Dried (*Zygophyllum coccineum* L) powder as an additive agent to some bakery products and its effect on blood sugar

مسحق نبات الرطريط المجفف (*Zygophyllum coccineum* L) كمادة مضافة الى بعض المخبوزات وتأثيره على سكر الدم

BY

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Abstract

Returning to nature is a crucial aspect of sustainable development, aimed at reducing the food gap and related diseases. Diabetes is a chronic disease that poses significant risks, and patients may need to avoid certain foods to prevent complications. One of these foods is bakeries which raise blood sugar levels, which causes serious health problems for patients with both types of diabetes. The goal of this study is to enhance the production of bran bread and rusks by incorporating *Z. coccineum* powder, which is known for its ability to lower blood sugar levels. In order to investigate the potential of *Z. coccineum* as an anti-diabetic agent, an *in vitro* assay was conducted to test its ability to inhibit α-amylase activity by spectrophotometric assay was employed with acarbose as the control, testing various concentrations 50, 100, and 200 mg/ml. The results showed a clear correlation between the concentration of *Z. coccineum* powder extract and the inhibition value of α-amylase, with the highest inhibition percentage of 76.90% achieved at a concentration of 200 mg/ml. Chemical composition and HPLC determination for polyphenolic compounds were done. For each of the bran bread and rusks, three treatments were made with the previous *Z. coccineum* powder extract concentrations, and then sensory evaluation was conducted. All treatments for both bakery products gained general acceptability. In conclusion, the study suggests that *Z. coccineum* may be used as a potential natural anti-diabetic agent in functional food industries.

المستخلص:

تعد العودة إلى الطبيعة جانبًا حاسمًا ومهما من جوانب التنمية المستدامة، التي تهدف إلى تقليص الفجوة الغذائية والأمراض المرتبطة بها. داء السكري هو

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مرض مزمن يشكل مخاطر كبيرة، وقد يحتاج المرضى إلى تجنب بعض الأطعمة لمنع المضاعفات المرتبطة به. ومن هذه الأطعمة المخبوزات بأنواعها المختلفة، والتي ترفع نسبة السكر في الدم، مما يسبب مشاكل صحية خطيرة لمرضى السكري بأنواعه. الهدف من هذه الدراسة هو تعزيز إنتاج خبز النخالة والبقسماط من خلال دمج مسحوق نبات الرطريط (*Z. coccineum*) المعروف بقدرته على خفض مستويات السكر في الدم. من أجل التحقق من إمكانات *Z. coccineum* كعامل مضاد لمرض السكري، تم إجراء اختبار قدرته على تثبيط نشاط أنزيم الألفا أميليز (α-amylase) عن طريق الفحص الطيفي الذي تم استخدامه مع الأسكربوز acarbose كعنصر تحكم. تم اختبار تركيزات مختلفة ٥٠، ١٠٠ و ٢٠٠ ملغم/مل من مستخلص مسحوق الرطريط، وأظهرت النتائج وجود علاقة واضحة بين تركيز مستخلص مسحوق *Z. coccineum* وقيمة التثبيط لإنزيم α-amylase، حيث سجلت أعلى نسبة تثبيط بلغت ٧٦,٩٠% عند التركيز ٢٠٠ ملغم/مل. تم إجراء التحليل الكروماتجرافي عالي الدقة HPLC لتحديد المركبات البوليفينولية في النبات. بالنسبة لكل من خبز النخالة والبقسماط، تم إجراء ثلاث معاملات مع التراكيزات السابقة من مستخلص مسحوق *Z. coccineum* ، ومن ثم تم إجراء التقييم الحسي. اكتسبت جميع المعاملات للمخبوزات محل الدراسة قبولًا عامًا. في الختام، تشير الدراسة إلى أنه يمكن استخدام *Z. coccineum* كعامل طبيعي محتمل مضاد لمرض السكري في تصنيعالأغذية الوظيفية.

1. Introduction

Zygophyllum coccineum L. is part of the *Zygophyllaceae* family, which contains approximately 285 species belonging to 27 genera. These plants are commonly found in desert areas. Folk medicine frequently uses *Zygophyllum* species for their antidote properties against a variety of illnesses, including diabetes, spasms, eczema, diarrhea, histamine, hyperlipidemia, and infections. Many of these diseases can be counteracted by the components found in *Z. coccineum* (Amin *et al.*, 2010).

