Antimicrobial Activity and Some Physiochemical Properties of Pumpkin (*Cucurbita pepo* L.) Seed oil

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ABSTRACT

Pumpkin and squash plants grow in warm, humid regions, cannot withstand frost. Most of the pumpkin consumed in Sudan was grown in Kordufan (western sudan).The use of plants and their extracts as remedies for curing many diseases have stimulate studies for investigating the presence of effective antimicrobial substances in them The present study was investigating the physiochemical and the biological activity of the different solvent extracts of pumpkin (*Cucurbita pepo*) seed oil (hexane, ethanol, methanol, and water) against two fungi (*A. niger* and *P. italicum*) and two bacteria *(E.coli* and *Staph. aureus).*The inhibition zone method was used for bacterial tests and the mycelia weight method was used for the fungal study. The results of the physiochemical showed that the specific gravity of the oil was between 0.997–0.908. The Refractive index was between 1.46–1.470. The average iodine value was 117.97, the free fatty acids was between 0.2 and 0.55. The peroxide value was in the normal range (5.92–9 and the average saponification value was about 188. The biological study showed that the fresh and the dry weights of mycelia were highly reduced, although the reduction was only statistically different at the higher concentrations (75-100%).The fresh weights for the higher concentrations were 1.65 and 1.0 and for the lower concentrations were 3.1 and 3.7 for both *A. niger* and *P. italicum*, respectively. The inhibition zone of growth of both bacteria was far greater than that of the control treatment. The results indicated that the pumpkin oil extracts are more effective against bacteria compared to fungi. The hexane extract was giving a large inhibition zone (about 26.5 mm diameter) while, the ethanol extract was giving a less inhibition zone (about 14.2.mm diameter). On the other hand both methanol and the aqueous extracts were giving very low inhibition zones (13.5 and 13.6 mm diameter, respectively). However, more physiochemical characterizations need to be done and the antifungal and the antibacterial properties should verified in any further studies on the pumpkin seed oil.

Key words: Pumpkin seed oil, Physiochemical properties, Antimicrobial Activity.

INTRODUCTION

Pumpkin and squash plants grow in warm, humid regions, and can not withstand frost. They grow better on fertile, warm, well-drained light soils (Thompson and Kelly ,1987; Whitaker and Davis, 1982). The plant is grown abundantly in Austria, Hungary, Rumania, Yugoslavia, Southern Russia, U.S.A. and throughout the Indo-Pakistan sub-continent (Eckey, 1984; Butt,1985; Bastic *et al*, 1987).

Most of the pumpkin consumed in Sudan was at one time grown in Kordufan (western Sudan) where it contributes up to 39.1% (12,174 metrictons in the year 1980/81) of the total vegetable and fruits produced (Obeidalla and Riley, 1984). Pumpkins can produce fruits weighing up to 55 pounds (25kg) each. They are also one of the highest sources of protein. The pumpkin plant is indigenous plant of the American Indians. However, pumpkin fruits are not nearly as significant in man's economy as cereals or legumes, but in the tropics and subtropics they are crops of more than ordinary importance and used in the diet as a source of carbohydrates (Whitaker and Davis, 1982).

Pumpkin fruit was considered a good source of nutrients. Gangadharam and Sirsi (1985) found that the fruit of *C. pepo* contains almost all nutrients as it was found to contain 94.5% moisture, 0.97% protein, 0.90% fat, 3.98% carbohydrate, and 0.46% ash as a percentage of the whole fruit and 17.69% protein, 2.68% ash, on a dry weight basis. The chemical composition of naked pumpkin seed cake (*C. pepo*) was 8.01%, 55.6%,7.42% and 8.63% for moisture, protein, crude fibre, and ash, respectively (El-Gharbawi,1988). According to Kamel *el al*. (1982) the pumpkin seeds contain sizeable amount of protein (35%) and approximated (50%) fatty oil, whose fatty acid-profile is dominated by unsaturated fatty acids, namely linoleic and oleic acids. There are many trace constituents like tocopherols (0.1%) and phytosterols (0.1 to 0.5%). The seeds are rich in vitamins and beside their use in the production of edible oil, pumpkin seeds were recommended as protection against colworm, tap worm, sea sickness, and interruption of pregnancy, while, the extracted oil has been used in medicine by physicians all over the world (Butt ,1985; Markovic and Bastic ,1996).

