NUTRITIONAL, PHYSICO-CHEMICAL, ANTIMICROBIAL AND ANTIOXIDANT SCREENING OF SEED AND SEED OIL OF *CUCURBITA PEPO* GROWN IN KPK, PAKISTAN

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خلاصہ

مندرجہ بالا تحقیق میں صوبہ خیبر پختونخواہ، پاکستان میں اُگنے والی کدو کے بیج اور اس کے تیل کی غذائی ساخت، حیاتیاتی سر گرمیوں اور تکسیدی صلاحیت کو جانچا گیا۔ نتائج نے ظاہر کیا بیج کے حیاتین، معدنی راکھ، غذائی ریشہ، نشاستہ اور تیل کا تناسب خوردنی تیل کے لیے تجویز کردہ غذائی ساخت کے عین مطابق ہے۔ کدو کے بیج میں کافی مقدار کیلشیم (12.36± 0.8 ملی گرام)، لوہا (3.89 ± 4.8 ملی گرام)، زنک (11.39 ± 0.9 ملی گرام) پائے گئے جبکہ اس کے تیل میں غیر سیر شدہ فیٹی ایسڈز جیسا کہ لینولئیک ایسڈ (43.65 فیصد)، پالمیٹک ایسڈ (14.94 فیصد)، اولینک ایسڈ (19.95 فیصد) اور لائنولینک ایسڈ (5.22 فیصد) بھی کافی مقدر میں پائے گئے۔ تیل کے اعلیٰ آیوڈین ویلیو، سیپونیفیکیشن ویلیو، اور کم پر آکسائیڈ کا حامل ہے۔ اینٹی مائکرو بئیل اثرات نے ظاہر کیا کہ نا صرف تیل کے اندر مختلف اقسام کے بیکٹیریا اور پھپوندی کے خلاف مدافت کی صلاحیت موجود ہے بلکہ ڈی۔ پی۔ ایچ فری ریڑیکل عمل کے ذریعے تکسیدی عمل کو روکنے اور ختم کرنے کی اہلیت بھی رکھتا ہے۔ موجودہ تحقیق نے ظاہر کیا کہ علاقائی سطح پر کاشت کی جانے والی کدو کے بیج اعلیٰ معیار کی غذائی ساخت رکھتے ہیں جس کو نا صرف بطور غذا استعمال کیا جاسکتا ہے بلکہ ان کو دواسازی اور صنعتی پیمانے میں مختلف اشیاء میں بھی استعمال کیا جاسکتا ہے۔

Abstract

The present study focused on the nutritional composition and biological activity of *Cucurbito pepo* (Pumpkin seed) seed and seed oil. Data indicated the contents of seeds protein, ash, fiber, carbohydrates, and percent oil fell well within the ranges specified for edible seeds. The seeds were found to be rich source of Calcium (12.36 ± 0.08 mg/100g)), iron (3.89 ± 2.8 mg/100g)), and zinc (11.39 ± 0.9 mg/100g) indicating its utility as a dietary mineral source. Seed oil was found to be rich in unsaturated fatty acids (67.33%) and was found to be a good source of lenoleic acid (43.65%) , followed by palmitic acid (14.94%), Oleic acid (19.95%) and lenolenic acid (5.22%), respectively. The higher Iodine value, saponification value, the low peroxide value and acid value and other physic-chemical parameters were indicators of the vast utility of the oil. The antimicrobial effects of pumpkin seed oil showed 51.04%, 69.61%, 79% and 85.93% activity against *paecilomyces, pythillum, Rhizopus stolorife*r and *Trichoderma harzianum,* respectively. The oil also showed good antibacterial properties (8.6 – 0.16mm) against bacterial strains and a good antioxidant DPPH radical scavenging activity in different oil: n-hexane and oil: Methanol concentration ratios. The study showed that pumpkin seed has a good nutritional, pharmaceutical, and industrial utility.

Introduction

Cucurbitaceae, Cucurbita pepo, (commonly known as Pumpkin) is a medium sized *cucurbit* grown for its fruits and edible seeds and used as food and in herbal formulations. (Elinge *et al*.,2012). *Cucurbita Pepo* has wider variation and greater utility in diet and edibility (Gemrot *et al*., 2006). The quality of the seed oil has been shown to exhibit less deterioration (Murkovic *et al*., 1999). A large body of work has been done on the composition of seed and oil characteristics of the pumpkin seeds from different regions (Lazos 1986, Stevenson *et al*., 2007). Raw and roasted pumpkin seeds are used as snack items around the world. The seed kernel is used as flavor enhances, as a nutrient supplement and as a functional agent (Tsakins *et al*., 1997; El-Adawy and Taha, 2001).

