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IN VITRO ANTIOXIDANT ACTIVITY AND GC-MS ANALYSIS OF PEEL AND PULP EXTRACTS OF *DIMOCARPUS LONGAN*

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ABSTRACT

Waste substrates such as peels and seeds are accumulated with several bioactive compounds that impart various pharmacological properties. Longan is a sub tropical fruit that is either consumed as fresh, frozen, canned or dried. In spite of being rich in several bioactive and non-nutritive compounds, the peels and seeds of this fruit is usually discarded. The objective of the present study was to compare the antioxidant activity of peel and pulp extracts of *Dimocarpus longan*. Total phenol and flavonoid content was estimated using Folin – Ciocalteau and Aluminum Chloride method respectively. Antioxidant activity was determined using DPPH,

FRAP and Phosphomolybdenum assay. All the assays were carried out in triplicates. Bioactive compounds were analyzed using Gas Chromatography-Mass Spectrometry. Results indicated that the peel extract exhibited greater potential to scavenge DPPH free radicals when compared to pulp extract thereby indicating the use of waste substrates as free radical inhibitors.IC_{50} value of peel and pulp extracts were found to be 57.07µg/mL and 256.21µg/mL. Results of reducing power assays showed that peel extract exhibited greater reducing capacity than the pulp extract. Gas Chromatography-Mass Spectrometry analysis showed the presence of 14 bioactive compounds in peel extract and 12 bioactive compounds in pulp extract. Findings of the study indicate that peel extract possessed relatively higher antioxidant activity thereby highlighting the possibility of utilizing fruit peels as a natural source of antioxidant.

Keywords: fruit peel, waste substrates, polyphenols, antioxidant activity

INTRODUCTION

Fruit is one of the basic food groups that provide essential micronutrients such as vitamins, minerals, dietary fibre and phytochemicals. Fruit industry is one of the leading industries in terms of production, consumption, export and expected growth. Tropical and sub tropical fruits are rich in non-nutritive components, essential organic acids, pectin, cellulose and dietary fibre that impart several nutritional and therapeutic health benefits **[1]**. Longan (*Dimocarpus Longan* L.) is a subtropical fruit that is native to southern China and belongs to the family Sapindaceae. In India, it is widely distributed in south western parts such as Assam and Bengal. The fruit weighs about 5-20g and is either conical or spherical in shape. The colour of the fruit varies from greenish yellow to yellowish brown depending upon maturity **[2]**. It is consumed in different forms such as fresh, canned, frozen and dried. Longan fruit is used to treat stomach disorders, help to relieve insomnia, functions as an antipyretic agent, act as a vermifuge and possess anticancer activity **[3, 4]**. Peels and seeds are the major waste by-products obtained during processing of fruits and vegetables. Exploiting the usage of fruit peels in different industries has gained importance due to the presence of various natural bioactive compounds that is responsible for pharmacological and therapeutic properties **[5]**. Yang *et al*. **[6]** reported that longan pericarp and seed as by-products account for 16-40% of the whole fruit that can be potentially utilized as a readily accessible source of natural antioxidant. The hazardous effects of synthetic antioxidants have revived

the search for natural sources that have potent antioxidant properties. Studies focusing on antioxidant activity of biological waste substrates such as peels, seed residues and pericarp of longan fruit, particularly peels are limited. Based on this, the objective of the present study was to compare the antioxidant activity of peel and pulp extracts of longan fruit and to exploit the use of these waste fractions in different industries.

MATERIALS AND METHODS

Preparation of extract: The peels were separated manually and shade dried for 4-5 days at room temperature. The dried peels were cut into small pieces approximately 1x1 cm. The dried peels (50g) and the fresh pulp (50g) was soaked in 100 mL of ethanol separately for 72 hours by maceration technique. The supernatant obtained from both the extracts were filtered and concentrated using rotary evaporator. The dry residue was preserved at 5°C until further use.

Screening of phytochemicals: The extracts were screened for the presence of various phytochemicals such as alkaloids, glycosides, saponins, phenols, flavonoids, terpenoids, steroids, quinones and tannins **[7-9]**.

Estimation of total phenol content: The total phenolic content was estimated using Folin-Ciocalteau reagent method **[10]**. Hundred µL of the extracts (1mg/mL) were made up to 1 mL using methanol and mixed with 1 mL of Folin Ciocalteu reagent (1:10 diluted with distilled water). After 5 minutes, 1 mL of 20% sodium carbonate (Na_2CO_3) solution was added. The mixture was incubated at room temperature for 30 minutes and the absorbance was measured at 760 nm using a UV visible spectrophotometer. A calibration curve was prepared using gallic acid as standard and the total phenolic content was expressed in terms of gallic acid equivalent (µg/mg).

