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Comparative evaluation of proximate composition and biological activities of peel extracts of three commonly consumed fruits

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Abstract

For the proper utilization of the fruits peel, investigation on their biological potential is needed. The present study was designed to determine the proximate composition and biological activities of the fruit peel extracts of three common fruits namely *Citrullus lanatus, Punica protopunica* and *Pyrus pashia.* The methanolic fruit peel extracts (FPEs) were analyzed for phytochemical composition and antioxidant activity in terms of free radical scavenging capacity (FRSC), antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* and antifungal activity against *Aspergillus niger* and *Fusarium oxysporum*. The fruit peels were found to be statistically different ($p < 0.05$) in ash, moisture, crude protein, crude fiber content. A statistically significant difference ($p < 0.05$) was observed in ascorbic acid and total phenolic content, and antioxidant, antibacterial and antifungal activates of the selected FPEs. Each of the FPE showed a concentration-dependent significant linear increase in FRSC. However, the antibacterial and antifungal activity of the FPEs against each of the selected bacterial and fungal strains was found to be a logarithmic function of the extract concentration. The FPE of *P. pashia* was found to be the best among the selected plants due to comparatively higher values of carbohydrate, crude fiber and ascorbic acid content, FRSC, antibacterial and antifungal activity.

Keywords: antibacterial activity; antifungal activity; free radical scavenging capacity; *Punica protopunica*, *Pyrus pashi*a, *Citrullus lanatus*.

Practical Application: Several investigations on fruit peels have indicated the existence of essential elements that can be exploited in pharmacological or medicinal applications. Numerous components with biological activities such as antibacterial, antioxidant as well as those with nutritional importance have been isolated from the peel of various fruits. This study has explored the nutritional and pharmaceutical potential of the peel from the three commonly consumed fruits that will provide a base for designing the studies to extract the biologically active components to be utilized in pharmaceutical industry.

1 Introduction

Fruits have an important role in providing valuable nutritional components for maintaining human health. However, due to the lack of awareness and information regarding the nutritional composition of different fruits peel, a great part of the fruit in the form of peel is wasted without proper utilization. Fruits peels have been reported as an important source of various bioactive and nutritional components including tannins, flavonoids, phenols and anthocyanins (Ani & Abel, 2018; Orak et al., 2012) which possess antimicrobial and antioxidant activities (Barathikannan et al., 2016; Maniyan et al., 2015).

Citrullus lanatus commonly known as watermelon is a popular species of the family *Cucurbitaceae* originated from southern Africa (Olamide et al., 2011). Investigation of the watermelon fruits revealed that it is a good source of different beneficial chemical constituents (Tlili et al., 2011). This plant has been also reported for holding different pharmacological activities including anti-inflammatory, antiplasmodial, antibacterial, analgesic, hepatoprotective, antidiabetic and antioxidant (Kumawat, 2017).

Punica protopunica known as pomegranate tree or Socotran pomegranate originated from the island Socotra (Yemen), differ from the common pomegranate (*P. granatum*) in color and sweetness. These are the two sole species of the genus *Punica* from the family Lythraceae, where the species *granatum* got more attention for investigation of its chemical constituents and biological activities than *protopunica* (Al-Huqail et al., 2018). Different biological activities such as antioxidant, anti-

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inflammatory, anti-cancer, anti-viral, and anti-angiogenesis have been reported in *P. granatum*, which could be attributed to the presence of diverse types of metabolites including alkaloids, flavonoids and phenols (Rahimi et al., 2012).

P. pashia locally known as wild Himalayan pear, is a deciduous tree of small to medium height bearing small toothed oval to crown ovate leaves, white attractive flowers with red anthers pear-like small fruits (Sheikh, 1992). It is widely distributed in different regions of Pakistan like Chitral, Swat, Kaghan, Hazara, Muree, Poonch and Mirpur districts. This plant is famous for its medicinal and nutritional importance (Janbaz et al., 2015). This plant has also been reported to contain bioactive phytochemical constituents with antioxidant potential (Petkou et al., 2002). This plant has been used to treat conjunctivitis in human and eye infections in cattle (Kanwar & Yadav, 2005; Siddiqui et al., 2015). Other medicinal uses of *P. pashia* include the treatment of gastrointestinal disorders, fever, headache, body sweating (diaphoretic), hysteria and epilepsy (Petkou et al., 2002).

The fruit peel of these commonly used fruits is usually discarded as waste due to unawareness about its potential benefits. Although, most of the studies have been reported on the nutritional and medicinal importance of fruit pulp or seeds of these plants yet the fruit peel has remained unexplored for its phytochemical composition and biological activities (Barathikannan et al., 2016; Negi & Jayaprakasha, 2003; Sajjad et al., 2015). The present study was, therefore, planned to carry out a comparative analysis of the proximate composition, antioxidant phytochemicals and three different biological activities of the fruit peel from *C. lanatus, P. protopunica and P. pashia*. The concentration-dependent response of biological activities of peel extract of the selected plants was also studied by regression analysis. The applied regression model provides a good statistical relationship between the selected input factors and the observed response for such type of studies (Nawaz et al., 2018).

