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Antioxidant and Antimicrobial Properties of Pumpkin (Cucurbita maxima) Peel, Flesh and Seeds Powders

Ashiq Hussain[1]* Dr. Tusneem Kausar[1] Dr Ahmad Din[2] Dr Anjum Murtaza[1]
Muhammad Abdullah Jamil[1] Saima Noreen[1] Muhammad Azhar Iqbal[1]
1.Institute of food science and Nutrition, University of Sargodha, Sargodha, Pakistan
2.National Institute of Food Science and Technology, University of Agriculture Faisalabad, Faisalabad, Pakistan

Abstract
This research work was designed to investigate and utilize all three parts (peel, flesh and seeds) of pumpkin for their antioxidant and antimicrobial activities. Pumpkin parts were separated, dried, grinded to powder and extracted by using 80% methanol. Percentage yield of pumpkin peel, flesh and seeds extracts, was found 12.37±0.10, 8.84±0.07 and 3.53±0.06% respectively. DPPH free radical scavenging activity (mg AAE/100 g) of pumpkin peel, flesh and seeds extracts was found 13.00±0.08, 10.58:0.06 and 16.53±0.09 respectively. All three types of extracts exhibited prominent antifungal activities against four fungal strains *Candida albicans, Fusarium oxysporum, Mucor miehei* and *Trichoderma spp.* Pumpkin seeds extracts exhibited greater zone of inhibition against these fungal strains as compared to pumpkin peel and flesh extracts. For antibacterial study four bacterial strains *Salmonella typhi, Escherichia coli*, *Bacillus subtilis* and *Streptococcus aureus* were used. Pumpkin flesh extracts exhibited greater antibacterial activities as compared to pumpkin peel and seeds extracts.
Keywords: Pumpkin, Extracts, Antibacterial, Antifungal, Zone of inhibition, Free radicals
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INTRODUCTION

Plants are natural sources of many components, responsible to cure various kinds of diseases. In the modern lifestyle, use of synthetic drugs has gained importance but their adverse effects are always present on human beings. The role of plants, especially fruits and vegetables are highly recognized towards good health and reduced risk of diseases (Mala and Kurian, 2016). Waste generated by fruits and vegetables processing industries is an excellent source of bioactive compounds, which can be directly extracted or transformed into high value-added products (Rico *et al.,* 2020). Antioxidants play a very important role in different ways, during different types of chronic diseases, in the body of living by protecting the cells from oxidation and scavenging the free radicals produced in the body (Skandrani *et al.,* 2010). Antioxidants inhibit the oxidizing chain reactions in the living body. Antioxidants can be natural or synthetic. Synthetic antioxidants are restricted to be used because of their carcinogenic effects (Velioglu *et al.,* 1998). Some important synthetic antioxidants are butylated hydroxytoluene, butylated hydroxy anisole and tertiary butylhydroquinone, which are used for the preservation of fats and oils. But now research provided evidences of adverse effects of these synthetic antioxidants (Yesilyurt *et al.,* 2008). Therefore, interest of scientists in natural antioxidants has grown up because of their beneficial health effects. Fruits and vegetables are believed to have protective mechanism due to presence of phenolic antioxidants. Polyphenols present in fruits, vegetables and herbs and also in products made from these plant materials like cocoa, wines and beverages, are important naturally occurring antioxidants (Aouidi *et al.,* 2011). In the last few years phenolic compounds from natural plant-based materials have attracted the attention of researchers because they play important role in human health by protecting the body tissues from oxidative stress (Chiou *et al.,* 2007).

