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Isolation and Characterization of Bioactive Compounds from Fruit Wastes

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ABSTRACT

The current study was aimed at utilizing fruit wastes generated after pectin extraction for assessing their antimicrobial and antioxidant properties. Total soluble proteins (TSP) and heat-stable proteins (HSP) were extracted from wastes of *Musa* sp., *Citrus limetta*, *Citrullus lanatus*, *Solanum lycopersicum* and *Psidium* sp. The HSP from *S. lycopersicum* waste could suppress the growth of *Escherichia coli* whereas *Musa* sp. and *C. limetta* HSP could inhibit the growth of *Pseudomonas* sp. *C. limetta* HSP was most effective in suppressing the growth of *Fusarium oxysporum* relative to the other test samples. No pathogens responded towards the HSP of *C. lanatus*. High antioxidant activity [Ferric Reducing Antioxidant Power (FRAP)] along with high phenolic levels were observed in *Psidium* sp. and *Musa* sp. fruit residues. Adopting appropriate extraction methods for active biomolecules from biodegradable wastes may pave the way for neutriceutical and pharmaceutical applications.

Keywords: fruit waste, pectin, antimicrobial and antioxidant activity, phenolic content
Abbreviations: FRAP, ferric reducing antioxidant power; **HSP**, heat-stable protein; **TSP**, total soluble protein; **GA**, gallic acid; **EDTA**, ethylene diamine tetra acetate; **TPTZ**, 2,4,6-tripiridyl-s-triazine

INTRODUCTION

Fruit and vegetable wastes are the resourceful components for generating bioactive compounds like antimicrobials and antioxidants (Maatta-Riihinen *et al.* 2004; Gautam and Guleria 2007; Ayoola *et al*. 2008; Reddy *et al.* 2011). Pulp, rind and seed extracts of *Musa sapientum*, *M. paradisiaca* (cv. 'Bontha') and *Musa* sp. were found to be antimicrobial against various human pathogens (Fagbemi 2009; Jain *et al.* 2011; Rao *et al.* 2012). Biomolecules isolated from different parts of pomegranate (*Punica granatum*) and other fruits exhibited antioxidant and antimicrobial properties (Kabuki *et al.* 2000; Singh *et al.* 2002; Negro *et al.* 2003; Li *et al*. 2006; Prasad *et al.* 2010; Yehia *et al*. 2011). Antolovivich *et al.* (2004) opined that natural phenolic compounds in plants need to be investigated for their antioxidant mechanisms and biological functions.

The present study was focused on recovery of the total soluble proteins (TSP) and heat stable proteins (HSP) after the extraction of pectins from the fruit wastes collected from the market yards. The HSP of different fruits were tested individually for their probable antimicrobial activities against selected pathogens and their phenolic contents to assess the antioxidant properties. The study has relevance to utilize biodegradable matter as bioresource material.

MATERIALS AND METHODS

Fruit peels from *Musa* sp. (cv. 'Cavendish') and *Citrus limetta* (cv. 'Sweet lime'), rind of *Citrullus lanatus* (watermelon) and putrefied fruits of *Solanum lycopersicum* (cv. 'Roma tomato') and *Psidium* sp (cv. 'Red Indian') were homogenized using de-ionized water (1: 1.5, w/v). Lemon juice was added to homogenate to adjust the pH to 2-2.5. The autoclaved homogenate was filtered to recover the pectin from the filtrate (Schemin *et al*. 2005). The residue was sun dried and further used utilized for protein extraction.

Protein extraction from the residue

All the chemicals and reagents used for the study were purchased from Sigma-Aldrich.

The protein was extracted from the residue using the extraction buffer (1M Tris HCl pH 7.6, EDTA 0.5 M pH 8.0, ascorbic acid and β-mercaptoethanol). Mixture was agitated on a magnetic stirrer for 20 min at 4°C. It was centrifuged at 10,000 rpm at 4°C. Supernatant containing TSP was heated 10 min. at 70°C. It was further centrifuged at 10,000 rpm at 4°C to obtain HSP. The amount of protein was quantified by UV-visible spectrophotometer (Shimadzu UV-1700) at 280 nm. The HSP recovered from different fruit wastes were tested against the pathogens for their antimicrobial properties.

Antimicrobial assay

The well diffusion method (Shobha and Kale 2008) was followed to assess the antimicrobial activity of HSP. Cultures of *E. coli* and *Pseudomonas* sp. were swabbed uniformly on nutrient agar plates. The concentrations tested included 50, 75 and 100% in 100 µl of the original protein levels in the samples. Sterile distilled water was used as control in all the plates. Evaluation of antifungal activity against *Fusarium oxysporum* (on Potato Dextrose Agar plates) was carried out similarly. All the antimicrobial assays were done in triplicates.

