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Total Phenolic, Flavonoid Contents and Antioxidant Activity of Ethanolic Extracts (Leaves and Fruit) of *Cucurbita maxima* Duch. ex Lam

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ABSTRACT

Background: *Cucurbita maxima* known as pumpkin belongs to the family *Cucurbitaceae*, widely used in traditional medicine.

Methods: The present work evaluates the antioxidant activity of the different parts (leaves and fruit) of *Cucurbita maxima.* Antioxidant activity was assessed through the DPPH test and the β-carotene bleaching test.

Result: The results showed that the ethanolic extract of fruit and leaves are rich in polyphenols, flavonoids and tannin with values, respectively (50.13±4.49 mg EAG/g dry extract), (0.75±3.64 mg EQ/g dry extract) and (477.05±6.67 mg EAT/g dry extract) for the fruit, (27.37±0.95 mg EAG/g dry extract), (1.83±0.28 mg EQ/g dry extract) and (481.69±5.77 mg EAT/g dry extract) for leaves. The ethanolic extract showed scavenger activity IC_{50} is 0.41±1.20 µg/ml fruit and 0.18±0.06 µg/ml leaf compared with 0.01±0.01 µg/ml BHT (Butylated hydroxytoluene). The results obtained in the β-carotene bleaching test of the ethanolic extracts of the leaves and fruit of *Cucurbita maxima* is of the order (70.13±13.84%) and (67.69±13.82%) respectively.

Key words: Antioxidant, Cucurbitaceae, Polyphenols, Pumpkin, Tanin.

INTRODUCTION

The genus Cucurbita is a member of the Cucurbitaceae family which includes five major species (*Cucurbita moschata*, *Cucurbita pepo*, *Cucurbita argyrosperma*, *Cucurbita maxima* and *Cucurbita ficifolia*). Hundreds of varieties and cultivars of Cucurbita are grown around the world and are major agricultural commodities. The most important Cucurbit genus members include; gourd, melon, cucumber, squash and pumpkin, which are used as food and industrial crops (El-Adawy *et al*., 2001; Acquaah, 2004; Leffingwell *et al*., 2015). Pumpkin is cultivated worldwide for its nutritional and medicinal importance. Each pumpkin part contains a significant amount of antioxidants, tocopherols and carotenoids (Kim *et al.,* 2012). Pumpkin pulp can be consumed both raw and after being processed, *e.g*., cooked, or as compotes, jams, purees and juices (Paris *et al.,* 2006). Polyphenolic compounds are secondary metabolites and constitute the largest group of phytochemicals that promote health. It is known that these compounds have important antioxidants, exhibit anti-glycemic, anti-viral, anti-cancer and anti-inflammatory activities, anti-allergic and anti-microbial properties (Manach *et al*. 2004). The antioxidant activity of phenolic compounds depends on the number and position of their hydroxyl groups, which can easily donate their H^+ to reactive oxygen species (ROS). ROS are generated due to the result of environmental effects, such as UV radiation, cigarette smoke and air pollutants (Okmen *et al*., 2009). Therefore, the main aim of study is to determine the antioxidant activity of ethanolic extracts of *Cucurbita maxima.*

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MATERIALS AND METHODS

Plant material

This study was carried out on both parts: leaves and fruits of a species of the family Cucurbitaceae called Cucurbita maxima. The plant was harvested in November 2018 in the region of N'gaous in Batna, The identification of the species was made by Dr Nouioua Wafa (Laboratory of Phytotherapy Applied to Chronic Diseases).

Preparation of ethanolic

The ethanolic extract of leaves and fruit was obtained by maceration in water/ ethanol mixture (20:80) for 5 days in the dark with agitation every 24 hours. The resultant extract was filtered through Wattman paper and the solvent was removed by rotary evaporator under reduced pressure at 45°C (Lakić *et al*., 2010).

Determination of total phenolic content

Folin Ciocalteu method was used for determination of poylphenol content (Li *et al*., 2007). The samples (0.2 mL) were mixed with 1 mL of the Folin-Ciocalteu reagent previously diluted with 10 mL of deionized water. The solutions were allowed to stand for 4 minutes at 25°C before 0.2 mL of a saturated sodium carbonate solution (75 mg/mL) was added. The mixed solutions were allowed to stand for another 120 minutes before the absorbance were measured at 765 nm. Gallic acid was used as a standard for the calibration curve. The total phenolic content was expressed as mg equivalent of Gallic acid per gram of extract (mg EAG/GE).

