Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(3): 2891-2894
Received: 15-03-2018
Accepted: 20-04-2018

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Total phenol and flavonoids in by-product of Indian litchi: Difference among genotypes

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Abstract
An investigation was carried out to determine the differences in phenolic and flavonoids profiles on by-product of (*Litchi chinensis* Sonn.) from thirty genotypes available in India at ICAR-NRC on Litchi, Muzaffarpur, Bihar during 2017. Litchi by-product contains significant amounts of phenol and flavonoids which have been found to exhibit diverse biological activities. The results indicated that the total by-product in litchi fruit varied from 19.85 per cent in genotype IC-0615587 to 59.54 per cent in genotype IC-0615595. The maximum seed and pericarp per cent was found in genotype Coll.38 (22.58%) and genotype IC-0615595 (36.96%), respectively. A wide variation in phenolics content has been observed in the present study. The maximum phenol in pericarp was found in genotype IC-0615613 (62.2 mg GAE/g) followed by IC-0615588 (55.77 mg GAE/g) whereas, lowest phenol was found in genotype IC-0615606 (7.5 mg GAE/g) followed by Coll. 35 (8.75 mg GAE/g) and maximum phenol in seed was found in genotype IC-0615597 (85.57 mg GAE/g) followed by IC-0615603 (75.3 mg GAE/g whereas, the minimum phenol in seed was recorded in IC-0615608 (23.01 mg GAE/g) followed by IC-0615606 (24.53 mg GAE/g). The maximum pericarp flavonoid content was observed in four genotypes *viz*., IC-0615592, IC-0615595, IC-0615613 and Coll.37 (96.62 mg CE/g) followed by IC-0615598 (59.93 mg CE/g) while, the minimum flavonoid in pericarp was found in genotype IC-0615600 and IC-0615610 (0.73 mg CE/g) followed by IC-0615602 (0.86 mg CE/g). The maximum flavonoid content in seed was observed in genotype IC-0615586 (27.50 mg CE/g) followed by IC-0615602 (20.79 mg CE/g) whereas, the minimum flavonoid content in seed was found in genotypeIC-0615587 (2.41 mg CE/g) followed by IC-0615591(2.92 mg CE/g) and IC-0615592 (3.20 mg CE/g). The correlation study showed that total phenol in pericarp had significant positive correlation with flavonoids content in pericarp (0.413) and had highly significant negative correlation with per cent of seed (-0.635) in fruit. Similarly, flavonoids in pericarp had significant negative correlation with flavonoids in seed (-0.436) but had positive correlation with per cent of pericarp (0.341). Total phenol in seed had positive correlation with per cent of pericarp (0.336) in fruit at harvesting.

Keywords: genotype, by-product, pericarp, seed, phenol, flavonoids

1. Introduction
The chronic diseases, such as cardiovascular diseases and cancer are the leading causes of death in many developed and developing countries. Many researches have suggested that diets rich in fruits and vegetables are associated with reduced risk of these diseases. Phytochemicals in fruits and vegetables are known to be associated with many health benefits. Therefore, interest of research on phenolics compound has been increasing day by day. Litchi is one of the best source of phenolics in which all parts of litchi contains phenol and flavonoids. Litchi (*Litchi chinensis* Sonn.), an evergreen tree belonging to the Sapindaceae family, has been widely cultivated for fruit in tropical and subtropical areas of the world. Litchi fruit is popular in domestic and foreign markets because of its juicy and sweet aril and attractive red pericarp. The annual output of fresh fruit in India exceeds 580 (000 MT) and the pericarp and seed accounts for approximately 20% and 15% of the whole fruit in fresh weight. Litchi aril is consumed fresh or as processed products, while the pericarp and seed is discarded. Phytochemical investigation of the pericarp and seed is necessary to utilize it as a resource. The pericarp is usually discarded as waste is however now being considered as new source of bioactive phenolics and flavonoids (Zhao *et al.*, 2006) [17].
Different parts of litchi can be used for improvement of human health due to its nutritious compounds such as amino acids, dietary fibers, linoleic acid, trace elements, vitamins, and added unsaturated fatty acids (Wills and Lim 1986, Wall 2006) [11, 13]. In addition, the peel and seed are rich in antioxidants such as ascorbic acid, phenolic compounds including gallic acid, flavonoids (procyanidin B4, procyanidin B2and epicatechin), and anthocyanins (cyaniding 3-rutinoside, cyanidin-3-glucoside, quercetin 3-rutinoside and quercetin 3-glucoside). Pharmacological studies indicate that the by-products of the litchi have various effects