Pumpkins are classified under the family "*Cucurbitaceae*" On the basis of stem shape and texture, pumpkins are grouped into *Cucurbita maxima*, *Cucurbita pepo*, *Cucurbita mixta* and *Cucurbita moschata* (Xanthopoulou *et al.,* 2009). Pumpkin is a versatile vegetable having identical position among all vegetables, due to its peel, flesh and seeds, each possessing outstanding phytochemicals applicable in treatment and prevention of medical disorders (Sharma *et al.,* 2020). Pumpkin different parts are rich source of biologically active compounds like phenolics, flavonoids, flavones, tocopherols and tocotrienols (Asif *et al.,* 2017). Pumpkin flour exhibits high total antioxidant activity (Que *et al.,* 2008). Pretreatment of pumpkin slices by blanching followed by pulsed vacuum osmotic dehydration and then connective drying results in good quality pumpkin flour (Junqueira *et al.,* 2017). Kvapil *et al.* (2020) analyzed that osmotic dehydration and proper packaging improved the quality of dried pumpkin. Pumpkin seed kernel (PSK) flour can be considered as a potential source of important nutrients for food enrichment due to its high protein (31.96%), oil (49.87%), oleic (44.78%), and linoleic acid (39.40%) content (Ozturk and Turhan, 2020). Pumpkin peel and seeds are good sources of bioactive compounds which can be extracted maximally by adopting latest technologies (Massa *et al.,* 2019). Bochnak and Swieca (2020) reported that pumpkin powder is good source of potentially bio accessible phenolics and antioxidant capacities. Jukic *et al.* (2019) reported that flour obtained from pumpkin seed oil press cake can be successfully used as a functional and nutritionally valuable food ingredient.

According to different studies, pumpkin acts as hypertensive due to its antioxidant activities (Schiffrin, 2010). Antioxidant activity of various extracts of pumpkin fruits could play an important role in individuals with vascular injury, diabetics and pre-diabetics as these extracts of pumpkin fruits will protect cellular damage. Xia and Wang (2006) conducted their research on streptozotocin-induced diabetic animals to demonstrate the antidiabetic as well as antioxidative effect of pumpkin fruit extract. They observed the cell protecting action of pumpkin fruits extract. Vitamin E (tocopherol) which is an antioxidant is found in high contents in pumpkin seeds and pumpkin seeds oil which contains substantial amount of vitamin E is a good part of Japanese diets (Tokudome *et al.,* 1999). Aquas-methanol (80%) extracts of pumpkin (*Cucurbita maxima*), exhibits appreciable antioxidant activities (Kulczynski *et al.,* 2020)

Pumpkin is a potential crop of generating income and overcoming food insecurity. However, spoilage of horticultural produce results due to microbial attack. Microbial spoilage is the major factor limiting shelf-life of fresh produce and processed products. Preservation of pumpkin by drying is best way to prevent microbial spoilage (Kiharason and Isutsa, 2019). Bacteria, viruses, fungi and other parasites causes diseases in humans and due to these diseases, death, disability, social and economic problems for millions of people took place. Although safe and effective medicines are available for treatment of such diseases but still a number of individuals lack safe, healthy and economic access to prevent and treat these diseases. Pathogenic micro-organisms have developed resistance towards drugs which is a sign of alarm for the scientists to develop new more effective drugs for these infectious micro-organisms. That is why natural sources are considered best option to create new formulations and to isolate new anti-microbial components. Pumpkin has provided various broad spectrum anti-microbial components to the researchers. Pumpkin oil inhibits *Staphylococcus aureus, Acinetobacter baumanii, Aeromonas veronii, Escherichia coli, Salmonella enterica, Enterococcus faecalis, Pseudomonas aeruginosa,* bio group *Sorbia, Candida albicans, Klebsiella pneumoniae* and *Serratia marcescens* at the concentration of 2.0 % (v/v) (Hammer *et al.,* 1999). The extracted substances from pumpkin exhibited antimicrobial activity against both gram-positive and gram-negative bacteria (Tadee *et al.,* 2020). Extracts from different parts of pumpkin, contain biologically active components, which show antidiabetic, antibacterial, hypocholesterolemic, antioxidant, anticancer, antimutagenic, immunomodulatory and other miscellaneous effects (Krimer-Malesevic, 2020). Alabassi *et al.* (2020) demonstrated that pumpkin fruit is a powerful antibacterial and antioxidant agent. Various parts of pumpkin plants contain various antibiotic components including antibacterial and antifungal agents. Some of these agents are proteins such as α and β-moschins, myeloid antimicrobial peptide (Ng *et al.,* 2002; Xiong, 2000; Vassiliou *et al.,* 1998). Recently a new protein named as Pr-1 was isolated from pumpkin by Park *et al.* (2010) which exhibits strong antifungal effect without any harmful or toxic effect on human erythrocytes. This protein is quite stable towards high temperature up to 70 ^{0}C. Skin, seeds and leaves of pumpkin possess antioxidant and antibacterial constituents (Dissanayake *et al.,* 2018). Pumpkin seed oil showed remarkable antioxidant and antibacterial activities (Amin *et al.,* 2020). Phyto-therapy is being used to discover new herbal medicines but only a few herbal plants have got importance to cure diseases. It is very important to test the herbal plant and its therapeutic agents scientifically by experiments and to validate their results before their commercial use as a number of plants have toxic agents with their side effects (Yadav *et al.,* 2010).

