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# Chemical Composition and Antioxidant Activity of Stevia leave Aqueous Extract

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*Key words:*
*Stevia rebaudiana, Total phenols, Flavonoids, Antioxidant activity, DPPH and reducing power.*

*Accepted*

## ABSTRACT

Stevia rebaudiana (SR), is a natural sweetener or substitute for sucrose in many foods and beverages. This study aimed to estimate chemical composition, phenol content and antioxidant activity of aqueous extract of Stevia rebaudiana. The results indicated that aqueous extract significant (P≤0.01) improvement antioxidant activity (reducing power and radical scavenging activity). Additionally, results show that chemical composition of aqueous extract of SR. leaves (ash, lipid, protein, total carbohydrates and fibers content were 6.48, 8.24, 7.44, 29.16 and 17.5%, respectively). However, reducing sugar, total phenolic and flavonoid contents were found to be 10.58%, 2.07(mg/ml extract) and 0.94 (mg/ml extract), respectively. Reducing power of SR. leaves aqueous extract was increased (1.33%) compared to ascorbic acid. Free radical scavenging capacity of SR. leaves aqueous extract was (IC50; 3.19 µg /mL) comparable with ascorbic acid (IC50; 2.56 µg/ml). Total antioxidant capacity (TAC) of SR. leaves aqueous extract was (0.608) compared to ascorbic acid (1.785)..

## INTRODUCTION

Stevia rebaudiana is a perennial herb and there are about 200 species in the Stevia Genus, which belongs to the Asteraceae Family. Stevia rebaudiana, formerly known as Eupatorium rebaudianum Bertoni, is one of the representatives of Stevia Genus (Yadav et al., 2011).

Stevia rebaudiana is produced commercially worldwide as a natural sweetener. The stevia plant is used as a natural substitute for sucrose to improve the taste. Stevia can be used in both foods and beverages. A well-known natural sweetener, which is easily accessible in markets all over the world, stevioside is the main sweetener of stevia leaves. The biological activity of Stevia rebaudiana, may be due to its antioxidant, antibacterial and antifungal qualities in addition to non-toxicity and physiological safety characteristics (Kumari et al., 2021).

Stevia rebaudiana (SR) contains diterpene glycoside;stevioside (C38H60O18), which is a common sweetener of the leaf. Because of its high sweetness (250–300 times sweeter than sucrose), stevioside has gained popularity and is used as a non-caloric sweetener in several nations (Chatsudthipong and Muanprasat, 2009). As a substitute for sugar, the stevia plant's stevioside has also been used to prevent cancer, diabetes, obesity, hypertension, and inflammation (Sambra et al., 2022). Alkaloids, Flavonoids, xanthophylls, chlorophylls, hydroxycinnamic acids such as chlorogenic, caffeic, lipids, free sugars, amino acids, oligosaccharides and trace elements are all found in the dry aqueous extract of stevia leaves. In addition, extracts from S. rebaudiana sweeteners may have positive effects on human health; including anti-hypertensive, anti-hyperglycemic and anti-rotavirus properties (Muanda et al., 2011).

Stevia leaf extract has been shown to include active and useful compounds with antiviral activities and therapeutic efficacy in the treatments of lumbago, neuralgia, anemia, dermatitis, eczema and rheumatism. In addition, anti-amnesic, antioxidant activities, antifungal and antibacterial properties were discovered. These properties work in concert with immune modulatory actions that are anti-diarrheal, anti-hyperglycemic, anti-inflammatory, anti-hypertensive, anti-tumor and diuretic to prevent the development of chronic degenerative diseases. Additionally, its components might help the human immune system fight COVID-19 (Raspe et al., 2022).

This investigation aims to study the efficiency of Stevia rebaudiana leaves aqueous extract and its components. In addition, evaluation of polyphenols, total flavonoids, reducing power, radical scavenging activity (DPPH) and total antioxidant capacity.

