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Comparative Analysis of Phenolic Contents in Litchi and Pomelo Fruit Peel

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ABSTRACT

Fruit processing industry produces large quantities of fruit peels as waste material which causes environmental pollution if not recycled carefully. There is a vast scope for using fruit peels in animal feed industry, as it contains antioxidants *i.e.* phenolics and flavonoids compounds that may be used for producing functional feed for animal in improving their health. The main aim of present investigation is analyze the phenolic and flavonoid profile of two fruit peels- litchi and pomelo. For this purpose, Shed dried peels were grounded and dissolved in 80% methanol (v/v) for extraction and were subsequently analysed by HPLC-DAD to estimate their antioxidant capacity. Results revealed that litchi peel contains significantly ($P<0.05$) high level of total phenolic content than pomelo peel. Detailed report was obtained from the phenolic and flavonoid finger printing. It was observed that s*yringic acid* and *kaempferol* were the principal phenolic component in litchi peel, where as pomelo peel predominantly contains n*aringin, kaempferol*and m*yricetin*. The study clearly indicate that both litchi and pomelo peels contain various antioxidant molecules that could be strategically used in feed designed for better growth promotion, disease prevention and overall wellbeing of animals.

Key Words: Antioxidants, Flavonoid, Litchi, Peels, Phenolic.

INTRODUCTION

Cellular oxidation is a natural outcome of cell metabolism Reactive Oxygen Species (ROS) from cellular oxidation cause oxidative damage to the living system leading to negative performance (Fathi *et al*, 2011; Ismail *et al*, 2013). Synthetic antioxidants like ethoxyquin, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are widely used in poultry feed industry; however their long term use may pose vital organ dysfunctions in poultry and may enter into the food chain (Tan, 2009). Due to the growing concerns about synthetic antioxidants, various research works have been carried out so far on dietary manipulation of different herbal extracts that would be equally effective as synthetic antioxidants (Zhao *et al*, 2011; Loetscher *et al*, 2013; Settle *et al*, 2014). Again, supplementation of herbal extracts to animal feed is known to produce anti-microbial and anti-inflammatory actions apart from anti-oxidative effects (Khan *et al*, 2012; Allen *et al*, 2013). These unique properties of natural antioxidants have recently attracted researchers worldwide to identify phenolic compounds in fruit peel extracts. Polyphenol compounds particularly phenolic acids and flavonoids present in the fruit peels are potent anti-oxidants (Babbar *et al,* 2011) by virtue of their ability to accept electron from ROS and thus may act as additive in animal feed.

In this context, European food safety authority has already recommended use of *naringin*, a flavanone glycoside, in the animal feed as additive (EFSA, 2011). Different flavones like *myricetin, naringin, catechin, rutin, quercetin, and kaempferol* were proved to confer beneficial effects on animals when used as additives in ruminal diets (Oskoueian *et al*, 2013). In India, Litchi (*Litchi chinensis*) and pomelo (*Citrus grandis*) are two important fruits available in the market during summer (April-June) and post monsoon (August-October) respectively. Many authors (Queiroz *et al*, 2015; Zefang *et al*, 2016) opined that these two fruits as rich source of

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phenolic acids and flavonoids. However, no reports have been documented so far on detail profile of phenolic acid/flavonoid components from litchi and pomelo peels in local cultivars, so the present research work was undertaken to examine the different phenolic and flavonoids content in the in the litchi and pomelo fruit peels.

MATERIALS AND METHODS

Procurement of raw material

Litchi and Pomelo fruits (Table 1) were collected from local orchards of Baruipur, West Bengal, India during the month of June-August, 2017 and stored in cold condition for 1-2d. Peels were collected in sterilized plastic bins.

Estimation of Physio-chemical properties

Moisture

Moisture percentage of peels was determined by a moisture meter (Mettler-Toledo, Switzerland) and peels were subsequently washed with distilled water and air dried under shade for five days. The peels were chopped into smaller pieces and then made it into coarse powder using an electrical grinder. The dried powder of peels was packed into air tight containers in refrigerated condition (4˚C) for preparation of liquid extracts.

