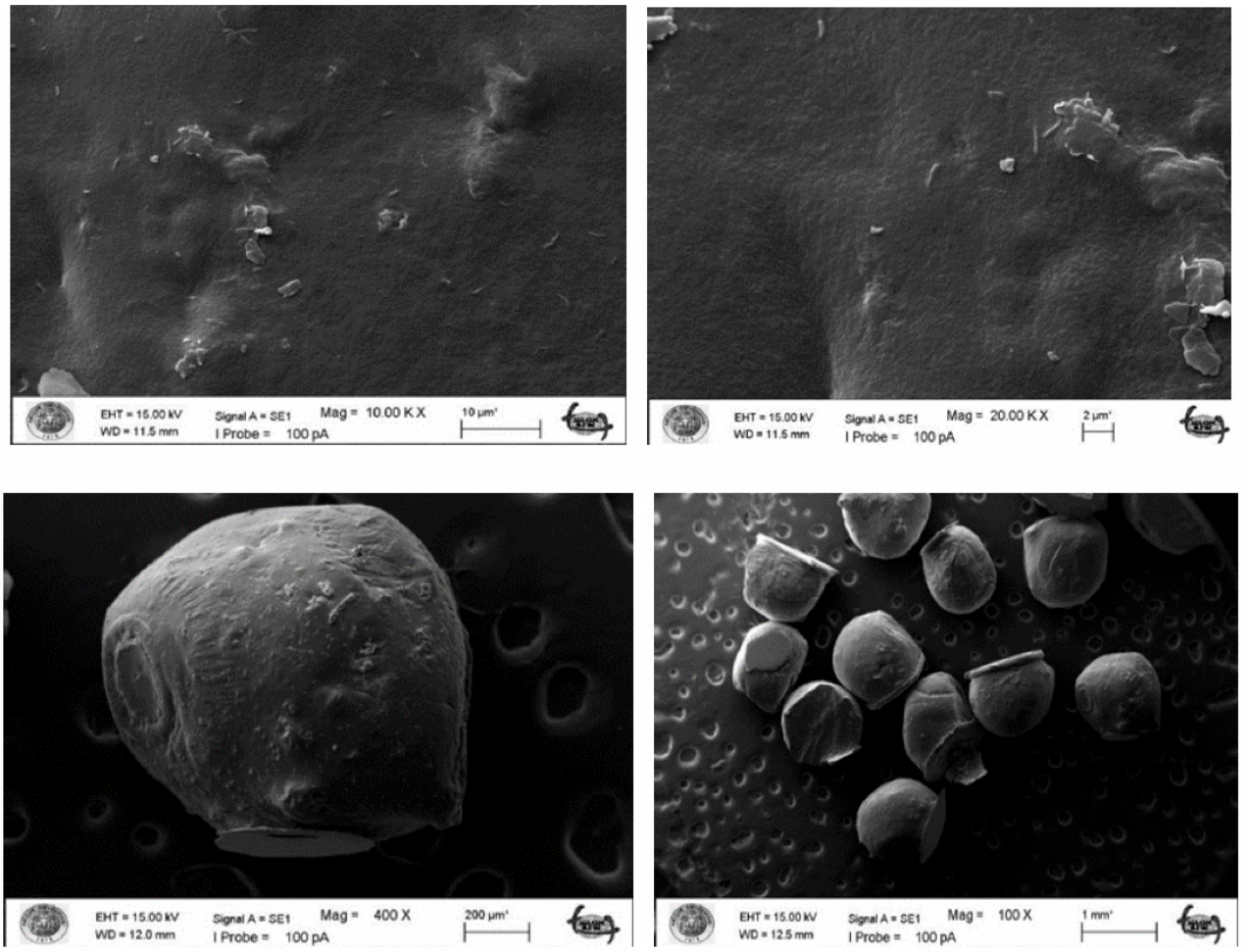

Fig. 1. Scanning Electron Microscopy (SEM) images of *A. maculatum*-chitosan microcapsules.