Estimation of total flavonoid content: Total flavonoid content was determined using the Aluminium Chloride method **[11]**. The extracts (500µg/mL) were made up to 1 mL using methanol and was mixed with 0.5 mL of 5% sodium nitrate ($NaNO_2$) solution and allowed to stand for 5 minutes. Later, 0.3 mL of 10% aluminium chloride ($AlCl_3$) solution was added and the mixture was allowed to stand for 5 minutes. Finally, 1 mL of 1M sodium hydroxide (NaOH) solution was added, and the volume was brought to 5 mL using distilled water. The mixture was incubated for 15 minutes at room temperature and the absorbance was measured at 510 nm using UV visible spectrophotometer. A calibration curve was prepared using quercetin as standard and the

total flavonoid content was expressed in terms of quercertin equivalent (µg/mg).

Antioxidant activity

DPPH assay: DPPH radical scavenging activity was determined by following the method described by Blois **[12]**. Briefly, 1 mL of 0.1 mM of DPPH solution (dissolved in methanol) was added to various concentrations of the extracts. The setup was left in dark at room temperature and the absorption was measured after 30 minutes. Absorbance was read at 517nm using UV – visible spectrophotometer. The results were expressed in terms of percentage inhibition of DPPH using the following formula:-

$$\frac{Absorbance\ in\ control - Absorbance\ in\ sample}{Absorbance\ in\ control} x\ 100$$

Absorbance control was the absorbance of DPPH and methanol

Absorbance sample was the absorbance of DPPH and the test sample.

FRAP assay: Various concentrations of the extracts were mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1mL of 1% potassium ferricyanide. The mixture was incubated at 50^{o}C for 20 minutes. Later, 1 mL of 10% trichloroacetic acid was added to the mixture. Then 1 mL of 0.1% of freshly prepared ferric chloride was added and the absorbance was measured at 700nm using UV visible spectrophotometer. The reducing power was expressed as the absorbance value at 700 nm **[13]**.

Phosphomolybdenum reduction assay: Various concentrations of the extracts were mixed with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 minutes. The tubes were cooled to room temperature and the absorbance was measured at 695 nm against blank. The blank solution contained 1 mL of the reagent solution along with 1mL of the solvent used for dissolving the sample was also incubated under the same condition. The reducing power was expressed as the absorbance value at 695 nm **[14]**.

Gas Chromatography – Mass Spectrometry (GC-MS): The extracts were injected into a HP-5 column (30 m X 0.25 mm id with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. The following chromatographic conditions were used. Helium was used as the carrier gas at a constant flow rate of 1 mL/min and the injector was operated at 200°C. The column oven temperature was maintained at 50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV, ion source temperature of 250°C, interface

temperature of 250°C and mass range of 50-600 mass units. The database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for the interpretation on mass spectrum of GC-MS. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components were ascertained.

Statistical analysis: The experiments were carried out in triplicates. Data are expressed as mean ± standard deviation of triplicates. IC_{50} value was calculated using Graph Pad Prism software version 5.0.

RESULTS

Total phenol and flavonoid content: Qualitative phytochemical screening showed that terpenoids, phenols, flavonoids, glycosides, tannins, quinones and steroids were present in both the extracts. However, saponins were present only in the pulp extract. In the present study, the total phenol and total flavonoid content was found to be higher in the peel extract **(Table 1)**.

Antioxidant activity: DPPH free radical scavenging activity of peel and pulp extracts of *Dimocarpus longan* were found to be concentration dependent. The peel extract exhibited greater radical scavenging activity when compared to pulp extract thereby indicating the use of biological waste substrates as natural free radical inhibitors. IC_{50} value of peel and pulp extracts were found to be 57.07µg/mL and 256.21µg/mL respectively. The IC_{50} value for ascorbic acid was 2.89µg/mL. The difference observed in scavenging free radicals between the extracts might be due to an unequal distribution of plant metabolites **(Table 1)**. Findings of the study indicate that ethanolic peel extract of longan fruit showed the highest reducing capacity that increased with increase in concentration **(Table 2 and Table 3)**.

