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Changes of Antioxidant Activity in Different Forms and Meal of *Arum maculatum* in Kahramanmaras Province from Turkey

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The meal of *Arum maculatum* is known in traditional therapy that has many therapeutic effects on human health. In this study, it has been aimed to study the changes in the antioxidant activity of *Arum maculatum* in different process and consumption models. Also, the antioxidant effect in varying leaf, different forms and the meal of *Arum maculatum* was investigated. Plants were collected for four forms as fresh (group 1, 2, 3 and 4) and as meal. Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) in various leaf forms and meal were measured spectrophotometrically. The activities of SOD and CAT in fresh leaves of *A. maculatum*'s from various locations and their meal were measured. In the study, the results of SOD and CAT of the fresh samples were found as follows: 17.73 ± 7.48 U/mg protein and 13.55 ± 4.30 U/mg protein for group1, 25.05 ± 12.28 U/mg protein and 21.65 ± 4.96 U/mg protein for group 2, 19.86 ± 6.78 U/mg protein and 22.07 ± 4.01 U/mg protein for group 3 and 19.57 ± 6.89 U/mg protein and 22.38 ± 3.41 U/mg protein group 4, respectively. At the same time, CAT and SOD values of the meal,were determined as 20.59 U/mg protein and 62.77 U/mg protein, respectively. SOD activity of the meal was almost three-fold higher than the leaves of *A. maculatum*. Additionally, CAT activity of the meal was similar to the leaves. The best values for CAT and SOD in the leaves were obtained at -20 °C for 30 days. As a result, the meal and leaves of *Arum maculatum* could be possessed of antioxidant activity and beneficial effects on human health and nutrition.

Key Words: *Arum maculatum*, Antioxidant activity, Catalase, Superoxide dismutase.

INTRODUCTION

Turkey is a fairly rich country in respect to potential of endemic plants. *Arum maculatum*, among these, is a common woodland plant species of the Araceae family. It is widespread across temperate southern Turkey and is known common names including Wild arum, Lords and Ladies, Jack in the Pulpit, Devils and Angels, Cows and Bulls, Cuckoo-Pint, Adam and Eve, Bobbins, Naked Boys, Starch-Root and Wake Robin. The plant has used in traditional therapy of the diseases including the rheumatic pain, abscesses, convulsions, plague sores, gout, fever[1] for a long time and has many therapeutic effects on human health. Additionally, it has pro-inflammatory effect, antimicrobial and antifungal activities[2].

According to beliefs of some local inhabitants, most of these plants has functional properties in point of human health. Therefore, it is utilized by local people from seeds, oils, leaves, fruits and flowers of these plants for different purpose for long years. In previous years, specially, it has been extensively made scientific researches on some plants well known and being famous in point of functional properties among these. Thus, it has been tried to give an attribution of academic and scientific instead of these rumors. Nowadays, pharmaceutical and food industries are within attempting to attribute industrial property for a lot of products from this scope.

As known, *Arum maculatum* is a beet with poison and it can not consume by human directly. Also, this beet is fundamental compound of tirsik (*A. maculatum*) meal that known and famous in the Kahramanmaras area, Turkiye, in Andirin area (county of Kahramanmaras) specially as well. The meal of *Arum maculatum* and its leaves are commonly consumed in this region. It is also extensively believed that the meal has functional properties which provides benefits in point of human health. When tirsik's meal "named as the doctor of Andirin" is done, primarily finely chopped beet is mixed with some food components such as "yarma" (split wheat) and yoghurt and this is fermented 8-10 h as covered by flour surface of the mix (for providing an aerobic medium), then this mixture is goodish cooked. This fermentation is a synergy fermentation that lactic acid bacteria from yoghurt and fermentative yeasts from flour work together. As a result of this fermentation, the mixture possesses a plesantly acid and tart taste and some metabolites that facilitate digestion (*e.g.*, lactic acid) occur. These metabolites have some functional properties in point of human health and nutrition.