The aim of this study is to evaluate the effect of *Z. coccineum* on diabetes disease through inhibiting α-amylase. Diabetes mellitus (DM), is a metabolic disorder characterized by elevated levels of blood sugar. It includes a wide range of conditions that affect the body's ability to process glucose efficiently. DM is classified into different types, including type

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1, type 2, gestational diabetes, diabetes in newborns, and secondary causes resulting from endocrine disorders or the use of cortisol medications (Alam *et al.*, 2014). Alpha (α)-amylase an enzyme that digests polysaccharides by breaking down molecules into smaller molecules such as glucose and maltose. Furthermore, the enzyme causes postprandial hyperglycemia or raising blood sugar levels. α-amylase is a familiar therapeutic target for the treatment and maintenance of postprandial hyperglycemia. Various enzyme inhibitors, such as acarbose, and others have been found to be effective in blocking this enzyme, encouraging researchers to interest in developing powerful inhibitory molecules for α-amylase (Kaur *et al.*, 2021). Hence the trend of the current study is to use plants like *Z. coccineum* that may have the ability to inhibit this enzyme.

The phytochemical composition of *Zygophyllum* species has been extensively studied and found to contain triterpenes, flavonoids, saponins, sterols, phenolic compounds, and esters. These compounds demonstrate remarkable potential in the treatment of diabetes (Shawky *et al.*, 2019). These effects make it a suitable ingredient that can be included in functional foods for diabetes conditions.

2. Material and Methods

2.1. Materials

Z. coccineum L. plant was provided from the stations of the Desert Research Center DRC, Egypt. An *in vivo* study was done in the Global lab in Egypt. Proximate analysis and HPLC polyphenolic determination were done in the Regional Center of Mycology and Biotechnology, Al-Azhar Uni., Egypt. Basic ingredients for bran bread and rusks were purchased from the local central markets in Cairo, Egypt.

2. Methods

2.1. Preparation of *Z. coccineum* powder

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The plant parts were washed and all undesirable materials such as dust and insects were removed, then dried in a hot air oven at 60 ^{o}C till they completely dried, followed by grinding to obtain the powder. To prepare *Z. coccineum* powder extract, 70% ethanol was used.

2.2. Determination of proximate compositions of *Z. coccineum* powder

It was carried out by AOAC (2000) for the determination of contents of moisture, protein, ash, crude fiber, and total carbohydrates. The fat content of the sample was determined by Pearson (1976).

2.3. HPLC determination of polyphenols contents of *Z. coccineum* powder

HPLC analysis for quantification of phenolic acids extraction was characterized by the method of Goupy *et al.*, (1999) and flavonoids were carried out Flavonoids were determined by HPLC according to the method of Pirjo *et al.*, (2000).

2.4. Effect of *Z. coccineum* powder ethanolic extract on α-Amylase Inhibition Activity

To study the effect of *Z. coccineum* powder ethanolic extract on α-amylase inhibition activity, the in vitro study was carried out based on the spectrophotometric assay using acarbose as the positive control (Gella *et al.*, 1997). Concentrations of 50, 100, and 200 mg/ml were prepared by dissolving *Z. coccineum* powder ethanolic extract sample in DMSO. The enzyme α-amylase solution was prepared by mixing 3.246 mg of α-amylase in 100 mL of 40 mM phosphate buffer, pH 7. Then three concentrations of acarbose (25, 50, and 100 mg/ml) were added to 30 µL of α-amylase enzyme and incubated at 37°C for 10 minutes, and then 120 µL of ethylidene-pNP-G7 (*E-pNP-G7*) as the substrate was added, mixed and incubated at 37°C for 8 minutes. The absorbance was measured at 405 nm and the

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control reaction was carried out without the sample. Percentage Inhibition was calculated by the following expression:

PI= [(Absorbance Control–Absorbance Test)/Absorbance Control] ×100

2.5. Preparation of *Z. coccineum* powder bran bread (ZBB) and *Z. coccineum* powder rusks (ZR)

Three treatments of bran bread with the *Z. coccineum* powder were prepared according to the procedure described by the AACC Method (American Association of Cereal Chemists, 2000), using the following formula with some adjustments as described in Table (1) for bran bread, and Table (2) for rusks:

Table (1). Bran read formulas prepared with *Z. coccineum* powder (100 g for one loaf)

Ingredient	Treatments			
	Control	50 mg/ml of *Z. coccineum* powder	100 mg/ml of *Z. coccineum* powder	200 mg/ml of *Z. coccineum* powder
Wheat flour + *Z. coccineum* powder (g)	60 + 0	55 + 5	50 +10	40 +20
Bran (g)	20	20	20	20
Sugar (g)	5	5	5	5
Corn oil (g)	10	10	10	10
Table salt (g)	2.5	2.5	2.5	2.5
Dry yeast (g)	2.5	2.5	2.5	2.5
Water (ml)	As needed	As needed	As needed	As needed