## MATERIAL AND METHODS

### 2.1. Collection and Preparation aqueous extract of Stevia rebaudiana (SR) leaves

Stevia rebaudiana fresh leaves were obtained from local farm in Zagazig City, Alsharqia governorate, Egypt. Stevia rebaudiana leaves were cleaned thoroughly, then were dried

at room temperature, and crushed by using electric grinder into a fine powder. These samples filled in polyethylene bags of self-locking then preserved at 4°C until use. Weighing out (5 g) of Stevia rebaudiana powder, soaking in 200 ml of distilled water at 50°C for 10 minutes, and vigorously stirring with a glass rod that produced the aqueous extract. The solution was filtered and concentrated using muslin clothe three times, after which a clear aqueous extract of the plant was extracted. Then the extract was filtered using Whatman no.1 filter paper. The extract was kept at 4°C until being used (Bishnoi et al., 2018).

**2.2. Chemical analyses of Stevia rebaudiana (Bertoni)**

**2.2.1. Chemical composition of Stevia rebaudiana leaves**:

Moisture, ash, lipid, fibers, and protein content were measured in S. rebaudiana leaves according to standard protocol method of AOAC (2012). Total carbohydrates content of Stevia rebaudiana powder was evaluated using the phenol-sulfuric acid method described by (Dubois et al., 1956).

**2.2.2. Phytochemical screening:**

S. rebaudiana extract was subjected to the following tests:

Terpenes and flavonoids were measured as described by Finar (1968) and Geissman (1962), respectively. Detection of tannins and resins were analyzed in S. rebaudiana extract by using a method Harborne (1998). Moreover, Saponins were quantitatively described by Trease (1961).Total polyphenolic content was determined in S. rebaudiana using gallic acid as a standard phenolic compound and a Folin-Ciocalteu reagent, according to the procedure described by (Li et al., 2007). Additionally, total flavonoid content and reducing sugar content were measured by colorimetric method according to (Chang et al., 2002) and Somogyi (1952).

**2.2.3. Determination of antioxidant activity:**

**2.2.3.1. Determination of radical scavenging activity %**

Determination of radical scavenging activity DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was

determined according to the method described by **(Rekha*et al.*, 2012)**. Sample solution was mixed with 2.0 ml of 100 µM DPPH at room temperature for 30 minutes in dark. The antioxidant converts the stable radical DPPH, whose maximum absorption wavelength is 517 nm, to the yellow compound diphenyl-picrylhydrazine. Decolorization of DPPH was determined by the following equation.

Determination of radical scavenging activity

$\% = (A_0 - A_1/A_0) \times 100$

$A_0$: Absorbance without extract (blank)

$A_1$: is the absorbance in the presence of the extract or standard sample.

Reducing power of *S. rebaudiana* extract was determined according to the method of **(Ferreira *et al.*, 2007)**. Additionally, total antioxidant capacity was measured using the phosphomolybdenum method described **(Prieto *et al.*, 1999)**.

## RESULTS AND DISCUSSION

**3.1. Chemical composition of *Stevia rebaudiana* leaves aqueous extract:**

The moisture, ash, fibers, crude lipid, crude protein and total carbohydrate were determined in *Stevia rebaudiana* leaves aqueous extract. The results are shown in **Fig (1).**