2 Materials and methods

2.1 Experimental design

The present study was designed to determine the proximate composition and some biological activities of the fruit peel of three commonly used fruits including *C. lanatus, P. protopunica* and *P. pashia* grown in Sawat, Khyber Pakhtunkwa, Pakistan. The fruit peel powder of the selected plants was subjected to proximate analysis. The dried samples were also extracted in methanol and the extracts (10 mg/100 mL) were analyzed for antioxidant, antibacterial and antifungal activities in comparison with some standard antimicrobial drugs. The antioxidant activity was determined in terms of free radical scavenging capacity against 2,2-Diphenyl-1-picryl hydrazyle (DPPH) radical. The antibacterial and antifungal activity was determined against two pathogenic bacterial strains including *Escherichia coli* and *Staphylococcus aureus* and two pathogenic fungal strains including *Aspergillus niger* and *Fusarium oxysporium*, respectively. The concentration-dependent behavior of the studied biological activities was determined by regression analysis using a series of extract concentrations as 50, 100, 250, 500, and 1000 µg/mL.

2.2 Sample collection

The fresh fruits of the selected plants were collected in August-September 2016 from village Jambel, District Swat, transported to the Department of Biochemistry, Hazara University, Mansehra Pakistan. The fruits were washed with distilled water, peeled with a sharp knife and the peel was dried under shade in airflow at room temperature. The dried samples were ground using an electrical grinder (Geepas, China) to a fine powder and sieved through a muslin cloth to obtain fine particle size (<100 µm). The powdered samples were stored in airtight glass containers at standard laboratory conditions until analysis.

2.3 Proximate analysis

The moisture content of the fruit peels was determined gravimetrically. The sample (1 g) was taken in a pre-dried, pre-weighed crucible and then subjected to moisture removal by drying at 105 ± 1 °C in a dry heat till constant weight was obtained. The ash contents of the peels were determined as performed previously by Sing and Sharma (Singh & Sharma, 2010). Determination of the crude fiber was performed by the method as described previously by Indrayan et al. (2005). Crude protein was determined through Kjeldahl method as total nitrogen after digestion of the samples using the previously reported method as described by Official Methods of Analysis of AOAC International, 17th Edition (Association of Official Analytical Chemists, 2000). Fats were determined using Soxhlet apparatus as performed previously (Nielsen, 2003). Carbohydrate estimation was performed using the weight difference method (Offor et al., 2014).

2.4 Antioxidant analysis

For antioxidant, antimicrobial, and antifungal analysis, the fruit peels powders were extracted in analytical grade aqueous methanol (85% v/v), in a large closed container at room temperature for 24 h with infrequent shaking. The solvent was evaporated to dryness and the blackish crude extracts obtained were stored at 20 °C, until used for further analysis. The dried extract (1 g) was dissolved in water (100 mL) and proceeded for the analysis of antioxidant phytochemicals including phenolics and ascorbic acid and biological activities including antioxidant, antimicrobial, and antifungal activities.

Total phenolic content

The total phenolic content (TPC) of the extracts was determined using Folin-Ciocalteau's reagent as reported earlier (Muhammad Aslam Shad, 2012). The peal extract (1 mL) was mixed with Folin reagent (0.5 mL) followed by addition of saturated solution of Na_2CO_3. The absorbance of the reaction mixture was noted at 720 nm using a spectrophotometer (Jenway 6505). TPC was calculated as mg/g of extract using regression equation obtained from the standard curve of Gallic acid ($R^2 = 0.9725$).

Ascorbic acid content

The ascorbic acid from the fruit peel of the selected plants was extracted in *m*-phosphoric acid and the content was

determined by redox titration against 2, 6-Dichloroindophenol using previously reported methods (Association of Official Analytical Chemists, 2000; Stan et al., 2014). The ascorbic acid content (AAC) was calculated as mg/g of extract.

Free radical scavenging potential

The antioxidant activity of the extracts was determined in terms of their free radical scavenging potential against stable DPPH radical as performed previously (Muhammad Aslam Shad, 2011). The fruit peal extract (10 mg/mL) was mixed with methanolic solution of DPPH radical (3 mL), allowed to stand for 30 min and the absorbance of the reaction mixture was noted at 517 nm. The DPPH radical scavenging capacity was calculated in terms of percent inhibition of the DPPH radical using following Expression 1.

$$\text{DPPH RSC}(\%) = \frac{Absorbance\,of\,control - Absorbance\,of\,sample}{Absorbance\,of\,control} \times 100 \quad (1)$$

2.5 Antibacterial activity

Antibacterial activities of the FPE were tested against two bacterial strains *i.e. Escherichia coli* and *Staphylococcus aureus*, kindly provided by Microbiology Lab, Department of Pharmacy, University of Malakand, Khyber Pakhtunkhwa, Pakistan, following the Agar-well diffusion method reported earlier (Akinnibosun et al., 2009). The standard antibiotics Erythromycin and Azithromycin and methanol were taken as positive and negative controls respectively. The fruit peel extracts (10 mg/mL) and the solution of standard antifungal drug (10 mg/mL) were applied to agar plates consisting of the selected bacterial strains. All of the plates were incubated at 32 °C for one week. The antibacterial activity of the extracts was measured in terms of zone of inhibition of bacterial growth (mm).

2.6 Antifungal activity

Antifungal activity of the FPE was determined by well diffusion method (Akinnibosun et al., 2009) against *Aspergillus niger* and *Fusarium oxysporium* fungal strains. Griseofulvin, used as a positive control, was poured into the central well of each Petri plate. The fruit peel extracts (10 mg/mL) and the solution of standard antifungal drug (10 mg/mL) were applied to agar plates consisting different fungal strains. All of the plates were incubated at 26 °C for one week. The antifungal activity was measured in terms of inhibition of fungal growth (mm). The methanol

2.7 Statistical analysis

Results were expressed as mean± standard deviation (SD) of three separate determinations. The data was statistically analyzed using the statistical program (Origin Version 5.1). The significant mean differences were calculated by a one-way Analysis of Variance (ANOVA) using Duncan's multiple range test at 95% confidence level ($p < 0.05$). The concentration-dependent behavior of the response variables was determined by regression analysis and the suitability of the applied regression model was checked by correlating the experimental values versus predicted values.

