

Scaling up the Phytochemical Contents in the Leaves of Beetroot Using a Formulated Antioxidant Gawda®

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ABSTRACT

Beetroot (*Beta vulgaris* L.) have been used for many years as a food supplement to support treating a number of diseases. It is also classified as medicinal plant because of its excellent source of phytochemicals including antioxidants, minerals, vitamins and amino acids. Beetroot are vegetable belong to the family Chenopodiaceae. In food industry they are formulated in edible powder and energetic juice. In some regions of the world beetroot leaves are cut off and discard in spite of their health benefit because of lack of knowledge among the local people regarding their health benefit. So far, this investigation aimed to evaluate the possible role of the formulated antioxidant GAWDA® on enhancing the phytochemicals of the content of beetroot leaves. P.P, Proline, Potassium and the antioxidant activity were determined. Beetroot seeds were soaked in water solution of GAWDA® formulation (2g/L for 12 hours) pre sowing vs tap water in case of the check. The target physiological characters were recorded in leaves of 3 month old plants. The results showed that soaking seeds in water solution of GAWDA® formulation (2g/L for 12 h) before sowing significantly increased the contents of Ch. A, B, carotenoids, total phenolic compounds, proline, potassium and antioxidant activity in the plant leaves. The potential of the formulated antioxidant (GAWDA®) for scaling up the contents of the phytochemicals studied beetroot leaves was concluded.

Keywords: Beetroot (table beet), formulated antioxidant GAWDA®, p.p phenols, proline, potassium, carotene.

INTRODUCTION

Since several decades the use of food supplements has increased day after another mainly in forms of crude extracts¹. Many leafy vegetables contain ascorbic acid, beta-carotene, minerals, phenols, fibers and others Negi and Roy (2001), which increases the immunity against several diseases including cardiovascular diseases, cataracts, and brain and immune dysfunction Vinson and Hontz (1995). Beetroot (*Beta vulgaris*) which is a member of the family Chenopodiaceae, consists a good number of important compounds which support the body against many diseases. This plant is commonly used in the Mediterranean basin since 1000 B.C. Vali *et al.* (2007). A number of studies are handling their biological activities including the potent antioxidants and free radical scavengers Marja *et al.* (1999). In addition, to their chelation potential Ramarathnam *et al.* (1997); Kuo *et al.* (1998). Both, roots and leaves of beetroot consist high level of phenolic compounds Marja *et al.* (1999). They are used in some areas of the world as a remedy for kidney and liver diseases Kanner and Granit (2001). As beetroot leaves are highly and cheap source of nutrients. The present study aimed to evaluate the role of formulated antioxidant GAWDA® on enhancing the constituent of the phytochemical compounds in the leaves of beetroot plant.

MATERIALS AND METHODS

This investigation was carried out at the laboratories and experimental field plots of Plant Pathology Department, Mansoura University during growing season of 2016-2017. Beetroot seeds cv. Detroit superene Hybrid used in this study was obtained from Bakker Brothers Co., Holland.

Experimental design

One Way Completely Randomized design with 6 replicates was followed.

Formulated Antioxidant used

GAWDA® formulation, Patent No. 23798 (consists of calcium borate 5 mM + calcium chloride 6 mM+ magnesium chloride 5 mM + tartaric acid 2 mM +

hydroxyquinoline 1 mM) was used in this investigation. Before sowing, beetroot seeds were soaked in water solution of GAWDA® formulation at a rate of (2g/L). Tap water was used as a control. Soaking period was 12h. Leaves from three month old plants were harvested to assess the concentrations of the target constituents.

Chemical assay:

1- Photosynthetic pigments

The third upper leaf of the beetroot plants was used for chl. a, b as well as carotenoids were investigated, while ten mL methanol (90%) was determination according to Mackinney (1941): methanol was used for extraction.

2- Total phenols

The content of the total phenols in the lower leaves of the plants was determined using Foline-ciocalteau reagent Singleton and Rossi (1965)

Results were calculated as mg catechol/100 g fresh weight (fw).

3- Proline content

Proline content was also determined in the lower leaves following the method of Bates *et al.* (1973).

4- Antioxidant activity

The assessments of the antioxidants were measured in the forms of radical scavenging ability to use stable free radicals (diphenylpicrylhydrazyl) (DPPH) radical Fradovich (1986); Daivies (2000) according to the method of (Teraos *et al.* (1988).

5- Total potassium

The concentration of total potassium content (mg/100g dry weight) were determined in leaves using the method of Chapman and Pratt (1961; 1982).

Statistical analysis

Data was statistically analyzed using COSTAT (2005) software Gomez and Gomez (1984). The means were compared using the least Significant Difference (LSD) of the means and compared at $p = 0.05$ as outlined by Duncan(1955). While the means followed by different letter(s) in the all columns of the tables show significantly different according to Duncan's multiple range test at $p = 0.05$.

RESULTS AND DISCUSSION

Results

Effect of the formulated antioxidant (GAWDA®) on the photosynthetic pigments:

Table(1) shows that soaking the seeds in the water solution of GAWDA® formulation at 2 g/L before sowing significantly increased the content of total chlorophylls as they recorded (2.741 mg/g F.W), ch A (1.828 mg/g fw), ch B (0.913 mg/g F.W) and carotenoid (0.306 mg/g F.W). In the check, total chlorophyll recorded (2.415 mg/g F.W), ch A (1.700 mg/g F.W), ch B (0.715 mg/g F.W), while carotenoid recorded 0.133 mg/g F.W.