Vegetable oil is an integral part of human diets. Although broad range of edible oils is available these conventional sources may become insufficient to meet the increasing demands (Idouraine *et al*., 1996). Several

studies have shown that seed oil from *curcurbitaceae* can be a potential source of edible oils in the future (Esuoso *et al*., 1998).

The dark green colour pumpkin seed oil is a common salad oil and is naturally rich in proteins and vitamins. The oil is also a rich source of Omega-6 fatty acid, which has a number biological applications such as being anti inflammatory, hypolipidemic, and helps in the maintenance of healthy blood vessels, nerves and tissues (Makni *et al*., 2011). These qualities of the oil are attributed to its inherent triterpenes, lignins, phenolic compounds and phytosterols, caratenoids, and tocopherol, (Fu *et al*., 2006). The oil is also a rich source of Palmitic, stearic, oleic and lenoleic acids. The seed has been used traditionally in herbal, therapeutic and clinical applications, i.e. as a deworming agent, diuretic agent and as a nerve tonic (Ravishankar *et al*., 2012).

Inter varietal and Intraregional differences in plants quality are natural phenomena. The current study aimed to determine the nutritional composition of the seeds, physicochemical and antimicrobial and antioxidant activity of the crude oil of *C. pepo* grown in KPK region of Pakistan.

Materials and Methods

Pumpkin seeds Procurement

Ripened fruits of a local variety of *Cucurbita pepo* were purchased from the local vegetable market. The seeds were separated and were washed several times with water to remove plant and other foreign material. Later the seeds were dried in an electric oven (at 101 °C) until constant weight was achieved. The dried seeds were stored in airtight jars for further analyses.

Oil Extraction

Dried seeds were grinded in an electric grinder. The ground seed powder was soaked in n-hexane for 24 h. After soaking, the extract was filtered through filter paper and was then concentrated and distilled in rotary evaporator under reduced pressure at 40-45^{o}C. Percent oil was calculated and the cakes were directly used for quality parameters.

Proximate Composition and Mineral Content Determination

Using AOAC official methods (1990) total crude protein content of the seed residues/cake was estimated by a micro Kjeldhal apparatus. Crude oil was estimated by Soxhlet method while crude fiber and ash contents were determined by the ISO methods. In order to perform minerals' analysis samples were first subjected to acid digestion and later were analyzed by Atomic Absorption Spectrophotometric methods (AOAC, 2012).

Oil Analysis

The physical and chemical parameters of the crude oil were determined as per AOAC official methods (AOAC, 1990). Perfective index, density, specific gravity, surface tension, acid value, free fatty acid value, saponification value iodine value, color and peroxide value were carried out.

Fatty acids Analysis

Preparation of FAMES

About 25-40 mL of oil sample was taken in a FAMEs tube and to it 1.5 mL methanolic Sodium hydroxide (0.5 N) was added. The tubes were Stoppard with screw capes. The mixture was heated in a boiling water bath for 05 minutes. Tubes were cooled to room temperature and 2.5 mL BF_3 (10 % in MeOH) was added. Tubes were again heated in boiling water bath for half an hour. Cooled at room temperature and 5 mL brine solution + 1 mL n-hexane was added. The tubes were shaken vigorously on vortex and then allowed the layers to separate. The upper (hexane) layer was taken through pasture pipette. About 1 mL n-hexane was added and these steps were repeated thrice. The volume was adjusted to 2 mL and was filtered through 45 μm membrane filter. The filtrate was transferred to GC vial for further injection into GC-MS.

Identification of fatty acids by GC-MS

The methyl esters of the oil were analyzed for the respective fatty acid composition by Gas Chromatography Mass Spectrometry. The equipment used for this purpose was Shimadzu GC-MS- QP 2010 Plus using a capillary column TRB FRAP (30 mm x 0.25mm). The programming temperature of the column oven was set as 50 ^{o}C – 220 ^{o}C with rise of 5 ^{o}C/ min. Helium was used as the carrier gas and its total flow was adjusted to 77.1 mL/min while column flow was 3.29 mL/min at split ratio of 20.0. The temperatures of injector, interface and ion source were set at 240 ^{o}C, 240 ^{o}C and, 250 ^{o}C, respectively. The peaks were identified by comparison of their retention time with those of the standard methyl esters (FAMEs standard mix, 37 components, Sigma Aldrich) analyzed under the same conditions.