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Table (2). Bran read formulas prepared with *Z. coccineum* powder (100 g of rusk dough)

Ingredient	Treatments			
	Control	50 mg/ml of *Z. coccineum* powder	100 mg/ml of *Z. coccineum* powder	200 mg/ml of *Z. coccineum* powder
Wheat flour + *Z. coccineum* powder (g)	60 + 0	55 + 5	50 +10	40 +20
Oat bran (g)	20	20	20	20
Sugar (g)	10	10	10	10
Corn oil (g)	15	15	15	15
Table salt (g)	2.5	2.5	2.5	2.5
Dry yeast (g)	2.5	2.5	2.5	2.5
Water (ml)	As needed	As needed	As needed	As needed

The mixer is used to mix ingredients to prepare bran bread and rusk dough. Next, the dough is shaped, cut, and baked at 225°C. The bread is then cooled. For the rusk crumbs, after cooling, are cut into slices again, and the final baking is done (150°C/15 minutes) to obtain the crispy crumbs.

2.6. Sensory evaluations of *Z. coccineum* powder bakeries

Organoleptic evaluations of *Z. coccineum* powder samples were performed by ten panelists. Color, odor, taste, texture, and overall accessibility were among the characteristics tested. All sensory items were scored out of ten.

2.6. Statistical analysis

Results were analyzed by (ANOVA) using the SAS (1999) statistical package of the general linear model (GLM). The results average was based on three replicates ($p \leq 0.05$) (SAS Statistical Analysis System, 1999).

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3. Results and Discussion

According to the findings, *Z. coccineum* L. had a high moisture content (34.72%), and the crude fiber content of the *Z. coccineum* L sample was (5.78%), implying a strong correlation between moisture content and fiber, which could be of interest to human health because fibers are important for digestion processes, as reported by Hussain et al., (2009). The ash value in *Z. coccineum* L was found to be relatively high (14.89%); determining ash contents is important because mineral contents may be the cause of a pharmacological effect (Lee, 2005).

The sample of *Z. coccineum* L contained 6.94% protein. The protein content of *Z. coccineum* L samples was low. The fat content of *Z. coccineum* L was found to be (1.74%), which is relatively low. Fat is an excellent source of energy, helps in the transfer of fat-soluble vitamins, insulates and protects internal tissues, and participates in important cell processes (Pamela *et al*., 2005).

Carbohydrates are an essential component of many foods, and digestible carbohydrates are regarded as an important source of energy. As shown in Table (3), the percentage of total carbohydrate composition in the *Z. coccineum* L sample was relatively high (35.93%) calculated by difference.

Table (3). Proximate compositions of *Z. coccineum* L powder

Components	(%)
Moisture	34.72
Ash	14.89
Crude protein	6.94
Fat	1.74
Crude fibers	5.78
Total carbohydrates	35.93

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Phenolic acid and flavonoid compounds were detected according to their retention time and their peaks compared with authentic use. HPLC analysis showed a higher content of bioactive compounds in *Z. coccineum* L powder ethanolic extract (70% conc.). HPLC was used to identify flavonoids in the *Z. coccineum* L powder ethanolic extract according to the authentic used. The results presented in Figure (1. a) showed that Naringenin 9.48 µg/g, Rutin 11.12 µg/g, Quercetin 6.78 µg/g, luteolin 10.04 µg/g, and Apigenin 6.55 µg/g. Phenolic compounds namely Chlorogenic acid 2.11 µg/g, Caffeic acid 5.33 µg/g, Syringic acid 8.06 µg/g, Gallic acid 7.98 µg/g, Ellagic acid 6.54 µg/g, and Cinnamic acid 3.26 µg/g as represented in figure (1. b).

The best polyphenol compound that manages blood sugar levels is Quercetin, a flavonoid that has received the most research attention for its *in vivo* and *in vitro* anti-diabetic models, followed by, luteolin, and naringenin. Also, phenolic acids including Gallic acid, Ellagic acid, Caffeic acid, and Chlorogenic acid have positive direct effects on blood sugar levels. This result was in agreement with Naz *et al.,* (2023). Polyphenols have been shown in animal models and a small number of human studies to reduce hyperglycemia and improve acute insulin secretion and insulin sensitivity. Reduced glucose absorption in the intestine, inhibition of carbohydrate digestion, activation of insulin production, control of glucose release from the liver, reactivation of insulin receptors and absorption of glucose in insulin-sensitive tissues, control of intracellular signaling pathways, and gene expression are some of the potential mechanisms (Aryaeian *et al.,* 2017). This result encourages us to utilize *Z. coccineum* L powder as a supplement for diabetes mellitus cases.