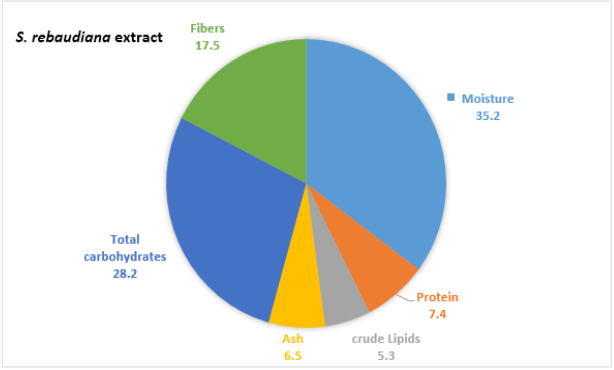



*Figure 1. Chemical composition values of S. rebaudiana extract*

**Fig (1)** illustrated that the moisture, ash, fibers, crude lipid, crude protein, and total carbohydrate were 35.2, 6.5, 17.5, 5.3, 7.4 and 28.2 % ,respectively. Stevia has been found to possess an incredible amount of protein, making it a useful source for the growth of the body's structure and a variety of physiological processes. In this investigation, crude protein was found to be 7.4%. In addition, lipids are vital components of the human body that are biologically active, essential for storing energy, creating parts of cell membranes, and controlling physiological processes. In this study, lipids were found to be 5.3%. Moreover, the principal sources of energy are carbohydrates. they are detected as structural parts of cellular components, and in the current investigation, carbohydrates are found to be 28.2%. Additionally, crude fibre was 17.5%. Thus, The chemical composition of *Stevia rebaudiana* which in concert with several studies. **Gasmalla *et al.*, (2014)** reported that the the percentage of the crude protein and fat were (10.73%) and (6.13 %). **Chughtai *et al.*, (2019)** found that crude protein and crude fat were 10.64% and 5.47%. In addition, **Yen (2021)** mentioned that the percentage of protein, lipid, fibers, and ash were (10.67 %, 4.16 %, 14.67 % and 7.26% ,respectively).. **3.2. Phytochemical screening of *Stevia rebaudiana* leaves aqueous extract**

Terpenes, tannins, flavonoids, , and saponins are all present while resins are not detected, as seen in Table (1) that

showed positive result for tannin, terpenes, flavonoid, and phenolic glycosides. These results are in agreement with those of **Mali *et al.*, (2015)** and **Gunasena *et al.*, (2021)** who stated that terpenes, tannins, flavonoids, saponins, and phenolic glycosides were detected in *Stevia rebaudiana*.

**Table (1). Phytochemical screening of *Stevia rebaudiana* extract:**

| *S. rebaudiana* extract | | | |
|---|---|---|---|
| Terpenes | ++ | Flavonoids | + |
| Tannins | +++ | Saponins | ++ |
| Resins | - | Phenolics glycosides | ++ |

(++) strong (+) medium (-) absence

The qualitative phytochemical screening of SR. leaves revealed that Terpenes, tannins, flavonoids, phenolic glycosides, and saponins were present in aqueous extract, while resins were absent.

3.3. Total phenolic, flavonoid compounds and reducing sugar of Stevia rebaudiana leaves aqueous extract

The purpose of the current study was to determine the total phenolic, flavonoid, and reducing sugar contents of the aqueous extract of SR. leaves. Results are shown in Fig.2.

Total phenolic content of SR leaves extract was 2.07 mg of gallic acid per ml of extract. And flavonoid content of the SR leaves extract was 0.94 mg quercetin per ml extract. Furthermore, reducing sugar of SR. leaves aqueous was 10.58%.