Total Phenolic Contents

One gram of sample peel powder was extracted with 25 ml of 80% (v/v) HPLC grade methanol (Merck, Germany) in 27°C. The mixture was kept in an orbital shaker for 4 h with 110 rpm. The extracts were filtered through Whatman No.1 filter paper. The residues were re-extracted again with the same procedure under the same condition and filtered in the same way. The two extract fractions were pooled and final volume was adjusted to 50 ml in a volumetric flask. Total Phenolic Contents (TPC) was measured by using Folin-Ciocalteu method (Singleton *et al*, 1999) with slight modifications. Briefly 500 µl of extracts were mixed with 2.5 ml of Folin-Ciocalteu reagent and incubated for five minutesat 25°C in dark. Then 2.5 ml of 7.5% Na_2CO_3 solution was added and the mixture was incubated again at 25°C for 15 min. Then absorbance of the samples were determined at λ_{max} = 765 nm using an automated UV/Visible spectrophotometer (Shimadzu 1800-UV, Japan). Standard curve was constructed by using 10, 20, 30, 40 and 50 mg/ml solutions of gallic acid. Total phenolic content was expressed in terms of gallic acid equivalent (mg of GaA/g of dry extract).

Total Flavonoids Content (TFC)

Total Flavonoids Content (TFC) was measured according to Pal *et al (* 2009) and Naskar *et al* (2011). The assay was determined using 0.5 ml of each extract solution and each dilution of standard quercetin taken separately in test tubes. To each test tube 1.5 ml methanol, 0.1 ml aluminium chloride solution, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. The mixture was incubated for 30 min in 27˚C and absorbance was measured at λ_{max} = 415 nm by UV/Visible spectrophotometer (Shimadzu 1800-UV, Japan). Quercetin solution (concentration range 10-60 mg/ml) was used to make a standard curve. Results were expressed in mg of quercetin equivalent (mg of QE/g of dry weight).

Table 1. Description of collected fruits.

Sr. No.	Fruit	Ripening stage of collection	*Moisture % (at time of collection)*	*Cultivar*	Peeling Method
1	**Litchi** (*Litchi chinensis*)	Mature	*73.89*	*Bombai*	Hand peeling
2	**Pomelo** (*Citrus Grandis L. Osbeck*)	Mature	*75.33*	*White fleshed-Indigenous*	Hand peeling

Estimation of antioxidants via HPLC

Fingerprinting of 80% (v/v) methanolic extracts of peels was performed using HPLC method (Seal *et al*, 2016) using Dionex Ultimate 3000, Thermo Scientific, USA equipped with quaternary pump (LPG 3400 SD) for solvent delivery, 20µl loop for injection and PDA detector (DAD 3000) and Chromeleon 6.8 system manager as data processor. The separation was achieved using reverse phase column, AcclaimTM 120 C18 column (250mm x 4.6mm, 5µm). Individual peel extractswere further diluted with aquous-methanol (20:80) at a concentration of 1 mg/ml and filtered through 0.2 µm PDVF filter. Standard polyphenols like gallic acid, protocatechuic acid, p-hydroxy benzoic acid, catechin, chlorogenic acid, caffeic acid, vanilic acid, syringic acid, p-coumaric acid, ferullic acid, sinapic acid, salicylic acid, naringin,, rutin, ellagic acid, myrectin, quercetin, apigenin and kaempferol were prepared in aquous-methanol (20:80) at concentration 1 mg/ml as stock solution. Further dilutions were made for calibration of each standard. The mobile phase contains methanol (Solvent A) and 1% acetic acid solution (Solvent B), the column was thermostatically controlled at 28˚ C. The gradient elusion was 10 % A and 90% B with flow rate 1ml/min to 0.7 ml/min in 27 min, from 10 % to 40% A and 90% to 60% B with flow rate 0.7ml/min for 28 min, 40 % A and 60% B, with flow rate 0.7 to 0.6 ml/min for 5 min, from 40 to 44 % A and 60% to 56% B with flow rate 0.6 to 0.3 ml/min in 5 min, 44 % A and 56% B with flow rate 0.3 to 0.6 ml/min in 5 min. The mobile phase composition back to initial condition of 10% A and 90% B and allowed to run for another 8 min, before another injection of sample. The detection of compounds was performed using detector at 280 nm. Each compound was identified by its retention time and by spiking with standards under the same conditions. The quantification of the sample was done by the measurement of the integrated peak area and the content was calculated using the calibration curve of the respective standard sample.