Antimicrobial Assay

The extracted oil from pumpkin seeds was tested against different strains for anti-infective potential. Four types of bacteria; *Bacillus subtitles, Escherichia coli, Proteus mirabilis,* and *Xanthomonas campestris* while four pathogenic fungi including *Paecilomyces lilacinus, Phytium ultimum, Rhizopus stolonifer,* and *Trichoderma harzianum* were tested. The pure microbial strains were obtained from Department Agricultural Chemistry lab, KPK Agriculture University, Peshawar. Agar Well Diffusion method was used for fungi and Streaking method was used for bacteria.

DPPH Radical Scavenging Activity

DPPH radical scavenging activity was performed by following the method of Rauf *et al.*(2013). About 25mg of oil was dissolute in distilled methanol and was diluted up to 50 ml. from the stock solution 10, 20, 40, 60, 80, 100, mg/ mL were prepared. 5 ml of each solution was added along 1 mL of 0.01 M of DPPH solution in a test tube. They were kept in a dark for test minutes along a control (5 methanol+ 1 mL DPPH solution). After the incubation for antioxidant activity optima U-V- visible spectrophotometer was used at wave length of 517 nm. The experiments were performed in three replicates. Quercetin was used as a standard. Percent radical scavenging activity was calculated as.

% DPPH= Control absorbance- extract absorbance × 100/Control absorbance

Results and Discussion

Proximate and Mineral Composition

Results of the chemical composition of pumpkin seed are presented in Table – 1. The seeds contained 7.9 ± 0.4% moisture, 31.20 ± 0.72% oil, 27.41 ± 0.72% proteins, 4.96% fiber, 2.25 ± 0.04% ash, and 26.28 ± 1.79 carbohydrates. The moisture content indicated the seeds to be safe for longer duration and lower susceptibility to microbial attack as suggested by Ajay (2006). The percent oil content of the seed fall well within the ranges reported by Stevenson *et al* (2007). However were lower than those reported by Murkovic *et al*. (1999) and El-Abawy and Taha (2001) but higher than that reported by Younis *et al*. (2000). The protein content are in good agreement with the findings of Al-Khalifa (1996), Achu *et al* (2005) and Arabili *et al* (2011). Total carbohydrate content calculated is higher than that found by Lazos (1986). The total Ash content was closer to the values reported by Younis *et al* (2000). Idoeraine *et al*. (1996), Alfawaz (2004), and Maryam *et al*.(2012). The mineral concentration (mg/100 on dry weight basis) of the pumpkin seed showed it being a good source of dietary minerals particular manganese (0.97± 1.3mg/100), Zinc (11.39 ± 0.9) Copper (1.34 ± 1.57) and a fair source of calcium (12.36 ± 0.08) and Iron (3.89 ± 2.8). The results were in close agreement to the values reported by Elinge *et al*. (2012) and Glew *et al*. (2006).

Table 1: Proximate and Elemental Composition of *Cucurbita pepo* Seeds.

Parameter	Content*
Proximate values (g/100g)	
Moisture	7.09 ± 0.4
Percent oil	31.20 ± 1.31
Crude protein	27.41 ± 1.72
Crude fiber	4.96 ± 1.20
Ash	2.25 ± 1 0.4
Carbohydrates	26.28 ± 1.79
Mineral (mg/100mg)	
Calcium	12.36 ± 0.08
Iron	3.89 ± 2.8
Copper	1.34 ± 1.57
Zinc	11.39 ± 0.9
Manganese	0.97 ± 0. 13

* Means of triplicate analysis.

Physico-chemical properties of the crude oil are presented in Table 2. The solvent extracted oil content of the *C. pepo* was found to be 31.20%. The value fell within the oil range of 9.8-to-51.2% of the different varieties reported by Stevenson *et al* (2007). However this percent value is much lower than reported for European varieties by Murkovic *et al*. (1999), Egyptian varieties by El-Adawy and Taha (2001) but higher from African varieties reported by Younis *et al*. (2000) reported to be 54.9%, 51.0%, and 21.9 - 35.6 % respectively. This variability in the seed oil content can be attributed to genetic diversity, differences in climatic temperature,

humidity, soil nature and other climatic factors pertaining to the site of cultivation. It has been reported that seed oil content tends to be lower being grown at low altitudes where temperature is high. The brown with an intense green tint of the *C. Pepo* oil are in agreement with the findings of Ardabili *et al*. (2011).

Table -2: Physicochemical Properties of Pumpkin seed (*C. Pepo*) Oil.

