Original article

Physicochemical Properties, Fatty Acid Profile, and Antioxidant Activity of Seeds Essential Oil of *Cucurbita pepo* from the Sudan

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Abstract

In this study, the dried seeds of *Cucurbita pepo* available in Khartoum Central Market were extracted using maceration method in n- hexane with a yield of (45.05%), and the resultant oil was subjected to sensory, physical and chemical evaluation, it has a special pleasant odor, slightly palatable taste and dark red in color. The physiochemical properties of oil were carried out using standard analytical methods. The density, refractive index and viscosity were (0.905 g/ml), (1.467) and (28.66 poise) respectively. The acid value, saponification value, iodine value, peroxide value, ester value and free fatty acid were in order (22.44 mg KOH/g, 138.2 mg KOH/g, 90 g/100g oil, 00.04 meq /kg, 6.33mg KOH/g, 11.29 mg KOH/g), respectively. The essential oil of the seeds of *C. pepo* was analyzed by gas chromatography-mass spectrometry (GC-MS) which revealed the presence of 47 compounds were completely identified and the major compounds were unsaturated fatty acids (58.81%) while saturated fatty acids were(37.02%). The dominant unsaturated fatty acids of *Cucurbita pepo* seeds oil were oleic acid (15.61 %) and linoleic acid (36.61%), while the saturated fatty acids were stearic acid (12.64%), palmatic acid (20.64%) and archidic (1.74%).

The antioxidant activity using DPPH radical-scavenging method was applied for essential oil, petroleum ether, chloroform and methanolic extracts obtained by extraction the seeds powder successively in Soxhlet apparatus of *C. pepo* seeds. The results showed that the oil extract gave highest activity (66 %) at 0.1 mg/ml. The present study revealed that pumpkin seed oil can be a valuable source of edible oil.

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Introduction

Plant products, such as fruit, are characterized by the presence of various substances and different effects on the human body (Barceloux, 2008). The genus Cucurbita is a member of the Cucurbitaceae plant family which includes five major species which comprise the majority of pumpkins and squashes – *Cucurbita pepo*, *Cucurbita moschata*, *Cucurbita maxima*, *Cucurbita argyrosperma and Cucurbita ficifolia*. Many hundreds of varieties and cultivars of these are grown around the world and are major agricultural commodities. Some species of the genus Cucurbita L. are also interesting for studying. In addition to the investigation for possible use in the production of biodiesel (Schinas *et al.*, 2009). The pumpkin is also interesting for the use in food technology and for therapeutic use (Adams *et al*. 2012).The pumpkin seed is

valued in regard to nutritional points. Several studies have reported the chemical composition and oil characteristics of the pumpkin seed from different origins and varieties (Stevenson, *et al.* 2007). The four fatty acids presented in significant quantities are palmitic, stearic, oleic, and linoleic acids (Stevenson, *et al*. 2007). The pumpkin seed is a good source of potassium, phosphorus and magnesium, and also contains moderately high amounts of other trace minerals (calcium, sodium, manganese, iron, zinc, and copper) and these elements make pumpkin seed valuable for food supplements (Lazos, 1986). Ethno-pharmacological studies show that *C. pepo* is used in many countries for treating numerous diseases, e.g., as an anti-inflammatory, antiviral, analgesic urinary disorders, anti-ulcer and anti-diabetic (Rosa, 2016). antioxidant (Masuda, *et al.* 2003) antimicrobial, (Noumedem *et al.* 2003), Anticancer/anti-tumour effects (Chen *et al*. 2012), Anti-inflammatory activity (Kikuchi *et al*. 2015), hypoglycemic (Shan *et al*. 2015), antidiabetic (Boaduo *et al.* 2014). The seeds are used as a vermifuge to treat problems of urinary system, hypertension, prevents the formation of kidney stone and alleviates prostate disease (Martha and Gutierrez, 2016).

EXPERIMENTAL

2. Materials and Methods

2. 1 Plant material

The seeds of *Cucurbita pepo* were purchased from the local market at Khartoum state, Sudan.

2.2 Extraction methods:

The method used in this study was described by Harbone (1984) with slight modification.

2.2 .1 Successive Extraction:

A dried *C. pepo* seeds powder (50 g) was successively and separately extracted using a Soxhlet extractor with pet. ether (300 ml), chloroform (300 ml), and methanol (300 ml). The solvent was carefully evaporated from each extract and the extractability of each solvent was determined.

2.2 .2 Maceration Extraction:

Sample of the dried seeds powder (25 g) was transferred into a beaker and (200 ml 95% methanol) was added. The contents of the beaker were left at room temperature for three days with frequent shaking. The extract was filtered. The solvent was carefully evaporated and the extractability of solvent was determined. The same procedure was repeated using (25 g) seeds powder and 200 ml distilled water.