including anti-inflammatory, anti-hyperlipidemic, anti-hyperglycemic, hepatic and cardioprotective, as well as having high antioxidant activity (Jiang *et al*., 2012, Xu *et al*., 2011) [3, 14].

Litchi pericarp contains large amounts of phenolics (Zhang *et al*., 2000) [15]. The biological activity of the phytochemical extracts was associated with their composition and contents of activity ingredient. Moreover, little is known about the complete profiles of phenolic compounds and antioxidant activity of LFP of different varieties. There is scanty information on phenolic content in Indian litchi cultivars. However, many workers have been reported from different countries. Therefore, it is important to determine the phenolic contents and characterize individual bioactive compounds of Indian litchi genotypes. The aims of this study thus were: (1) to determine phenolic profiles, (2) to compare the varietal differences of phytochemicals, and (3) to investigate the correlations between total phenolics and flavonoids.

2. Materials and Methods

Thirty litchi germplasm of thirteen years old planted in Active National Germplasm Site at ICAR-NRC on Litchi, Muzaffarpur with uniform vigour and size were selected for the investigation during 2017. These trees were given uniform cultural practices and three tree of each germplasm were considered for recording the observations. The experiment was conducted in RBD with three replications. The analysis of variance and correlation coefficient was estimated.

2.1 Litchi genotypes collected from different sources

Source of collection	Litchi accessions
Sabour, Bhagalpur Bihar	IC-0615585, IC-0615586, IC-0615587, IC-0615588, IC-0615589, IC-0615590, IC-0615591
Pantanagar, Udham Singh Nagar (UK)	IC-0615592, IC-0615593, IC-0615594
Palandu, Ranchi (JH)	IC-0615595, IC-0615596, IC-0615597, IC-0615599, Coll. 39, IC-0615600, IC-0615601, IC-0615602
Gurdaspur, Punjab	IC-0615603, IC-0615604, IC-0615605, IC-0615606
Mohanpur, Nadia, (WB)	IC-0615608, Coll. 38
Muzaffarpur, Bihar	IC-0615610, IC-0615611, IC-0615613, Coll. 35
Surguja (CG)	Coll. 36
Bhuvaneshar, Odisa	Coll. 37

2.2 Per cent of seed and pericarp

The weight of seed and pericarp were obtained from 20 fruits and per cent of seed and pericarp was calculated against fruit weight.

Seed (%) = (Seed weight/Fruit weight) x 100

Pericarp (%) = (Pericarp weight/Fruit weight) x 100

2.3 Total phenol (mg/g)

The total phenolic content was determined by the method given by Sethi *et al.* (2013) [10]. Weigh exactly 200 mg of the sample and grind it with pestle and mortar in 10 ml of 80% ethanol. Centrifuged the homogenate at 10,000 rpm for 10 minutes and extract the supernatant. Pipetted out 20 microlitre in attest tube and make up the volume to 3 ml with distilled water and then added 0.5 ml of 1N of FCR (Folin ciocalteau reagent). After three minutes, added 2 ml of 20% of Na_2CO_3 solution to each tube. The sample was mixed thoroughly and the absorbance was taken at 750 nm. Standard curve was established using various concentrations of gallic acid and results were expressed as gallic acid equivalent (GAE). Total Phenolic content was calculated as gallic acid equivalent (mg/100g).