Parameters	Content*
Percent oil (%w/w)	31.20%
Color	Green brown
Refractive index at 25^0C	1.4696 ± 0.004
Density (20^0C Kg/m^3)	0.9182 ± 0.018
Specific gravity	0.906 ± 0.7
Surface tension	2.75 ± 0.08
Viscosity (21^0C)	72 ± 0.58
Kinematic viscosity (mm^2/Sec)	32.22± 0.178
Acid value (mg KoH/g)	1.6 ± 0.8
Free fatty acid (g/100g)	0.8 ± 0.91
Saponification value (mg KoH/g of oil)	185.3 ± 1.8
Iodine value (g of I_2 /100g)	132.7 ± 0.5
Peroxide value (meq of O_2 Kg of oil)	1.5 ± 0.5

*Means determined from triplicate analysis.

Refractive index was 1.4696 which is similar to the findings of Younis *et al*. (2000). Density of the oil from *C. Pepo* was 0.9182 at 20^0C Kg/$m^3$and this value is in agreement with the findings of Tsakins *et al* (1997). Specific gravity (0.906) of the oil is also the same as being reported by El-Adawy and Taha (2001). Niyam *et al*. (2009) reported similar results for the surface tension (2.75) and viscosity (72 at 21^0C. Bello *et al* (2009) also reported similar results for kinematic viscosity of *C. Pepo* seed oil. The acid value of the extracted oil was 1.6mg kOH / g which is higher than being reported by Tsaknis *et al*. (1999) as 0.97±0.075. Similarly the free fatty acid value (0.8g / 100g) was lower than 10419/100g of El-Adawy and Taha (2001). Saponification value of the current C. Pepo seed oil was 185.3mg KoH/g was higher than reported by Younis *et al*. (2000) and lower than the findings of El-Adawy and Taha (2001) being 132.33 and 206 mg KOH/g respectively. This value is also comparable to those of other common vegetable oils indicating the presence of high content of high molecular weight tri acylglycerols (Kailas, 2012). The Iodine value fall inside the interval range of the value mentioned by Tan *et al* (2002). Peroxide value (1.5 meq of O_2 / Kg of oil) is much lower than being reported by Ec-Adawy and Taha (2001) and Tsakins *et al*. (1997) as 3.60 and 9.20 meq of O_2/Kg respectively. This value is towards the lower end as defined by Codex Alimentarious Commission (1982) indicating the oil being of good quality.

Table -3: Fatty acid profile of C*ucurbita pepo* seed Oil.

Fatty Acids	% Concentrations*
Hexanoic Acid C_6:0	0.074±0.05
Caprylic Acid C_8:0	0.014±1.23
Capric Acid C_{10}:0	0.014±1.23
Lauric Acid C12:0	0.035±0.06
Myristic Acid C_{14}:0	0.274±0.13
Palmitic Acid C16:0	14.942±0.90
Palmiteic Acid C_{16}:1	0.235±0.28
Stearic Acid C_{18}:0	6.474±0.82
Oleic Acid C_{18}:1	19.945 ±0.06
Lenoleic Acid C_{18}:2	43.650±0.90
Octadecadienoic acid C_{18}:2t6	0.403±0.32
q-Linolenic acid C_{18}:3n6	5.218±0.82
Linolenic acid C_{18}:3n3	0.282±1.22
Arachidic Acid C_{20}:0	0.443±0.90
Behenic Acid C22:0	0.121±0.13
Liqnoceric acid C_{24}:0	0.06±0.02
Total saturated acid	22.32±0.02
Total unsaturated acid	67.733±0.82
16 / 18 ratio	0.2000±1.22
20 – 24 ratio	0.6374±0.406