Table 1. Impact of the antioxidant GAWDA® formulation on the concentration of photosynthetic pigments in the 3rd upper leaf of beetroot plants.

Treatment	Ch A mg/g Fresh weight	Ch B mg/g Fresh weight	Total chlorophyll mg/g Fresh weight	Carotenoid mg/g Fresh weight
GAWDA® formulation	1.828 a*	0.913 a	2.741 a	0.306 a
Check	1.700 b	0.715 b	2.415 b	0.133 b

Effect of GAWDA® formulation on total phenols in the leaves:

Results revealed in Table (2) show that soaked seeds in the water solution of GAWDA® formulation at 2 g/L significantly increased the content of total phenols in the plant leaves. It recorded 244.97 mg catechol/100g fw. Whereas 183.96 mg catechol/100g fw in the check.

Table 2. Impact of GAWDA® formulation on the total phenols content in the leaves of beetroot plant.

Treatment	Total phenol mg catechol/ 100 g fresh weight
GAWDA® formulation	244.97 a*
Check	183.96 b

Impact of GAWDA® formulation on proline:

Results revealed that soaking seeds in the water solution of GAWDA® formulation increased the content of total proline in the plant leaves (Table3). It recorded .193 0µM/g fw and 0.167µM/g fw in the treated and untreated plant, respectively.

Table 3. Impact of GAWDA® formulation on proline content in leaves of beetroot plants

Treatment	Proline (µM/g ⁻¹ tissue)
GAWDA® formulation	0.193 a*
Check	0.167 b

Impact of GAWDA® formulation on antioxidants activity (DPPH):

Table 4 shows that DPPH % activity was significantly increased to record 65.62 %. While in the check it recorded 25.24 %.

Table 4. Effect of GAWDA® formulation on antioxidant activity (DPPH) in the leaves of beetroot plants.

Treatment	DPPH radical Scavenging activity (%)
GAWDA® formulation	65.62 a*
Check	25.24 b

Impact of GAWDA® on Potassium content:

The content of potassium also presented a highly significant increases to reached 1672.54 mg/100g while, the check recorded 312.41 mg/100g (Table 5).

Table 5. Impact of antioxidant (GAWDA® formulation) on Potassium content level in the leaves of beetroot plants.

Treatment	Potassium content mg/100g(dry weight)
GAWDA® formulation	1672.54 a*
Check	312.41 b

Discussion

Results revealed that soaking seeds in GAWDA® formulation at 2g/L before sowing increased in the content of photosynthetic pigments, total phenols, proline, antioxidant activity (DPPH) and potassium in the beetroot leaves. Amtmann *et al* (2006) indicated that potassium is a vital element in the life of human and has a role in the metabolism, enzyme activity and polarization of cell membran. It has also a profitability role in the physiological processes in plants i.e.: photosynthesis, photorespiration and its growth. Rai *et al.* (2002); Marschner (2012) This riches might attributed to the antioxidant efficiency as assimilator for enhancing N uptake, regulation of cellular pH, activating phyto-hormone and enzymatic systems. This research indicated that using antioxidants as a seed treatment for sure enhance the contents of phenolic compounds and increase the level of potassium in the vegetative parts of the plants. Moreover, it's a promising tactic for scaling up the useful nutrients in beetroot plants.

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زيادة محتويات المواد الكيميائية النباتية في أوراق الشمندر باستخدام مركب GAWDA® المضاد للأكسدة
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إستخدام الشمندر لسنوات عديدة كمكمل غذائي لعدم علاج عدد من الأمراض. كما يتم تصنيفه كنبات طبي نتيجة محتواة الممتاز من المواد الكيميائية النباتية بما في ذلك مضادات الأكسدة والمعادن والفيتامينات والأحماض الأمينية. ينتمي الشمندر إلى العائلة الرمامية . يتم استخدام الشمندر في صناعة المواد الغذائية حيث يتم وضعه في مسحوق الطعام والعصائر الحيوية. وفي بعض مناطق العالم ، يتم قطع أوراق الشمندر وتجاهلها على الرغم من فوائدها الصحية بسبب نقص المعرفة بين السكان المحليين بما يتعلق بفائدتها الصحية. يهدف هذا البحث إلى تقييم الدور المحتمل لمركب GAWDA® المضاد للأكسدة في تعزيز محتوى المواد الكيميائية النباتية في أوراق الشمندر. حيث تم تقدير صبغات البناء الضوئي ، البرولين ، البوتاسيوم ونشاط مضادات الأكسدة. تم نقع بذور الشمندر في محلول مائي من مركب GAWDA® بمعدل 2 جم / لتر لمدة 12 ساعة قبل الزراعة مع إستخدام ماء الصنبور في حالة معاملة المقارنة. تم تقدير الصفات الفسيولوجية المستهدفة في أوراق نباتات عمرها ثلاثة أشهر. وأظهرت النتائج أن نقع البذور في محلول مائي من مركب GAWDA® بمعدل 2 جم / لتر لمدة 12 ساعة قبل الزراعة زاد بشكل كبير من محتويات صبغات البناء الضوئي، محتوى الفينولات الكلاية، البرولين ، البوتاسيوم ونشاط مضادات الأكسدة في أوراق النبات. توصى الدراسة بإمكانية إستخدام مركب GAWDA® المضاد للأكسدة لزيادة محتويات المواد الكيميائية النباتية في أوراق الشمندر.