Journal of Agricultural Science and Food Technology
Vol. 3 (6), pp. 103-109, November, 2017
ISSN: 2465-7522
Full Length Research Paper
http://pearlresearchjournals.org/journals/jasft/index.html

Evaluation of Antioxidant potential and comparative analysis of Antimicrobial activity of Various Extracts of *Cucurbita pepo* L. Leaves

Parsa Dar[1*], Muhammad Farman[1], Amara Dar[2], Zaman Khan[3], Rebecca Munir[1], Amina Rasheed[1] and Usama Waqas[4]

Accepted 12 November, 2017

[1]Department of Chemistry, Quaid-i-Azam University, Islamabad, Pakistan.
[2]Center for Undergraduate studies,University of the Punjab, Lahore 54590, Pakistan.
[3]University institute of medical laboratory technology, University of Lahore 54590, Pakistan.
[4]Government College University Lahore 54000, Pakistan.

ABSTRACT

"Thy food is thy medicine" this statement led us to explore the antimicrobial and antioxidant potential of *Cucurbita pepo* L. The plant belongs to family Cucurbitaceae. Cucurbits have a lot of folkloric medicinal uses. In this study, *in vitro* spectroscopic protocols were executed for the determination of total phenolic content, the concentration of flavonoids, total antioxidant capacity as well as free radical scavenging potential of leaves extract of *Cucurbita pepo* L. (CPL) in solvents i.e., n-hexane, chloroform, ethyl acetate, n-butanol, ethanol, and water. Ethyl acetate extract of CPL. exhibited the highest flavonoid concentration, whereas *n*-Butanolic extract was found to contain highest total phenolic content and both extracts also displayed a strong antioxidant activity. The antioxidant activity of extracts was expressed as percentage of DPPH radical inhibition and IC_{50} values (µg/ml). A significant linear correlation was observed between the values for the total phenolic content, flavonoid concentration, phosphomolybdate assay and percentage antioxidant activity of all the extracts. The higher concentration of phenolic compounds was inferred to be a major contributor of antioxidant activity. Antimicrobial potential of various extracts of CPL leaves were explored. Aqueous, n-Butanolic and ethyl acetate extracts were found active against Bacillus subtilis, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Therefore, *Cucurbita pepo* L. *CP*L leaves can be considered as an auspicious candidate for natural plant source of especially antioxidants and antimicrobial agents.

Keywords: *Cucurbita pepo* L., Total phenolic content, Flavonoids, Total flavonoid content, DPPH, antimicrobial agent.
Corresponding author email id: parsadar@yahoo.com. Mobile.+92 3355913432.

INTRODUCTION

The Cucurbitaceae family also referred as cucurbits, melons and pumpkins consist of approximately 130 genera and 800 species, which are cultivated worldwide and according to archeologists, it is one of the world's earliest domesticated species (Martha and Gutierrez, 2016; Adam et al., 2011). Different vegetal parts of *Cucurbita pepo* plants such as flowers, fruit, seeds and young leaves are used for eating purpose. Seeds, leaves, sap, and pulp have long been used for medicinal purposes including treatment of intestinal worms, urinary issues, and poultices for burns. The vines and fruit are used as food for livestock, and gourds used for a vast array of ornamental, traditional and functional purposes (http://eol.org/pages/584410/details; Zuhair et al., 2000). Different pharmacological studies revealed that *Cucurbita pepo* has a cure for cancer and hypertension-related

ailments (Jian et al., 2005; Nawirska et al., 2013). These species have been reported to possess antidiabetic (Boaduo et al., 2014), anti-inflammatory and antioxidant potential (Martha and Gutierrez, 2016; Berdick, 1972).The seeds oil used as a base, antioxidant, antibacterial and ingredient in cosmetic, pharmaceutical and food industries (Gunstone, 2008; Hammer et al.,1999).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; Noumedem et al.,2013). The use of antioxidants is very indispensable to scavenge different free radical species, cause cell damage and that may lead to ruinous health disorders and cancer. Vegetables and fruits are considered as rich dietary sources of natural antioxidants. Due to afore said properties, the plant extracts of *Cucurbita pepo* caught our research interest. Antimicrobial studies have been carried out on fruit and leave methanolic extracts (Dubey et al.,2010; Slinkard and Singleton, 1977).The present study is, therefore, an attempt to have acomparative analysis of antimicrobial activity and antioxidant potential of various extracts of CPL leaves.

