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Phytochemical screening and *in-vitro* pharmacological evaluation of three vegetable peel extracts

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Abstract

Fruits and vegetables are used for nutritive purpose and also for disease prevention and treatment but peels remain un-utilized and usually thrown away as waste material. The purpose of this study was to evaluate peel extracts of three vegetables (*Allium sativum*, *Brassica rapa* and *Solanum tuberosum)* for phytochemical constituents, antibacterial and antioxidant activity *in-vitro.* The phytochemical analysis of vegetable peel extracts was conducted according to standard procedures. The antibacterial activity was performed by disc diffusion method. Total phenolics (TFC), total flavonoids (TFC) contents, DPPH radical inhibition activity and reducing activity assay were used for the estimation of antioxidant potential. The phytochemical results showed that *Solanum tuberosum* peel extracts have more bioactive constituents than *Allium sativum* and *Brassica rapa.* Antibacterial activity of *Allium sativum* and *Brassica rapa* peel extracts was more prominent against gram negative bacteria while *Solanum tuberosum* peel extracts activity was higher against gram positive bacteria. The overall antioxidant activity in three different vegetable peels was highest due to TPC as compared to other antioxidants. The *Brassica rapa* peels was found to contain highest TPC followed by *Allium sativum and Solanum tuberosum.* So, it was concluded that peels of three vegetables have important bioactive constituents and pharmacological activities. Future perspectives are isolation and characterization of pharmacologically active constituents from vegetable peels for human/veterinary medicine.

Keywords

Peels
Antioxidant activity
Antibacterial activity
Allium sativum
Brassica rapa
Solanum tuberosum

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Introduction

Frequent scientific inquiries point at repeated rich sources of antimicrobials, mainly in vegetables and fruits, and rarely include by-products of vegetables and fruits. Mostly by-products of fruits and vegetables i.e. Peels and seeds fed to livestock or thrown in the waste (Reddy et al., 2015). Fruit waste can be used for the treatment of different diseases. The main advantages of use of fruit waste include low cost, high availability and capacity of good absorption (Pathak et al., 2015). In developing countries, about 80% population use traditional medicine in primary medical care (Patel et al., 2010). The medicinal plants extract has biologically active compounds that can retard microbial growth (Sivakumar & Venkataraman, 2010). Fruit and vegetables are the major functional foods as these are main source of nutraceuticals such as phenolic compounds, vitamins and minerals (Borek, 2001).

Garlic belongs to family *Liliaceace* and its botanical name is *Allium sativum* L. It is broadly classified into two varieties hark neck (*ophioscordon*) and soft neck (*sativum*). It has strong folkloric awareness and traditionally famous and common spice for flavoring the food. Odor and taste of *Allium sativum* L. contributed due to aromatic sulphur based

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compounds (Akinmusire et al., 2014). The ethanolic extract of garlic bulb found to contain secondary metabolites such as tannins, saponins, glycosides, alkaloids and flavonoid (Lalitha et al., 2009). Allicin is the main component which exhibit anti-viral, anti-microbial, anti-parasitic and anti-fungal activity (Akinmusire et al., 2014). Phytochemicals present in garlic contribute to important role in disease prevention and maintenance of human health (Butt & Sultan, 2009).

Turnip is a root vegetable in the *Brassicaceae* or *Cruciferae* family (Oxford Encyclope, 2004). However, it is not a root vegetable. Actually, it is a swollen stem which grows beneath the soil surface (Hirst & Gillian, 2006). Turnip is almost round in shape, have white flesh and rough, thin leaves which are covered by prickly hairs (The Cambridge World History of Food, 2000). Turnips are effective in reducing the risk of diabetes, obesity and hypertension and in the prevention and control of many types of cancer such as pancreas, breast, stomach, bladder and lungs cancer (Ambrosone & Tang, 2009). Different parts of turnip contain important minerals, vitamins, β carotene, amino acids, proteins and various phenolics (Anwar et al., 2007).

Potato (*Solanum tuberosum* L.) belongs to *solanaceae* family. Potato is an herbaceous, perennial crop that grows up to 60 cm high. 30 to 40 % potatoes are wasted during peeling process. In 19th century, British first introduced potato in indo-Pak subcontinent. Almost 320.67 million tons potatoes are produced every year throughout the world. The major producer of potato is china which produces 72 million tons potatoes every year. Production of potato in Pakistan is almost 2.5 million tons and the area under potato cultivation is 149.1 thousand hectares. Potato crop globally is the fourth largest productive crop after wheat, rice and corn. Potatoes are rich in carbohydrates and also provide essential vitamins, proteins and minerals (Ali et al., 2015). Pharmacologically, potatoes are very important because of analgesic, antioxidant, antibacterial, muscle strengthening, blood pressure lowering and appetizing activities (*Amanpour* et al., 2015).