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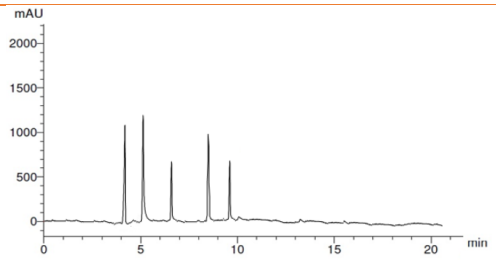
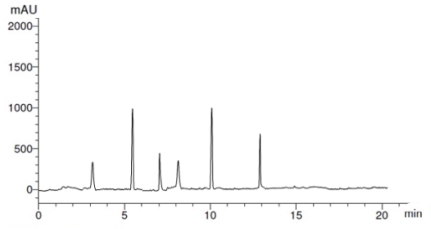


RT#	Compound	Concentration µg/gm
4.0	Naringin	9.84
5.2	Rutin	11.12
6.8	Quersestin	6.78
8.8	Luteolin	10.04
9.9	Apegenin	6.55

Figure 1.a. HPLC analysis for determination of flavonoids content in *Z. coccineum* powder extract.



RT#	Compound	Concentration µg/gm
3.0	Chlorogenic	2.11
5.2	Syringic	8.06
7.0	Cinnamic	3.26
8.0	Caffeic	5.33
10.0	Gallic	7.98
13.0	Ellagic	6.54

Figure 1.b HPLC analysis for determination of phenolic acids content in Z. coccineum powder extract

Alpha-amylase is an enzyme that breaks down carbohydrate molecules to produce a variety of products such as small polymers composed of glucose units that cause elevated glucose levels and the development of type II diabetes (Güder *et al.*, 2015). The ability of an ethanolic extract of *Z. coccineum* L powder to inhibit the activity of α-amylase, a carbohydrate digestive enzyme secreted by the saliva glands and pancreas, was tested *in vitro*.

Numerous synthetic drugs are used as potent α-amylase inhibitors; however, natural α-amylase inhibitors play a role in reducing hyperglycemia after eating by suspending carbohydrate digestion and, thus, glucose intake. Figure (2) shows that the bioactive components of *Z. coccineum* are an interesting α-amylase inhibitor with an inhibition level of 76.90±1.63% at a concentration of 200 mg/ml. is comparable to that of Acarbose. It should be noted that acarbose has been used to treat post-

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prandial hyperglycemia, but it has been linked to a number of negative health effects. (Hanefeld, 2007)

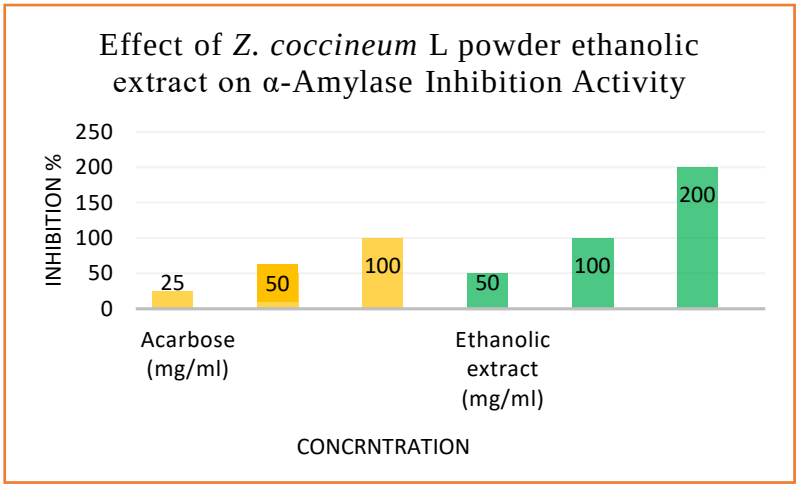

Figure 2. Effect of Z. coccineum L powder ethanolic extract on α-Amylase Inhibition Activity (orange color represent acarbose concentration, green color represent Z. coccineum L powder extract concentration)

Bakery products play a vital role in human nutrition. Wheat bakery products are generally regarded as a good source of energy and essential nutrients for the human body. This is particularly true for whole-grain products. Bakery products bread made from polished flour is nutritionally deficient and fails to meet the requirements for many macro- and micronutrients. But for diabetes mellitus cases bakery products are still prohibited because they contribute to raising blood sugar levels. For that, the idea of functional foods is currently popular.