MATERIALS AND METHODS

Chemicals

All the chemicals used were of analytical grade. Ammoniummolybdate, Aluminium chloride, Ascorbic acid DPPH, Folin Ciocalteu reagent, Gallic acid, Methanol, Quercitin Sodium carbonate, Sodium hydroxide, Sodium nitrate, Sodium phosphate, Sulfuric acid. The chemicals and solvent used in the present work were purchased from Sigma Aldrich, Germany.

Test microorganisms

Different clinically important bacterial strains such as *Pseudomonas aeroginosa, Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and a fungal strain *Candida albicane*were used to explore the potential antibacterial and antifungal potential of CP*L* leaves extract. The test microbes were isolated from industrial waste waters of different localities *viz*. Sattokatla drain of Kot Lakh pat, District Lahore, and ChotiDeg, Degnullah, Bhednullah of District Sheikhupura, Punjab, Pakistan.

Collection and preparation of the plant extract

The CPL plant was collected from District of Pakpattan, Pakistan. Leaves were dried under shade for two weeks. The dried leaves were ground. 30 g of ground leaves were soaked in 250 mL of n-hexane, chloroform, ethyl acetate, n-butanol, and water separately. Filtration was carried out. This process was repeated twice to ensure the maximum extraction. The extract was concentrated under reduced pressure on a rotary evaporator (BüchiiRotavapor R-200, Switzerland) and the leaves extract was obtained in semi-solid form.

ANTIOXIDANT PROPERTIES

Total phenolic content

Total phenolic contents (TPCs) of various extracts were estimated by the method proposed by Slinkard and Singleton (1977). The sample of each extract was prepared by taking 4.3 mg extract in 10 mL of ethanol and irradiated with ultrasonics for 5 min to obtain a homogenized solution. Later, 0.3 mL was taken in a test tube and 1 mL methanol; 3.16 mL of distilled water; 0.2 mL of Folin-Ciocalteu reagent were added. The test tube mixture was then incubated for 8 min at room temperature, thereafter 0.6 mL solution (10%) of sodium carbonate was added in a test tube, covered with aluminum foil and incubated in hot water bath for half an hour at 40°C. An equal volume of methanol was used to prepare the blank solution by the same procedure used for the preparation of the sample. The absorbance of all the samples was determined using a UV visible spectrophotometer (UV-1700 Shimadzu) at 765 nm.

Total flavonoid content

The total flavonoid content (TFC) was determined using a reported protocol by sahreen etal. (2011). A clear solution was obtained by taking 0.3 mL extract (0.3 mg/mL in ethanol) and 3.4 mL of aqueous ethanol (30%) in a test tube. Then 0.15 µL of aqueous sodium nitrite solution (0.5 M) was added followed by 0.15 µL aluminum chloride solution (0.3 M). After a time of 5 min, 1 mL NaOH solution (1 M) was added and the contents were mixed together before measuring its absorbance using UV visible spectrophotometer (UV-1700 Shimadzu) against a blank sample at 506 nm. The blank sample was prepared using the same procedure. In preparing the blank sample an equal volume of methanol was used to replace the plant extract.

Total antioxidant capacity

The total antioxidant capacity of all the extracts was estimated using a protocol devised by Prieto et al. (1999). An aliquot of 0.2 ml (500 µg/ml) of the sample solution was mixed with 2 ml of the reagent solution (28 mM sodium phosphate, 600 mMsulphuric acid and 4 mM ammonium molybdate). The reaction mixture was incubated for 60 min at 95°C and absorbance were recorded at 695 nm against a blank sample containing 2 ml of reagent solution.

DPPH TLC autograph assay

DPPH TLC autograph assay was performed by applying small amount of different concentrations of all extracts on TLC plates. The TLC plates were sprayed with 0.2 %

Table 1: Yield of various plant extracts.

Extract	Yield (g)	% age yield
Ethanolic	5.97	19.9
Aqueous	5.22	17.4
n-Butanolic	2.93	9.7
n-Hexane	2.65	8.8
Ethyl acetate	2.2	7.3
Chloroform	1.75	5.8

DPPH solution (2 mg/mL) and observed after 30 min. The emergence of light yellow color on purple background indicated the positive results of this assay (Brand-Williams et al.,1995).