However, Gangadharam and Sirsi (1985) mentioned the medicinal properties of pumpkin seed oil with particular reference to its antibacterial activity.

The antimicrobial activity of essential oils has been recognized for many years. However, few investigations have compared large numbers of oils and extracts using methods that are directly comparable. Food borne illness resulting from consumption of contaminated food products with pathogenic bacteria has been of concern to public health. Controlling pathogenic bacteria would reduce food borne out breaks and assure consumer a continuing safe, whole some and nutrition food supply. Essential oils, were found to inhibit the growth of a wide variety of pathogenic microorganisms (Abdel-Rahim *et al*., 2009; Aljali *et al*., 1997).

The present study was conducted to investigate the physiochemical and the antimicrobial activities of the pumpkin seed oil.

MATERIALS AND METHODS

Physical and Chemical Tests

The specific gravity, the iodine value, the moisture content and the saponication value was obtained according to the A.O.C.S. Official method (1993), using a pycnometer.

The A.O.C.S. Official Method (2003) was used to determine the refractive index, using an able refractometer (Model ATAGO Rx -7000α) and to determine the free fatty acid which was the number of milligrams of potassium hydroxide required to neutralize the free fatty acid in one ml of oil, and to determined the acid value as well as the peroxidise value.

Effects of the extracts on bacterial growth

The cup plate method was used, using Nutrient Agar (NA). In the method 2ml of a standardized bacterial cell suspension ($1.0x10^5$) of *E. coli* or of *staphylococcus aureus* were thoroughly mixed with 200 ml of sterile molten nutrient agar, then the medium was distributed into sterile Petri-dishes and was left to solidify at room temperature for 24 hours. Sterile Whatman glass fiber discs (No.5) were saturated with the oil, then allowed to dry and transferred centrally on the surface of the solidified medium in each plate.

The plates were then incubated at room temperature for 72 hours and the inhibition zones were measured as described by Barry et al (1970) and Cruickshank et al (1975). Three replicates were made for each treatment.

Effects of the extracts on fungal mycelial weigh

The method used was as described by Abdel-Rahim *et al.* (2002). The Potato Dextrose Broth (PDB) medium was prepared and then dispensed in 100 ml volume in conical flasks (250 ml). The oil solution was added to each flask, sterilized in an autoclave at 121^0C (15-Ib/in^2) for 15 minutes, and then allowed to cool at room temperature, before inoculation. Each flask was inoculated by three discs (5.0 mm diameter), taken from an edge of an actively growing culture on a solidified PDA medium. Inoculated flasks were incubated at room temperature (28–30^0C) for 8 days. After incubation mycelia were collected by filtering the culture through a Whatman No. 1 filter paper and the fresh weight was recorded. The mycelia mats were then dried at 80^0C for 24 hours, before being reweighed. All treatments were done in triplicates.

RESUTS AND DISCUSSIONS

Physicochemical properties

The results of some physicochemical studies made in the present work are shown in Table (1). The moisture content of the pumpkin oil samples was in the range (0.001 -0.129). Although no similar results were found, Ebrahim (2009) and Asiedu, (1990) were studied the moisture content of the groundnut oil and found it almost similar to that. The specific gravity of the pumpkin oil was determined at a temperature of 20^0C as was recommended by the British Institutions (Codex Alimentation, 1969). The results (Table, 1) showed that the specific gravity of the oil was

between 0.997 – 0.908. However, this was in agreement with many reports (Tckey, 1984; Batt, 1985; . Markovic and Bastic, 1996 and El-Gharbawi (1987). Table (1) is also showing the Refractive index of the pumpkint oil. The results showed that the Refractive index of the sample was in the normal ranges as compared with the results reported before (Ebrahim, 2009 and Musa, 2001). The iodine value for the pumpkin seed oil was also carried out in the present study. The results are also shown in Table (1).