Tables 4 and 5 display the sensory assessments of the two bakery products containing *Z. coccineum* powder (ZBB, ZR), covering taste, odor, color, texture, and overall acceptability. It is crucial that these results are thoroughly analyzed and taken into

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consideration in any decision-making processes regarding the production and sale of these products. The tongue distinguishes taste, which can be influenced by food texture. For the ZBB and ZR products, taste scores extended from 7.17±0.17 (with 50 mg/ml of *Z. coccineum* powder) to 6.35±0.23 (with 200 mg/ml of *Z. coccineum* powder) compared to the control bread sample (7.34±0.23) for ZBB, and 7.15±0.16 (with 50 mg/ml of *Z. coccineum* powder) to 6.57±0.13 (with 200 mg/ml of *Z. coccineum* powder) compared to the control rusks sample (7.23±0.22) for ZR. The samples with 200 mg/ml of *Z. coccineum* powder differed significantly from the control samples ($p<0.05$) for both products. However, this difference is still acceptable given the benefits of adding *Z. coccineum* powder to these products.

Based on the data presented in Tables 4 and 5, there is no notable difference in odor among the different treatments applied to both products and controls. However, upon closer examination of color character samples of bread and rusks enhanced with *Z. coccineum* powder, a remarked difference ($p<0.05$) was observed between samples that contained a concentration of 200 mg/ml as compared to the controls. This is believed to be a result of the interaction between reducing sugars and proteins at temperatures at or above 150°C, which plays a significant role in determining the flavor and aroma of bread (Zidan and Eldemery, 2016).

The quality of bakery products is heavily influenced by their texture. According to a study, adding *Z. coccineum* powder at a concentration of 200 mg/ml to ZBB and ZR products resulted in texture scores of 6.50±0.08 and 6.61±0.09, respectively, which were significantly different from the control samples. Mechanical properties like hardness, softness, and resiliency are critical in assessing bakery products as they reveal freshness and appeal to consumers (Zidan and Eldemery, 2016).

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In general, the bread and rusks that were made using Z. coccineum powder were considered acceptable, but they received lower ratings when compared to the control samples. These observations are supported by the study conducted by Khalil *et al.*, (2015), where it was found that incorporating different types of fibers into the production of pan bread resulted in a decrease in its sensory properties.

Table (4): sensory evaluation of *Z. coccineum* powder bran bread (ZBB)

Sample / Constituent %	Control	*Z. coccineum* powder bran bread (ZBB)		
		50 mg/ml of	100 mg/ml	200 mg/ml
Taste	7.34±0.23	7.17±0.17	6.50±0.17	6.35±0.23
Color	8.00±0.01	7.62±0.32	7.16±0.39	6.22±0.32
Odor	7.81±0.21	7.44±0.32	7.56±0.31	7.62±0.74
Texture	8.00±0.11	7.21±0.21	7.30±0.45	6.50±0.08
Overall acceptability	7.82±0.37	7.45±0.5	7.10±0.78	7.11±0.37

Each value is the mean ±SD, each column values are significantly different at $p < 0.05$

Table (5): sensory evaluation of *Z. coccineum* powder rusks (ZR)

Sample / Constituent %	Control	*Z. coccineum* powder rusks (ZR)		
		50 mg/ml of	100 mg/ml	200 mg/ml
Taste	7.23±0.22	7.15±0.16	6.71±0.19	6.57±0.13
Color	8.00±0.01	7.82±0.33	7.26±0.4	6.32±0.22
Odor	7.81±0.21	7.55±0.34	7.64±0.21	7.70±0.62
Texture	8.00±0.01	7.32±0.22	7.41±0.35	6.61±0.09
Overall acceptability	7.71±0.26	7.56±0.6	7.22±0.68	7.10±0.27

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Each value is the mean ±SD, each column values are significantly different at $p < 0.05$

4. Conclusion

In summary, the research indicates that adding *Zygophyllum* powder to food products can act as a valuable dietary supplement, effectively reducing the risk of elevated blood sugar and its related health issues. Introducing such bakery products as functional food with the capacity to decrease blood sugar levels is a secure choice for individuals with diabetes, making them a desirable alternative.

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