DPPH radical scavenging assay

The radical scavenging ability of plant extracts was measured using a DPPH assay by Brand-Williams et. al. (1995). Ethanol solution (1.0 ml) of all the extract of different concentrations (0.01 to 1.5 mg/ml) was added in 1.0 ml (0.2 mg/ml) methanol solution of DPPH and placed in dark. The absorbance was recorded using UV visible spectrophotometer (UV-1700 Shimadzu) and a decrease in absorbance value was recorded after 30 min at 517 nm. The % scavenging of radical was determined using the formula:

$$\% \text{ DPPH} = 100x\ [\text{DPPH}]_{\text{extract}}/[\text{DPPH}]_{\text{standard}}$$

Antimicrobial activity

Pure microbial cultures were acquired from the Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore. The inoculum of *P. aeroginosa, B. subtilis, E. coli, S. aureus* and *C. albicane*were prepared on agar Petri plates and incubated for 24 hours. Agar well diffusion method was used to determine the growth inhibition ability of plant extracts. Antibacterial and antifungal activity was performed using potato dextrose and Mueller-Hinton agar. Each plant sample was prepared in 100% DMSO(1mg/ mL of DMSO).

The potato dextrose and Mueller-Hinton agar were melted and then cooled to 48°C to 50°C. Standardized inoculum (1.5x108 CFU/mL); 0.5 McFarland (1.17% $BaCl_2.2H_2O$ + 1% H_2SO_4) of each microorganism was added separately to the molten agar. The mixture was then decanted into a Petri plate and allowed to solidify.

A sterilized cork borer of 8.5 mm diameter was used to form wells on each plate. The leaves extracts were decanted into each plate and labeled accordingly. The Petri plates were then incubated for 24 h at 37°C and 28°C for bacterial and fungal strains respectively (Bardaa et al.,2016).The diameters of the inhibition zones were measured in mm. Amoxil was used as a positive control.

Data Analysis

All determination of zones of inhibition and % inhibition activity was carried out at least three times. Statistical analysis was performed using Microsoft Excel 2013, and results were expressed as mean ±SEM.

RESULTS AND DISCUSSION

Various leaves extracts were prepared alike by following the similar protocol to prepare the extracts in various solvents. The percentage yield and calculated results are shown in Table 1.

The low yield of Chloroform and Ethyl acetate shows that the plant material has a low concentration of partially polar compounds. The n-Butanolic extract shows the presence of slightly high polarity compounds might be flavonoids and phenolic acids. n-Hexane with 2.65 g extract also shows that non-polar compounds are although present in excess than partially polar compounds, but the polar content is the highest. The high values of extracts i.e., Ethanolic and Aqueous, shows that compounds like glycosides, polar flavonoids and saponins are present in higher concentration in the leaves of CPL.

Total phenolic contents (TPC), total flavonoid contents (TFC) and total antioxidant capacity (TAC) of various extracts were investigated and results are shown in Table 2. Phenolic compounds are usually found in both non-edible and edible plants, which exhibit several biological effects including antioxidant activity (Ahmed et al.,2016). The presence of total phenolic contents was expressed in terms of Gallic acid equivalent (GAE). The slope of standard curve obtained was: y = 0.00x+0.160, R2= 0.96. The total phenolic contents in the studied extracts ranged from 12.5-92.62 mg GA/g.

The n-butanolic extract exhibited the highest concentration of phenols and n-hexane extract showed the lowest contents. Total phenolic contents depend on the kind of extract i.e. the polarity of used solvent for extraction. It was observed that high solubility of phenols in polar solvents delivered a higher concentration of these compounds in polar solvents.

The flavonoid concentration (TFC) of *Cucurbita pepo* L. was spectrophotometrically determined with aluminum chloride. The total flavonoid contents were expressed in

Table 2: Results of Total phenolic content (TPC mg/ g GAE), Total flavonoid content (TFC mg/ g QE), Total antioxidant capacity (TAC mg/g AAE).