The objectives of the present research work were to investigate all three parts (peel, flesh and seeds) of pumpkins cultivated in subcontinent, for their antioxidant and antimicrobial potential, in order to utilize the waste streams of pumpkin fruits, as good source of functional and medicinal ingredients, during their consumption and processing.

MATERIAL AND METHODS

Collection of raw materials and chemicals

Mature pumpkins (n=40) with an average weight of 5±0.5 kg, were collected from the local market of district Sargodha, Pakistan. Specimen was submitted in Department of Botany, University of Sargodha for identification. All the chemicals used in this research work were of reagent grade purchased from Sigma-Aldrich chemicals. Bacterial and fungal strains were obtained from department of Biotechnology, University of Sargodha.

Preparation of pumpkin peel, flesh and seeds powders

Pumpkins were washed with fresh running water and then with distilled water to remove any foreign material attached and separation of three fractions (peel, flesh and seeds) was done manually with the help of knife. These three parts, were cleaned properly from fibrous material attached and placed in stainless steel containers under sunlight for a time period of 48 hours. Pumpkin fractions were dried through conventional hot air-drying method. Powder of each of these three fractions was obtained firstly by drying the pumpkin parts in hot air oven (BIOBASE HAS-T105 China) at 60°C till constant weight and secondly by grinding of dried parts with grinder (NIMA NM-8300 Japan), as described by Pongjanta *et al.* (2006). Resulting final powders were stored at ambient conditions in polyethylene bags.

Preparation of pumpkin peel, flesh and seeds powder's extracts

Extracts of three types of pumpkin powders were prepared by following the procedure given by Asif *et al.* (2017). According to these guidelines, 20 gm of powders (peel, flesh and seeds) sample was taken and 80% methanol was used as solvent for each type of extract. Twenty grams of each sample powder was soaked in solvent (200 ml) and continuous stirring was done at 200 rpm in orbital shaker (Biosan ES-20 Japan), for a time period of 120 h at 25°C. Separation of this mixture was done with the help of Whatman filter paper. Concentration of this filtrate was done through vacuum rotary evaporator (BIOLAND RE-5000A China). Five ml raw 80% methanolic (80% MeOH) extract of each fraction was stored for further analysis at 4°C temperature. Yield of extraction was measured through following equation in terms of percentage:

$$Yield\ (\%) = \frac{Solvent\ free\ extract\ weight\ (g)}{Weight\ of\ dried\ extract\ (g)} \times 100$$