2.2 .3 Extraction of essential oil

Extraction of essential oils from seed of (20 g) was carried out by maceration in n-hexane (200cm^3) for 72 hr. The n-hexane was removed by evaporation at room temperature.

2.3 Physicochemical analysis of essential oil

The physical parameters *C. pepo* seed essential oils such as density, refractive index and viscosity were estimated according to the method described by A.O.A.C (1990). While chemical parameters: Acid Value (AV), Saponification Value (SV), and Iodine Value (IV) Peroxide Value (PV) Ester Value (EV) and Free Fatty acid (FFA) were determined according to A.O.A.C (1990).

2.4. GC-MS analysis of the essential oil

The seed essential oils of *C. pepo* were analyzed by GCMS-QP2010 Ultra (Japan) (Shimadzu); RTX-5MS capillary column (30m x 0.25mm; 0.25 mm film thickness); The temperature program was set up from 60°C to 300°C with 3°C/min, both the injector and detector temperatures were 300°C and helium was used as carrier gas with a flow rate of 3ml/min. -2-picrylhydrazyl (DPPH) free radical by adopting

2. 5 DPPH free radical scavenging assay

The antioxidant activity of the seed plant extracts was

assessed on the basis of the radical scavenging effect of the stable 1,1-diphenyl.

Modified method of (Hue, *et al*. 2012), 0.1 mM solution of DPPH was prepared in ethanol and 0.5 ml of this solution was added to 1.5 ml of *C. pepo* oil solution in ethanol, (15-45 g/ml). These solutions were vortexed thoroughly and incubated in dark. Half an hour later, the absorbance was measured at 517 nm against blank samples, and the activity was calculated by antioxidant activity. Inhibition percentage of DPPH activity (%) = [(A-B)/A)] *100

Where A= Absorbance of the blank solution and B=

Absorbance of the tested solution, The IC50 was calculated.

3. Results and Discussion

The hexane (mac), water (mac), methanol (sox), and methanol (mac) extracts were evaluated for their *in vitro* antioxidant activity using DPPH radical-scavenging assay and the results have been shown in **Figure (1).**

The results in **Table (1)** revealed that the hexane (mac) extract of seed exhibited highest antioxidant activity with inhibition percentage of 61% followed by water (mac) extract with inhibition percentage of 50%, while methanol (mac) extract exhibited moderate antioxidant activity with inhibition percentage of 37.5% and methanol (sox) extract shown as aweak antioxidant activity with inhibition percentage of 34.8% at the same concentration 0.1mg/ml. The DPPH radical scavenging activity of seeds crude extracts were concentration dependent. Our preliminary data shows that the plant has the percentage of radical scavenging activity and reductive capabilities found to be increased (66% at 4.5mg/mL) (64% at 3.0mg/mL) (61% at 1.5mg/mL) (45% at1.0mg/mL) and (25 at0.5mg/mL). Efficient antioxidant activity and also, with the increase in the concentration

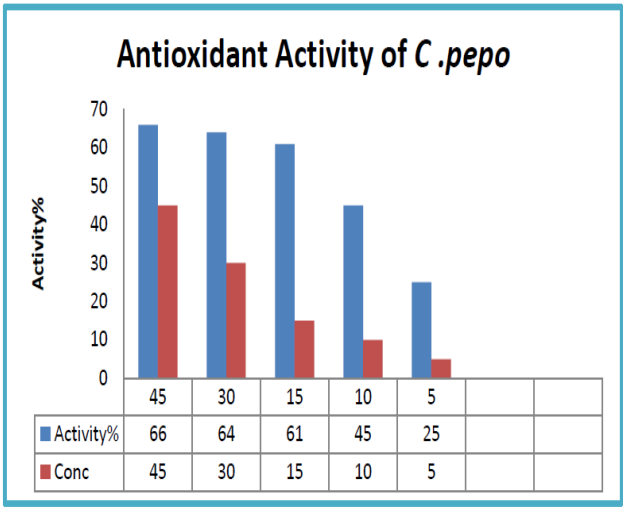

Figure (1): Antioxidant Activity of Essential Oil Extract of *C. pepo*

Table (1): Physical Properties of Hexane Extract

Properties	Results	Properties	Results
Oil yield	45.05%	Density (g/cm^3)	0.905
Taste	Palatable	Refractive index	1.467
Odour	Pleasant	Viscosity	28.66
Colour of oil	Dark red		

Table (2): Chemical Properties of Hexane Extract and amino acids in *C. pep*

The maximum antioxidant effect of hexane extract was observed in the highest concentration 66 %. The present results are in disagreement with earlier results (Chen et al.,

Sample	Conc. mg/ml	Activity% S ± /D	IC_{50} S ± D
Hexane (mac)	0.1	61 ± 0.042	10.1019 ± 0.016
Water (mac)	0.1	50 ± 0 .008333	_
Methanol (mac)	0.1	37.5 ± 0.104	_
Methanol (sox)	0.1	34.8 ± 01333	_