Extract	TPC mg/ g GAE	TFC mg/ g QE	TAC mg/ g AAE
EtOH	40.37	50.76	211.25
Aqueous	40.12	38.46	236.75
n- BuOH	92.62	67.69	484.75
Ethyl acetate	85.12	76.92	383.50
Chloroform	21.25	53.07	139.72
n-Hexane	12.50	31.50	86.25

Table 3: Free radical % inhibition of various extracts of *C. pepo L*.

Concn. mg/ mL	Aqueous	Ethyl acetate	Chloroform	*n*- Hexane	*n*- Butanol	Ethanol
50	11.48±0.70	19.9±0.00	4.65±1.76	2.8±0.00	3.04±1.76	6.04±0.02
100	22.84±0.02	27.1±0.00	9.04±1.60	6.3±0.00	11.45±1.41	14.62±0.70
150	30.94±0.00	40.6±0.00	16.04±0.35	15.4±0.00	23.04±0.70	21.83±1.41
250	42.92±2.34	56.71±1.41	28.2±0.00	25.9±0.00	40.45±0.00	30.4±0.00
500	59.96±0.70	79.44±0.70	47.56±0.35	40.5±0.00	68.9±0.00	54.66±1.41
IC_{50} (µg/ mL)	371.17	372.9	-	-	359.16	494.6

terms of quercetin equivalent. The slope equation of standard curve was: y = 0.001x + 0.249R^2 = 0.981. The values obtained were in the range of 31.50- 76.92 mg/ g of quercetin.

The *n-b*utanolic extract exhibited the highest flavonoid content whereas, lowest flavonoid contents were observed in the *n*-hexane extract. Flavonoids have polyphenolic groups that make them polar and increases their affinity for polar solvents. N-hexane being nonpolar will have hydrocarbons, fatty acids steroidal compounds in it probably as they are less polar or nonpolar. In total flavonoid assay aluminum ion (Al^{3+}) forms complexes with Carbon 4 keto and either C-5 or C-3 hydroxyl or with the *ortho* hydroxyl groups in the A or B ring (Ahmed et al.,2016). Higher total phenolic content values of aqueous, *n*-butanol and ethyl acetate extracts are supporting the fact that most of the phytoconstituents are of phenolic nature. So, on the basis of aforesaid facts, it is proposed that *n*-butanol and ethyl acetate were the best solvents used for the extraction of both phenolics and flavonoids.

Phosphomolybdate assay was performed to determine the total antioxidant capacity (TAC) of the plant extracts. The assay was based on the fact that molybdenum (VI) was reduced to molybdenum (V) in the existence of a reducing agent. A green phosphomolybdate (V) complex is formed which can be evaluated spectrophotometrically at 765 nm (Ahmed et al.,2014). This assay involves a mechanism of electron transfer. Various natural products including flavonoids and phenolic acids can be a source of reduction. In this assay, *n*-Butanolic extract showed maximum activity while the *n*-hexane extract exhibited the least antioxidant activity. The results for TAC were observed in the range 86.25- 484.75 mg/ g of ascorbic acid. The order of activity from polar ethanolic to non-polar *n*-hexane extract was quite usual because the polar solvents have a stronger capacity to dissolve and therefore, extract polar phytochemicals. More or less, thesame trend was observed in case of total phenolic and total flavonoid contents.

DPPH TLC autograph showed that even very small amount of plant extract is active to scavenge free radical by turning the spots on TLC yellow against purple background after half an hour. The evaluated results are shown in Table 3. The phytochemicals that are more soluble in water retain a stronger capacity to scavenge DPPH free radicals. The DPPH radical also has a capacity to accept an electron and a hydrogen atom, but the data supports the later mechanism predominantly (Huda-Faujan et al.,2009; Ahmed et al.,2015). Ethyl acetate extract exhibited highest DPPH activity. Ethylacetate showed the highest potential to neutralizethe DPPH radicals, which was 50% of free radicals at the concentration of 250 mg/mL.The *n*-butanolic, aqueous and ethanolic extract showed moderate activity whereas, the least activity was observed for chloroform and *n*-hexane extracts. The slightly higher free radical scavenging activity of the *n*-Butanolic, aqueous extract indicates the presence of a higher content of flavonoids (Shah et al.,2013; Liang and Kitts, 2014).

