Tropical Journal of Pharmaceutical Research September 2017; 16 (9): 2221-2229 ISSN: 1596-5996 (print); 1596-9827 (electronic) © Pharmacotherapy Group, Faculty of Pharmacy, University of Benin, Benin City, 300001 Nigeria. All rights reserved.

> Available online at http://www.tjpr.org http://dx.doi.org/10.4314/tjpr.v16i9.25

Original Research Article

Antioxidant, antimicrobial and antiproliferative activities of peel and pulp extracts of red and white varieties of Ipomoea batatas (L) Lam

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Sent for review: 19 June 2016

Revised accepted: 10 August 2017

Abstract

Purpose: To investigate the antioxidant, antibacterial and anticancer potentials of methanol and ethanol extracts of the peel and pulp of red and white species of Ipomoea batatas (L.) fruit.

Methods: Total phenolic contents and flavonoids were determined using chemical assays. Antioxidant studies were carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, inhibition of linoleic acid peroxidation assay and reducing power assay. Antibacterial and antiproliferative activities of extracts were determined using disc diffusion and MDBK cancer cell line inhibition methods, respectively.

Results: The extract of peels of red specie (PERS) showed total phenolic contents (TPC) 8.9 mg gallic acid equivalent (GAE)/g dry extract and flavonoids 6.5 mg catechin equivalent (CE)/g dry extract. The extract of PERS also showed promising DPPH free radical scavenging activity, inhibition of linoleic acid peroxidation and reducing power activity. However, mild antibacterial and anti-proliferative activities were noted except that the extract showed significant inhibition of Bacillus subtilis growth.

Conclusion: The results indicate that the peel and the pulp of red sweet potato (SP) specie are rich in antioxidants and can potentually be processed as antioxidant food supplements.

Keywords: Ipomoea batatas (L.) Lam, Sweet potato, Phenolic content, Antioxidants, Antibacterial activity, Antiproliferative activity

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The role of antioxidants is important in fixing the conditions such as heart, cancer, chronic inflammation and Alzheimer's diseases which arose due to the oxidative cell damage. Inflammation alone may initiate variety of diseases including vasculitis, lupus erythematous, glomerulonephritis, adult respiratory diseases syndrome, and arthritis. Oxidative stress or cell damage may also involves in many other kinds of chronic diseases

such as ischemic diseases (stroke, heart diseases. intestinal ischema), acquired immunodeficiency syndrome (AIDS), hemochromatosis, hypertension emphysema, gastric ulcers, organ transplantation, preeclampsia, neurological disorder (muscular Alzheimer's disease, Parkinson's dystrophy, disease), smoking-related diseases, and alcoholism [1].

Antioxidants can effectively reduce the oxidative stress and consequently the risk of chronic

diseases. Antioxidants, which are mainly obtained from two major sources i.e., extraction from natural sources and by chemical synthesis are being used as preventive or therapeutic medicines. The later source may cause severe toxicity such as genotoxicity, carcinogenicity or hepatotoxicity [2].

The reports of several studies have demonstrated that natural antioxidants offer high potential in the prophylaxis and treatment of some oxidative damage diseases, resulting in the reduction of mortality rate [3]. Therefore, the search for antioxidant, antibacterial and anticancer potential of natural plant components and isolation of natural antioxidants is of fundamental interest of natural product chemistry scientists. Extraction, isolation and purification of natural products have been translated into a drug process. development About 50 % pharmaceuticals currently in use are derived from natural products [4]. The discovery of anticancer drugs such as vinca alkaloids, etoposide, paclitaxel, and variety of antimicrobial drugs encourage drug-discovery programs [5].

Ipomoea batatas (L.) Lam (SP) is an important fruit belonging to the family Convolvulaceae, and is well known to consumers for its nutritional values and sweet taste [6]. Initially, it was cultivated in Central America but later on due to its wide adaptability to various climates and farming systems, it has now been cultivated throughout the world and being studied for its medicinal potential [6,7]. In view of the nutritional benefits of SP, the aim of this study was to investigate the antioxidant, antibacterial and anticancer activities of red and white varieties of SP. Therefore, the aim of this study was to assess the antioxidant, antibacterial and anticancer potentials of the methanol and ethanol extracts of peel and pulp of red and white varieties of SP