3 Results and discussions

The fruits of *C. lanatus, P. protopunica*, *and P. pyshia*, the commonly grown indigenous plants in Swat valley, are frequently used by the local population. The peel of these fruits, a considerable portion of the biomass, is usually discarded as waste. Previously, the fruit juice and pulp have been investigated for their biological activities (Indrayan et al., 2005; Rahimi et al., 2012; Siddiqui et al., 2015) while the fruit peel of these plants is still unexplored and remained underutilized. The present study covers the analysis of some phytochemical antioxidants and their antioxidant, antibacterial, and antifungal activities. The statistical model applied in this study will open new avenues to determine the concentration-dependent behavior of biological activities of plant materials.

The current work reports the proximate and phytochemical composition and antioxidant, antibacterial and antifungal activities of the peels of the selected fruits. The proximate composition is an important criterion for the determination of nutritional values and quality of food (Qayyum et al., 2012). The findings for the proximate composition of *C. lanatus*, *P. protopunica* and *P. pashia* fruits peel are listed in Table 1. The ash and moisture content (g/100 g dw) of the fruit peel of the selected fruits ranged from 1.88 ± 1.10 to 5.50 ± 0.96 and 11.00 ± 1.02 to 21.10 ± 1.13, respectively. *C. lanatus* peel was found to be high in ash content while fruit peel of *P. pashia* contained comparatively highest moisture content. The total carbohydrate and crude fat, protein and fiber content (g/100 g dw) ranged from 46.50 ± 1.15 to 48.77 ± 1.03 and 2.20.00 ± 1.05 to 4.10 ± 1.08, 8.75 ± 1.00 to 14.37 ± 0.95 and 31.5 ± 1.16 to 35.6 ± 0.15, respectively. *P. protopunica* was found to be high in crude protein and crude fat content while fruit peel of *P. pashia* contained the comparatively highest amount of total carbohydrate and crude fiber content. A statistically significant variation ($p < 0.05$) in ash, moisture, crude protein, and crude fiber content was observed. The fruit peel of *P. pashia* was found to be best among the selected plants regarding the total carbohydrate and crude fiber content while *P. protopunica* fruit peel was comparatively rich in crude fat and protein content. However, the fruit peel of *C. lanatus* was found to be low in organic matter but high in ash content which indicates the presence of a relatively higher amount of minerals in *C. lanatus* peel. These findings also suggest an inverse correlation of inorganic matter with the organic one. Contradictory, a previous study has reported that fruit peel of *C. lanatus* is lower in ash content (5.03 ± 0.80 g/100g) compared to that in pomegranate (6.07 ± 0.07 g/100 g). Similarly, the fruit peel of *C. lanatus* was found nutritionally better than that of the *P. granatum* in containing a higher quantity of the crude protein, lipids, and fiber (Feumba Dibanda Romelle, 2016). This difference could be attributed to the different species (*Ggranatum*) of the *Punica* used in their study. Comparative biochemical analysis of the fruit juice and peel of another plant species i.e. *Citrus maxima* indicated that peel has more nutritional value than the juice of the fruit (Ani & Abel, 2018).

Table 1. The proximate composition (g/100 g dw) of fruits peel of the selected plants.

Nutritional content	*C. lanatus*	*P. protopunica*	*P. pashia*	*P value*
Ash Content	5.50 ± 0.96*	3.53 ± 1.01	1.88 ± 1.10	0.014
Moisture	12.40 ± 1.11	11.00 ± 1.02	21.10 ± 1.13	0.000
Carbohydrate	48.30 ± 1.05	46.50 ± 1.15	48.77 ± 1.03	0.089
Crude Fat	3.50 ± 1.03	4.10 ± 1.08	2.20 ± 1.05	0.158
Crude Protein	08.75 ± 1.00	14.37 ± 0.95	11.55 ± 0.98	0.001
Crude Fiber	33.80 ± 1.52	31.50 ± 1.16	35.60 ± 0.15	0.011

*The values are expressed as mean ± SD of three parallel determinations on dry weight (dw) basis.

3.1 Antioxidant potential

Total phenolic content

Gallic acid equivalent total phenolic content (TPC) in fruit peel extract (FPE) of *C. lanatus*, *P. protopunica*, and *P. pashia* was found to be 1.11 ± 0.08, 1.24 ± 0.10 and 0.88 ± 0.07 mg/g of extract. A statistically significant difference ($p < 0.05$) was observed in TPC of FPE of the selected plants that was found to be comparatively higher in *P. protopunica* and low in *P. pashia* (Figure 1A). A recent study reported that fruit peels of both the *Punica* species (*Protopunica* and *Granatum*) are a good source of phenolic components responsible for the antioxidant activity of these plants. Although reports on the chemical analysis of the *P. protopunica* fruit peel are scarce, several studies reported the fruit peel of *P. granatum* as a good source of phenolic contents (Feumba Dibanda Romelle, 2016; Negi & Jayaprakasha, 2003; Orak et al., 2012).