The antioxidant potential of different extracts of *Cucurbita pepo* L. leaves was expressed in terms of percentage inhibition (%) and IC_{50} values (µg/mL). Figure 1 shows the comparative results of % inhibition of various extracts of CPL. The experiment was run in duplicate.

The correlation was studied between total phenolic content and total flavonoid content, total phenolic content

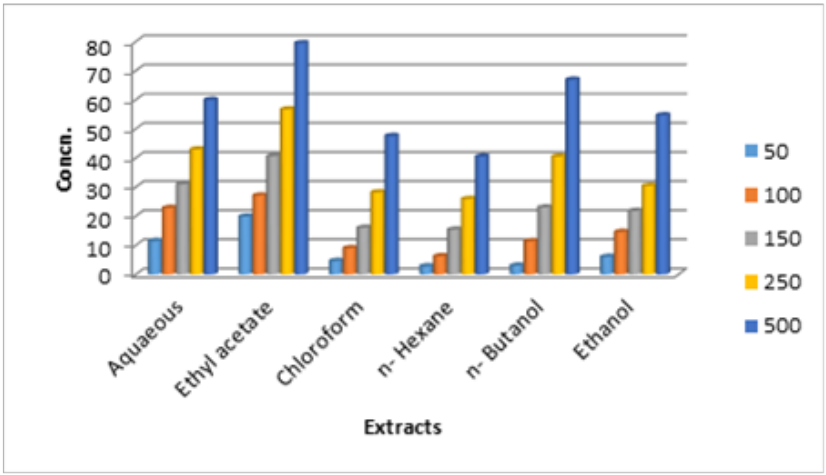

Figure 1: % inhibition of various extracts of *Cucurbita pepo* L. leaves at different concentrations in mg/ mL.

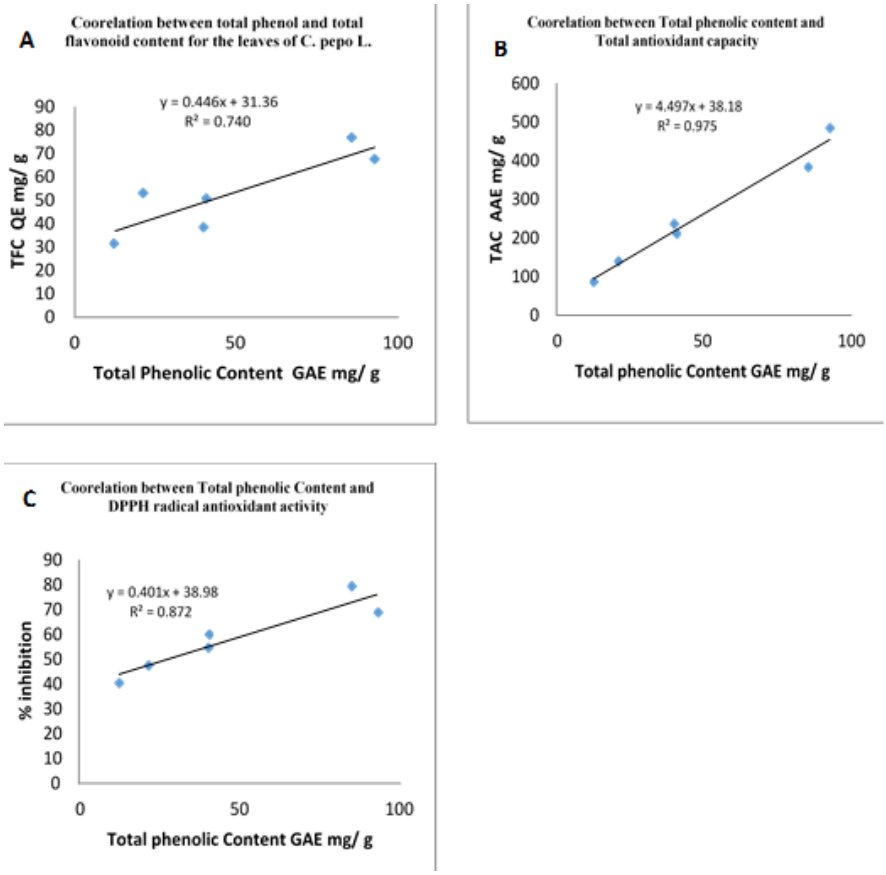

Figure 2. A. Weak Correlation between total phenol and total flavonoid content for the leaves of C.pepo L. **B**. Significant Correlation between total phenolic content and total antioxidant activity for the leaves of C. pepo L. **C**. Good Correlation between total phenolic content and DPPH activity showed by leaves of *C. pepo* L.

and total antioxidant content and total phenolic content and free radical scavenging activity by linear regression analysis. There was a high correlation between TPC and TAC (R^2= 0.975) and DPPH (R^2= 0.872) and a weak correlation was found between TPC and TFC (R^2= 0.740) (Figure 2).