The average iodine value was 117.97, which is similar to the results reported by Eckey (1984), for European pumpkin oil and to the results reported by (Alhelo, 2006), for the Sudanese pumpkin oil. The results also showed that the free fatty acids of the samples were between 0.2 and 0.55. (Table (1)).

Table (1) Some physicochemical properties of the extracted from pumpkin seeds.

characteristics	Treatments			
	1	2	3	mean
Specific gravity	0.897	0.908	0.907	0.891
Refractive index	1.471	1.47	1.46	1.467
Iodine value	117.86	118.2	117.86	117.97
Free fatty acids	0.92	0.90	0.89	0.90
Peroxide value	7.92	7.85	9.90	8.56
Saponification value	187.3	189.9	188.6	188.6
Moisture content	0.0129	0.001	0.001	0.004

The acid values were in the range (0.39–1.09) for the crude oil. However the Codex Alimentation (1969) indicated that the free fatty acids value of some oils was about 0.6. On the other hand Ebrahim (2009) was reported a higher value for the free fatty acids. The higher value in their study was due to direct addition of water to the seeds before pressing. However , in our study no water was added, that is why our values are lower. The peroxide values were in the normal range (7.92 - 9.90 for the crude oil (Table, 1) and the result was comparable to what which was reported by Codex Alimentation (1969) and Ebrahim (2009). The average saponification value of the oil extracted from the pumpkin seeds was found to be about 188.6. This was almost similar to the value reported by Markovic and Baslic (1986) for the Yugoslavian pumpkin seed oil and by Alhelo, (2006), for the Sudanese oil .

The biological activities

The present study was also conducted to investigate the biological activity of the oil of pumpkin (*Cucurbita pepo*) against two fungi (*A. niger* and *P. italicum*) and two Bacteria (*E. coli* and *Staph. aureus*).

The effects of the pumpkin oil on mycelial weights of *A. niger and P. italicum*, are shown in Table (2) and Table (3) as well as on Plate (1). From the results it is clear that the oil was highly effective in reducing both the fresh and the dry weights of both fungus. However, both the fresh

and dry weight of the two fungi were decreasing with increasing oil concentration. On the other hand *P. italicum* was found more tolerant to the oil compared to *A. niger*. Extracts of many plant species were reported to have antifungal activities (Bullerman, 1974, Abdel-Rahim *et al*. ,1989, Al-Jali *et al.*,1997). Extracts and essential oils of both clove and cinnamon were found to inhibit growth and aflatoxin production by *Aspergillus flavus* and *A .parasiticus* (Abdel-Rahim *et al*., 2002, Sulieman *et al.*, 2008, and Abdel-Rahim *et al*., 2010). According to Al-Jali et *al*.,(1997) essential oils were more effective than plant extracts. On the other hand, Zainal *et al.*, (1988) reported a pronounced effect of the leaf litter extracts of mesquite (*Prosopis juliflora*) on *A. niger* and *Condida albicans*. Lai and Joy (2004) reported that many herbs and spices can yield medicinal compounds. Spices and herbs have been used for thousands of years by man in traditional medicine.

The effects of the pumpkin oil extract on the inhibition zone of *E.coli* and *Staph. aureus* are also studied in the present study. The results in Tables (4,5) and Plate (2) show the effect on both *E.coli and staph. aureus* . From the results it was found that hexane extract was giving a large inhibition zone (about 26.5 mm diameter) while, the ethanol extract was giving a less inhibition zone (about 14.2.mm diameter). On the other hand both methanol and the aqueous extracts were giving lower inhibition zones (13.5 and 13.6 mm diameter, respectively). However, The lower concentration (25%) of the hexane extract gave about 8.5 cm diameter and that of the ethanol extract gave about 6.5 mm diameter. The lower concentrations of both methanol and water were giving almost similar results (8 and 7.5 mm diameter, respectively.