Determination of total flavonoids content

The flavonoids content in crude extract were estimated by the Aluminium chloride solution according to the method described by Bahorun *et al*. (1996). Briefly, 1 mL of the methanol solution of the extract was added to 1 mL of 2% $AlCl_3$ in methanol. After 10 minutes, the absorbance was determined at 430 nm. Quercetin was used as a standard. Results were expressed as mg equivalent Quercetin per gram of extract (mg EQ/GE).

Determination of tannins content

The capacity to precipitate haemoglobin was determined by using bovine fresh blood according to the method described by Gharzouli *et al*. (1999). Briefly, a volume of leaves and fruits extracts were mixed with an equal volume of hemolysed bovine blood (absorbance=1.6). After 20 min, the mixture was centrifuged at 4000 rpm for 10 min and the absorbance of the supernatant was measured at 756 nm. Results were expressed as mg equivalent tannic acid per gram dried weight (mg TAE/g DW).

Evaluation of antioxidant activity

DPPH assay

The donation capacity of extract was measured by bleaching of the purple-coloured solution of 1, 1-diphenyl-2-picrylhy drazyl radical (DPPH) according to the method of (Hanato *et al*. (1998). One mL of the extract at different concentrations was added to 0.5 mL of a DPPH-methanol solution. The mixtures were shaken vigorously and left standing at room temperature for 30 minutes in the dark. The absorbance of the resulting solutions were measured at 517 nm. The antiradical activity was expressed as IC_{50} (micrograms per millilitre). The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where:

A_0: Absorbance of the control at 30 minutes.

A_1: Absorbance of the sample at 30 minutes. BHT was used as standard (Bettaieb *et al.,* 2012).

Reducing power

The reducing power was determined according to the method of (Oyaizu, 1986). The extract (2.5 mL) was mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 10 mg/mL potassium ferricyanide. The mixtures were incubated at 50°C for 20 minutes; after cooling, 2.5 mL of 100 mg/mL trichloroacetic acid were added and the mixtures were centrifuged for 10 minutes. The upper layer (5 mL) was mixed with 5 mL of deionized water and 1 mL of 1 mg/mL ferric chloride and the absorbance was measured at 700 nm against a blank. A higher absorbance indicates a higher reducing power. EC_{50} value (mg extract/mL) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis. BHA (Butylated hydroxytoluene) was used as standard (Huang and Mau, 2006).

β-carotene bleaching assay

In this test, the antioxidant capacity of the The ethanolic extract of leaves and fruit extract is determined according to the method of Dapkevicius *et al*. (1989). Briefly, A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml of chloroform and 25 µl linoleic acid and 200 mg Tween 40. Chloroform was completely evaporated and then, 100 ml distilled water saturated with oxygen (30 min, 100 ml/min) were added with vigorous shaking. 2500 µl of this reaction mixture were dispensed into test tube and 350 µl of seeds extract, prepared at 2 mg /ml concentrations, were added and the emulsion system was incubated for 48 h at room temperature. The same procedure was repeated with synthetic antioxidant BHT as positive control and blanks (MeOH and H_2O). The absorbance of the mixture was measured at 490 nm after; 0, 1, 2, 4, 6 and 24 hour. The antioxidant activity was measured in terms of successful bleaching of β-carotene by using the following equation:

$$AA\% = \frac{A\ \text{sample}}{A\ \text{BHT}} \times 100$$

A sample: Absorbance in the presence of the extract.

A BHT: Absorbance in the presence of positive control BHT.

RESULTS AND DISCUSSION

The total flavonoids content of *Cucurbita maxima* extracts was determined using the aluminium chloride method and the total amount of flavonoid compounds was determined in mg of quercetin equivalents/gin (Table 1). The extract of the fruit has given the highest rate in phenolic compounds which is of the order (50.13±4,49 EAG/g of dry extract), relative to the leaves (27.37±0, 95 mg EAG/g of dry extract). A similar study conducted by Hashash *et al*. (2017) showed that the total polyphenols of the ethanolic extract of the fruit of the same family was (49.80 0.44 mg EAG/g of extract), as higher than that of our study. Studies carried out by

Table 1: Total polyphenols, flavonoids and tannins contents in ethanolic extracts leaves and fruit of *Cucurbita maxima.*

Matériel végétale	Extracts	Yield%	Polyphenols (mg EAG//mg)	Flavonoid content (mg EQ/g)	Tannin content (mg EAT/g)
Leaves	Ehanolic	20%	27.37±0.95	1.83±0.28	481.69±5.77
Fruit	Ethanolic	16%	50.13±4.49	0.75±3.64	477.05±6.67

GAE: Gallic acid equivalent, QE: Quercetin equivalent andmg tannic acid equivalent/g of dry extract. Each value represents the mean±SD (n=3).