*means of duplicate analysis

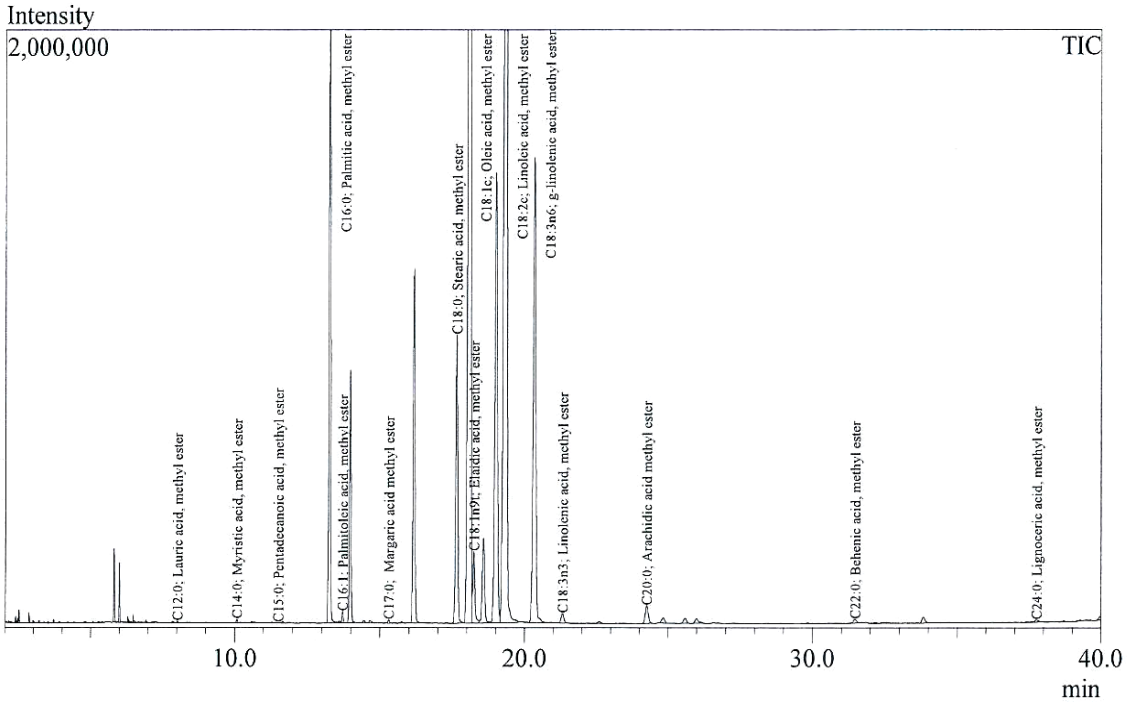

Fig.1. GC MS Chromatgram of Fatty acids from *Curcurbita pepo* Seed oil

Fatty acid composition of the pumpkin seed oil is presented in Table 3 and Figure 1. Five major fatty acids contributed to the makeup of the oil; lenoleic acid, oleic acid, palmitic acid, stearic acid, and *a* lenolenic acid. The oil contained 22.32% total saturated acid the major being palmitic acid (14.942%) and stearic acid (6.474%). The total unsaturated fatty acids content was 67.733%. The main unsaturated fatty acids were linoleic acid (43.650%) followed by oleic acid and *a* – linolenic acid (19.945%, 5.218%) respectively. The 16 /18 ratio was 0.20 while 20-24 ratio was 0.638. The % saturated fatty acids concentration is in agreement with the findings of Stevenson *et al* (2007). The percentage of linoleic aid and oleic acid are closer to the values reported by Lazos (1986). El-Adway and Taha (2001) of pumpkin seed oil (43.1 – 55.6%). However Ardobili *et al*. (2011) reported lower % concentration of lenoleic acid (39.84%) and higher % concentration of oleic acid (38.42%). The linolenic acid percentage was quite lower (0.282%) along with other fatty acids which are similar to the results of Stevenson *et al*. (2007).

Radical Scavenging Activity

The scavenging activity of the Methanolic extract of the pumpkin seed oil is depicted in Figure 2. The DPPH scavenging activity was comparable to that of Standard Quercetin at 80µg/mL concentration. This reducing activity may be attributed to the high content of tocopherol and other inherent total phenolic compound making the oil more acceptable lending high stability and improved safety making it more suitable for pharmaceutical purposes and requiring little addition of synthetic antioxidants during the refining and processing of the oil. Results are comparable to the findings of Amutha *et al*. (2014) and Sigar *et al*. (2008).

ANTIMICROBIAL ACTIVITY:

The antimicrobial activities of Pumpkin seed oil in five different concentration of oil are presented in Table – 4 and Table – 5. As shown the antibacterial activity of the oil was:

Escherichia coli > Bacillus subtilus >Xanthomanas *campestris* > *Proteus mirabilis*

As the concentration of oil increased in n-hexane inhibitory activity of the oil increased. Results showed that the values obtained for E-Coli were closely related to the findings of Willis *et al*. (2009) and Abd EL-Aziz and Abd EL-Kalek (2011) who showed 1-to-11mn inhibitory growth.

Similarly the antibacterial property of the present oil was in agreement to that of the findings of Ravishankar *et al*. (2012) and AbdEL-Aziz and Abd EL-Kalek (2011). Kirbaslar *et al*. (2012) and Ravishankar *et al*. (2012) also reported similar inhibitory activity against both proteins and xanthomanas Strains. Results of the standard against Eschericlia Coli (10.36mm), Xanthomanas (10mm), Bacillus Subtilis (6.46mm) and Proteus (6.46mm) respectively against zero growth in the control.