EXPERIMENTAL

Materials

Red and white SP tubers were collected from local farm houses situated in countryside of Faisalabad (Punjab), Pakistan. The tubers of both varieties were collected in December 2015. The authentication of the samples was carried out by Dr. M. Naeem (Taxonomist & Associate Professor, Department of Botany, GC University, Faisalabad-Pakistan). The voucher specimens (No: GCUF/Bot-102 & 103) were kept at herbarium of Department of Botany, GC University, Faisalabad, Pakistan. DPPH, linoleic acid, BHT were purchased from Sigma-Aldrich, Germany. All other chemicals were purchased from BDH Chemicals UK. All chemicals were of analytical grade. Bacterial strains and MDBK cancer cell line were obtained from the University of Health Sciences, Lahore, Pakistan. Spectroscopic analyses were performed using double beam Spectrophotometer (Hitachi U-2000, Japan) and Elisa reader (Bio-Rad Model 680, Hercules, CA, USA).

Preparation of extracts

The tubers of red and white varieties of SP were washed properly after collection with distilled water to remove soil contents. The peel and pulp of the fruit was separated and subjected to freeze drying. The dried material were stored at 4 °C for extraction processes. The biological components of peel and pulp were extracted in 95 % methanol and ethanol solvents. The extraction was carried out by mixing 20 g freeze dried sample in 120 mL of extraction solvent in 250 mL conical flasks followed by shaking (200 rpm) at room temperature for 24 h. After 24 h, the contents of the flasks were filtered using Whatman no. 1 filter paper. The residue was again subjected to extraction process and the both filtrates were combined. The filtrates were concentrated using rotary evaporator at reduced pressure. The concentrated raw extracts were stored at -4 °C for further analysis.

Determination of total phenolics

Total phenolic contents were determined using method described by Chaovanalikit and coworkers [8]. Briefly, dry extract (50 mg) was mixed with 0.5 mL of Folin-Ciocalteu reagent and 7.5 mL of deionized distilled water in a test tube. The dissolved contents were kept at room temperature for 10 min, followed by the addition of 1.5 mL NaCO₃ solution (20 %). The solution was then heated at 40 °C for 20 min and then cold in an ice bath. The absorbance of the cold solution was noted at 755 nm using a double beam UV-Vis spectrophotometer. The amount of TPC was estimated using a calibrated curve of gallic acid. The results were presented as GAE / g dry extract. The experiments were performed in triplicate (n=3) for each sample.

Determination of total flavonoids

Total flavonoid contents (TFC) were determined using the method described by Dewanto *et al.* with slight modification [9]. Briefly, dry extract (0.01 g) was dissolved in 1 mL of ethanol in a volumetric flask followed by the subsequent addition of 6.1 mL distilled water and 0.3 mL NaNO₂ solution (5 %). The mixture was

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incubated for 5 min, after which 0.6 mL of $AICl_3$ (10 %) was added into the flask, gently shaked and then 2 mL of NaOH solution (1 N) was added. The absorbance of the solution was noted at 510 nm by a double beam UV-Vis spectrophotometer. The amount of TFC was expressed as CE / g dry extract.

Determination of antioxidant activity

The antioxidant potential of peel and pulp extracts was determined using DPPH free radical scavenging assay, inhibition of linoleic acid peroxidation assay and reducing power assay.

Determination of DPPH free radical scavenging potential

DPPH free radical scavenging potential of peel and pulp of SP extracts was assessed according to the method reported by Yen & Chen [10]. To the 1 mL of DPPH solution (0.1 mM in ethanol) added 3 mL extract solution of varying concentration (20, 40, 60, 80 and 100 µg/mL) and the mixture was incubated in the dark for 30 min. Thereafter, the absorbance was measured by double beam UV-Vis spectrophotometer at 517 nm. Synthetic antioxidant, BHT was taken as reference compound. Solution without extract was taken as negative control. DPPH free radical scavenging (D) was calculated using Eq 1.

where A_1 and A_0 are the absorbance of sample and blank solutions, respectively. The results are expressed as mean \pm standard deviation (mean \pm SD, n = 3).