GCMS analysis

GC-MS analysis of peel extract of *Dimocarpus longan*: Spectral analysis of ethanolic peel extract of *Dimocarpus longan* showed the presence of 14 peaks. The retention time, name, molecular weight, molecular formula and structure of the detected components is depicted in **Figure 1** and given in **Table 4**.

GC-MS analysis of pulp extract of *Dimocarpus longan*

Spectral analysis of ethanolic pulp extract of *Dimocarpus longan* showed the presence of 12 peaks. The retention time, name, molecular weight, molecular formula and structure of the detected components is depicted in **Figure 2** and given in **Table 5**.

Table 1: Total phenol and total flavonoid content

Total phenol content	
Peel extract	**Pulp extract**
192.8 ± 9.90 µg/mg GAE	**48.75 ± 0.78 µg/mg GAE**
Total flavonoid content	
Peel extract	**Pulp extract**
582.79 ± 1.42 µg/mg QE	**215.51 ± 2.45 µg/mg QE**

Table 2: Antioxidant activity of peel extract of *Dimocarpus longan*

Concentration µg/mL	DPPH assay	FRAP assay	Phosphomolybdenum assay
	% inhibition	Absorbance at 700 nm	Absorbance at 695 nm
20	**16.02 ± 0.15**	**0.48 ± 0.01**	**0.41 ± 0.03**
40	**40.98 ± 0.93**	**0.56 ± 0.01**	**0.61 ± 0.01**
60	**52.75 ± 4.47**	**0.59 ± 0.03**	**0.68 ± 0.04**
80	**63.08 ± 1.17**	**0.63 ± 0.01**	**0.77 ± 0.01**
100	**78.70 ± 0.68**	**0.66 ± 0.01**	**0.79 ± 0.02**
120	**81.74 ± 0.73**	**0.69 ± 0.01**	**0.84 ± 0.02**

Values are the mean of triplicates

Table 3: Antioxidant activity of pulp extract of *Dimocarpus longan*

DPPH assay		FRAP assay		Phosphomolybdenum assay	
Concentration µg/mL	% inhibition	Concentration µg/mL	Absorbance at 700 nm	Concentration µg/mL	Absorbance at 695 nm
100	**22.48 ± 1.98**	**50**	**0.15 ± 0.03**	**20**	**0.58 ± 0.04**
200	**41.28 ± 1.92**	**100**	**0.19 ± 0.02**	**40**	**0.71 ± 0.06**
300	**58.87 ± 6.21**	**150**	**0.34 ± 0.04**	**60**	**0.76 ± 0.04**
400	**74.82 ± 2.58**	**200**	**0.41 ± 0.04**	**80**	**0.81 ± 0.03**
500	**82.66 ± 1.78**	**250**	**0.49 ± 0.04**	**100**	**0.83 ± 0.02**
600	**90.42 ± 2.18**	**300**	**0.53 ± 0.02**	**120**	**0.84 ± 0.03**