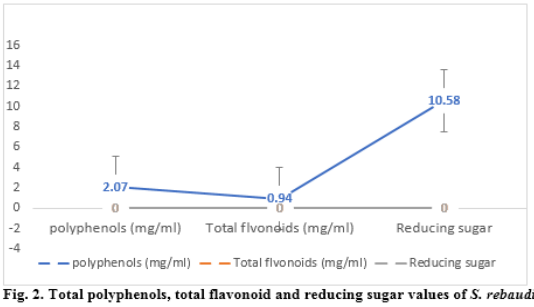



**Fig. 2. Total polyphenols, total flavonoid and reducing sugar values of *S. rebaudiana* extract**

**Fig. (2)** showed that total phenolic, flavonoid compounds and reducing sugar of *Stevia rebaudiana* leaves aqueous extract were (2.07mg/ml, 0.94 mg/ml and 10.58%). These results almost agree with those reported by **Garcia-Mier *et al.* (2021)**. They mentioned that total polyphenols and total flavonoid were $0.948 \pm 0.157$ and $0.165 \pm 0.030$ (µg/mg), respectively. **Javed *et al.* (2018)** found that polyphenols and total flavonoid were 3.13 and 2.02 (µg/mg), respectively. In addition, **Yen (2021)** mentioned that the percentage of reducing sugar was 7.20 ±0.87 %. These findings suggest that the higher levels of antioxidant activity were due to the presence of phenolic and flavonoid components.

The scavenging capacity of phenols, which is linked to their hydroxyl (-OH) groups and the methyoxy (-OCH3) substituent in the molecules, makes them an essential component of plants. The phenolic chemicals may directly contribute to the antioxidative effect, and they may also inhibit the development of heart disease and cancer. The attention in phenolics is increasing in the food industry because these substances retard the oxidative degradation of lipids, thereby enhancing both the quality and the nutritional value of the food **Kim *et al.* (2011)**.

**3.4. The DPPH radical scavenging activity:**

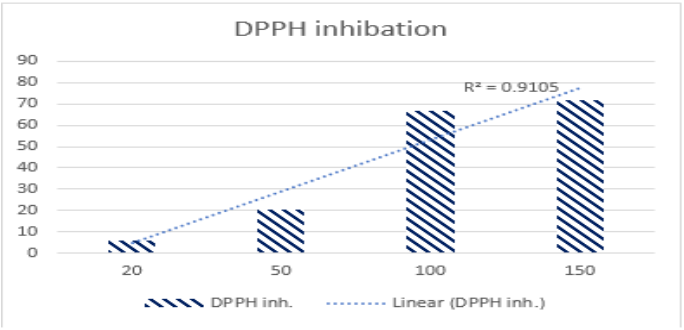
Antioxidant activity can be determined using a variety of methods. The DPPH method is frequently used to evaluate the radical scavenging capacity of any antioxidant since it is a quick, accurate, and reproducible test. It is frequently used to test a compound's capacity as a hydrogen donor or free radical scavenger, as well as to assess the antioxidant activity of extracts.

**Table. 2. Regression statistics of DPPH values of *S. rebaudiana* extract**

| *Regression Statistics* | | | | |
|---|---|---|---|---|
| Multiple R | 0.952382 | | | |
| R Square | 0.907031 | | | |
| Adj. R Square | 0.860547 | | | |
| St. Error | 12.2662 | | | |
| ANOVA | | | | |
| Regression | 1 | 2935.86 | 2935.86 | 19.51261 |
| Residual | 2 | 300.9192 | 150.4596 | |
| Total | 3 | 3236.779 | | |
| | *Coeffs.* | *St. Error* | *t Stat* | *P-value* |
| Intercept | -2.531 | 11.65651 | -0.216998 | 0.848334 |
| Conc. | 0.547337 | 0.123907 | 4.417308 | 0.047618 |