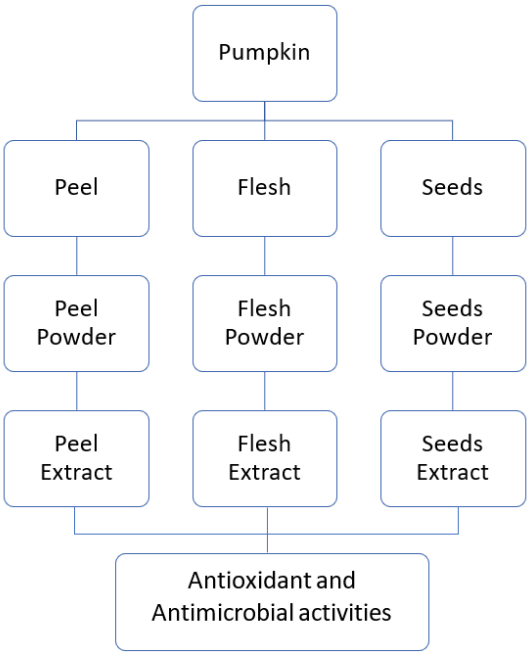

Figure 1. Overview of research work plan

Determination of antioxidant activity by DPPH free radical scavenging Method

Free radical scavenging activity of pumpkin (peel, flesh and seeds) powders was analyzed through DPPH assay as illustrated by Brand-Williams *et al.* (1995), with slight modifications. An amount of 0.01 g of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was taken after weighing and transferred into a 25-mL volumetric flask having solvent (80:20 methanol/water (v/v). Flask was filled with solvent, up to the marking level. A calibration curve for ascorbic acid was also developed. An amount of 100 µl from each type of 80% MeOH extract was collected in microplates, then an amount of 2.0 ml solvent and 250 µl DPPH reagent were added. These microplates were shaken and kept in darkness at ambient temperature for a time period of 20 mint. Reduction in DPPH absorbance by spectrophotometer (Hitachi U-2900 Japan) at 520 nm was witnessed every 5 min intervals unless this absorbance was stabilized after a time period of 30 mints. Methanol played its role as blank solution, while DPPH solution with no test samples acted as control. All samples analyses were done in triplicate. The DPPH radical scavenging activity of pumpkin powder's methanolic extracts was shown as mg of ascorbic acid equivalent (AAE) /100 g powder in a reaction time of 30 mints.

Antimicrobial activities of pumpkin peel, flesh and seeds powders

Pumpkin peel, flesh and seeds powder's 80% MeOH extracts were used to study their antimicrobial activity against bacterial and fungal strains.

Antifungal Activity of pumpkin peel, flesh and seeds powders

Agar well diffusion method was used to determine the antifungal activity of pumpkin peel, flesh and seeds extracts as described by Khaing, (2011), with little modifications. Firstly, fungal growth of four strains was taken in a

water-bath shaker in their respective broths at 25-30°C for 48 hours. Harvesting of strains, after incubation, was done and fugal suspensions (100 µl) were taken through swab on a certain media called SDA (Sabouraud‟s Dextrose Agar). On agar plates equally spaced wells were produced through punching out agar and after that these were heavily seeded with test organisms. These extracts were dissolved in methanol, filled in the wells and Diflozon was used as control. After filling, inoculation of plates was done for 24 hours at 37°C and measured zones of inhibition in millimeter by following the guidelines given by APHA (2001).

Antibacterial activity of pumpkin peel, flesh and seeds powders

Antibacterial activity of pumpkin peel, flesh and seeds powder's extracts was determined by disc diffusion technique as described by Marinova *et al.* (2005), with slight modifications. Test bacteria like gram positive i.e., *Staphylococcus aureus, Bacillus subtilus* and gram negative i.e., *Salmonella typhi, Escherichia coli* were inoculated over the nutrient agar medium with help of sterile cotton buds. 80% MeOH extracts of pumpkin peel, flesh and seeds were used to determine antibacterial activity. These extracts were planted with the help of discs, at organisms-inoculated plates at identical distances and similarly, the control drug Ciprofloxacin. The incubation of bacterial planted dishes was carried out for 24 hours at 37°C. The diameter of inhibition zones was determined in mm as per guidelines given by APHA (2001).

RESULTS AND DISCUSSION

Preparation of pumpkin peel, flesh and seeds powder's extracts

The values of percentage yield of 80% MeOH extracts of pumpkin peel, flesh and seeds powders has been presented in Table 1, from where it was clear that among peel, flesh and seeds, highest percentage of extract yield was found for pumpkin peel powder and lowest percentage of extract yield was found for pumpkin seeds powder. Extracts of pumpkin peel, flesh and seeds were prepared using most friendly and effectively working solvents i.e., Methanol and water in 80:20 ratio due to the reason that all volatile and non-volatile components get extracted by two types of solvents mixture. Higher yield of extracts of pumpkin flesh and peel might be due to more active components present because these are places of more metabolism (Asif *et al.,* 2017). Asif *et al.* (2017) conducted research to investigate antioxidant potential of 65, 80 and 99.9% methanolic extracts of pumpkin peel and puree and results for percentage yield of extracts were; Pumpkin peel extract 65% MeOH (15.61%), 80% MeOH (16.23%) and 99.9 MeOH (18.45%) whereas, pumpkin puree extract 65% MeOH (10.52%), 80% MeOH (12.41%) and 99.9 MeOH (14.10%). From these results it was clear that yield of extracts was greater from peel fraction as compared to puree and by decreasing the concentration of methanol yield of extracts was reduced for both peel and puree. Wanna, (2019) gave results for ethanolic extracts yield (%) for pumpkin peel and flesh as 4.29% and 6.78% respectively. Singh *et al.* (2016) extracted different parts of Cucurbit fruits with different solvents and gave results for pumpkin peel and pulp 70% methanolic extracts as 7.37% and 5.43% respectively. Jarungjitaree and Naradisorn (2019) evaluated pumpkin peel by using different types of solvents and also their different concentrations and gave results for yield of pumpkin peel extracts of 95%, 70% and 50% methanol extracts as 14.05, 15.75 and 20.00% respectively. These results were in close resemblance with our research work. Xanthopoulou *et al.* (2009) used four different types of solvents to extract four different types of pumpkin seeds groups and determined their yield as g of extract per 100 g of pumpkin seeds. Methanol extracts of four groups of pumpkin seeds resulted extracts yield range 1.54-3.4 g/100 g.