Values are the mean of triplicates

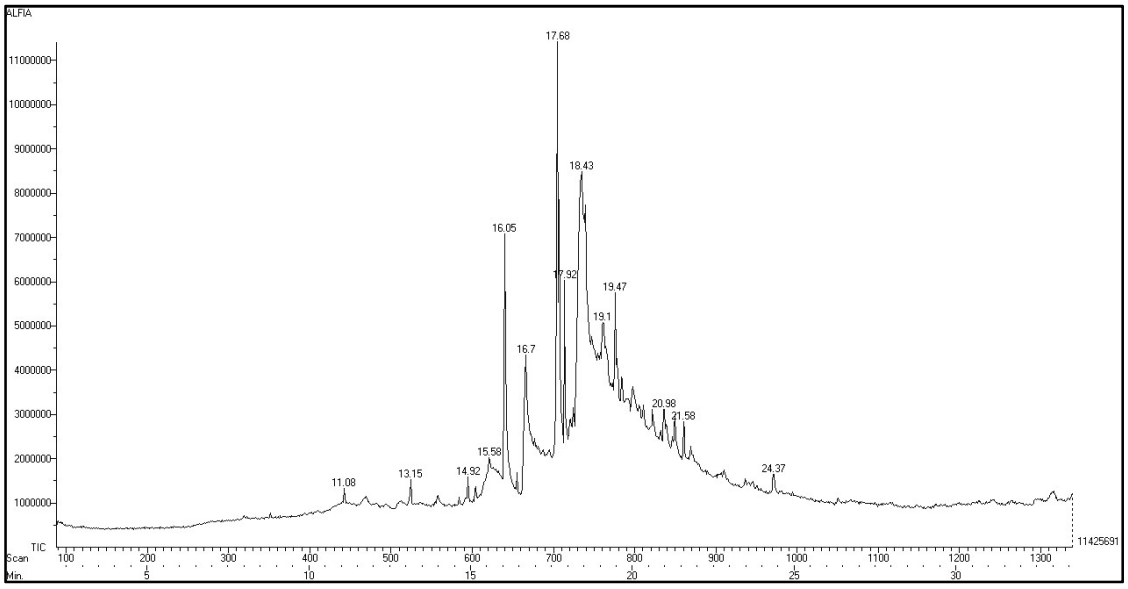
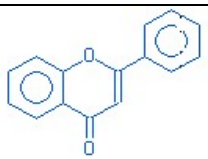
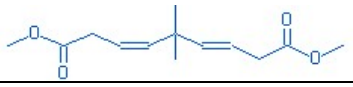
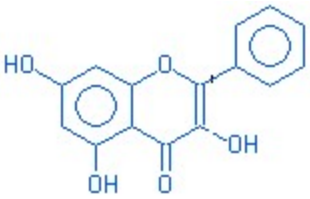
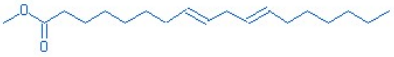
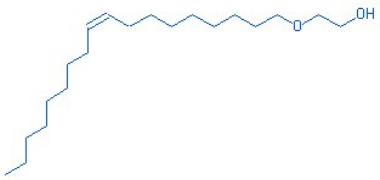
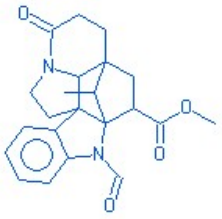


Figure 1: GC-MS analysis of peel extract of *Dimocarpus longan*

Table 4: GC-MS analysis of peel extract of *Dimocarpus longan*

Retention time	Name	Structure	Molecular weight	Molecular formula
11.08	Naphthalene,1,2,3,5-tetrahydro-2-methyl-		146	$C_{11}H_{14}$
13.5	Flavone		222	$C_{15}H_{10}O_2$
14.92	3-pentadecanone		226	$C_{15}H_{30}O$
15.58	3,6- Nonadienedioic acid,5-5-dimethyl,-dimethyl ester		240	$C_{13}H_{20}O_4$
16.5	4H-1-Benzopyran-4-one,35,7-trihydroxy-2-phenyl		270	$C_{15}H_{10}O_5$
17.68	8,11-octadecadienoic acid, methyl ester		294	$C_{19}H_{34}O_2$
17.92	2-octadecenoic acid, methyl ester		296	$C_{18}H_{34}O_2$
18.43	9-octadecenamide		281	$C_{18}H_{35}NO$
19.1	E,E,Z-1,3,12-Nonadecatriene-5,14-diol		294	$C_{19}H_{35}O_2$
19.47	10,13-Eicosadienoic acid, methyl ester		322	$C_{12}H_{36}O_2$
20.98	Ethanol,2-(9-octadecenyloxy		312	$C_{20}H_{40}O_2$
21.58	13-Docosenoic acid, methyl ester		352	$C_{23}H_{44}O_2$

Retention time	Name	Structure	Molecular weight	Molecular formula
24.37	2,20-cycloaspidospermidine-3-carboxylic acid		380	$C_{21}H_{24}N_2O_2$
16.7	Trans-2-Hexadecenoic acid		254	$C_{16}H_{30}O_2$