2.4 Total flavonoids (mg/100gm)

The total flavonoids content was determined by the method given by Sethi *et al*. (2013) [10]. A known volume (1ml) of the sample extract in 80% ethanol was added to make up the volume to 5 ml with distilled water and 0.3 ml of 5% sodium nitrite was added. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added and after 6 minutes, 2 ml of 1M NaOH was added to mixture. This was followed by adding distilled water to make a final volume to 10 ml and mix to appear pink to yellow colour. Absorbance was read at 510 nm against the blank (water) and flavonoids content was expressed as catechin (mg) per gram.

3. Results and Discussions

3.1 By-product in different litchi genotypes

From the Fig 1, it can be drawn that the total by-product in litchi varied from 19.85 per cent in genotype IC-0615587 to 59.54 per cent in genotype IC-0615595. The maximum seed per cent was found in genotype Coll.38 (22.58 %) followed by Coll. 35 (19.01 %) whereas, lowest seed per cent was found in IC-0615613 (6.96 %) followed by IC-0615604 (9.09 %). The maximum pericarp per cent was recorded in genotype IC-0615595 (36.96 %) followed by IC-0615589 (31.32 %) whereas, lowest per cent of pericarp was found in genotype IC-0615587 (12.89 %) followed by IC-0615606 (13.58 %).

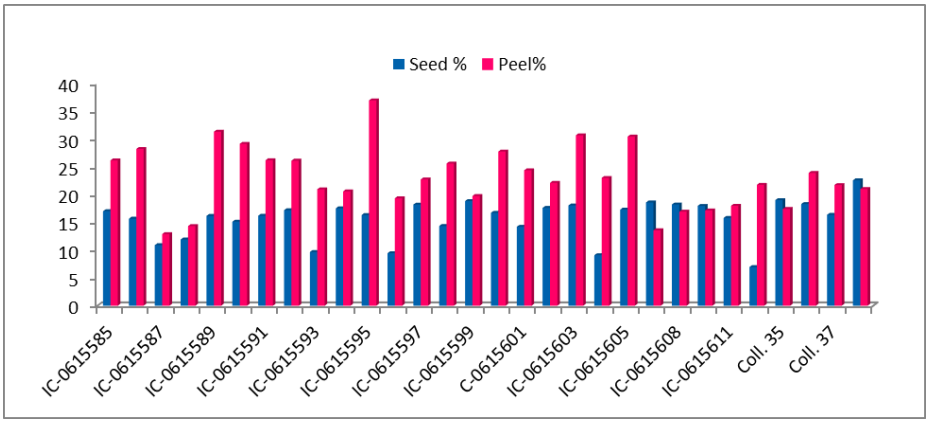

Fig 1: Per cent of by-product of fruit in 30 litchi genotypes

3.2 Total phenol and flavonoids in litchi by-product

Total phenol and flavonoids in by-product of litchi are presented in Table 2. The maximum phenol in pericarp was found in genotype IC-0615613 (62.2 mg GAE/g) followed by IC-0615588 (55.77 mg GAE/g) and IC-0615587 (54.9 mg GAE/g) whereas, lowest phenol was found in genotype IC-0615606 (7.5 mg GAE/g) followed by Coll. 35 (8.75 mg GAE/g) and IC-0615601 (10.19 mg GAE/g) and maximum phenol in seed was found in genotype IC-0615597 (85.57 mg GAE/g) followed by IC-0615603 (75.3 mg GAE/g) and IC-0615585 (64.89 mg GAE/g) whereas, the minimum phenol in seed was recorded in IC-0615608 (23.01 mg GAE/g) followed by IC-0615606 (24.53 mg GAE/g) and Coll. 35 (24.92 mg GAE/g). Prasad *et al.* (2009) [7] showed a difference in phenolic content in pericarp with 16 mg/g dry weight in Baila variety and by Ruenroengklin *et al.* (2008) [9] with 100 mg/g dry weigh in Feizixiao variety. The total phenolic contents of tested litchi genotypes were comparable to that of the grape seed with 1.4–22.3 mg GAE/g (Guendez *et al.*, 2005) [2] and skin with 4.9–13.8 mg GAE/g (Poudel *et al*., 2008) [6]. Therefore, litchi fruit pericarp is a good source for phenolic compounds. Reyes *et al*. (2016) **[8]** also found total phenol in fresh pericarp (16.5±0.91mg GAE/L) and dry pericarp (121.3 ± 3.2 mg GAE/L).