Table 1: Percentage of extracts yield of pumpkin peel, flesh and seeds powders

Pumpkin powders	Extract's yield (%)
Peel powder	12.37±0.10a
Flesh powder	8.84±0.07b
Seed's powder	3.53±0.06c

Means sharing similar letter in a column are statistically non-significant and means sharing different letter in a column are statistically significant (P>0.05).

Determination of Antioxidant Activity with the DPPH Radical Scavenging Method

Data of DPPH free radical scavenging activity of 80% MeOH extracts of pumpkin peel, flesh and seeds powders has been presented in Table 2. Significantly different values of DPPH free radical scavenging activity of each type of extract were obtained. Among three types of extracts, greater DPPH free radical scavenging activity was exhibited by pumpkin seeds extract and lower DPPH free radical scavenging activity was found from pumpkin flesh extract.

Phenolic compounds such as alkaloids, saponins, flavonoids and steroids present in pumpkin fruits parts especially seeds and pulp have been found good antioxidant agents ((Mala and Kurian, 2016)). Asif *et al.* (2017) conducted research to investigate antioxidant activity of 65%, 80% and 99.9% methanolic extracts of pumpkin peel and puree by using DPPH free radical scavenging assay and declared that pumpkin peel and puree extract (99.9% MeOH) exhibited promising antioxidant activity 68.79% and 69.56% respectively. They also concluded

that by decreasing the concentration of methanol antioxidant activity was reduced. Dissanayake *et al.* (2018) determined the antioxidant activities of three fractions skin, seeds and leaves of Sri Lankan variety of pumpkin by using three different types of solvents through DPPH free radial method and concluded leaves presented greater antioxidant activity as compared to seeds and skin while skin showed greater antioxidant activity as compared to seeds. Acetone extracts gave least values and ethyl acetate extracts higher values for antioxidant activity whereas methanol extracts values were in between of both these. Tyan *et al.* (2018) determined antioxidant activity of three plants extracts of Cucurbitaceae family by using DPPH free radical scavenging assay and concluded that all the extracts showed an increase in antioxidant activity by increasing the concentration of dose. They suggested that pumpkins could reduce the diseases related towards oxidation of lipids such as cardiovascular diseases. Singh *et al.* (2016) extracted different parts of Cucurbit fruits with different solvents to determine their antioxidant activity by DPPH free radical scavenging method and values of antioxidant activity for 70% methanolic extract of pumpkin peel and pulp were 43.79% and 38.56% respectively.

Table 2: DPPH free radical scavenging activity of pumpkin peel, flesh and seeds powders

Pumpkin powders	DPPH free radical scavenging activity (mg AAE /100 g)
Peel powder	13.00±0.08b
Flesh powder	10.58±0.06c
Seed's powder	16.53±0.09a

Means sharing similar letter in a column are statistically non-significant and means sharing different letter in a column are statistically significant (P>0.05).