**** Y= -2.53+0.55X**

Y= DPPH inhibition X= Concentration extract



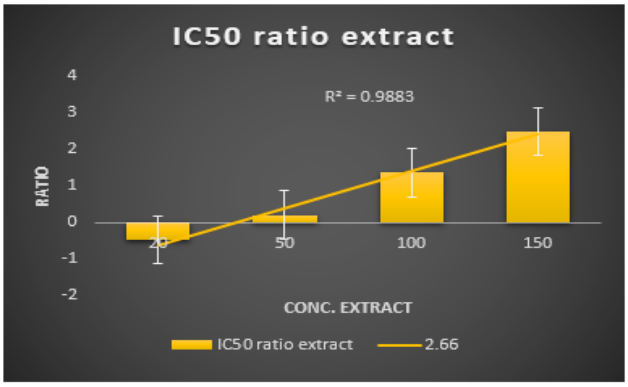




**Fig.3.** ($IC_{50}$) of *S. rebaudiana* leaves aqueous extract comparable with ($IC_{50}$) ascorbic acid; 2.56 µg/ml)

**Table (2)** displayed the ability of *Stevia rebaudiana* aqueous extract to act as antioxidant and scavenge DPPH free radical. In addition, **fig. (3)** show that free radical scavenging capacity of SR. leaves aqueous extract was ($IC_{50}$; 3.19 µg/ml) compared to ascorbic acid ($IC_{50}$; 2.56 µg/ml). Results showed that DPPH had a maximum absorbance at 517 nm and that the initial concentration of DPPH required to be reduced by 50% by an antioxidant concentration ($IC_{50}$). These results agree with those presented by **Ahmad *et al*. (2010), Myint *et al*. (2020) and Garcia-Mier *et al.* (2021)**.

**3.5. Reducing power and total antioxidant capacity of *Stevia rebaudiana* aqueous extract.**

The measuring of reducing power was made by the reduction of $Fe^{+3}(CN)_6$ to $Fe^{+2}$ $(CN)_6$. The result was made visible by forming an intense Prussian blue color complex; a higher absorbance value indicates a stronger reducing power of the sample. Table (4) and figure (4) illustrate the effectiveness of *Stevia rebaudiana* as a reductant agent. According to the results of this experiment, it is possible to conclude that the extract has the capacity to donate electrons to reactive radicals, causing them more stable and inert species (**Moguel-Ordóñez *et al*. 2015)**. Total antioxidant capacity depends on the reduction of $Mo^{+6}$ to $Mo^{+5}$ by the extract or ascorbic acid, and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH. The absorbance was measured at 695 nm against a blank solution. In addition, ascorbic acid was used as a standard **(Prieto *et al*., 1999)**.

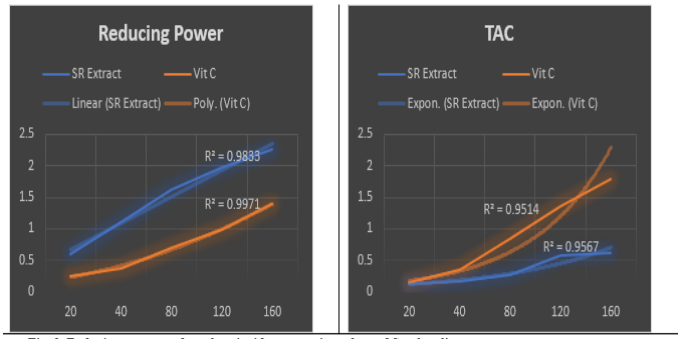



**Fig. 3. Reducing power and total antioxidant capacity values of *S. rebaudiana* extract**

Table (4) illustrated that various concentrations of SR. leaves aqueous extract were (20–40-80-120-160 µg/ml**)** showed absorbance (0.596, 1.106, 1.627, 1.966 and 2.255, respectively) at reducing power assay. Moreover absorbance of ascorbic acid was ( 0.247, 0.376, 0.698, 0.995 and 1.395 respectively) at the same concentrations of SR. leaves aqueous extract. Reducing power of the SR. leaves aqueous extract increased with concentration. Results of this study found that reducing power of SR. leaves aqueous extract was higher than reducing power of ascorbic acid. According to total antioxidant capacity at the same concentrations of SR. leaves aqueous extract , the absorbance was (0.119, 0.175, 0.274, 0.579 and 0.608, respectively) ,while the absorbance of ascorbic acid was (0.140, 0.348, 0.847, 1.354 and 1.785 respectively).