It has been found that phenolic compounds exist in significant quantities in cell walls of plants. These compounds, called bound phenolics, are covalently conjugated to cell wall components such as cellulose, pectin and polysaccharides through ester bonds (Naczk and Shahidi, 1989) [5]. Because of the conjugation with cell wall components, bound phenolics cannot be extracted through commonly used solvent extraction method, which results in underestimation also, to some extent, of total phenolic content. The variation in total phenol content in litchi was also observed by Feng *et al.* (2017) [1], Wang *et al.* (2011) [12], Zhang *et al.*, (2013) [16]. These varietal differences may be partly attributed to the different genotypes of these litchi cultivars and to different growing conditions. Phenolic content in plant depends on genetic, agronomic and environmental factors. These results indicated that the genotypes vary greatly in their capacity to synthesize phenolics.

The maximum pericarp flavonoid content was observed in four genotypes *viz*., IC-0615592, IC-0615595, IC-0615613 and Coll.37 (96.62 mg CE/g) followed by IC-0615598 (59.93 mg CE/g) and IC-0615585 (44.50 mg CE/g) while, the minimum flavonoid in pericarp was found in genotype IC-0615600 andIC-0615610 (0.73 mg CE/g) followed by IC-0615602 (0.86 mg CE/g) and IC-0615608 (0.91 mg CE/g). The maximum flavonoid content in seed was observed in genotype IC-0615586 (27.50 mg CE/g) followed by IC-0615602 (20.79 mg CE/g) and IC-0615600 (17.63 mg CE/g) whereas, the minimum flavonoid content in seed was found in genotypeIC-0615587 (2.41 mg CE/g) followed by IC-0615591(2.92 mg CE/g) and IC-0615592 (3.20 mg CE/g). The maximum flavonoid in seed was observed in genotype IC-0615586 (27.01 mg CE/g) followed by IC-06155602 (21.14 mg CE/g) whereas, the lowest flavonoids was found in genotype IC-0615597(2.26 mg CE/g) followed by IC-0615591 (2.74 mg CE/g). A great variation has been seen in respect of flavonoids content in litchi genotypes. The germplasm which contains low flavonoids have diverted to pericarp and seed. The partitioning of secondary metabolites during growth and development of fruits decides the phenol content in different parts of fruit. Li *et al.* (2012) [4] found that total flavonoids contents of nine varieties ranged from 7.12 to 23.46 mg CAE/g FW. Prasad *et al.* (2009) [7] reported that the major flavonoid compounds extracted from LFP were epicatechin and epicatechin gallate. Zhao *et al.* (2006) [17] identified the major flavonoid chemicals extracted from LFP as procyanidin B2, procyanidin B4 and epicatechin.