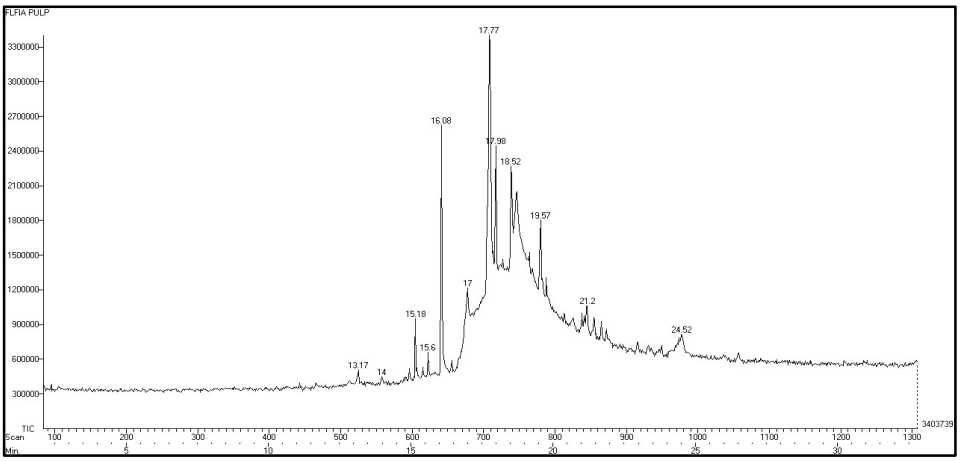
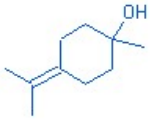
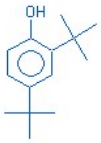
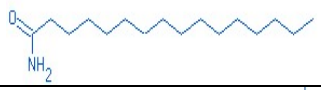
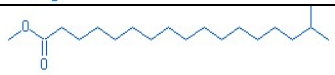
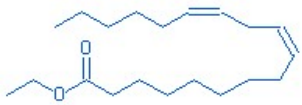
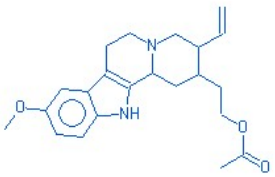
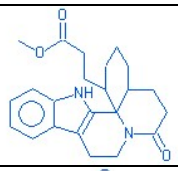
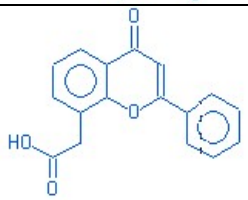


Figure 2: GC-MS analysis of pulp extract of *Dimocarpus longan*

Table 5: GC-MS analysis of pulp extract of *Dimocarpus longan*

Retention time	Name	Structure	Molecular weight	Molecular formula
13.2	Cyclohexanol,1-methyl-4-(1-methylethylidene)		154.2	$C_{10}H_{18}O$
14	3-Tridecanone		198	$C_{13}H_{26}O$
15.8	Phenol,2,4-bis(1,1-dimethylethyl)-		206	$C_{14}H_{22}O$
15.6	3,13- Octadecadien-1-ol		266	$C_{18}H_{34}O$
16.08	Cyclopentaneundecanoic acid methyl ester		268	$C_{16}H_{32}O_2$

Retention time	Name	Structure	Molecular weight	Molecular formula
17	Hexadecanamide		255	$C_{16}H_{33}NO$
17.98	Heptadecanoic acid, 16-methyl-,methyl ester		298	$C_{19}H_{38}O_2$
18.53	Linoleic acid ethyl ester		308	$C_{20}H_{36}O_2$
19.57	22-Tricosenoic acid		324	$C_{23}H_{44}O_2$
21.2	Corynan-17-ol, 18,19-didehydro-10 methoxy-, acetate(ester)		368	$C_{22}H_{28}NO_3$
24.2	Propanoic acid, 3-(7-oxo2,3,4,4a,5,6,7,9,10,15,-decahydro-1H-quindino(8a,1-a)-a-carbolin-iyl)-,methyl ester		380	$C_2H_5COOCH_3$
17.7	Mitoflaxone		280	$C_{17}H_{12}O_4$

DISCUSSION

In the present study, the total phenol and flavonoid content decreased from peel to pulp extract. Phenolic content of citrus fruit peels, seeds of avocado, jackfruit, and mango are 15% higher than the amount present in pulp **[15-17]**. Phenol content of pomegranate peel and pulp was 249.4 mg/g and 24.4 mg/g respectively **[18]**. Gonzalez *et al*. **[19]** reported that banana peel had higher concentration of catecholamines, dopamine and other phenolic compounds. The total phenol and flavonoid content of peel extract of *Dimocarpus longan* was found to be 192.8 ± 9.90 µg/mg GAE and 582.79 ± 1.42 µg/mg QE respectively. Findings of the present study indicate that the total phenol content and flavonoid of peel extract was found to be four times and two times higher than the pulp extract. Zhang *et al*. **[20]** reported the total phenol content of pulp extract of *Dimocarpus longan* to be 22.09 mg/100g GAE. The total polyphenol content of aqueous extract of *Dimocarpus longan* pericarp was 29.2 mg/g

GAE **[21]**. Liu *et al*. **[22]** documented the total phenolic content of leaf and bark extracts of *Dimocarpus longan* to be 132.47 mg/g GAE and 140 mg/g GAE. Thus polyphenol content in *Dimocarpus longan* is mostly present in the outer layers of the plant decreasing in the order of bark, leaves, pericarp and pulp. Studies show that vegetable peels are also rich in phenolic compounds that exhibit good antioxidant and antimicrobial activity **[23-25]**.