The second fundamental compound of this meal is yoghurt. Hence, biological value of this meal increases depending on animal protein taking part in the composition of yoghurt. Also, some vitamins and minerals taking part in the composition of this beet provide much more substantiality for human nutrition besides of its fibrous structure useful for human metabolism. In cases of addition of split wheat, content of B group vitamin increases and antinutritional factors decreases as a resulting of this fermentation. All these have showed that this food is seen a regional functional food.

All substances that protect foods against autoxidation should be called inhibitors of oxidation and only substances that inhibit oxidation by reaction with free radicals should be called antioxidants. The preventive inhibitors acting in the first defence line suppress the formation of free radicals and active oxygen species and the radical scavenging antioxidants are responsible in the second defence line and inhibit chain initiation and/or break the chain propagation[3-5]. Antioxidants such as catalase (CAT) and superoxide dismutase (SOD) protect the cells from damage caused by reactive oxygen species (ROS). *In vitro* and *in vivo* studies have shown that antioxidants help prevent the ROS damage that is associated with cancer and heart disease. Antioxidants can be found in most fruits and vegetables but also culinary herbs and medicinal herbs can contain high levels of antioxidants. Antioxidants are used in a

wide variety of food products and their activity may vary depending on the temperature, food composition, food structure and availability of oxygen. Temperatures at which antioxidant activity may be required range from 180-200 °C for frying oils, to about 5 °C for products such as margarine or mayonnaise that are stored in the fridge. Besides the processing and storage temperatures to which these products are exposed, the accompanying constituents including water, proteins, carbohydrates, vitamins, minerals and other food components vary and the physical structure of the food also varies. This can cause big changes in the activity of the antioxidant in different food systems[6-8].

We aimed to investigate the antioxidant activity of *A. maculatum* from various locations and their meal. The antioxidant activity of *Arum maculatum* has been reported here for the first time. Also, we investigated antioxidant enzyme activities such as CAT and SOD in leaves of *A. maculatum* for the best stored at +4 and -20 for 30 days.

EXPERIMENTAL

In the study, for determining of antioxidant activity of *Arum maculatum* plant and its meal, samples were collected from leaves (*Arum maculatum*) and their meal was prepared in the region. These leaves are categorized to four groups as fresh and stored at +4 and -20 °C for 30 days. Extracts were prepared from these leaves and meal was homogenized with 1.5 % KCl[1]. All chemicals used in antioxidant enzyme assays were analytical grade and were from the Sigma Chemical Company (St. Louis, MO, USA).

Preparation of meal from the leaves of *A. maculatum*: In making of this meal, firstly, the most fundamental compound, *A. maculatum* was finely chopped and mixed with yoghurt and then, the fermented mixture was applied to fermentation (for 10 h), then, this mixture was boiled until certain time (3 h). With this processing of fermentation and boling, poison effect of the beet plant was eleminated. Then, it is serviced for human consumption. The meal compounds were consist of "*A. maculatum*" plant, yoghurt (in sometimes, in manufacturing of this meal, it is added some slit wheat with yoghurt) and flour (for covering mix surface during fermentation).

Preparation of homogenates from the leaves of *A. maculatum*: The leaves of *A. maculatum* were homogenised with 3 volumes of ice-cold 1.15% KCI. The activities of antioxidant enzymes such as CAT and SOD were measured in the supernatant obtained from centrifugation at 14.000 rpm.

Biochemical analysis in leaves and meal extractions: Superoxide dismutase activity was measured in the extracts according to the method described by Fridovich[9]. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with *p*-iodonitrotetrazlium violet (INT) to form a red formazan dye which was measured at 505 nm. Assay medium consisted of the 0.01 M phosphate buffer, CAPS (3-cyclohexylamino-1-propanesulfonicacid) buffer solution (50 mM CAPS, 0.94 mM EDTA, saturated NaOH) with pH 10.2, solution

of substrate (0.05 mM xanthine, 0.025 mM INT) and 80 UL xanthine oxidase. SOD activity was expressed as U/mg protein.