Table 2: DPPH scavenging activity of *Cucurbita maxima*extracts and standards.

Extracts	IC_{50} µg/ml
Ethenolic leaves	0.41±1.20.
Ethenolic fruit	0.18±0.06
BHT (Butylated hydroxytoluene)	0.01±0.01

µg/ml. Each value represents the mean±SD (n=3).

Table 3: Antioxidant activities of *Cucurbita maxima* extracts at 24 hours of incubation measured by β-carotene bleaching method.

	Inhibition %
BHT (Butylated hydroxytoluene)	99.5±1.15
H_2O	72.09±4.88
Methanol	72.64±4.84
Ethenolic leaves	70.3±3.84
Ethanolic fruit	67.69±3.82

Each value represents the mean±SD (n=3).

Dissanayake *et al*. (2018) the total polyphenol content of the ethanolic extract of the leaves was (7.0±0.2 mg EAG/g of extract) is less than that found in this study and the flavonoid content was (4.5±0.2 mg Eq/g of extract), is small to that found in this study. The fruits and leaves are very rich in tannins and the results are very close and are of the order of (477.05±6.67 mg EAT/g dry extract) and (481.69±5.77 mg EAT/g dry extract). Studies by Ebana *et al*. (2019) have shown that flavonoids in the fruit and leaves of the same family are (1.58±0.02 mg EAT/g dry extract) and (0.22±0.01 mg EAT/g dry extract, respectively), which are very close to that obtained in this study.

Antioxidant activity evaluation

DPPH radical scavenging activity

The scavenging effect of the DPPH radical was evaluated spectrophotometrically according to the reduction of this radical which is accompanied by its passage of purple color to the measurable yellow color at 517 nm and the results are present in (Table 2). The antioxidant activity of ethanolic fruit extract (0.18±0.06 µg/ml) is higher than that found by (Hashash *et al*. (2017) from the same family (643.09±6.31 µg/ml). And leaves (0.41±4.20. 10-3 µg/ml) are superior to that found by Sayed *et al*. (2017) of the same family (625.02±8.80 µg/ml). Phenolic compounds used as antioxidants reduce the odd electron of the nitrogen atom in the DPPH radical by giving it a hydrogen atom (Contreras-guzman and Strong,1982). Flavonoids are one of the phenolic compounds found in plants, they are associated with various pharmacological activities including anti-inflammatory and antitumor properties and they are capable of acting as antioxidants that shield the cells from destructive effects of free radicals (Moriasi *et al*., 2020; Kibiti and Afolayan, 2015).

β-carotene/linoleic acid bleaching assay

In the present study, the potential of the plant to inhibit linoleic acid oxidation was evaluated using the β-carotene/linoleic acid test system the resultofa show in (Table 3). The ethanolic extracts of leaf and fruit of *Cucurbita maxima* demonstrated lower inhibition percentages compared to the positive control BHT (99.51±1.15). However The oxidation of β-carotene from the leaves (70.13±3.84%) is higher than that obtained from the fruit (67.69±3.82%). The inhibitory power of β-carotene oxidation in leaves is higher than that obtained by Marzouk *et al*. (2016) be the same family, which is equal to (31.92±2.30%). These results are related to the presence of phenolic compounds, the effect of either inhibiting the peroxidation of linoleic acid or trapping hydroperoxide radicals formed during peroxidation of linoleic acid (Tepe *et al*., 2005).

CONCLUSION

The ethanol extracts (leaves and fruit) of *Cucurbita maxima* found with the highest frequency in antioxidant activity. It is clear that they have a good potential to be used in therapies as well as leaf vegetables for daily consumption. The total phenolic content of this study supports the hypothesis that phenolic compounds are a significant contributor to total antioxidant capacity.

Conflict of interest

All authors declare that they have no conflict of interest.

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