Antibacterial activity of plant extracts are well documented (Alicia, 1981). Vlietinek *et al*.(1995) screened about 100 medicinal plants used by traditional healers in Rwanda, for their antibacterial, antifungal and antiviral properties. The study showed that 45% of the plants were active against *Staph. aureus*, 2%, against *E. coli*, 16% against *Pseud. aeroqenosa* and 7%

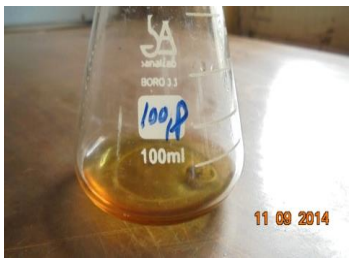
against *Candida albicans.* Moreover, about 27% of the plants tested exhibited antiviral properties.

Table (2) The effect of the different concentrations of the solvents on the fresh weight (gm) of both *A. niger* and *P. Italicum*.

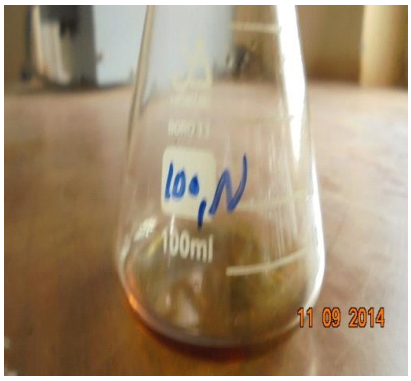
Fungi	Concentration				
	0%	25%	50%	75%	100%
A. niger	3.11	2.79	2.21	1.8	1.65
Pencillium	3.7	2.92	2.04	1.63	1

Table (3) The effect of the different concentrations of the solvents on the dry weight (gm) of both *A. niger* and *P. Italicum*.

Fungi	Concentration				
	o%	25%	50%	75%	100%
A. niger	1.9	1.1	1.2	0.9	0.8
pencillium	1.6	1.4	1.3	0.9	0.7



A
Control 100%



B
Control 100%

Plate (1): Different concentrations of pumpkin oil extrac on Fresh weights of the two fungi A= *P. italicum* and B= *A. niger*

Table (4). The effect of the extracts with diffeent solvents on *Staphylococous aureus* growth (inhibition zone)

Solvent	concentration			
	25%	50%	75%	100%
Hexane	8.5	19.2	21.1	26.5
Ethanol	6.5	9	11.5	14.2
Methanol	8	9.7	12	13.5
Water	7.5	11	19	13.6

Table (5) The effect of the different extracts with solvents on *E. coli* growth (inhibition zone)

Solvent	Concentrations			
	25%	50%	75%	100%
Hexane	8.5	11.2	13	17.4
Ethanol	6.5	8.3	9.5	12.7
Methanol	7	9	10	11.7
Water	7	9.5	10.4	12.1

A **B**

Plate (**2**): Different inhibition zones of *E. coli* (A) and *Staph. aureus* (B)

In Sumatra (Indonesia), 114 plant extracts were assayed for their antibacterial activity (Ahmed, 2002). About 82% of the extracts were active against *Staph. aureus* while 32% of them were active against *E. coli* . The extracts of plant leaves of *Tagetes minuta* were found to exhibit some activity against both Gram positive and Gram negative bacteria (Tereschuk *et al.*, 1997).

In Sudan many studies were carried out for testing the antimicrobial activity of some medicinal plants. Ahmed (2002) tested the extracts of 10 plants against Gram positive and Gram negative bacteria as well as *Candida albicans*. He found a marked effect against the Gram and positive *Staph. aureus* followed by *E. coli* and *Candida albicans*, respectively.