Statistical Analysis

The mean and standard error was calculated for each of the parameters examined and compared for significant differences between the groups by using a paired *t*- test (Excel, Microsoft). A *P* value of less than 0.05 indicated statistically significant difference.

RESULTS AND DISCUSSION

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

TPC gives an idea about antioxidant potential of the plant sample. Present study (Table 2) revealed that TPC in litchi peel was significantly higher ($P<0.05$) than that of pomelo peel, however no significant difference was observed in case of TFC. Kanlayavattanakul *et al* (2012) reported 90.547 mg of GaE/g DWof TPC in litchi pericarp from a different cultivar in Thailand. Queiroz *et al* (2015) observed 0.71 mg of GaE/g DW of TPC in dried litchi peel in Brazil. Such large variations of TPC in litchi peel as reported by various authors may be attributed to extraction procedure, geochemical variations and difference in cultivars. Wu *et al* (2011) reported that TPC of white variety pomelo peel of Taiwan was 9.99 mg GAE/g DW. Zefang *et al* (2016) reported a range of TPC between 6.04 to 11.53 mg of GaE/g DW in Chinese pomelo cultivars. This was in agreement with present study and revealed that litchi variety under study contains more quantity of the total phenolic compounds. Earlier works showed high degree of positive correlation between antioxidant activity and TPC (Jayaprakasha *et al,* 2008; Pilluza and Bullitta; 2011; Singh *et al*, 2016).

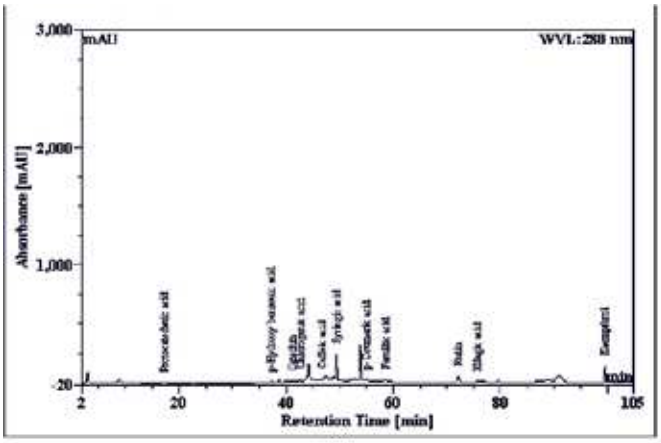
Flavonoids are strong bioactive compounds that act against various diseases, inflammatory conditions and oxidative stress (Yao, 2004). Present study (Table 2) indicated that both litchi and pomelo peel contains good amount of flavonoids. Pomelo peel contains slightly higher amount of TFC than litchi peel. Ghasemi *et al*., (2009) evaluated flavonoid content of 13 citrus species in Pakistan

Table 2.Total Phenolic Content (TPC) and Total Flavonoid Content (TFC).

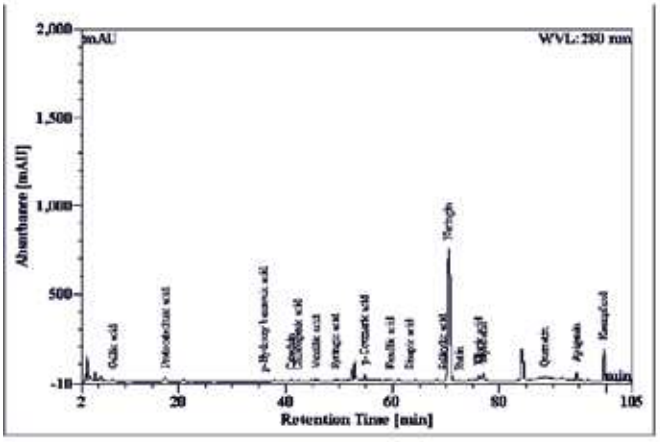
Total Phenolic Content (mg of GaE/g of dry weight) and Flavonoid Content(mg of QE/g of dry weight) in peel samples				
	Litchi Peel	Pomelo Peel	t stat	P Value
Total Phenolic Content	36.54 ±1.56	17.34±2.21	10.88	0.01*
Total Flavonoid Content	2.86±0.02	2.86± 0.28	-0.007	0.99
*Significant Difference at P<0.05; paired sample t test				

and TFC varied from as low as 0.3 to as high as 31.1 mg QE/g DW. In the present study, TFC of pomelo peel was in accordance withGhasemi *et al*., (2009). No comparable data was available for litchi peel.