The concentration used for the activity was 50mg/ mL. At this concentration ethylacetate, *n*- butanolic and aqueous

Table 4. Diameter of zone of inhibition (mm) shown by extracts of CPL against different pathogens.

	Diameter of Zone of Inhibition (mm)		
Extracts	*Bacillus subtilus*	*Staph. Aureus*	*P. aeruginosa*
Ethyl acetate	10.6±0.848	-	-
n-Hexane	-	-	-
Ethanol	-	-	-
Aqueous	-	-	15.5±0.707
n-Butanol	8.25±0.353	11.5±0.707	11.75±1.06
Amoxil	21.5±0.707	24.25±0.707	15.25±1.76

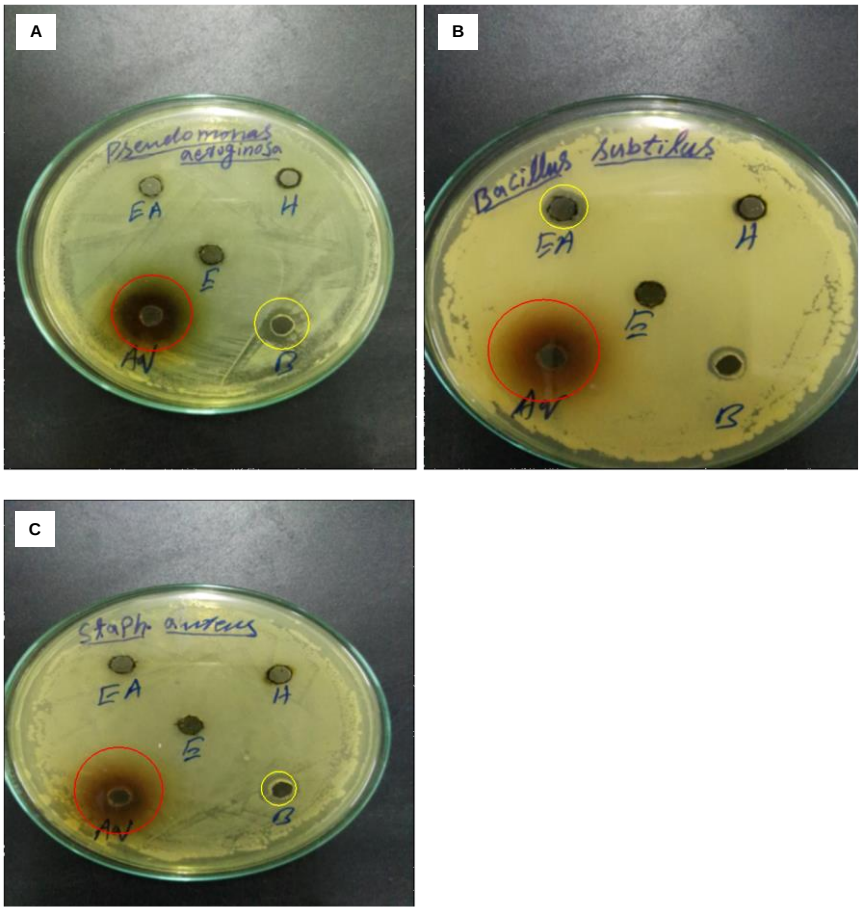

Figure 3.**A**. ZOI (mm) shown by various extracts of CPL against Pseudomonas *aeruginosa*. **B**. ZOI (mm) displayed against *Bacillus subtilis* by various extracts of CPL. **C**. ZOI indicated with red and yellow circle showing the activity of leaves extract against Staphylococcus aureus.