Antifungal Activity of pumpkin peel, flesh and seeds powders

Results of antifungal activities of pumpkin peel, flesh and seeds 80% MeOH extracts against four fungal species have been presented in Table 3. Antifungal activity of 80% MeOH extract of pumpkin peel was significantly different against four fungal species. Highest zone of inhibition 8.69±0.10 mm against *Candida albicans* and lowest zone of inhibition 3.11±0.05 mm was observed against *Trichoderma spp.* Similarly, antifungal activity of 80% MeOH extract of pumpkin flesh was significantly different against these fungal species. Highest zone of inhibition 7.80±0.08 mm against *Candida albicans* and lowest zone of inhibition 3.38±0.07 mm was observed against *Trichoderma spp*. Similar trend was also observed when pumpkin seeds 80% MeOH were used against these fungal species as highest zone of inhibition 9.34±0.06 mm against *Candida albicans* and lowest zone of inhibition 6.24±0.02 mm was observed against *Trichoderma spp.* Standard drug used in this study Diflozon exhibited zone of inhibition 8.52±0.04 mm against *Candida albicans*, 2.23±0.04 mm against *Trichoderma spp.,* 5.33±0.06 mm against *Mucor miehei* and 4.33±0.05 mm against *Fusarium oxysporum.*

Now when we compared antifungal activities of these three types of extracts against individual species one by one, significantly different values were obtained, as pumpkin seeds extract exhibited highest zone of inhibition against *Candida albicans*, pumpkin peel extract zone of inhibition was in between flesh and seeds and pumpkin flesh exhibited lowest zone of inhibition. Similarly, significant data was obtained against *Mucor miehei* as highest zone of inhibition was exhibited by pumpkin seeds extract, lower zone of inhibition by pumpkin peel extract and lowest zone of inhibition by pumpkin flesh extract. Data was also significant for antifungal activities of these three types of extracts against *Trichoderma spp.* Where also pumpkin seeds extract exhibited highest zone of inhibition but lowest zone of inhibition was observed by pumpkin peel extracts and intermediate zone of inhibition was observed by pumpkin flesh extracts. When antifungal activity against *Fusarium oxysporum* for pumpkin peel, flesh and seeds extracts was compared significant different values were obtained for each type of extracts. From these results it was also clear that higher antifungal activities were observed by pumpkin seeds extracts when compared with pumpkin peel and flesh extracts.

Although pumpkin contains less protein contents (less than 2.0% of dry matter weight) but there are very essential amino acids and proteins in pumpkin parts which acts as antifungal agents (Zhang, 2003). Phenolic compounds especially alkaloids and flavonoids present in fruits are found useful antimicrobial and anti-inflammatory agents (Waterman, 1992). Abd El-Aziz and Abd El-Kalek, (2011) extracted different proteins from pumpkin (*Cucurbita moschata*) peel, pulp and seeds and tested for their antifungal effect. Extracted crude proteins from pumpkin seeds exhibited zone of inhibition against *Candida albicans* 5.0 mm, against *Rhodotorula rubra* 9.0 mm, against *Aspergillus flavus (A)* 10.0 mm, against *Penicillium chrysogenum* 1.0 mm and against *Rhizopus spp.* 1.0 mm. Whereas no zone of inhibition was noticed against *Aspergillus niger, Aspergillus flavus (H), Trichoderma viride, Aspergillus fumigates* and *Aspergillus parasiticus*. On the other hand, extracted crude proteins from pumpkin rind exhibited zone of inhibition against *Candida albicans* 4.0 mm, against *Rhodotorula rubra* 6.0 mm, against *Aspergillus flavus (A)* 25.0 mm, against *Penicillium chrysogenum* 27.5 mm, against *Rhizopus spp.* 2.0 mm, against *Aspergillus niger* 5.0 mm, against *Aspergillus flavus (H)* 4.0 mm, against *Trichoderma viride* 4.0 mm and against *Aspergillus fumigates* 23.0 mm while no zone of inhibition was examined against *Aspergillus parasiticus*. Extracted crude proteins from pumpkin pulp exhibited zone of inhibition against *Rhodotorula rubra* 8.0 mm, against *Aspergillus flavus (A)* 4.0 mm, against *Aspergillus niger* 4.5 mm and against *Rhizopus spp.* 1.0

mm. Yadav *et al.* (2013) gave a list of antifungal proteins with their molecular weights isolated from different parts of pumpkin. PR-1 having molecular weight 40 was isolated from pumpkins rind and PR-2 having molecular weight 14.8 was also isolated from pumpkins rind and these proteins possess antifungal activity against different fungal species. PR-5 having molecular weight 28 was isolated from pumpkins leaves and exhibit antifungal activity against *Candida albicans*. Cucurmoschin having molecular weight 8 was isolated from pumpkins seeds and exhibit antifungal activity against *Fusarium oxysporum*, *Mycosphaerella oxysporum* and *Botrytis cinereal.*