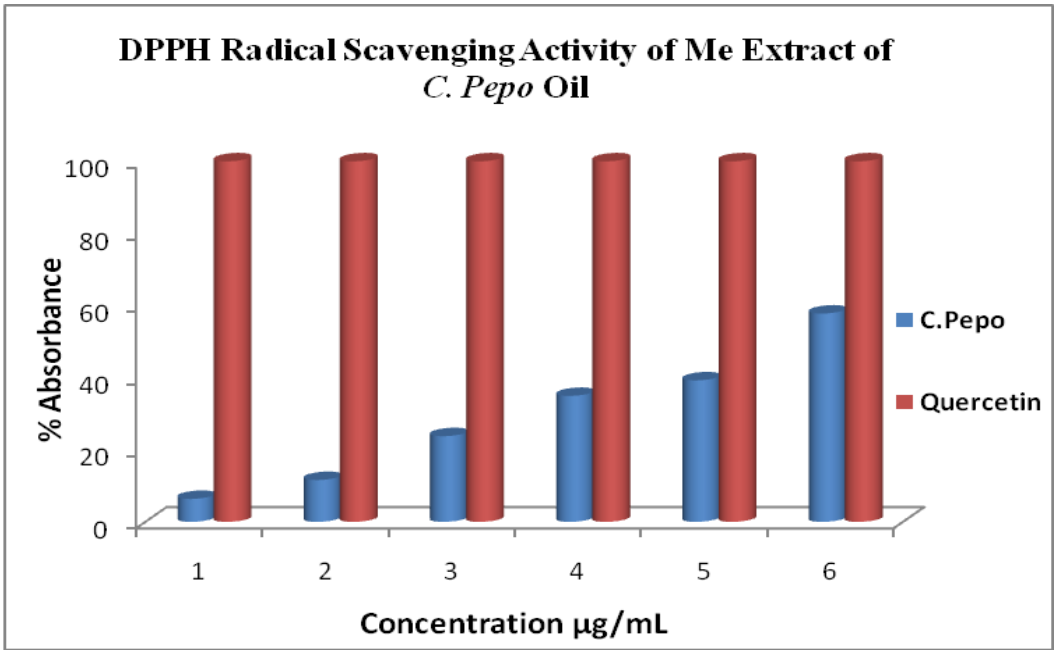

Fig.2, DPPH Radical Scavenging Activity of oil in Different Concentrations

The antifungal activity of *cucurbita pepo* seed oil against the pathogenic strains was in the following manner in 8:0 mg/µL concentration:

Rhizopus stolonifer > Trichoderma herzianum> Pythium ultimum> Paecilomyces lilacinus

The findings are closely related to the evidences reported by Vassiliou *et al*. (1998) for *Paecilomyces* and *Phythium*. Results obtained for the zone of growth of *Rhizopus* and *Trichoderma* in Pumpkin seed oil are similar to the findings of Abd EL-Aziz and Abd EL –Kalek (2011). The current study showed that local variety of *C.pepo* oil posseses a good antifungal potential in different concentrations with n-hexane.

Table – 4: Anti bacterial Potential of the Pumpkin Seed Oil.

Concentration mg/µL	Zone of inhibition (mm)			
	Escherichia coli	*Bacillus subtilis*	*Proteus Mirabilis*	*Xynthomonas Campestris*
2:1	1.2 ± 0.668	0.53 ± 0.368	0.076 ± 0.087	0.6 ± 0.402
4:4	2.2 ± 1.023	2.03 ± 0.047	0.16 ± 0.047	2.1 ± 0.081
5:3	4.43 ± 0.368	2.86 ± 1.342	0.53 ± 0.286	3.26 ± 0.20
6:2	6.8 ± 0.623	5.96 ± 0.047	1.13 ± 0.262	3.3 ± 0.748
8:0	8.6 ± 2.294	6.56 ± 0.047	2.46 ± 0.368	5 ± 0.81
Standard	10.36 ± 0.44	8 ± 0.81	6.46 ± 0.368	10 ± 0.081
Control	0	0	0	0

Table – 5: Antifungal Activity of Pumpkin seed Oil.