Antioxidant assay

Antioxidant activity of the fruit wastes was assayed by measuring Ferric Reducing Antioxidant Power (FRAP) (Benzie and Strain 1996). The residues of the five different fruit wastes were extracted using 50% methanol at room temperature and centrifuged at 10,000 rpm. The supernatants were filtered after washing with equal volumes of petroleum ether to remove oil content. The FRAP reagent (10 mmol/L TPTZ (2,4,6-tripiridyl-s-triazine) in 40 mmol/L HCL plus 20 mmol/L $FeCl_3$ and 0.3 mmol/L acetate buffer,

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Short Communication

pH 3.6) was warmed at 37°C and mixed with methanolic extract. After incubating the mixture for 10 min at 37°C, the reactant was measured at 593 nm.

Analysis of total phenolics

Total phenolics were determined colorimetrically (Velioglu *et al.* 1998) with slight modifications. The methanolic extracts of the residues obtained from the different fruit wastes were mixed individually with Folin Ciocalteau reagent (diluted 10-fold with distilled water) and incubated at 22°C, for 5 min. Sodium bicarbonate solution (60 g/L) was added and after 90 min at 22°C, absorbance was measured at 725 nm. Total phenols of unknown samples were quantified from the absorbance values of known concentrations of gallic acid. The total antioxidant assay and the analysis of total phenols were carried out in triplicates and the mean values were considered for statistical analyses.

RESULTS AND DISCUSSION

The fruit and vegetable wastes that are found to contain 12% crude protein form part of the diet of pigs and this reduces the quantum of waste entering landfills (Esteban *et al.* 2007). Mahopatra *et al.* (2010) reported that *Musa* sp. peel contains 1.8% protein. The results of the present study also suggest the presence of proteins in fruit wastes. It was found that TSP and HSP concentrations in *S. lycopersicum* waste was highest (2.17 and 1.48 mg/mL) and the lowest level was in *Musa* sp. (0.93 and 0.90 mg/mL) (**Table 1**).

Bacterial strains have developed resistance to many drugs and moreover the new generation drugs are to the reach of common man (Aibinu *et al.* 2003). Hence there is always a need to look for potent antimicrobials from other sources. The World Health Organization (WHO) has an estimate that four billion (80%) of the world's population presently use herbal medicine for primary healthcare. Pharmacologists, rather than using a whole plant, are engaged in synthesis of individual components (bioactive principles) to work against the pathogens (Rios and Recio 2005). The present investigation is an attempt to assess the antimicrobial activity of the HSPs isolated from the fruit wastes.

The suppressive property (zone of inhibition) of the extractable HSP against *E. coli* measured as clear zone around the well was found to be at the range of 2.26-2.36 and 2.73-3.13 cm, respectively for 50 and 100% concentrations. Undiluted HSP (100%) of *S. lycopersicum* waste showed the highest antimicrobial activity (3.13 cm) against *E. coli* whereas that of *Psidium* sp. was effective at a concentration of 50% (2.36 cm). HSP of *C. limetta* has shown the suppressive zone of 2.73 cm and from reports of Kumar *et al.* (2011), the zone of inhibition observed for crude extracts of peels and leaves of *C. sinensis* against *E. coli* was 9 mm for the aqueous extract and 8 mm for the ethanol extract. The results of the present study indicate that the specific isolates from the plant residues are more efficient than when tested as crude preparations (**Table 2**).

Zone of inhibition observed against *Pseudomonas* sp. was 2.40-2.70 and 2.80-3.33 cm at 50 and 100% concentrations, respectively of HSP. Jain *et al.* (2011) used different organic solvents like hexane, ethyl acetate and ethanol for extraction of active principles from peels of *Musa* sp. against *Pseudomonas aeruginosa.* They found that ethanol and ethyl acetate were the effective solvents that could extract the principles responsible for the inhibition of pathogen (12.0 and 16.5 mm, respectively) and hexane was not a favoured solvent for the purpose. The HSP (100%) from peels of *Musa* sp. and *C. limetta* showed the highest suppression of *Pseudomonas* sp. (3.33 cm). HSP from *Musa* sp. was highly effective though the recovered protein was comparatively low (**Tables 1, 2**).

The results of the study were indicative of importance of water soluble, temperature tolerant (non enzymic) proteins as antimicrobials to suppress the growth of pathogens.

HSP extracted from *C. limetta* waste (100, 75 and 50%) showed the maximum suppression of *Fusarium oxysporum* and the least was by HSP from *C. lanatus* (**Table 3**). HSP from *Psidium* sp. showed the lowest suppression of the plant pathogen at 75 and 50%, respectively. An antifungal peptide pomegranin isolated from pomegranate was found to suppress 50% of the population of *F. oxysporum* with an IC_{50} of 6.1 μM (Guo *et al.* 2009). It was found that HSP from *C. limetta* waste efficiently suppressed growth of *F. oxysporum*. The present study showed that the antifungal activity of the HSPs was concentration dependant unlike the antibacterial activity and showed an increase in growth suppression with increase in concentration (**Table 3**).

Table 1 Total soluble proteins and heat stable proteins extracted from various fruit wastes.