Table 2: Total phenol and flavonoids in by product of litchi

Genotypes	Total phenol (mg GAE/g)		Total flavonoids (mg CE/g)	
	Peel	Seed	Peel	Seed
IC-0615585	50.12	66.17	44.01	10.71
IC-0615586	40.19	37.29	8.42	27.50
IC-0615587	55.27	43.84	6.93	14.62
IC-0615588	56.44	43.38	10.24	14.76
IC-0615589	44.00	41.07	2.91	10.40
IC-0615590	48.65	34.01	40.93	4.23
IC-0615591	36.92	39.80	95.69	2.92
IC-0615592	37.72	35.85	40.31	3.20
IC-0615593	39.84	61.40	2.24	9.01
IC-0615594	14.24	44.49	1.02	9.74
IC-0615595	34.16	60.82	95.94	6.79
IC-0615596	40.08	61.47	19.14	9.13
IC-0615597	12.77	86.56	1.83	2.41
Coll. 39	34.92	61.16	59.17	10.23
IC-0615599	30.96	44.56	11.39	7.50
IC-0615600	13.28	26.33	0.79	17.63
C-0615601	11.19	50.26	1.37	13.71
IC-0615602	16.74	42.58	0.85	20.79
IC-0615603	35.88	74.41	19.85	9.84
IC-0615604	40.38	60.58	2.23	8.81
IC-0615605	28.56	61.25	12.24	8.91
IC-0615606	8.57	25.49	1.56	14.23
IC-0615608	12.85	24.12	0.97	8.98
IC-0615610	14.77	25.53	0.75	4.03
IC-0615611	27.46	44.91	44.48	4.07
IC-0615613	61.24	27.31	96.37	7.65
Coll. 35	9.30	23.68	2.33	11.34
Coll. 36	23.97	41.24	9.81	9.78
Coll. 37	29.76	48.75	96.49	4.04
Coll. 38	25.08	51.65	9.64	10.34
SEm ±	0.278	0.244	0.224	0.261
CD at 5%	0.789	0.692	0.637	0.742

Table 3: Correlation Coefficient

	Phenol in Pericarp	Phenol in Seed	Flavonoids in Pericarp	Flavonoids in Seed	Seed %	Pericarp %
Phenol in Pericarp	1	.165	.403*	-.028	-.628**	.169
Phenol in Seed		1	.057	-.213	-.076	.361
Flavonoids in Pericarp			1	-.443*	-.198	.341
Flavonoids in Seed				1	-.032	-.054
Seed %					1	..124
Pericarp %						1

* Correlation at 5% and ** Correlation at 1%

The correlation study was presented in Table 3 showed that the total phenol in pericarp had significant positive correlation with flavonoids content in pericarp (0.403) and had highly significant negative correlation with per cent of seed (-0.628)

in fruit. Similarly, flavonoids in pericarp had significant negative correlation with flavonoids in seed (-0.443) but had positive correlation with per cent of pericarp (0.341). Total phenol in seed had positive correlation with per cent of pericarp (0.361) in fruit at harvesting. The results indicated that phenol and flavonoids had positive correlation. A genotype having more phenol content in pericarp is also good source of flavonoids and genotype possessed maximum per cent of seed resulted low content of phenol in pericarp because phenols are diverted to seed and pulp. The flavonoids content in pericarp was inversely correlated with flavonoids content in seed. It means the genotype having more flavonoids in pericarp possessed low flavonoids in seed. This is mainly due diversion in flavonoids accumulation to seed from pericarp. The more per cent of pericarp in fruit had also good source of flavonoids.

4. Conclusion

Litchi by-products are good source of phenol and flavonoids exhibit diverse biological activities. The total by-product of a fruit varied from 19.85-59.54 per cent. Different genotypes displayed marked difference in the level of total phenol and flavonoids composition. The genotype IC-0615597 and IC-0615613 exhibited highest content of phenol in seed and pericarp, respectively, while lowest phenol content was found in seed of IC-0615608 and in pericarp of IC-0615606. Similarly, maximum flavonoid content in seed of genotype IC-0615586 and in pericarp of genotype IC-0615592, IC-0615595, IC-0615613 and Coll. 37. The total phenol in pericarp had significant positive correlation with flavonoids content in pericarp (0.403) and had highly significant negative correlation with per cent of seed (-0.628) in fruit. Similarly, flavonoids in pericarp had significant negative correlation with flavonoids in seed (-0.443) but had positive correlation with per cent of pericarp (0.341). Total phenol in seed had positive correlation with per cent of pericarp (0.361) in fruit at harvesting.

5. Acknowledgement

Authors are thankful to Department of Horticulture, JNKVV Jabalpur to allow the research at NRCL and ICAR-NRC on Litchi, Muzaffarpur for providing all the facility and chemicals to complete the study.

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