HPLC Analysis of Phenolic acids and Flavonoids



(A)



(B)

Fig. 1: HPLC Chromatogram of A) Litchi Peel and B) Pomelo Peel

Detailed profile of phenolic acids and flavonoids (Table 2) were characterised by HPLC-DAD using nineteen external standards for identification and quantification. Results revealed that syringic acid and kaempferol was the predominant phenolic acid and flavonoid respectively in litchee peel. Syringic acid is a hepatoprotective agent and kaempherol is widely known for its anti-metastatic and anti-inflammatory role in living system (Itoh *et al*, 2009; Chen and Chen, 2013). Significant amount of rutin was also observed in litchi peel which was in accordance with previous study (Li and Jiang, 2007). Rutin is a flavonoid and strong antioxidant that has positive action on several performance parameters on poultry (Peña *et al*, 2008) and dairy (Stoldt *et al*, 2016) due to their superoxide and hydroxyl radical scavenging activities.

Pomelo peel extract showed plethora of phenolic compounds upon analysis. Among them naringin was predominat flavonoid followed by Kaempferol and ellagic acid. Naringin is available from diverse source of citrus fruits. Present report validated earlier reports (Yusof *et al*, 1990) that showed high level of naringin in pomelo fruit peel. Naringin content of pomelo peel is much higher than that of orange peel (Omoba *et al*, 2015; Pereira *et al*, 2017).Therapeutic potential (Chen *et al*, 2015) of naringin may be harnessed using pomelo peel extract as additive in animal feed.

CONCLUSION

Both litchi and pomelo peel contains valuable bioactive compounds that could be used for various

Table 2. Profile of phenolic acids and flavonoids present in the peel samples.

Sr. No.	Phenolic Acid	Litchi Peel (mg/g)	Pomelo Peel (mg/g)	t stat	P value
1	*Gallic Acid*	ND	0.078±0.005	-	-
2	*Protocatechuic acid*	0.020±0.008	0.276±0.003	-105.455	0.000089*
3	*p-Hydroxy benzoic acid*	0.057±0.001	0.028±0.006	4.453	0.046*
4	*Ellagic acid*	0.066±0.001	0.324±0.01	-25.408	0.001*
5	*Chlorogenic acid*	0.152±0.013	0.067±0.011	11.725	0.007*
6	*Caffeic acid*	0.011±0.001	ND	-	-
7	*Vanilic Acid*	ND	0.057±0.002	-	-
8	*Syringic acid*	**0.623±0.018**	0.036±0.002	38.865	0.0008*
9	*p-Coumaric acid*	0.028±0.001	0.049±0.001	-13.56	0.005*
10	*Ferullic acid*	0.052±0.002	0.02±0.001	12.132	0.006*
11	*Sinapic Acid*	ND	0.005±0.001	-	-
12	*Salicylic Acid*	ND	0.058±0.001	-	-
	Flavonoids				
13	*Myrectin*	ND	0.36±0.019	-	-
14	*Quercetin*	ND	0.025±0.002	-	-
15	*Apigenin*	ND	0.174±0.013	-	-
16	*Kaempferol*	0.459±0.01	**0.749±0.016**	-27.764	0.001*
17	*Catechin*	0.057±0.001	0.104±0.011	-5.292	0.033*
18	*Naringin*	ND	**6.796±0.003**	-	-
19	*Rutin*	**0.457±0.011**	0.049±0.001	38.572	0.0006*
ND: Not Detected *Significant Difference at P<0.05; paired sample t test					

pharmacologic actions in animals. Pomelo peel extract particularly had large amount of naringin and thus could be used as additive in animal feed industry. However further research on antioxidant potential of theses fruit peel wastes should be carried out before recommending them as additive in animal feed in near future.

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