The purpose of the present study is to perform phytochemical screening and *in vitro* evaluation of anti-bacterial and antioxidant property of the peels of *Solanum tuberosum, Allium sativum* L. and *Brassica rapa* cultivated in Pakistan because this type of analysis has not been carried out on potato peels in our country.

Materials and Methods

Preparation of peel extract: Mature vegetables of *Allium sativum, Brassica rapa* and *Solanum tuberosum*were collected from local market of Faisalabad. These fruits were identified by experts of Institute of Horticulture Science, University of Agriculture, Faisalabad. Vegetables peels were separated, dried under shade and was ground to powder. The air-dried ground plant material was extracted with water, absolute and 80% ethanol for 5 hours in an orbital shaker at room temperature. The liquid portions of the extracts were filtered with the help of muslin cloth to separate it from the residues. The same solvent was used twice to extract the residues and then the extracts were combined. The combined extracts were concentrated at 45°C, using a rotary evaporator and dried by using freeze dryer. The dried crude concentrated extracts were stored in a refrigerator at 4^{o}C, until analysis.

Phytochemical analysis: The phytochemical constituents were determined qualitatively by reference methods for alkaloids, saponins, anthraquinones, steroids, proteins, coumarins, anthocyanins, tannins, phenols, cardiac glycosides, terpenoids, resins and fixed oils (Shabi et al., 2014).

Antioxidant activity: Antioxidant activity of different extracts of vegetables were tested by total phenolic content, total flavonoid content, DPPH (1, 1'–diphenyl–2-picrylhydrazyl) scavenging activity and reducing power.

Determination of total phenolic content: Amount of Total phenolic content was assessed by using the Folin-Ciocalteu method (Sultana et al. 2009). Briefly, the 50 mg of crude extract was mixed with 0.5 ml Folin-Ciocalteu reagent and 7.5 ml deionized water. Then this mixture was kept at room temperature for some 10 minutes, and 1.5 ml of 20% w/v sodium carbonate was added into it. This mixture was then heated at 40^{0}C for 20 min in a water bath and cooled in an ice bath. After that, absorbance of this mixture was read at 755 nm using a spectrophotometer. Amounts of total phenolic content were calculated by using gallic acid calibration curve (R2 = 0.9986). Three readings of each sample were taken and results averaged.

Determination of total flavonoid contents: The Total flavonoid content was measured with previously reported spectrophotometric method (Sultana et al. 2009). Briefly, 1 ml of extracts of each plant material was diluted with 4 ml of water in a volumetric flask. Initially, 0.3 ml of 5% NaNO2 solution was added to each flask and at 5 min, 0.3 ml of 10% AlCl3 was added into it and at 6 min, 2 ml of 1M NaOH was added into it. 2.4 ml of water was then added to the flask and mixed thoroughly. After that, absorbance of the mixture was read at 510 nm using a spectrophotometer. Amount of total flavonoid content were calculated by using catechin calibration curve (R2 = 0.98). Three readings of each sample were taken and results averaged.

Determination of reducing power: The reducing power of the extracts was determined according to the

procedure described earlier (Sultana et al. 2009). 10 mg of concentrated extract was mixed with 5 ml of 0.2M sodium phosphate buffer and 5 ml of 1% potassium ferricyanide and the reaction mixture was then incubated at temperature of 50°C for 20 min. Then 5 ml of 10% trichloroacetic acid was added and the mixture centrifuged at 980g for 10 min. The upper layer of the solution was removed and 5 ml of this layer was diluted with 5 ml of distilled water and 1 ml of 0.1% ferric chloride. After that, absorbance of this solution was read at 700 nm using a spectrophotometer. The solution without extract was used as control. All the tests were performed in triplicate. BHT was used as reference standard.

DPPH radical scavenging assay: 1, 1'–diphenyl–2-picrylhydrazyl (DPPH) free radical inhibition activity of the extracts was checked by using the earlier reported procedure (Sultana et al. 2009). Briefly, 1 ml of extract containing 25 µg/ml of dry matter in solvents was mixed with 5 ml of 25 mg/L of freshly prepared solution of DPPH. Absorbance of this solution was read at 517 nm using a spectrophotometer. Low reaction mixture absorbance showed high radical scavenging activity. The solution without extract was used as control. All the tests were performed in triplicate. BHT was used as reference standard.