Another study by Pandey *et al.* (2010) proved that pumpkin seeds oils are good antifungal agents. Volatile constituents from essential oils of *Cucurbita maxima* seeds were tested against 3 fungal strains and was found growth inhibition (%) against *Aspergillus niger* 51.2%, against *Aspergillus flavus* 46.2% and against *Candida albicans* 41.1%. Kabbashi *et al.* (2014) conducted in vitro study to prove antifungal effects of *Cucurbita maxima* seeds ethanolic extracts against two standard fungal strains. At a concentration of 12.5 mg/ml pumpkin seeds ethanolic extracts exhibited zone of inhibition 15 mm against *Candida albicans* and 16 mm against *Aspergillus niger*. Further they found increased zone of inhibition by increasing the amount of extract concentration. They also tested standard antifungal drugs Nystatin and Clotrimazole as standard.

Badar *et al.* (2011) determined the chemical composition and biological activity of ripe pumpkin fruit parts and found that pumpkin seed oil exhibited strong antifungal activities against fungal specie *Saccharomyces cerevisiae*. Zone of inhibition against fungal strain *Mucor miehi* was 16 mm from pumpkin flesh extract and 15 mm from pumpkin rind extract.

Table 3: Antifungal activity of pumpkin peel, flesh and seeds powders

Extracts (80% MeOH)	Zone of inhibition (mm)				Overall Means
	Fusarium oxysporum	*Trichoderma spp.*	*Mucor miehei*	*Candida albicans*	
Pumpkin peel extract	5.07±0.06h	3.11±0.05k	6.13±0.05e	8.69±0.10b	5.75±0.61b
Pumpkin flesh extract	5.72±0.05f	3.38±0.07j	5.13±0.09h	7.80±0.08c	5.51±0.48c
Pumpkin seeds extract	7.80±0.06c	6.24±0.02e	6.57±0.09d	9.34±0.06a	7.49±0.37a
Reference (Diflozon)	4.33±0.05i	2.23±0.04l	5.33±0.06g	8.52±0.04b	5.10±0.68d
Overall Means	5.73±0.39B	3.74±0.45C	5.79±0.18B	8.59±0.17A	

Means sharing similar letter in a row or in a column are statistically non-significant and means sharing different letter in a column are statistically significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Antibacterial activity of pumpkin peel, flesh and seeds powders

Data of antibacterial activities of three types of pumpkin powders 80% MeOH extracts against four bacterial species has been presented in Table 4. Significantly different values were obtained for antibacterial activity of pumpkin peel, flesh and seeds extracts against four bacterial species. Against bacterial specie *Salmonella typhi,* all three types of extracts, exhibited significant antibacterial activity, very comparable to standard antibacterial drug used. Against bacterial species *Escherichia coli* and *Bacillus subtilis* pumpkin flesh extract exhibited greater zone of inhibition as compared to peel and seeds extracts. Against bacterial specie *Streptococcus aureus* pumpkin peel, flesh and seeds extracts exhibited non-significant activity whereas, standard drug Ciprofloxacin exhibited significant inhibition.