Plants may represent a potential source of antibiotics as evidenced by the huge number of studies dealing with antimicrobial activities (Alderman and Marth, 1976, Pelcazar, *et al.*, 1977 and Pratt, 1977). The use of plants and their extracts as remedies for curing many disease have stimulate studies for investigating the presence of effective antimicrobial substances in them (Ahmed, 1983, Abdel Daim, 2001, Sulieman *et al.*, 2008, Abdel-Rahim *et al*., 2010)

CONCLUSIONS

The physiochemical properties of the pumpkin seed oil tested in this study were almost similar to those reported for the same oil in Sudan and elsewhere. The pumpkin oil has an excellent antifungal properties, it almost completely inhibited mycelial growth of the two tested fungi (*Aspergillus niger* and *Penicillium italicum*). The oil was also found to has a good antibacterial activity. It formed a large inhibition zone on growth of the two tested bacteria (*E. coli* and *Staphylococcus aureus*).

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الملخص

نباتات القرع والكوسة تنمو في المناطق الدافئة الرطبة لكنها لا تتحمل الجليد. معظم القرع المستهلك في السودان كان ينمو في كردفان (غرب السودان) حيث وصلت النسبة المئوية الى (39.1% و 1–17% طن متري في الموسم 1980–1981) من الانتاج الكلى للخضروات والفاكهة المنتجة. استخدام النباتات ومستخلصاتها لمعالجة بعض الامراض حفزت الباحثين بدراسة المواد بها. تم في هذا البحث دراسة الخواص الفيزيائية والكيميائية والنشاط البيولوجي لزيت القرع باستخدام مذيبات مختلفة (هكسين– ايثانول – ميثانول – ماء) وذلك على نمو اثنين من الفطريات (*P. italicum* and *A. niger*). (*E.coli and Staph aureus*). استخدمت طريقة المنطقة المثبطة لاختبارات البكتيريا وطريقة وزن الميسوليوم لدراسة الفطريات. أشارت نتائج الاختبارات الفيزوكيميائية الثقل النوعي للزيت كان 9.7–7.97 وكان معامل الانكسار بين 1.465– 1.470 وكانت قيمة اليود 117.97 وكانت قيمة الأحماض الدهنية الحرة بين 0.2– 0.55 وقيم البيروكسيد في المعدل العام 7.92– 9.90 وكان متوسط قيمة التصبن 188.6. واشارت النتائج الى ان مستخلصات زيت القرع كانت فعالة ضد الفطريات حيث انخفض الوزن الرطب والجاف كثيراً على الرغم من أن الانخفاض كانت له فروقات معنوية فقط على التركيزات العالية ولكن اعطت التركيزات العالية فقط فروقات معنويه. تثبيط نمو البكتيريا كان أكبر من معاملة المقارنة. وكان الوزن الرطب للتركيزات العالية 1.0– 1.65 وللتركيزات المنخفضة كان 3.1–3.7 لكلا الفطرين (*P. italicum* and *A. niger*) بالتوالي. وكانت قيمة المنطقة المثبطة لنمو كلا البكتيريا أكبر بكثير من معاملة المقارنة. اثبتت النتائج ان مستخلصات القرع أكثر فعالية على البكتيريا مقارنه بالفطريات. واعطى مستخلص الهكسين منطقة تثبيط اكبر حوالي 26.5 مم للقطر، بينما اعطى مستخلص الاثانول منطقة مثبطة حوالي 14.2 مم للقطر. ومن الناحية الأخرى فقد أعطت كلا مستخلصات المثانول والماء مناطق مثبطة صغيرة 13.6 و13.5 مم للقطر على التوالي. ومع ذلك لابد من اجراء مزيد من دراسة الخواص الفيزيائية الكيميائية وكذلك لابد من دراسة تفصيلية للمواد المثبطة للفطريات والبكتيريا في أي دراسات لاحقه على زيت بذور القرع.