Antibacterial activity: The bacterial strains (*Staphylococcus aureus, Bacillus subtilis, Escherchia coli and Pseudomonas* aeruginosa) were identified by the microbiologist Dr. Shahid Mehmood. Solution of dried extracts were prepared in DMSO and antibacterial activity of these extracts of fruit peels were checked by using well established Disc diffusion method (DDM) against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* (Cruickshan, 1975: Onyeagba et al., 2004). Sterilized petri plates that already contained nutrient-agar were inoculated with 0.01 ml of bacterial culture medias (105-106 bacteria/ml) and discs injected with 50 mg/ml concentration of aqueous, absolute ethanol and 80% ethanol extracts of banana and papaya peel were placed on the semisolid agar medium with gentle tap. These petri plates were placed at 4^0C for 2 hours and then incubated for 18-24 hours at 37^0 C. After incubation, zone reader was used to measure the zones of inhibition on the nutrient agar media. All the test tests were performed in triplicate and results were averaged. Commercially available chloramphenicol antibiotic disc (30 µg) was used as positive control.

Results

The presence of different phytochemical compounds in 3 tested plant peels extracts are shown in Table 1.

Anti-bacterial activity: The results of anti-bacterial activity of three different vegetables peel extracts are shown in Table 2.

Antioxidant activity: Results of antioxidant activity of *Allium sativum, Brassica rapa* and *Solanum tuberosum* peels, using three different solvents (aqueous, absolute ethanol and 80% ethanol) are shown in figures 1-4.

Table 1: List of Phytochemical bioactive constituents present in plant peel extracts.

Sr. No.	Constituents	*Allium sativum*			*Brassica rapa*			*Solanum tuberosum*		
		DW	Absolute alcohol	80% alcohol	DW	Absolute alcohol	80% alcohol	DW	Absolute alcohol	80% alcohol
1	Alkaloids	-	-	-	-	-	-	-	-	-
2	Saponins	-	-	-	+	-	-	+	-	-
3	Anthraquinone	-	-	-	-	-	-	-	-	-
4	Steroids	-	-	-	+	+	+	+	+	+
5	Proteins	-	-	-	-	-	-	+	+	+
6	Coumarins	-	-	-	-	-	-	-	-	-
7	anthocyanins	-	-	-	-	-	-	+	+	+
8	Tannins	-	-	-	+	+	+	+	++	+
9	Phenols	+	+	+	+	++	+	+	+	++
10	Cardiac glycosides	+	++	+++	+	+	+	+	+	+
11	Terpenoids	-	-	-	+	+	+	+	+	+
12	Resins	-	-	-	-	-	-	-	-	-
13	Fixed oils and fats	-	-	-	+	+	+	+	+	+

Table 2: Mean ± SEM of Anti-bacterial activity of plant peel extracts (DW, 80%E, 100%E) and chloramphenicol (Standard).

Sr. No.	Bacteria	*Allium sativum*			*Brassica rapa*			*Solanum tuberosum*		
		DW	Absolute alcohol	80% alcohol	DW	Absolute alcohol	80% alcohol	DW	Absolute alcohol	80% alcohol
1	*E.coli*	9.66	13.66	10	7	19	14	1	11	3
2	*P.aeruginosa*	9	13.33	8.33	6	15	13	0.1	7	5
3	*B.subtilis*	3	7	4.66	5	13	11	9	13	7
4	*S.aureus*	6.66	8	4	6	14	12	4	15	12

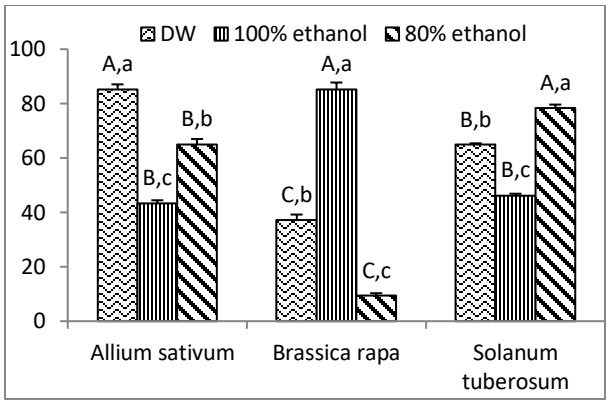

Figure 1: Total phenolic contents (mgGAE/g of extract) in *Allium sativum*, *Brassica rapa* and *Solanum tuberosum* peel extracts.