Ascorbic acid content

The AAC of FPE of the selected plants ranged from 13.20 ± 1.13 to 20.00 ± 1.23 mg/g extract. A statistically significant variation ($p < 0.05$) in AAC was observed with the comparatively highest value for fruit peel of *P. pashia* followed by fruit peel of *P. protopunica* and *C. lanatus* (Figure 1B). Previously, no investigation on the AAC of FPE of the selected plants has been reported in the literature. However, the AAC of the selected FPE were found to be higher than that reported for *P. granatum* peel (Ani & Abel, 2018).

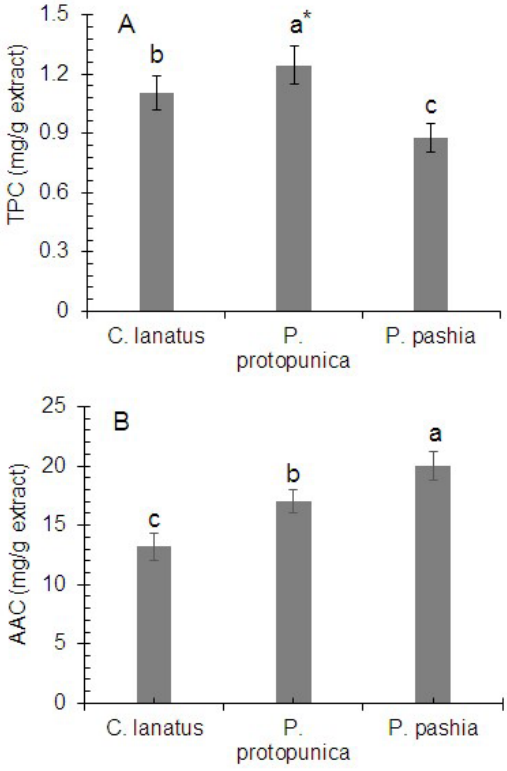

Figure 1. Phytochemical composition of fruit peel extracts of the selected plants. TPC: Total phenolic content, AAC: Ascorbic acid content. *The bars and error bars represent the mean values and standard deviations of three parallel replicates. The bars labelled with different alphabets are statistically different at $p \leq 0.05$ using Duncan's multiple range test.

DPPH radical scavenging capacity

The free radical scavenging capacity of FPE of *C. lanatus*, *P. protopunica* and *P. pashia* at 10 mg/100 mL concentration was found to be 58.50 ± 2.93, 51.5 ± 2.575, and 62.5 ± 4.34% respectively (Table 2). A statistically significant difference ($p < 0.05$) was observed in the radical scavenging activity of the extracts. *P. pashia* fruit peel was found to possess comparatively higher scavenging potential among the selected fruits. The high antioxidant potential of the *P. Pashia* and *C. lanatus* may be attributed to the identified flavonoids and non-flavonoids phenolic compounds from different parts of these plants (He et al., 2015; Zamuz et al., 2021), indicating the presence of such bioactive compounds in fruit peel of the plants.

A concentration-dependent behavior of DPPH-RSC was also studied by taking the regression curve at various concentrations of the extracts. The regression analysis of the data showed that the radical scavenging capacity of each extract was a linear function of extract concentration (Figure 2A). The concentration-dependent increase in the radical scavenging capacity of the extracts was explained by the following generalized regression Equation 2:

$$\text{DPPH RSC}(\%) = RSC_{SC}E_c + RSC_0 \quad (2)$$

where RSC_{SC} is the radical scavenging capacity sensitivity coefficient, E_c is the extract concentration and RSC_0 is the radical scavenging capacity at negligible concentration.

The predicted values of DPPH RSC at different levels of extract concentration were calculated by putting the values of RSC_{SC}, E_c and RSC_0 in the regression equation. The predicted

Table 2. Biological activities of FPE of the selected plants and the standard drugs at 10 mg/100 mL concentration.

Activity	Free Radical/ Strain	Fruit peel extracts			Standard drugs		*p-value*
		C. lanatus	*P. protopunica*	*P. pashia*	Erythromycin	Azithromycin	
FRSC (%)	DPPH·	58.50 ± 2.93	51.5 ± 2.58	62.5 ± 4.34			0.000
Antibacterial activity (ZOI: mm)	*Escherichia coli*	11.60 ± 2.15	6.50 ± 1.12	5.60 ± 1.15	30 ± 1.51	ND	0.001
	Staphylococcus aureus	10.50 ± 2.01	9.03 ± 2.01	11.60 ± 1.17	ND	28 ± 1.43	0.004
Antifungal activity (IOG: (%)					Griseofulvin		
	Aspergillus niger	20.10 ± 2.41	13.00 ± 1.14	25.5 ± 1.27	40 ± 2.00		0.000
	Fusarium oxysporium	13.50 ± 1.62	15.25 ± 2.11	18.3 ± 0.92	46 ± 2.3		0.001

FRSC: Free radical scavenging capacity; ZOI: Zone of Inhibition, IOG: Inhibition of growth, ND: Not determined.