Concentration mg/µL	Zone of growth (mm)			
	Paecilomyces lilacinus	*Phytium Ultimum*	*Rhizopus Stolonifer*	*Tricoderma Harzianum*
2:1	17.8 ± 0.216	18.96 ± 0.776	21.06 ± 0.754	24.9 ± 0.531
4:4	12.5 ± 0.408	16.5 ± 0.079	20.7 ± 0.081	23.1 ± 0.941
5:3	10.6 ± 0.081	15.9 ± 0.047	20 ± 0.006	20.86 ± 0.65
6:2	9.5 ± 0.081	14.3 ± 0.059	18 ± 0.006	15.7 ± 0.26
8:0	8.5 ± 0.081	9.6 ± 0.0810	17.16 ± 0.124	12.46 ± 0.339
Standard	0	0	0	0
Control	19.3 ± 0.816	25.8 ± 0.163	22.7 ± 0.880	26.6 ± 0.432

Conclusion

Local pumpkin seed and seed oil possess good physico- chemical, nutritional, and antimicrobial potential. The local variety of *C.pepo* can serve as better substitute as an edible oil fats besides good pharmaceutical, cosmetic, and industrial utility.

References

A. O. A. C. (2012). International. Official Methods of Analysis of Association of the Official analytical International OMA. 19th ed. p24-52. Retrieved www.aoac.org/..../AOAC/ …/ Official Methods-of – Analysis/AOAC…/OM

A.O.A.C. (1990). Official Methods of Analysis of the Association of the Official Analytical Chemists. 15th Ed. Suite 400, 2200 Wilson Boulevard, Arlington, Virginia, 22201, USA. p951-972

Abd El-Aziz. A.B and Abd El-Kalek. H.H. (2011). Antimicrobial proteins and oil seeds from pumpkin (*Cucurbita moschata*). *Nature and Science*. 9(3): 105-119

Achu, M.B., Fokou, E., Tchiegang, C., Fosto, M., and Tchouanguep, M.F. (2005). Nutritive value of *Cucurbetaceae* oil seeds from different regions in Cameroon. *African J Biotech*; 4: 1329-1334

Alfawaz, M.A. (2004). Chemical Composition and oil characteristics of pumpkin (*Cucurbita maxima*) Seed kernels. Res Bult (129), *Food Sci Agric Res.* Center King Saud Univ. 5-18.

Al-Khalifa. A. (1996). Physicochemical Characteristics, fatty acid composition and lipoxygenase activity of crude pumpkin and melon seed oil. *Journal of Agricultural and Food Chemistry*. 44: 964-966

Amutha, M., Geetha, A., and Monika, P. (2014). Determination, Metabolites, LD_{50} value and antioxidant activity of seed extract of *Cucurbita Pepo. Linn*. *Asian J Pharm Clin Res;* 7(3): 173-177.

Ardabili. G.A., Farhoosh. R. and Khodaparast. H.M. (2011). Chemical Composition and Physicochemical Properties of Pumpkin Seeds (*Cucurbita pepo* sub sp. *pepovar.styriaka*) grown in Iran. *J Agri Sci Tech*. 13:1053-1063

Bello, E.I., Anjorin, S.A., and Agge, M. (2009). Production of biodiesel from fluted pumpkin (*Telfairia accidentalis Hook F*) seed oil. *International Journal of Mechanical Engineering*; 2(1): 22-31.

Codex Alimentarius Commission.(1982). Recommended Internal Standards edible fats and oils. FAO/WHO, Italy, Rome. 11(1).

El-Adway, T.A and Taha, K.M. (2001). Characteristics and composition of water melon, pumpkin, and paprika seed oil and flour. *J Agri Food Chem*; 49: 1253-1259

Elinge.C.M., Muhammad.A., Atiku.F.A., Itodo.A.U., Peni.I.J., Sanni. O.M., and Mbongo .A.N. (2012). Proximate, mineral, and antinutrient composition of pumpkin (*Cucurbita Pepo L*) seed extract. *International Journal of Plant Research*. 2(5): 146-150

Esuoso, K., Lutz, H., Kutubuddin, and M., Bayer, E. (1998). Chemical composition and potential of some underutilized tropical biomass. I: Fluted pumpkin (*Telfairia occidentalis*). *Food Chem*;61; 487-492

Fu, C. L., Shi, H., and Li, Q.H. (2006). A review on pharmacological activities and utilization technologies of pumpkin. *Plant Food Hum Nutr.* 61: 73-80

Gemrot, F., Barouh, N., Vieu, J. P., Pioch, D. and Montet, D. (2006). Effect of Roasting on Tocopherols of Gourd Seeds *(Cucurbita pepo*). *Gras. Aceit.* 57: 409–414.