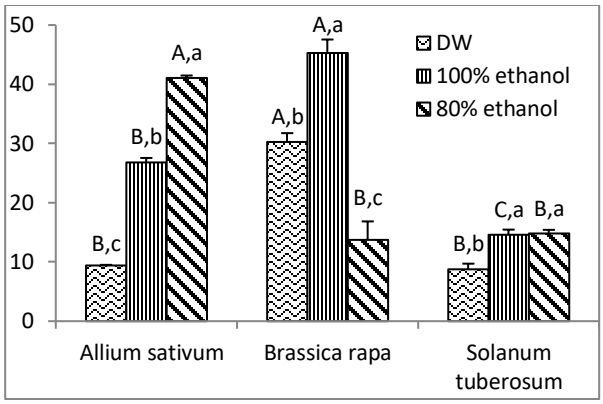

Figure 2: Total flavonoid contents (mgCE/g of extract) in *Allium sativum*, *Brassica rapa* and *Solanum tuberosum* peel extract

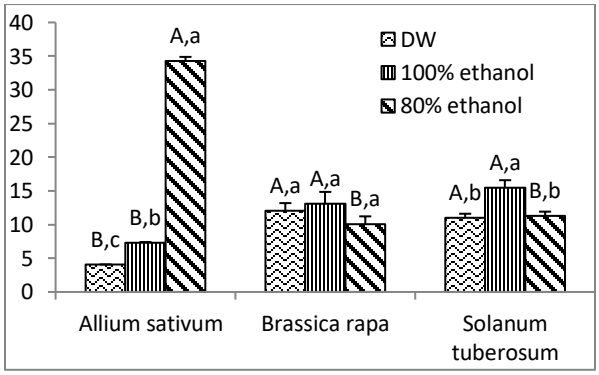

Figure 3: Percentage DPPH radical inhibition of *Allium sativum*, *Brassica rapa* and *Solanum tuberosum* peel extracts.

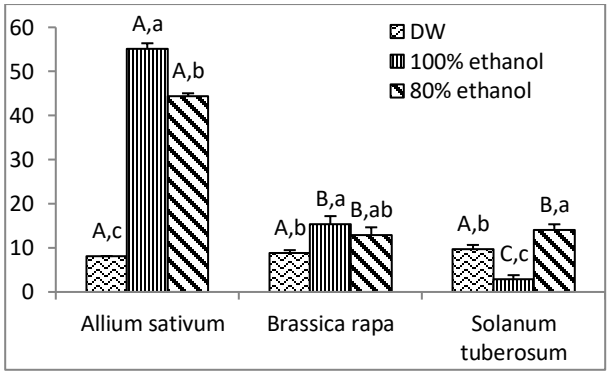

Figure 4: Ferric reducing power activity of *Allium sativum*, *Brassica rapa* and *Solanum tuberosum* peel extracts.

Capital alphabetic letters indicates comparison of antioxidant activity among three different vegetable peel extracts. While small alphabetic letters indicate comparison of antioxidant activity among three different extracts of peels within same vegetable.

Discussion

Highly positive (+++) cardiac glycosides are present in 80% ethanolic extract of *Allium sativum.* Aqueous, 80% and 100% ethanolic extracts of *Brassica rapa* and *Solanum tuberosum* also showed positive results for cardiac glycosides. Aqueous, 80% and 100% ethanolic extracts of *Allium sativum, Brassica rapa* and *Solanum tuberosum* showed positive results for phenols. While highly positive (++) phenols showed in absolute ethanolic extract of *Brassica rapa* and 80% ethanolic extract of *Solanum tuberosum.* Steroids, tannins, terpenoids, fixed oils and fats showed positive results in all three extracts of *Brassica rapa* and *Solanum tuberosum* peel. Penecilla & Magno, (2011) performed phytochemical analysis on twelve different plants extracts. Garlic clove was one of them on which they have performed phytochemical tests. According to their study, alkaloids, flavonoids, glycosides, tannins, terpenoids and saponins are present in garlic clove.