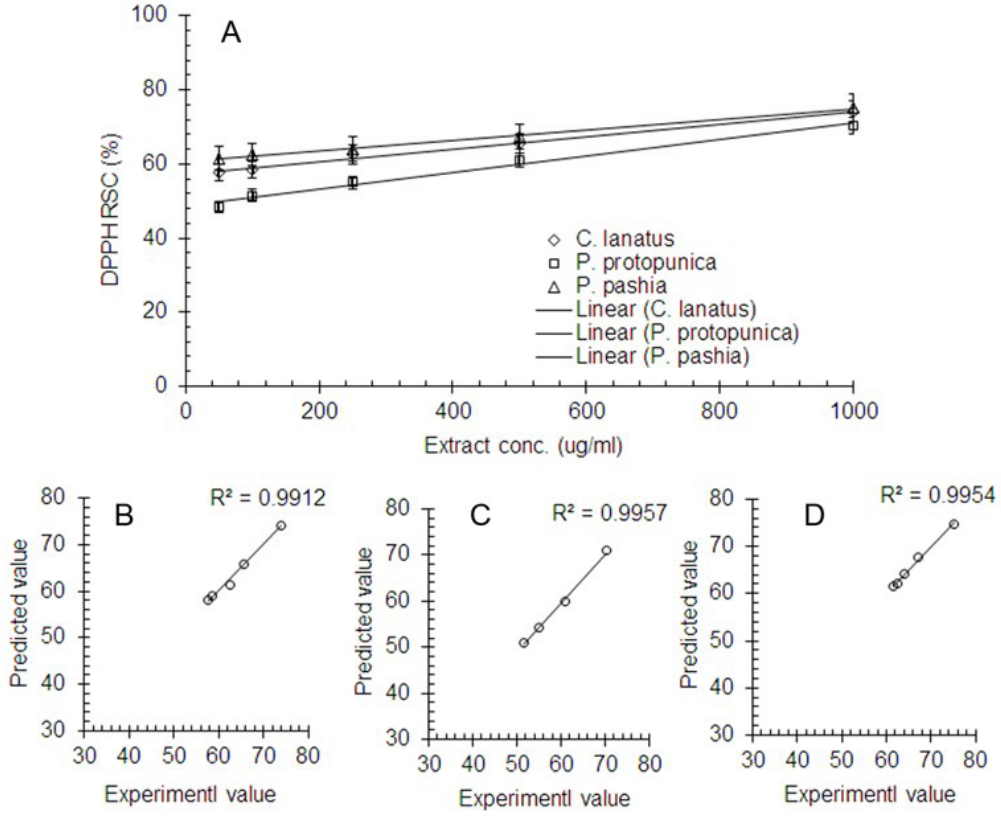

Figure 2. (A) The concentration-dependent response of antioxidant potential of fruit peel extracts of the selected plants in terms of free radical scavenging capacity and (C-D) the plots of actual values versus predicted values obtained by regression analysis. (B) *C. lanatus*, (C) *P. protopunica*, (D) *P. pashia*.

values, thus obtained, were used to determine the accuracy and applicability of the suggested regression model. The plot of the experimental values versus predicted values of DPPH RSC showed that the suggested model is applicable with a high value of coefficients of determination (R^2 = 0.9912-0.9957) to study the concentration-dependent variation in the free radical scavenging capacity of the FPE of the selected plants (Figure 2B-D). The regression equations and regression coefficients obtained from the suggested model and p-values of each sample are presented in Table 3.

Free radical scavengers are the antioxidants compounds which possess donate-able hydrogens in their structure. The phenolic are the strong antioxidants as free radical scavengers due to the presence of the donate-able hydrogens in the form of hydroxyl groups (Kulkarni et al., 2004; Razali et al., 2008). However, the present results showed an inverse correlation between the TPC and DPPH RSC for *P. protopunica* and *P. pashia* fruit peels. Although being high in TPC, *P. protopunica* was found to show lower free radical scavenging capacity than *P. pashia*. This may be attributed to the presence of phenolic compounds

Table 3. Regression equations, regression coefficients and *p-values* obtained from the regression curve of biological activities of FPE as a function of extract concentration.

Activity	Sample	Free Radical/Strain	Regression equation	Regression coefficient (R^2)	*p-value*
Free radical scavenging	*C. lanatus*	$DPPH^{\cdot}$	FRSC (%) = 0.017x + 57.175	0.9912	0.002
	P. protopunica	$DPPH^{\cdot}$	FRSC = 0.0221x + 48.859	0.9957	0.000
	P. pashia	$DPPH^{\cdot}$	FRSC = 0.014x + 60.716	0.9954	0.005
Antibacterial	*C. lanatus*	*E. coli*	ZOI (mm) = 2.5434ln(x) - 0.3365	0.9937	0.002
		S. aureus	ZOI (mm) = 2.4944ln(x) - 2.0903	0.9501	0.005
	P. protopunica	*E. coli*	ZOI (mm) = 4.0564ln(x) - 11.535	0.9685	0.010
		S. aureus	ZOI (mm) = 3.6414ln(x) - 9.9809	0.9436	0.003
	P. pashia	*E. coli*	ZOI (mm) = 3.716ln(x) - 6.2659	0.9731	0.024
		S. aureus	ZOI (mm) = 3.4457ln(x) - 10.078	0.9786	0.004
Antifungal	*C. lanatus*	*A. niger*	IOG (%) = 19.845ln(x) - 67.219	0.9552	0.000
		F. oxysporium	IOG (%) = 22.025ln(x) - 82.283	0.9888	0.000
	P. protopunica	*A. niger*	IOG (%) = 16.656ln(x) - 60.698	0.9896	0.000
		F. oxysporium	IOG (%) = 13.361ln(x) - 48.137	0.9892	0.000
	P. pashia	*A. niger*	IOG (%) = 25.142ln(x) - 90.097	0.9849	0.000
		F. oxysporium	IOG (%) = 17.475ln(x) - 60.686	0.9928	0.000
	Griseofulvin	*A. niger*	IOG (%) = 26.21ln(x) - 82.898	0.9942	0.000
		F. oxysporium	IOG (%) = 22.988ln(x) - 62.816	0.9888	0.000

containing a relatively higher number of donate-able hydrogens or electron-donating groups on their structure in *P. pashia* and vice versa in *P. protopunica*.