In plant leaves and different parts of fruits phenolic compounds are present especially alkaloids and flavonoids which have been reported as useful antimicrobial agents (Waterman, 1992). Phytochemicals such as saponins, tannins, flavonoids, alkaloids and steroids present in pumpkin fruit parts might have been playing their role as antibacterial agents (Chonoko and Rufai 2011). Asif *et al.* (2017) conducted research to investigate antibacterial activity of 65%, 80% and 99.9% methanolic extracts of pumpkin peel and puree against four bacterial strains E. *coli*, P. *multocida*, S. *aureus* and B. *subtilis.* Zone of inhibition of pumpkin peel and puree methanolic extracts against P. *multocida* was greater than 15 mm whereas against other three bacterial strains zone of inhibition was in the range of 10 to 15 mm. Dissanayake *et al.* (2018) used three bacterial strains S. *aureus,* B. *subtilis* and E. *coli* to determine the antibacterial activity of pumpkin skin, seeds and leaves extracts by using three types of solvents; acetone, methanol and ethyl acetate. Methanol extract of pumpkin skin gave zone of inhibition of 7.58 mm against S. *aureus* and 4.83 mm against B. *subtilis* whereas no zone of inhibition was observed against E. *coli.* On the other hand, methanolic extracts of pumpkin seeds gave no results as antimicrobial agent but ethyl acetate extract of pumpkin seeds showed 6.61 mm inhibition zone against S. *aureus.* Jun *et al.* (2006) extracted pectic polysaccharides from pumpkin peel and studied their growth promoting effect on good intestinal bacteria L. *brevis*, B. *bifidum* and B. *longum* and growth retarding effect on bad intestinal bacteria E. *coli* and C. *perfringens*. Tyan *et al.* (2018) tested seven bacterial strains to check the antimicrobial activity of pumpkins extracts and found that *Cucurbita moschata* extracts showed antimicrobial activity against all bacterial strains. Chonoko and Rufai (2011) conducted research on phytochemical screening and antimicrobial activity of pumpkin and reported an inhibition zone in the range of 7-10 mm of both ethanolic and methanolic extracts of pumpkin peel against bacterial

specie S. *aureus* and 6-12 mm range of zone of inhibition against S. *typhi*. Badar *et al.* (2011) determined the chemical composition and biological activity of ripe pumpkin fruit parts and found that pumpkin rind and flesh extracts exhibited moderate antimicrobial activities against Gram positive bacteria B. *subtilis* and B. *cereus*. Both pumpkin rind and flesh extracts showed a zone of inhibition 17 mm against bacterial strain S. *viridochromogenes*.

Antibacterial activity of pumpkin extracts is related to antibacterial proteins from pumpkin parts and seed oils from pumpkin seeds (Caili *et al.,* 2006). Phenolic compounds present in fruits and vegetables are strongly responsible for antibacterial activity as discussed in previous reports that polar isopropyl functionality of phenolic components may involve in bacteriostatic activities (Farag *et al.,* 1989). Antibacterial actions of phenolic compounds have been associated with reaction of these compounds with cellular components resulting in leakage of nucleotides and proteinaceous material into extracellular areas (Degre and Sylvestre 1983).

Table 4: Antibacterial activity of pumpkin peel, flesh and seeds powders

Extracts (80% MeOH)	Zone of inhibition (mm)				Overall Means
	Streptococcus aureus	***Bacillus subtilis***	***Escherichia coli***	***Salmonella typhi***	
Pumpkin peel extract	6.60±0.26f	4.43±0.13g	7.03±0.23ef	13.41±0.21c	7.87±1.01b
Pumpkin flesh extract	6.67±0.41f	6.40±0.26f	8.63±0.20d	15.18±0.09a	9.22±1.08a
Pumpkin seeds extract	6.43±0.23f	3.70±0.15h	5.07±0.27g	14.40±0.19b	7.40±1.26c
Reference (Ciprofloxacin)	7.60±0.26e	3.00±0.17i	6.43±0.18f	14.97±0.05ab	8.00±1.32b
Overall Means	6.83±0.19B	4.38±0.39C	6.79±0.40B	14.49±0.22A	

Means sharing similar letter in a row or in a column are statistically non-significant and means sharing different letter in a row or column are statistically significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

CONCLUSION

Pumpkin is one of the important, nutritious and medicinal vegetables, which has been consumed from ancient times to cure different remedies. In subcontinent pumpkin is cooked as vegetable and during preparatory operations of pumpkin, peel and seeds are discarded as waste material, but these seeds and peel are good source of phytochemicals responsible for antioxidant and antimicrobial activities to play important role in the field of medicine. Extracts from pumpkin constituents' parts when tested, exhibited remarkable antioxidant and antimicrobial activities.

RECOMMENDATIONS

Pumpkin peel, flesh and seeds can be converted into powders and extracts which become more concentrated source of nutrients and can be utilized in medicines and novel food products. Phytochemicals and proteins present in pumpkin constituent parts can be extracted to develop safe and novel antioxidant and antimicrobial drugs. After approval from scientific community pumpkin based nutraceuticals and cosmetics can be commercially marketed for the well-being of humans.

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CONFLICT OF INTEREST

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