Camire et al., (2009) reported that *Solanum tuberosum* is balanced human food due to rich source of carbohydrates and phytochemicals like anthocyanins and polyphenols. The antibacterial activity of *Allium sativum* and *Brassica rapa* peel extracts were more against gram -ve bacteria as compared to gram +ve bacteria. While, antibacterial activity of *Solanum tuberosum* peel extracts was more prominent against gram-positive *S. aureus* and *B. subtilis* as compared to gram-negative *E. coli* and *P. aeruginosa*.

Penecilla and Magno, (2011) performed antibacterial activity on garlic clove. The ethanolic extract of garlic clove have antibacterial activity against both gram +ve and –ve bacteria.

Several studies have emphasized the potential importance of extracts from *Brassica* species as the bases of polyphenolics (phenolic acids, flavonoids and the related analogues), able to exercise antimicrobial effects (Beltagy, 2014).

Amanpour et al., (2015) reported that ethanolic extract of *Solanum tuberosum* peel had antibacterial activity and this activity was more on gram-positive bacteria. While, ethanolic extract of *Solanum tuberosum* peel had antibacterial effect against some gram-negative bacteria e.g. *P. aeruginosa.*

Present study showed that total phenolic contents were present in three tested vegetables namely *Allium sativum, Brassica rapa* and *Solanum tuberosum.* Aqueous extract of *Allium sativum* found to contain highest TPC, followed by *Solanum tuberosum* and *Brassica rapa.* Comparison of three aqueous extracts showed significant difference among total phenolic contents of tested vegetable peels. Absolute ethanolic extract of *Brassica rapa* found to contain highest TPC. While, non-significance difference of TPC of absolute ethanolic extract was observed between *Allium sativum* and *Solanum tuberosum.* 80% ethanolic extract of *Solanum tuberosum* found to contain highest TPC, followed by *Allium sativum* and *Brassica rapa.* Comparison of three 80% ethanolic extracts showed significant difference among total phenolic contents of tested vegetable peels. Aqueous extract of *Brassica rapa* found to contain highest TFC. While, non-significance difference of TFC of aqueous extract was observed between *Allium sativum* and *Solanum tuberosum.* Absolute ethanolic extract of *Brassica rapa* found to contain highest TFC, followed by *Allium sativum* and *Solanum tuberosum.* So, there was a significant difference among them. 80% ethanolic extract of *Allium sativum* found to contain highest TPC and there was non-significant difference between *Brassica rapa* and *Solanum tuberosum*. 80% ethanolic extract of *Allium sativum* found to contain highest DPPH radical scavenging activity and there was non-significant difference between *Brassica rapa* and *Solanum tuberosum*. Absolute ethanolic extracts of *Brassica rapa* and *Solanum tuberosum* showed non-significant results for DPPH radical scavenging activity. While, *Allium sativum* showed significant difference with *Brassica rapa* and *Solanum tuberosum.* Aqueous extracts of *Brassica rapa* and *Solanum tuberosum* showed non-significant results for DPPH radical scavenging activity. While, *Allium sativum* showed significant difference with *Brassica rapa* and *Solanum tuberosum*.

Absolute ethanolic extract of *Allium sativum* found to contain highest Ferric reducing power activity, followed by *Brassica rapa* and *Solanum tuberosum.* Aqueous extracts of *Allium sativum, Brassica rapa* and *Solanum tuberosum* showed non-significant results for Ferric reducing power activity. 80% ethanolic extracts of *Allium sativum* found to contain highest Ferric reducing power activity. But there was non-significant results of Ferric reducing power activity between *Brassica rapa* and *Solanum tuberosum.*

Antioxidant actions of *Brassica rapa chinensis* has been reported in goat liver model with H_2O_2 induced free radicals formation. This antioxidant action is attributed to the occurrence of secondary metabolites like phenolic complexes such as flavonoids, and the other phytochemicals (Mayilsamy & Krishnaswamy, 2015).

Conclusion: The phytochemical results showed that *Solanum tuberosum* peel extracts have more bioactive constituents than *Allium sativum* and *Brassica rapa.* The antibacterial activity of *Allium sativum* and *Brassica rapa* peel extracts were more against gram negative bacteria. While, antibacterial activity of *Solanum tuberosum* peel extracts was more prominent against gram-positive bacteria. The overall antioxidant activity in three different vegetable peels was highest due to TPC as compared to other antioxidants. While *Brassica rapa* peels was found to contain highest TPC followed by *Allium sativum*, while *Solanum tuberosum* has lowest TPC. Thus, it is suggested that tested vegetable peel constituent be determined and to determine the exact mechanism of its antibacterial and antioxidant properties, and more comprehensive research be done to apply it clinically.

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