Evaluation of inhibition of linoleic acid peroxidation

Inhibition of linoleic acid peroxidation was measured using ammonium thoicyanate assay [11]. According to this assay, an aliquot of 0.2 mL of linoleic acid emulsion (25 mg / mL in 99 % ethanol) and 0.4 mL of 50 mM phosphate buffer (pH 7.4) was subsequently added to 0.2 mL sample solution (100 µg / mL in ethanol). The mixture was then incubated in dark at 40 °C for 24-h. At the end of incubation period, 0.1 mL reaction mixture was taken in the test tube and then added 3 mL ethanol (70 %) and 0.1 mL ammonium thiocyanate (30 % w / v) solutions. The reaction mixture was then gently shaked and left to stand for 3 min at ambient temperature followed by then addition of 0.1 mL of ferrous chloride solution (20 mM in 3.5 % HCl). Then the absorbance was immediately recorded spectrophotometrically at 500 nm. The absorption of reaction mixture was noted after every 24-h unless the absorbance value of control reached to maximum value. A solution without extract was taken as negative control while BHT was used as reference.

Determination of reducing power

In this study, the reducing power of sample extracts was measured following the protocol described by Khan et al [11]. Briefly, the sodium phosphate buffer solution (5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5 mL) was added to 100 ug drv extract and left the mixture for incubation at 50 °C for 20 min. After that, 10 % aqueous solution of trichloroaceic acid (5 mL) was added and the mixture was centrifuged at 980 g for 10 min at -5 °C. The upper layer (~5 mL) of the mixture was separated in another test tube followed by the addition of 5 mL distilled water and 1 mL ferric chloride solution (0.1 %) which turned the color of the solution, yellow to green. The absorbance of the solution mixture was noted at 700 nm. All samples were analyzed thrice (n = 3).

Antibacterial assay

Antibacterial activity was assessed using disc diffusion method. [12]. The extracts was screened against four bacterial strains: Escherichia coli. Pasturella multocida, Staphylococcus aureus, and Bacillus subtilis. In order to prepare the petri plates of microbes; nutrient agar (28 g/L) was suspended in distilled water, mixed well and autoclaved at 121 °C for 15 min. Then the inoculums (100 μ L / 100 mL) were added to the medium and poured in sterilized petri plates. As the bacterial medium turned semisolid, the circular filter paper discs (~1.5 mm in diameter) loaded with 100 µL of sample (2 mg / mL in DMSO) were laid flat on the growth medium. DMSO and ciprofloxacin antibiotic were taken as negative and positive control, respectively. The petri plates were then incubated at 37 °C for 24 h. The extracts having antibacterial activity showed clear zones of inhibitions. The zones of inhibition were measured in millimeters using a zone reader.

Antiproliferative assay

Antiproliferative assay was performed using calorimetric method as described by Mosmann [13]. According to this method, 50 mg of sample extract was dissolved in 500 μ l of Glasco Modified Eagle Medium (GMEM). The solution was thoroughly mixed in an eppendorf tube, vortexed and filtered with 0.4 μ m filter

membrane. The filtered solution (20 µL) was diluted to 200 µL using GMEM in an eppendorf (solution A). In an each well of 96 well plate, 100 µL of media was poured using a micropipette followed by the addition of 100 µL solution A in the 1st well. This was mixed to form solution B. From the 1^{st} well, 100 µL of solution B was transferred to the 2^{nd} well and the process was repeated to make subsequent dilutions. In this way two fold dilutions was prepared up to the 5th well. The 6th well of each row was used as a negative control. Following the dilution process, 100 µL of cell suspension (MDBK) was poured in all wells with a multichannel micropipette and placed in 5 % CO₂ incubator at 37 °C for 24-h. After 24-h, 20 µL of 3-(4, 5-dimethyl thiazole-2yl)-2,5-diphenyl tetrazolium bromide (MTT) solution (5 mg / mL Phosphate Buffer Solution (PBS)) was added in all 96 wells. The plates were then placed in 5 % CO₂ incubator at 37 °C for 3 h for the formation of Formazon crystals. At the end of the incubation period the solution from each well was decanted with a micropipette and the crystals were dissolved using 100 µL of DMSO in each well resulting in a colored solution. The number of viable cells was directly proportional to the intensity of color. The absorption of the solution was recorded with ELISA reader (Bio-Rad Model 680) at 570 nm.

Statistical analysis

All experiments were carried out thrice and the results were calculated as mean of triplicate values $(n = 3) \pm$ standard deviation (SD).

RESULTS

The extraction yield of SP peel and pulp in methanol and ethanol was obtained in the range of 4.17 % to 15.96 % as shown in Table 1. The highest yield of extract was obtained with methanol from PURS while lowest yield was obtained with methanol from PEWS.