Fruit types	TSP (mg/mL)	HSP (mg/mL)
Musa sp.	0.93	0.90
Citrus limetta	1.73	1.38
Citrullus lanatus	1.84	1.40
Solanum lycopersicum	2.17	1.48
Psidium sp.	1.43	1.40

TSP = Total Soluble Protein; HSP = Heat Stable Protein

Table 2 Antibacterial activity of HSP extracted from different fruit wastes against *E. coli* and *Pseudomonas* sp. (n=3).

Fruit waste	Zone of inhibition Mean ± S.D (cm)			
	Escherichia coli		*Pseudomonas* sp.	
	HSP concentration			
	100%	50%	100%	50%
Musa sp.	2.93 ± 0.11	2.30 ± 0.10	3.33 ± 0.47	2.53 ± 0.25
Citrus limetta	2.73 ± 0.11	2.33 ± 0.06	3.33 ± 0.28	2.70 ± 0.17
Citrullus lanatus	2.73 ± 0.11	2.30 ± 0.17	2.80 ± 0.53	2.40 ± 0.45
Solanum lycopersicum	3.13 ± 0.32	2.26 ± 0.21	2.83 ± 0.21	2.43 ± 0.30
Psidium sp.	2.90 ± 0.10	2.36 ± 0.23	3.26 ± 0.40	2.70 ± 0.17

HSP = Heat Stable Protein

Table 3 Antifungal activity of HSP against *Fusarium oxysporum* (n=3).

Fruit waste	Zone of inhibition (cm) Mean ± S.D		
	HSP concentration		
	100%	75%	50%
Musa sp.	2.26 ± 0.31	1.86 ± 0.40	1.33 ± 0.35
Citrus limetta	2.70 ± 0.17	2.10 ± 0.17	1.60 ± 0.17
Citrullus lanatus	1.76 ± 0.05	1.46 ± 0.11	1.10 ± 0.17
Solanum lycopersicum	2.00 ± 0.20	1.53 ± 0.15	1.16 ± 0.15
Psidium sp.	1.93 ± 0.11	1.03 ± 0.25	0.33 ± 0.28

p = 0.01; Fcrit = 2.03742; Fcal = 19.91079

Table 4 Total phenolic content and antioxidant activity of various fruit wastes (n=3).

Fruit waste	Total phenolic content g GA/mL	FRAP value mmol Fe^{2+}/mL
Musa sp.	0.45	8.56
Citrus limetta	0.31	7.48
Citrullus lanatus	0.13	6.79
Solanum lycopersicum	0.09	2.95
Psidium sp.	0.63	8.75

GA = Gallic Acid; FRAP = Ferric Reducing Antioxidant Power

Table 5 ANOVA for total phenolic content and total antioxidant activity (n=3; p=0.01)

	F critical	F calculated
Phenolic content	3.47805	611.3818
Antioxidant activity	3.47805	54.63028

Total phenolic compounds and related antioxidant activity in the residual fruit waste

The growing interest in the substitution of synthetic food antioxidants by natural ones has fostered research on fruit and vegetable sources and the screening of raw materials for identifying new antioxidants. Polyphenols are the major

plant compounds with antioxidant activity (Moure *et al.* 2001). Biophenols have attracted increasing attention during the past few years due to their biological activities and natural abundance and are potential targets for the food and pharmaceutical industries (Hassan *et al.* 2005). The highest phenolic content was found in *Psidium* sp. (0.63 g GA/mL) and lowest phenolic content was found in *S. lycopersicum* residual waste (0.09 g GA/mL) (**Table 4**). Related research findings suggest that fruit peels and seeds, such as peels of grape, pomegranate, wampee and seeds of grapes and mango possess antioxidant properties (Kabuki *et al.* 2000; Singh *et al.* 2002; Prasad *et al.* 2010).

The highest FRAP value was obtained for the extract of *Psidium* sp. (8.75 mmol Fe^{2+}/mL), followed by *Musa* sp. (8.56 mmol Fe^{2+}/mL) and least for *S. lycopersicum* residual waste (2.95 mmol Fe^{2+}/mL) (**Table 4**).

Guo *et al.* (2003) have reported high FRAP values in *Psidium* sp. which is in accordance with the present study. The antioxidant activity of various varieties of *S. lycopersicum* were measured using both free radical quenching assay and FRAP assay and it was found to be higher in the hexane fraction containing lycopene than the methanol fraction containing phenolics (George *et al.* 2004). This could be a possible reason for obtaining low FRAP value where methanol extract of *S. lycopersicum* residual waste was considered in the current study. The total extractable phenols varied with the tested samples and were significantly different (**Table 5**).

There was an increase in the antioxidant activity with an increase in total phenolic content of the methanolic extracts of fruit wastes, except in case of *C. lanatus* waste extract, where the antioxidant activity was high even though the phenolic content was found to be low (**Table 4**). An earlier report also suggests that there exists correlation between the total antioxidants and the total phenolic content of the Iranian olive pulp and 97% of the antioxidant capacity results from the contribution of phenolic compounds (Hajimahmoodi *et al.* 2008). The present results are in accordance with it.

CONCLUSION

The potential of fruit and vegetable waste is not only limited to the production of value added products but could also be utilized in generating bioactive compounds like antimicrobials and antioxidants.

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