Free radicals or reactive oxygen species are highly reactive due to the presence of an unpaired electron. Free radicals such as single oxygen, superoxide, peroxyl and hydroxyl radicals are generated from endogenous as well as exogenous sources **[26]**. Inflammation, infection, mental stress and excessive exercise are endogenous sources of free radicals. Exogenous sources of free radicals include exposure to environmental pollutants, cigarette smoking, radiation and certain methods of cooking. When these exogenous compounds penetrate into the body, they get metabolized and free radicals are produced **[27-28]**. Substances that scavenge or neutralize the effect of free radicals are known as antioxidants.

DPPH free radical scavenging assay is one of the most popular methods used in determining the antioxidant capacity of a substance. In the radical form, DPPH which is dark purple in colour has an absorbance which can be measured at 517 nm. The absorbance decreases after the acceptance of an electron from an antioxidant molecule and gets converted to a less reactive form known as 1,1- diphenyl-2- picrylhydrazine **[29]**. The degree of decolorization indicates the radical scavenging potential. Greater the decolorization, greater is the antioxidant activity. On comparing the free radical scavenging of peel and pulp extracts, it is evident that peel extract exhibited greater radical scavenging activity. Higher free radical activity observed in peel extract might be due to the presence of high amount of phenolic compounds and flavonoids. In addition, GC-MS analysis of peel extract of *Dimocarpus longan* showed the presence of phenolic compounds, flavonoids, unsaturated fatty acids and esters of unsaturated fatty acid. Presence of these components could have also contributed to antioxidant activity. Pan *et al*. **[30]** reported that mechanically dried peels of *Dimocarpus longan* too showed potent radical scavenging activity (1.439 at 1 mg/mL). The results also indicated that peel possessed abundant phenolic content that significantly contributed to antioxidant activity and

thereby it is possible to use the peel of *Dimocarpus longan* as substitute for synthetic antioxidants in the food industry. Spectral analysis of ethanolic pulp extract of *Dimocarpus longan* showed the presence of flavonoids, saturated and unsaturated fatty acids.

Phenolic compounds exhibit antioxidant activity in various ways such as oxygen scavengers, free radical inhibitors, metal chelating agent, peroxide decomposers and also by modulating the action of endogenous antioxidant enzymes **[31-32]**. Flavonoids scavenge hydroxyl radicals, regulate mitochondrial functions and modulate inflammatory response **[33]**. IC_{50} value refers to the amount required to inhibit 50% of free radicals. Smaller the IC_{50}value, greater is the antioxidant activity. IC_{50} value of peel and pulp extracts was found to be 57.07µg/mL and 256.21µg/mL respectively. Kalpna *et al*. **[34]** studied the antioxidant activity of peel extracts of seven different fruits. Among them, the acetone and methanolic extracts of *Mangifera indica* exhibited potent DPPH free radical scavenging activity due to higher amount of phenolic comounds. The IC_{50} value of acetone and methanolic extracts was 16.5 µg/mL and 23.5 µg/mL. Findings of John *et al*. **[35]** showed that antioxidant activity observed in lemon peel was due to the presence of phenols and flavonoids.

Reducing power of Fe^{3+} (CN−) 6–to Fe^{2+} (CN−) 6 was measured by direct electron donation. Higher the absorbance value, stronger the reducing power **[4]**. The peel extract of longan fruit exhibited the highest reducing capacity at a concentration of 120µg/mL (absorbance rate= 0.69±0.01). The ability to reduce Fe^{3+} ferricyanide complex to Fe^{2+} ferrous form might be due to the presence of various reducing agents that act as reductants. Presence of reducing agents reacts with certain precursors of peroxides and prevents the formation of peroxides. Phosphomolybdenum assay is based on the reduction of Mo (VI) to Mo (V) in the presence of antioxidant compounds along with formation of bluish green colored phosphate Mo (V) complex at acidic pH. The results indicate that ethanolic peel extract of *Dimocarpus longan* exhibited good reducing capacity that increased with increase in concentration.

CONCLUSION

Thus the results of the present study indicate that peel and pulp of *Dimocarpus longan* can be put into use both nutritionally and therapeutically. Peels which are generally discarded can be used as a natural source of antioxidant agent and that in future its role in

food, cosmetic and pharmaceutical industries is promising.

Conflict of interest: Nil

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