3.2 Antibacterial activity

Antibacterial activity of FPE of *C. lanatus*, *P. Protopunica*, and *P. pashia* was determined in terms of zone of inhibition (ZOI) of growth of two bacterial strains known as *Escherichia coli* and *Staphylococcus aureus*. The ZOI (mm) of growth of *E. coli* and *S. aureus* under the influence of fruit peel extracts and standard antibiotics at 10 mg/100 ml concentration was found to be: *C. Lanatus* 11.60 ± 2.15 and 10.50 ± 2.01, *P. protopunica* 6.50 ± 1.12 and 9.03 ± 2.01 and *P. pashia* 5.6 ± 1.15 and 11.6 ± 1.17 mm respectively (Table 2). The FPE of *C. Lanatus* showed significantly higher activity ($p<0.05$) against *E. coli* while no significant difference was observed in antibacterial activity of the extracts against *S. aureus*. However, the antibacterial activities of FPE were found to be significantly lower than those of Erythromycin (ZOI: 30 mm against *E. coli*) and Azithromycin (ZOI: 28 mm against *S. aureus*) taken as standard antibacterial drugs.

The results are in agreement with those reported earlier that peel extracts of the selected fruit have potential antibacterial activity. *P. granatum* peel extract presented antibacterial activities against different pathogenic bacterias including *E. Coli* (Barathikannan et al., 2016; Sajjad et al., 2015). Similarly, *C. lanatus* fruit peel has been reported to harbor antibacterial activty against the bacterial species used in this study which provide the scientific basis of the use of the fruit peel in traditional medicine (Nessma Ahmed El Zawawy, 2015). Although, some parts of the *P. pashia* are reported for diverse biological activities including antimicrobial activities, the fruit peel of this plant is still unexplored for its biological activities (Zbigniew et al., 2014). Similarly, compounds such as flavonoids, saponin and tannins with antimicrobial activity were previously identified from *C. lanatus* (Zamuz et al., 2021), indicating the potential of the plant harboring such activity.

A concentration-dependent behavior of antibacterial activity was also studied by taking the regression curve at various concentrations of the extracts. The regression analysis of the data showed a logarithmic increase in the ZOI of growth of each bacterium under the influence of each extract with an increase in the extract concentration (Figure 3A, D, and G). The logarithmic increase in the ZOI as a function of extract concentration suggests that the bioactive phytochemical compounds present in FPE of the selected plants are highly active against the selected bacteria even at low concentrations (50-250 µl/ml). However, an increase in extract concentration up to 500 µl/ml or above resulted in very slow increase in antibacterial activity.

The concentration-dependent increase in antibacterial activity of the extracts in terms of ZOI was explained by the following generalized regression Equation 3:

$$\text{Zone of inhibition}\,(\text{mm}) = ZOI_{SC}\, ln\, E_c + ZOI_0 \quad (3)$$

where ZOI_{SC} is the zone of inhibition sensitivity coefficient, ln is the natural log, E_c is the extract concentration and ZOI_0 is the zone of inhibition at negligible concentration.

The predicted values of ZOI at different levels of extract concentration were calculated by putting the values of ZOI_{SC}, E_c and ZOI_0 in the regression equation and plotted against the experimental values to determine the accuracy and applicability of the suggested regression model. A good agreement was observed between the experimental and predicted values with