extracts were found active against *B. subtilis, S. aureus, and P. aeroginosa*. Table 4 shows the observed zone of inhibition in terms of diameter (mm). These results is in concordance with Bardaa et al., (2016) who investigated the antimicrobial potential with a concentration 100 mg/ mL. Alcoholic extract was found active against *B. subtilis* with ZOI 12 mm by disc diffusion method (Hammer et al.,1999). Al Ghazal (2012*)* reported results with extract of whole fruit with concentration of 20 mg/mL aqueous and alcoholic extracts showed amaximum zone of inhibition against *E. coli* and *S. aureus* (AL-Ghazal, 2012). Other concordant results were showed by Ubaid ulharyashawee in (2011) with the leaves and seeds extract of *Cucurbita pepo* L for concentration 300 mg/ mL (Ubaid ulharyashawee, 2011). Amoxil was used as a positive control.

Comparative study of the results obtained with already reported data showed the extracts of leaves of *C. pepo* L were found active against the *S. aureus, P. aeroginosa and B. subtilis* even at 50 mg/ mL. The clear zone of inhibition on agar plates shown by extracts of CPL is presented in Figure 3. The aqueous extract of leaves of

CPL was found to be the most potent against *P. aeroginosa* in the present study with a concentration of 50 mg/mL extract used. Hence it can be deduced from previous results and the findings of present work that even at this low concentration of 50 mg/ mL using agar well diffusion method the extract of leaves of Cucurbita have potential to show activity against the microorganism especially *P. aeroginosa*. No activity has been observed against *E. coli* and *C. albicane* at any concentration. The obtained results show that leaves of this plant have potential antimicrobial phytoconstituents that makes it a good candidate for further exploration regarding drug discovery.

CONCLUSION

The leaves of the CPL can be good sources for curing urinary tract infections, respiratory system infections, dermatitis, soft tissue infections *etc.* so we can suggest the aqueous extract of leaves of CPL as a potent source against *P. aeroginosa* on the already reported results and the obtained. Further isolation studies are to be done to get the actual compounds which act as asource of antibacterial agent and its concentration too to be evaluated.

The plants with high phenolic content showed greater potential to reduce free radicals in vitro than the plants with low phenolic content. CPL with higher phenolic content showed much stronger antioxidant activities than other selected plants. It should be noted that, other than phenolic compounds, these plants contain several other phytonutrients which possessed radical scavenging properties. These include nitrogen compounds, carotenoids, and ascorbic acid. In a hydrophilic oxidation model, ascorbic acid is a strong radical scavenger. Thus, the scavenging property of CPL could possibly be due to the activity of polyphenol and other substances like ascorbic acid.

REFERENCES

Adam GG, Imran S, Wang S, Mohammad A, Kok S, Gary DA, G. A. Channell, G. A. Morris, S. E. Harding, The hypoglycaemic effect of pumpkins as anti-diabetic and functional medicines, Food Res Int., 44, 862-867 (2011) doi: 10.1016/ j. fooders.2011.03.016

Ahmed D, Ejaz N, Saeed R and Dar P (2016). Cooking Effect on Anti-oxidative and Alpha-amylase Inhibitory Potential of Aqueous Extract of *Lagenariasiceraria* Fruit and its Nutritional Properties. Free Rad. Antiox. 6 (1): 44-50.

Ahmed D, Fatima K, Saeed R (2014). Analysis of phenolic and flavonoid contents, and the anti-oxidative potential and lipid peroxidation inhibitory activity of methanolic extract of *Carissaopaca* roots and its fractions in different solvents. Antioxidants, 3: 671-683. doi:10.3390/antiox3040671

Ahmed D, Khan MM, Saeed R (2015). Comparative Analysis of Phenolics, Flavonoids, and Antioxidant and Antibacterial Potential of Methanolic, Hexanic and Aqueous Extracts from *Adiantumcaudatum* Leaves. Antioxidants. 4:394-409. doi:10.3390/antiox4020394

AL-Ghazal AT (2012). Evaluation of Antibacterial Effect of Cucurbita pepo (Yakten) Extracts on Multi-antibiotic Resistance Bacterial Strains Isolated From Human Urinary Tract Infections. Raf. J. Sci. 23(2): 1-7.