Glew, R.H., Glew, R.S., Chuang, L.T., Huang, Y.S., Millson, M., Constans, D., and Vanderjaqt, D.T.(2006). Amino acids, mineral and fatty acid content of pumpkin seed (*Cucurbita spp*) and *Cyoerus esculentus* nuts in republic of Niger. *Plant Food Hum Nutr.* 6(2); 51-6

Idouraine, A., Kohlepp, and E.A., Weber, C.W. (1996). Nutrient constituent from eight lines of naked seed squash (*Cucurbita pepo L*). *J Agrc Food Chem*. 721-724

Kailas, M.T., Mahajan, D.T., and Masand, V.H. (2012). Study on physicochemical properties of vegetable oils and their blends use as a possible ecological lubricant. 2912. *Journal of Chemical and Pharmaceutical Research*; 4(12): 5139-5144.

Kirbaslar, G.F., Turker. G., Ozosy-Gunes, Z., Unal, M., Dulgar, B., Ertas, E., and Kizilkaya, B. (2012). Evaluation of fatty acids composition, antioxidant and antimicrobial activity, mineral composition and caloric value of some nuts and seeds from Turkey. *Records of Natural Products*; 6(4): 339-349.

Lazos, E.S. (1986). Nutritional, fatty acid and oil characteristics of pumpkin and melon seeds. *J Food Sci*; 51;1382-1283

Makni, M., Fetoui, H., Gargouri, K.N., Garoui, M.E., and Zeghal, M.M. (2011). Antidiabetic effect of flax and pumpkin seed mixture powder: Effect on hyperlipidemia and antioxidant status in diabetic rats. *Journal of Diabetes and its Complications.* 25: 339-345

Maryam.J., Goli.S.A.M., and Rahim. M.M. (2012). The chemical composition of the seeds of Iranian pumpkin cultivars and physicochemical characteristics of the oil extract. *Eur j Lipid Sci Techno.* 144:161-167

Murkovic, M., Hillebrand, A., Draxl, S., Wrinkler, J., and Pfannhauser, W. (1999). Distribution of Fatty acids and Vitamin E content in pumpkin seed (*Cucurbito Pepo L*) in breeding lines. *Acta Hortic.* 492: 47-55

Niyam, K.L., Tan, C.P., Lai, O.M., Long, K., and Che Man, Y.B.(2009). Physicochemical properties and bioactive compound of selected seed oil. *LWT-Food Science and Technology*; 42: 1396-1403.

Rauf, A., Khan, R., and Muhammad, N. (2013) Antioxidant studies of various solvent fractions and chemical constituents of *Potentilla Evestita. The Wolf African Journal of Pharmacy and Pharmacology.* 7(39): 2707-2710

Ravishankar, K., Kiranmayl, G., Reddy, A.G., Sowjanya, V., Sainadh, B., Durga, l., Prasad, S., Swaminaidu, P. and Prasad, T. (2012).Preliminary phytochemical screening and in- vitro antibacterial activity of *Cucurbita Maxima* extract. *IJRPC.* 2(1): 2231-2781

Sigar, A.,Nogala-Kalucka, M., and Lampart-Szczapa,E. (2008). The content and antioxidant activity of phenolic compounds in cold pressed plant oils. *Journal of Food Lipids.* 15(2): 137-49.

Stevenson, D.G., Eller, F.J., Wang, L., Jane, J.L., Wang, T., and Inglett, G.E. (2007). Oil and tocopherol content and composition of pumpkin seed oil in 12 cultivars. *J Agric food Chem* 55:4005-4013

Tan, C.P., Che Man, Y.B., Selamat, j., and Yusoff, M.S.A. (200). Comparative studies of oxidative stability of edible oils by differential scanning calorimetry and oxidative stability index methods. *Food Chem*; 76: 385-389.

Tsaknis, J., Lalas, S., and Lazos, E.S.(1997). Characterization of crude and purified pumpkin seed oil. *Gras. Asceit.* 48: 267-272

Vassiliou, A.G., Neumann, G.M., Condron, R., and Polya, G.M. (1998) Purification and mass spectrometry-assisted sequencing of basic antifungal proteins from seeds of pumpkin *(Cucurbita maxima). Plant Science Limerick,* 134(2): 141-162

Willis, l.M.B., Shukitt-Hale, B., and Joseph, J.A. (2009). Modulation of cognition and behavior in aged animals: role of antioxidants and essential fatty acids rich plant foods. *American Journal of Clinical Nutrition.* 89(5): 1602-1606.

Younis, Y.M.H., Ghirmay, S., and Al-Shihry, S.S. (2000). African Cucurbita Pepo L: Propeties of seed and variability in fatty acids composition of seed oil. *Phytochem.* 54: 71-75