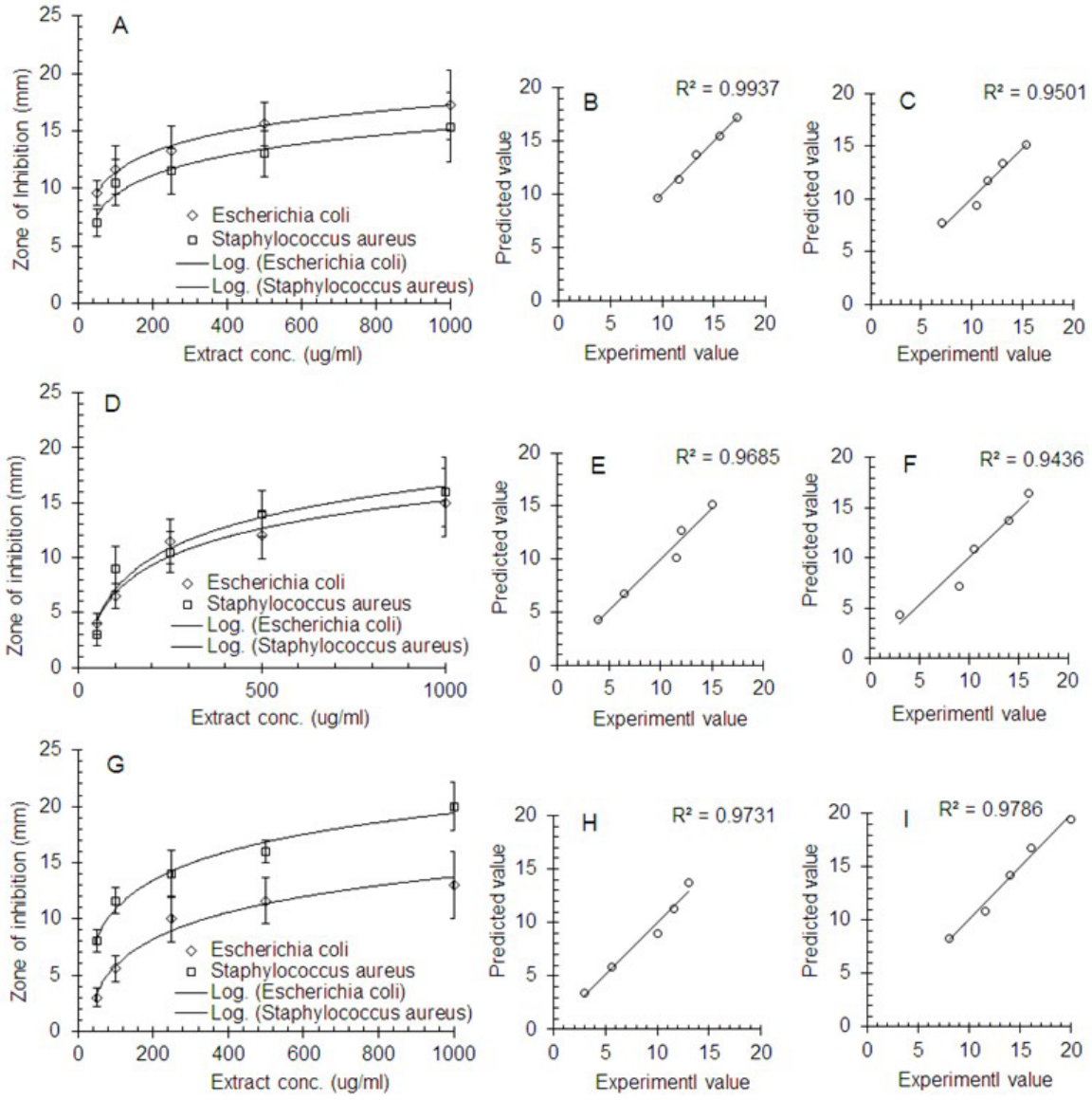

Figure 3. The concentration-dependent response of antibacterial activities, of fruit peel extracts of the selected plants in terms of zone of inhibition of bacterial growth and the plots of actual values versus predicted values obtained by regression analysis. (A-C) *C. lanatus*, (D-F) *P. protopunica*, (G-I) *P. pashia*.

high values of coefficients of determination (R^2 = 0.9436-0.9937) suggesting the applicability of the proposed model to study the concentration-dependent behavior of FPE of the selected plants (Figure 3B, C, E, F, H, and I). The regression equations and regression coefficients obtained from the suggested model and p-values of each sample against each of the bacterial strain are presented in Table 3.

3.3 Antifungal activity

The antifungal activity of methanolic extracts of fruit peel of *C. lanatus*, *P. protopunica* and *P. pashia* was determined in terms of percent inhibition of growth (IOG) of *Aspergillus niger* as well as *Fusarium oxysporium*. The IOG (%) of *Aspergillus niger*, as well as *Fusarium oxysporium* under the influence of FPE and Griseofulvin taken as standard antifungal drug at 10 mg/100 mL concentration, was found to be: *C. Lanatus* 20.10 ± 2.41 and 13.50 ± 1.62, *P. protopunica* 13.00 ± 1.14 and 15.25 ± 2.11, *P. pashia* 25.5 ± 1.27. and 18.3 ± 0.92 and Griseofulvin 40 ± 2.00 and 46 ± 2.3% respectively (Table 2). The fruit peel extract of *P. pashia* showed significantly higher activity ($p < 0.05$) than *C. Lanatus* and *P. protopunica* against both of the fungal strains. However, the antibacterial activities of fruit peel extracts were found to be significantly lower than that of Griseofulvin.

The results are in agreement with those reported earlier for *C. lanatus* where, in comparison to fruit peel of other plants, it showed lower antifungal activity against the fungal strains used here (Nessma Ahmed El Zawawy, 2015). Previously antifungal potential was attributed to the presence of flavonoids, saponin and tannins

(Zamuz et al., 2021). Antifungal activity for the fruit peel of the *P. protopunica* and *P. pashia* is not reported but the similar activities from the fruit peel of the other species (Barathikannan et al., 2016) of the genus endorse our findings that the fruit peels of the selected plant may contain phytochemicals with antifungal activities.

A concentration-dependent-behavior of antifungal activity was also studied by taking the regression curve at various concentrations of the extracts. The regression analysis of the data showed a logarithmic increase in the IOG of each bacterium under the influence of each extract and Griseofulvin with an increase in the extract/standard concentration (Figure 4A, D, G, and J). The logarithmic increase in the IOG as a function of extract concentration suggests that the bioactive phytochemical compounds present in the FPE of the selected plants are highly active against the selected fungal strains even at low concentrations