Table (4): Reducing power and total antioxidant capacity of *stevia rebaudiana* extract

| Conc. (µg/ml) | Reducing power | | Total antioxidant capacity | |
|---|---|---|---|---|
| | S. R. extract absorbance | Ascorbic acid absorbance | S. R. extract absorbance | Ascorbic acid absorbance |
| 20 | 0.596 | 0.247 | 0.119 | 0.140 |
| 40 | 1.106 | 0.376 | 0.175 | 0.348 |
| 80 | 1.627 | 0.698 | 0.274 | 0.847 |
| 120 | 1.966 | 0.995 | 0.579 | 1.354 |
| 160 | 2.255 | 1.395 | 0.608 | 1.785 |

*conc. :concentration * S. R. : *stevia reubadiana*

Total antioxidant capacity of SR. leaves aqueous extract decreased with concentration. Results of this study found that total antioxidant capacity of SR. leaves aqueous extract was less than total antioxidant capacity ascorbic acid.

## CONCLUSIONS

The nutritional value of Stevia leaves might be changed depending on the drying procedure, and the amount of common phytochemical components can significantly decrease. The plant leaf can be utilized as a starting point for the extraction and manufacturing of functional food ingredients as well as a source of nutrients important for human nutrition, such as carbohydrates, protein, crude fiber, and the various antioxidant properties that possessed. Moreover, these findings would be beneficial for some technological disciplines like pharmacology, biotechnology, food science, and chemistry.

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This research did not receive any funding.

**CONFLICT OF INTEREST:**

The authors declare that they have no conflict of interest.

**AUTHORS CONTRIBUTION**

Nabil A Azzaz; Sahar A Hamed; and Heba H Nekshara. developed the concept of the manuscript. All authors checked and confirmed the final revised manuscript.

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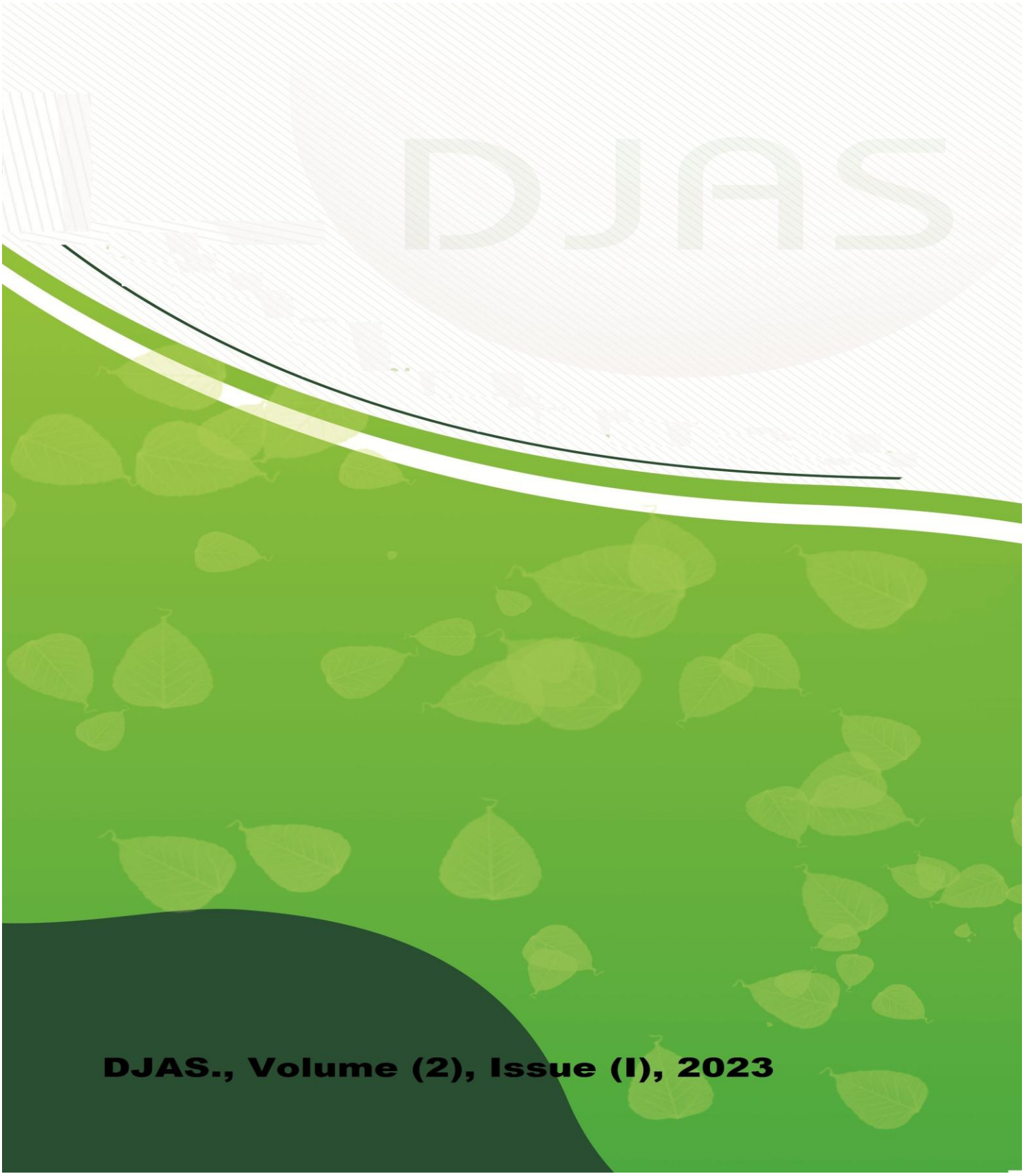
# التركيب الكيميائي وفاعلية تضاد الأكسدة لمستخلص الاستيفيا المائي