Bardaa S, Halima NB, Aloui F, Mansour RB, Jabeur H, Bouaziz M and Sahnoun Z (2016). Oil from pumpkin (*Cucurbita pepo* L.) seeds: evaluation of its functional properties on wound healing in rats. Biomed Central. 15:73.

Berdick M (1972). The role of fats and oils in cosmetics. J. Am. Oil Chemist Soc. 40(7):406- 408.

Boaduo NKK, Katerere D, Eloff JN, Naidoo V (2014). Evaluation of six plant species used traditionally in the treatment and control of diabetes mellitus in South Africa using in vitro methods. Pharm Biol. 52:756-61.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. LWT.Food Sci. Technol. 28:25-30.

Dar P, Ahmed D,Waqas U, Saeed R., Chaudhery R (2014). Comparative analysis of antimicrobial potential of peel and mesocarp of *lagenariasiceraria* fruit extracts in various solvents against clinically important pathogens.Pharmcol online. 3:100-105.

Dubey A, Mishra N, Singh N (2010). Antimicrobial activity of some selected vegetables. IJABTP 1: 995-999.

Gunstone F (2008). Oils and fats in food industry, Wiley Blackwell. ISBN: 9781-1-4051-7121-2

Hammer KA, Carson CF, Riley TV (1999). Antimicrobial activity of essential oils and other plant extracts. J. Appl.Microbiol. 86:985-90.

Huda-Faujan N, Noriham A, Norrakiah AS, Babji AS (2009). Antioxidant activity of plants methanolic extracts containing phenolic compounds. Afr. J. Biotechnol, 8 (3):484-489.

Jian L, Du CJ, Lee AH and Binns CW (2005). Do dietary lycopene and other carotenoids protect against prostate cancer? Int. J. Cancer. 113:1010-4.

Liang N, Kitts DD (2014). Antioxidant property of coffee Components: Assessment of methods that define mechanisms of action. Molecules,19: 19180-19208. doi:10.3390/molecules191119180.

Martha R, Gutierrez P (2016). Review of *Cucurbita pepo* (pumpkin) its phytochemistry and pharmacology, Med Chem. 6(1):2-21 (2016). doi: 10.4172/2161-0444.1000316.

Nawirska-OA, Kit A, Biesiada A, Sokot LA, Kucharska A (2013). Characteristics of antioxidant activity and composition of pumpkin seed oils in 12 cultivars. Food Chem.139:155-61. doi:10.1016/j. food chem. 02.069.Epub 2013 Feb16

Noumedem JAK, Mihasan M, Lacmata ST, Stefan M, Kuiate JR (2013). Antibacterial activities of the methanol extracts of ten Cameroonian vegetables against Gram-negative multidrug-resistant bacteria. BMC Complement Altern. Med. 13: 26-29.

Prieto P, Pineda M and Aguilar M (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269: 337–341.

Retrieved from: http://eol.org/pages/584410/details 24-11- 2016

Sahreen S, Khan MR. and Khan RA (2011). Phenolic compounds and antioxidant activities of Rumex hastatus D. Don- leaves. J.Med. Plants Res. 5 (13): 2755-2765.

Shah NA, Khan MR, Ahmad B, Noureen F, Rashid U, Khan RA (2013). Investigation on flavonoid composition and anti-free radical potential of Sidacordata. BMC Complement. Altern. Med. 13: 276.

Slinkard K, Singleton VL (1977). Total phenol analysis: Automation and comparison with manual methods. Am. J. Enol. Vitic. 28: 49-55.

Ubaid ulharyashawee R (2011). Comparison of Antimicrobial Activity of Both Seeds and Leaves Extract of Two Type of Cucurbita pepo L. (Iraqi &Chinese).J.Kerbala Uni. 9: 4.

Zampini IC, Ordoñez RM, Isla MI (2010). Autographic Assay for the Rapid Detection of Antioxidant Capacity of Liquid and Semi-solid Pharmaceutical formulations Using ABTS$^{\cdot+}$Immobilized by Gel Entrapment, AAPS. Pharm Sci. Tech. 11(3):1159-1163.

Zuhair HA, Abd El-Fattah AA, El-Sayed MI (2000). Pumpkin-seed oil modulates the effect of felodipine and captopril in spontaneously hypertensive rats, Pharmacol Res. 41:555-63.