3.2. Effect of solutions in different pH ranges on chitosan-A. maculatum microcapsules

In the study, the effect of solutions in different pH (1.0-9.0) ranges on chitosan-*A. maculatum* microcapsules were investigated. At pH 1.0, the beginning of dissolution was observed on the surface of the microcapsule in the 5th minute, and after 35 minutes, the microspheres completely lost their shape and formed a gel. While a small amount of dissolution occurred on the surface in the 5th minute at pH 2.0, the amount of dissolution increased at the 35th minute. While there was no dissolution at the 5th minute at pH 3.0, very little dissolution occurred at the 35th minute. No change was observed in the first 5 minutes from pH 3.0 to pH 9.0. No difference was observed in the pH 4.0-9.0 range. Since a herbal food product with pharmacological activity was encapsulated in our study, no chemical crosslinking agents (e.g., glutaraldehyde) were used (Kildeeva et al., 2009). Therefore, the prepared microcapsules were formed only by physical interactions and interactions between chitosan polymer chains (Desbrières and Babak, 2010). Consequently, it was dissolved at pH values lower than 4.0 and the chitosan-*A. maculatum* microcapsules were deformed. Considering the acidity of the stomach environment (pH: close to 2), this is a desirable property in the context of chitosan-*A. maculatum* microcapsules. These observations indicated that it is possible that chitosan-*A. maculatum* microcapsules can dissolve in the gastric environment.

3.3 Effect of salt concentrations on chitosan- A. maculatum microcapsules

The effects of NaCl solutions at different concentrations (1.0-0.5-0.1 M) on chitosan-*A. maculatum* microcapsules were investigated. No changes were observed in the light microscope images of the microcapsules at different NaCl concentrations (1.0-0.5-0.1 M). These observations showed that the chitosan-*A. maculatum* microcapsules were stable in ionic solutions and did not undergo deformation. There are reports in the literature that chitosan gel structures are stable in ionic solutions (Rinaudo et al., 1993). A more recent study reported that NaCl solutions did not affect the gelling of chitosan (Tang et al., 2010).

3.4 Stability of chitosan-A. maculatum microcapsules at different temperatures

No change was observed in chitosan-*A. maculatum* microcapsules at different temperatures (4, 20, and 37°C) in an incubator shaker (50 rpm). Although the temperature has a certain effect on the gelation of chitosan (Cho et al., 2005), the structural integrity of chitosan microcapsules gelled at average room temperature in our study was not impaired in the temperature range studied. Chitosan-*A. maculatum* microcapsules could maintain their structural integrity in a refrigerator at 4°C or room temperature at 20°C. It was also observed that the microspheres maintained structural stability at a body temperature of 37°C.

3.5 Content release from chitosan-A. maculatum microcapsules

The release profiles of chitosan-*A. maculatum* microcapsules were investigated at different pH values. In the release study, chitosan-*A. maculatum* microcapsules and *A. maculatum* tuber powder were placed in dialysis bags, and their release in pure water was followed by a UV-vis spectrophotometer. It was observed that the chitosan-*A. maculatum* microcapsules and *A. maculatum* tuber powder had the highest absorption at pH 1.0. Chitosan-*A. maculatum* microcapsules showed more absorption than tuber powder. Since the solubility of chitosan increases at low pH, the high absorbance may be due to dissolved chitosan polymer fragments. Absorbance values decreased considerably at pH 2.0 and pH 3.0, and it was observed that *A. maculatum* tuber powder absorbs more than chitosan-*A. maculatum* microcapsules. They gave very close absorbance values to each other at pH 4.0. A distinct peak between 200 and 300 nm was observed. This peak was observed in the spectra in the pH 4.0-9.0 range. This indicates that the release of some highly absorbent ingredients from *A. maculatum* tuber powder increases in the pH range of 4.0-9.0. *A. maculatum* tubers have been reported to have a rich and varied chemical composition (Allen, 1995).

4. Conclusions and Recommendations

This study showed that chitosan, which is biocompatible and has excellent gelling properties, can be used to encapsulate *A. maculatum* tuber powder, which is generally used in gastrointestinal disorders in folk medicine. This work is a preliminary study. In future studies, different systems should be investigated to determine the content of *A. maculatum* tuber powder by using biopolymers such as alginate in addition to chitosan.

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