**نبيل عبد الخالق عزاز وسحر السيد حامد وهبه حسن نقشارة**[1]

**قسم البيوتكنولوجيا الزراعية ، كلية الزراعة ، جامعة دمياط ، مصر**

الاستيفيا*Stevia rebaudiana* هوعشب يزرع على نطاق واسع على مستوى العالم ويستخدم كمحلي طبيعي او بديلا للسكروز في العديد من الاطعمة والمشروبات. تهدف هذه الدراسة الى تقييم التركيب الكيميائي والمحتوى الفينولي والنشاط المضاد للأكسدة للمستخلص المائي لاوراق نبات الاستيفيا. وأشارت النتائج الى ان المستخلص المائى لاورق نبات الاستيفيا له تأثير معنوى كمضاد للاكسدة وله قدرة اختزالية وفاعلية التخلص من الشوادر الحرة. تظهرالنتائج ان التركيب الكيميائي للمستخلص المائي لـأوراق نبات الاستيفيا (رماد،دهون،كان محتوى البروتين والكربوهيدرات الكلية والألياف ٦.٤٨ و ٨.٢٤ و ٧.٤٤. ٢٩.١٦ و ١٧.٥٪على التوالي). ومع ذلك،الحد من السكر،الفينولات الكلية و وجد أن محتويات الفلافونويد ١٠.٥٨٪، ٢.٠٧ (ملجم / مل مستخلص) و ٠.٩٤ (ملجم / مل مستخلص) على التوالي. وكانت القوة الاختزالية لمستخلص الاوراق المائي أعلي بمقدار 1.33%المقارنة بحامض الاسكوربيك. وفاعلية التخلص من الشوادر الحرة(DPPH($IC_{50}$; 3.19 µg/ml مقارنة بحامض الاسكوربيك($IC_{50}$; 2.56 µg/ml). وكانت السعة الكلية كمضاد للاكسدة Total antioxidant capacity (TAC) بقيمة (0.608)مقارنة بحامض الاسكوربيك( 1.785)

**الكلمات المفتاحية**: الفينولات الكلية، فلافونيدات، فاعلية تضاد الاكسدة، القوة الاختزالية DPPH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