2012). Apart from cultivar differences, other factors such as maturity, environmental conditions, and extracting solvent might affect the antioxidant capacity (lhami, *et al.* 2012), For instance, (Sulaiman *et al.* 2011) pointed out that the antioxidant activity of plant extracts would significantly be affected by the type of extracting solvent. Based on the and antioxidant results in Table the hexane (mac) extract was selected and carefully studied. Physiochemical properties and chemical composition of *C. pepo* hexane extract , were evaluated. The results were showed in **Table (2) and Figure**

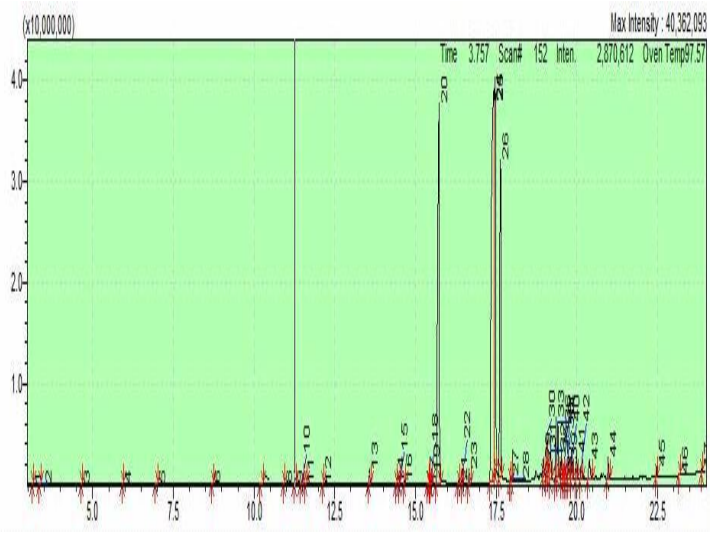

Figure (2): Gas Chromatogram of The Essential Oil Extract of *C. pepo*

Tables (2) showed the physico-chemical properties of *C. pepo* seeds oil. The oil extracted from *C. pepo* seeds has palatable taste, Pleasant odour and dark red colour. The density, refractive index and viscosity were (0.905 g/ml), (1.467) and (28.66 poise) respectively acid value (22.44 mg/g) which is higher than the Codex standard value for virgin vegetable oils ,it gives an indication about edibility of the oil. The high acidity value was obtained in the seeds, which is an indication of a unstable lipid at the room temperature thus enhancing the value of seeds as a best feeding source, While the high acidity could be attributed to the action of lipases as a result of prior wetting of the seed before oil extraction, it is true that such a process is needed to obtain a high yield of oil. As high levels of acidity make uneconomic the traditional process of Trans esterification, new approaches are necessary. The present findings were in disagreement with those of (Marianna, *et al.* 2009). The peroxide value was 00.04 Meq/kg. The value was lower than the codex standard value (10Meq/kg) for refined vegetable oil and lower than the maximum value (20Meq/kg) allowed for unrefined olive oil (FAO/WHO, 1993).

Saponification value gives an indication of the length chain free fatty acids present in the oil (11.29 mg KOH/g). The SV of the examined oil was (138.2 mg KOH/g) and ester value (6.33 mg KOH/g). The Iodine Value (I.V) gives a measure of the average degree of unsaturation of oils and fats is expressed as the grams of iodine absorbed per 100 grams of sample. The oil shows a high iodine value (90.42g/100g) due to its high content of unsaturated fatty acids, this value showed inconsistency with that obtained by (Marianna, *et al.* 2009). The GC/MS identification of compounds from derivatised *C. pepo* oil are presented in the Table (5) and Figure (2) Fourty seven compounds were characterized, unsaturated fatty acids represented (58.81%) while saturated fatty acids (37.02%). The fixed oil of *C. pepo L* contains oleic acid (15.61 %), linoleic acid (36.61%), palmatic acid (20.64%) and stearic acid (12.64%) derivatives. as the major compounds.

Conclusions

* Pumpkin seeds are the source of fatty acids, as well as other ingredients which, in addition to their antioxidant activity, are also the carriers of some other activities. *C. pepo* seed contains a healthy mixture of fatty acidssaturated and unsaturated.

* The major component of *C. pepo* seed oil was linoleicacid (36.61%), %), followed by palmitic acid (20.64) oleic acid (15.61 %), stearic acid (12.64%) and archidic (1.74%).

*The fatty acid profile plays an important role to the chemical properties therefore this is useful knowledge for further researches.

*The Pumpkin seed oil an excellentsource of essential fatty acids omega-6 (linoleic acid), omega-9 (oleic acid).

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