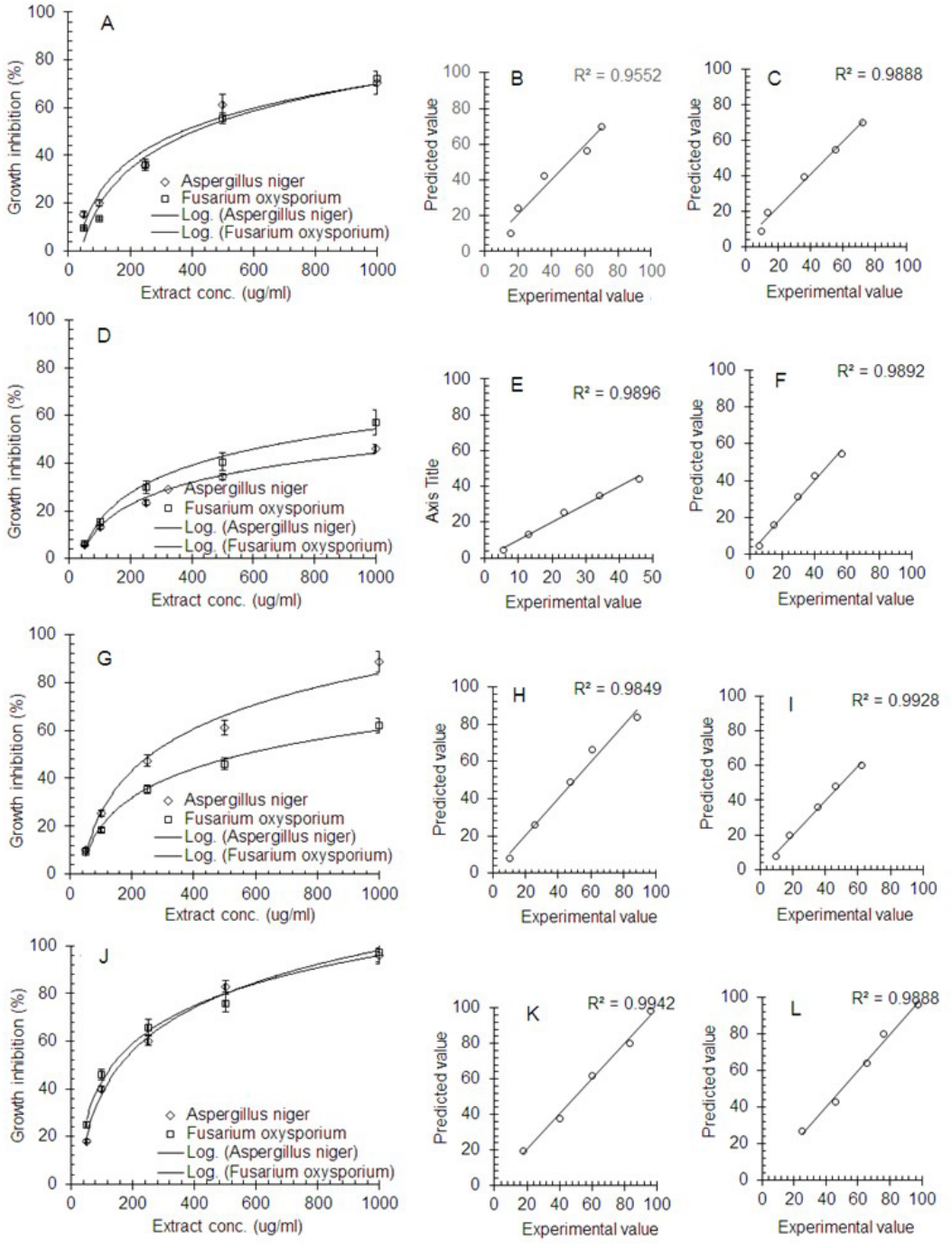

Figure 4. The concentration-dependent response of antifungal activities, of fruit peel extracts of the selected plants and Griseofulvin in terms of zone of percent inhibition of fungal growth and the plots of actual values versus predicted values obtained by regression analysis. (A-C) *C. lanatus*, (D-F) *P. protopunica*, (G-I) *P. pashia*, (J-L) Griseofulvin.

(50-250 µL/mL). However, an increase in extract concentration up to 500 µL/mL or above resulted in a very slow increase in antibacterial activity. The concentration-dependent increase in antibacterial activity of the extracts in terms of IOG was explained by the following generalized regression Equation 4:

$$\text{Inhibition of growth}(\%) = \text{IOG}_{SC} \ln E_c + \text{IOG}_0 \quad (4)$$

where IOG_{SC} is the zone of inhibition sensitivity coefficient, *ln* is the natural log, E_c is the extract concentration and IOG_0 is the zone of inhibition at negligible concentration.

The predicted values of IOG at different levels of extract concentration were calculated by putting the values of IOG_{SC}, E_c and IOG_0 in the regression equation and plotted against the experimental values to determine the accuracy and applicability of the suggested regression model. A good agreement was observed between the experimental and predicted values with high values of coefficients of determination ($R^2 = 0.9552$-0.9942) suggesting the applicability of the proposed model to study the concentration-dependent behavior of the FPE of the selected plants (Figure 3B, C, E, F, H, I, K, and L). The regression equations and regression coefficients obtained from the suggested model and p-values of each sample against each of the bacterial strain are presented in Table 3.

4 Conclusion

The fruit peels of *C. lanatus, P. protopunica*, and *P. pashia* possessed good proximate and phytochemical composition and biological activities. The selected FPEs were found to be significantly different in their proximate composition, studied phytochemical constituents, and biological activities. The peel extract of *P. pashia* was found to be the best among the selected plants due to comparatively higher values of carbohydrate, crude fiber, and ascorbic acid content, free radical scavenging capacity, antibacterial activity against *S. aurius* and antifungal activity against *A. nigar*. Each of the FPE showed a concentration-dependent linear increase in free radical scavenging capacity and a logarithmic increase in antibacterial and antifungal activities.

Conflict of interest

The authors declare no conflict of interest.

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