Catalase activity was determined by measuring the decrease in hydrogen peroxide concentration at 230 nm by the method of Beutler[2]. Assay medium consisted of 1 M Tris HCI, 5 mM Na_2EDTA buffer solution (pH 8.0), 10 mM H_2O_2 and tissue sample in a final volume of 1.0 mL. Catalase activity was expressed as U/mg protein. The protein concentration of the extracts was measured with Spectronic-UV 120 spectrophotometer by the method of Lowry[10].

Statistical analysis: The SPSS programme (Version 9.05) was used for student t test. Results were expressed as the means ± standard deviation (SD). The difference was considered significant when the probability was less than 0.05.

RESULTS AND DISCUSSION

Proclaimed benefits and announced argues without a scientific basis must be supported with scientific findings that will be obtained from experimental animals. This study showed that the meal of *Arum maculatum* and its leaves have antioxidant activities and they are exposed to changes in during storage.

As shown in Table-1, it was observed that CAT activity in group 1 was significantly lower than those found in leaves extracts ($p < 0.05$). On the other hand, SOD activity in group 2 was significantly higher compared to the others ($p < 0.05$). There are no significant differences in CAT and SOD activities of leaves between group 3 and 4 ($p > 0.05$). Figs. 1 and 2 showed the best stored for CAT and SOD activities in leaves of *A. maculatum* were found -20 °C. Also, CAT and SOD activities in the meal of *A. maculatum* were found as 20.59 U/mg protein and 62,77 U/mg protein, respectively. SOD activity in the meal was found almost 3-fold higher than the leaves of *A. maculatum*. However, CAT activity in the meal is similar to leaves of *A. maculatum*.

TABLE-1
CAT AND SOD ACTIVITIES IN VARIOUS FORM LEAVES OF *Arum maculatum*

The forms of *A. maculatum*	SOD Activity (U/mg protein)	CAT activity (U/mg protein)
Group1	17.73 ± 7.48	13.55 ± 4.30*
Group 2	25.05 ± 12.28**	21.65 ± 4.96
Group 3	19.86 ± 6.78	22.07 ± 4.01
Group 4	19.57 ± 6.89	22.38 ± 3.41

*There is a significant difference in CAT activity between group 1 and the other groups ($p < 0.05$). **There is a significant difference in SOD between group 2 and the other groups ($p < 0.05$).

In this study, it has been observed that revealed differences in point of antioxidant activity are significant and these differences may be change according as varieties in climatic conditions, ecological situation and soil properties, especially. Besides, this study is important in point of above mentioned themes in addition to being an original study in related field.

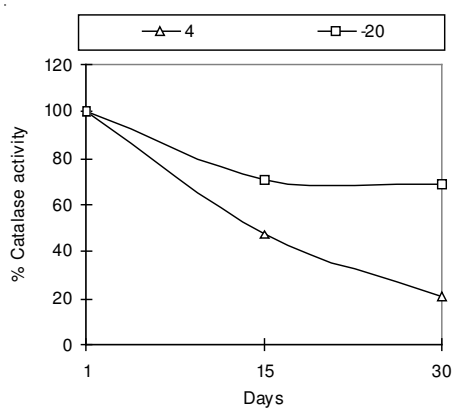

Fig. 1. Changes according to the days in CAT activity of *Arum maculatum*

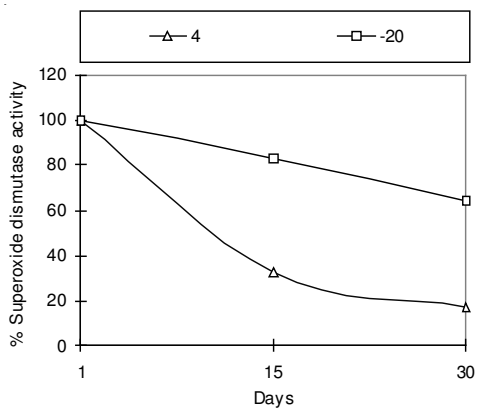

Fig. 2. Changes according to the days in SOD activity of *Arum maculatum*

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