Total phenolic and flavonoid contents

Table 2 shows TPC and TFC recorded in methanol and ethanol extracts of red and white variety of SP. Peels of red SP showed highest TPC and TFC, 8.9 mg GAE and 6.5 mg CE / g extract in ethanol, respectively. The same trend was also noted in methanol extracts of red SP peel which showed TPC and TFC, 4.6 ± 0.2 mg GAE and 3.8 ± 0.1 CE / g extract, respectively. Extracts of PEWS, PURS and PUWS in both solvents showed good quantity of TPC; however, TFC were found low in these extracts. The TPC data is in agreement with previously reported data on TPC recorded in roots extracts of SP [14]. Pochapski et al also reported TPC in the range of 1.9 to 11.6 mg GAE/g dry extract in leaves extracts of SP [15].

DPPH free radical scavenging potential

Table 2 shows the DPPH free radical scavenging potential of extracts. Most of the extracts showed promising free radical scavenging potential. Ethanol extract of PERS and methanol extract of PUWS showed high DPPH free radical scavenging potential i.e. 90.26 % and 88.59 %, respectively. The reference synthetic antioxidant, BHT showed 72.36 % scavenging potential. All other samples showed DPPH free radical scavenging potential less than BHT free radical scavenging potential.

Inhibition of linoleic acid peroxidation

The results of inhibition of linoleic acid peroxidation are shown in Figure 1.

The inhibition of linoleic acid peroxidation was investigated by measuring the absorbance of reaction mixture at 500 nm. Greater the absorbance greater would be the inhibition of linoleic acid peroxidation. It was observed that

Table 1:	Extraction	yield ((%) of	various	extracts	of SP

	Extraction yield (%)					
Extraction -	Red variet	y of SP	White variety of SP			
Solvent -	Peel	Pulp	Peel	Pulp		
Methanol	4.73	15.96	4.17	9.01		
Ethanol	8.44	7.68	6.45	7.72		

Table 2: TPC and 7	TFC of methanol a	ind ethanol extracts of	red and white variety of SP
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	Extract							
Biologics	PERS	PERS	PEWS	PEWS	PURS	PURS	PUWS	PUWS
	MeOH	EtOH	MeOH	EtOH	MeOH	EtOH	MeOH	EtOH
TPC (mg/g)	4.6±0.2*	8.9±0.2	2.7±0.3	2.6±0.1	3.1±0.3	1.5±0.0	2.9±0.2	1.5±0.2
TFC (mg/g)	3.8±0.1	6.5±0.1	0.7±0.0	1.6±0.1	1.9±0.1	0.8±0.1	0.5±0.1	0.2±0.0

Data shown are mean \pm SD (n=3)

 Table 3: DPPH free radical scavenging activity of methanol and ethanol extracts of red and white variety of SPs at different concentrations

Extract	Concentration (µg/mL)							
	20	40	60	80	100			
PERS MeOH	8.34±1.23*	11.73±1.70	13.86±0.78	15.02±1.34	17.44±1.21			
PERS EtOH	54.15±1.95	65.67±2.16	77.79±2.09	87.76±3.72	90.26±4.32			
PEWS MeOH	43.84±2.54	46.65±1.24	53.48±2.56	55.47±2.12	60.38±2.58			
PEWS EtOH	7.86±0.99	16.25±1.03	25.32±1.33	34.55±1.94	43.54±1.96			
PURS MeOH	2.90±0.18	4.02±0.53	4.44±0.71	5.32±0.63	6.75±0.93			
PURS EtOH	1.28±0.31	3.16±0.69	5.04±0.43	6.92±0.89	9.47±1.07			
PUWS MeOH	54.86±2.31	65.67±2.52	77.19±3.75	86.69±3.83	88.59±3.52			
PUWS EtOH	5.70±1.07	7.02±0.71	8.55±1.16	9.26±1.03	10.09±0.84			
BHT	58.79±2.39	62.81±2.41	65.32±3.58	68.34±2.17	72.36±3.61			

 $MeOH = methanol; EtOH = ethanol; *Values are mean \pm SD (n=3)$



Figure 1: Inhibition of linoleic acid peroxidation activity of methanol and ethanol extracts of pulp of SP (a) PURS MeOH (♦), PURS EtOH (■), PUWS MeOH (▲), PUWS EtOH (×), BHT (*) and peel of SP (b) PERS MeOH (♦), PERS EtOH (■), PEWS MeOH (▲), PEWS EtOH (×) BHT (*)

extract of PERS in ethanol and methanol showed high absorption, i.e., 1.12 and 1.11, respectively as compared to other sample extracts. Pulp of white SPs and red SPs also showed promising inhibition of linoleic acid peroxidation. BHT showed absorbance 1.2, which was slightly greater than the extract of PERS.

Reducing power activity

Reducing power is measure of strength to neutralize the free radical moieties. Natural products are commonly assessed for its medicinal values on the bases of their reducing power potential. Figure 2 shows the reducing power of extracts of SP; reducing power of sample is directly proportional to the absorption of visible light at 700 nm. Methanol extract of PEWS showed highest absorbance i.e. 1.45 which is higher than any other extract absorption. However, the methanol and ethanol extracts of PERS showed absorption in the range of 1.19 to 1.45. BHT, which is taken as reference compound showed absorption 1.45 (Figure 2a) while methanol extracts of PUWS and PURS showed absorption 0.58 and 0.2, respectively (Figure 2b).

Antibacterial activity

The results of antibacterial activities of peels and pulp of SP extracts against Gram-negative and Gram-positive bacterial strains are shown in Table 4. Multi drug resistant bug, *S. aureus* was found susceptible to methanol extract of PERS while *B. subtilis* was found susceptible to ethanol extract of PERS. Similarly, promising bactericidal activity of methanol and ethanol extracts of PEWS and PURS were recorded against Grampositive *P. multocida* bacteria.

Most extracts were found least active or completely inactive against Gram-negative bacteria. However, none of the extracts were able to inhibit *E. coli* bacterial growth.



Figure 2: Reducing power activity of methanol and ethanol extracts of pulp of SP (a) PURS MeOH (\diamond), PURS EtOH (\blacksquare), PUWS MeOH (\blacktriangle), PUWS EtOH (\times), BHT (*)) and peel of SP (b) pulp PERS MeOH (\diamond), PERS EtOH (\blacksquare), PEWS MeOH (\bigstar), PEWS EtOH (\times), BHT (*)

 Table 4: Antibacterial activity of methanol and ethanol extracts of peels and pulp of red and white variety of SP against Gram-negative and Gram-positive bacteria

	Bacterial growth inhibition activity								
Bacterial strain	PERS	PERS	PEWS	PEWS	PURS	PURS	PUWS	PUWS	Positive
	MeOH	EtOH	MeOH	EtOH	MeOH	EtOH	MeOH	EtOH	control
Staphylococus aureus	+++	+	-	+	-	-	+	-	+5
Bacillus subtilis	+	+++	+	-	-	+	-	-	+5
<i>Escherichia</i> coli	+	+	+	+	+	+	-	-	+5
Pasteurella multocida	+	+	++++	++++	+++	+++	-	+	+5

Activity: - = inactive, + = mild (2-5 mm), ++ = week (5-10 mm), +++ = satisfactory (10-18 mm); ++++ = good (18-25 mm), +5 = Strong (25-32 mm)

Antiproliferative activity

The results of antiproliferative activity using MDBK cancer cell line are shown in Figure 3. Five different concentrations were used to evaluate the antiproliferative activities of peel and pulp extracts of SP. Paclitaxal, an anticancer drug of plant origin which is commonly used in chemotherapeutic procedures of cancer treatment, was taken as positive control. As compared to reference drug, antiproliferative activity of SP extracts were found minimal with some exception such as ethanol extracts of PEWS and PURS that showed anti-proliferative activity at high concentrations.

DISCUSSION

Many reports that have been published previously demonstrate the medicinal values in terms of antioxidant, antibacterial and antiproliferative potential of plant extracts. In this study, the medicinal potential of two varieties of SP such as white and red SP were investigated for its antioxidant, antibacterial and antiprolifera-



Figure 3: Antiproliferative potential of methanol and ethanol extracts of peel and pulp of red and white variety of SP against MDBK cancer cell line; PERS EtOH (\blacklozenge), PEWS EtOH (\blacksquare), PURS EtOH (\blacktriangle), PUWS EtOH (\bigstar), PERS MeOH (\bullet), PURS M

tive activities by extracting bioactive components in methanol and ethanol solvents. Most of the diseases in the human body initiated by the production of free radicals in cell mitochondria during the production of ATP. Free radicals produced as a result of ATP production form reactive oxygen species (ROS) such as the superoxide anion and hydroxyl radicals.

Free radicals play vital role in biological tissues i.e. beneficial and harmful role. Controlled production of these radicals stimulate signaltransduction pathways. phagocytosis and induction of drug detoxification pathways. However, overexpression in the living body can directly influence the cell growth and development, and increase the chance of aging, cancer, pathogenesis of atherosclerosis and several other conditions, including inflammatory disease [16,17]. The risk of free radical, however, can be reduced either through avoiding the substances that produce free radicals for polyunsaturated example fats or caloric restriction (depression in ATP production) or increase in intake of antioxidant foods/supplements. Phenolic and flavonoid compounds are known with antioxidant properties [18]. Peels of red SP variety showed promising contents of phenolic and flavonoid compounds which were higher than previously reported for typical potato extract [19,20]. Lewis et al reported that the flavonoids content of peel higher than pulp which is in agreement with our study [21]. Antioxidant potential of plant extracts are assessed using different chemical based invitro assavs such as DPPH free radical scavenging assay, inhibition of linoleic acid peroxidation assay, reducing power assay, trolox-equivalent antioxidant capacity (TEAC) assay, ABTS assay, and FRAP assay. We in this study picked the DPPH free radical scavenging assay, inhibition of linoleic acid peroxidation assay and reducing power assay to assess the antioxidant activity of extracts. DPPH free radical scavenging potential of PERS in ethanol showed high free radical scavenging activity (90.26 ± 4.32 % at 100 µg/mL) which is in agreement of previously reported study [22].

Regarding inhibition of linoleic acid peroxidation, the PERS extracts in ethanol and methanol of both varieties showed > 90 % inhibition whereas standard reference compound, BHT showed 100 % inhibition which indicates the promising antioxidant potential. It has also been clearly documented that inhibition of linoleic acid peroxidation values as an index of antioxidant capacity of natural sources. It is also reported that purple-leave of SP are abound in quercetin and myricetin, which strongly inhibits lipid peroxidation in addition to scavenge DPPH, hydroxyl, and superoxide free radicals [23].

The reducing power assay is commonly employed to evaluate the electron donation ability of an antioxidant. In human body reduction of oxidants reduces the chance of physical degradation of tissues. Similarly, reducing power activity of PERS extracts in ethanol and methanol revealed promising ability to reduce Fe^{+3} to Fe^{+2} which is measured by recording the absorption of the solution at 700 nm. Absorption intensity is directly associated with reducing ability of phenolics or flavonoids.

Microbial infection is a major cause of mortality throughout the world. Natural products have shown critical role in fighting against infection diseases. Screening of antimicrobial potential of plant materials is a key area of phytochemical research. The antibacterial activity of medicinal plants is mainly due to diterpenes, sesquiterpene lactones, alkaloids, flavonoids, triterpenes or naphthoquinones [24]. In this study P. multocida was found most susceptible bacterial strain to white and red SP. Multi drug resistance bacterial strain S. aureus was found susceptible to PERS extract in methanol while other two strain B. subtilis and E. coli were found non-susceptible to all extracts except PERS in ethanol which showed promising *B. subtilis* inhibition potential.

The antiproliferative potential of extracts using MDBK cancer cell line indicates negligible activity; however in anticancer campaign natural dietary components have drawn a great attention in reduction of cancer risk. According to one report approximately 60 % anticancer drugs (currently used for cancer therapy) have been developed from natural products [25]. In-vitro biological screening of crude extracts of plant materials or its isolated fractions is a crucial step in finding or developing more effective agents to reduce the oxidative stress, bacterial infection and cancer diseases.

CONCLUSION

This study reveals that the ethanol peel extract of SP extract delivers greater antioxidant benefits than the pulp although the phenolic and flavonoid contents were found in good quantity in all extracts. Antibacterial activity is satisfactory against *P. multocida* but none of the extracts showed promising antiproliferative activity. Thus, the consumption of peel and pulp extracts of SP may afford health benefits, and also has a potential for development commercially as a nutritional supplement.

DECLARATIONS

Acknowledgement

The authors are thankful to Government College University, Faisalabad and HEC for supporting this study. Furthermore, the authors are grateful to Dr. Nasir Mahmood (Assistant Professor; University of Health Sciences, Lahore) for providing facilities for the anticancer studies. The authors are also grateful to John Mather and Bhatti Information and Management Solutions for editing the manuscript.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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