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Anti-oxidant activities and polyphenolic compounds of Longan (*Dimocarpus longan* Lour) peel and seed extracts

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Keywords: Longan (*Dimocarpus longan* Lour), anti-oxidant activity, polyphenolic compounds

Objectives: The objectives of the present study were to investigate the antioxidant activity and determine major polyphenolic compounds from Longan (*Dimocarpus longan* Lour) peel and seed extracts.
Methods: Longan peels and seeds were extracted with 20% and 95% ethanol. The combined filtrates of ethanol solution were evaporated under reduced pressure at room temperature. Anti-oxidative activity was measured using the 2,2-Diphenyl-1-picrylhydrazin (DPPH) radical scavenging assay compared with ascorbic acid. Phytochemical screening was investigated using reversed-phase TLC technique. Major components of polyphenolic compounds were determined by HPLC technique.
Results: The IC_{50} of antioxidant activity of 20% and 95% ethanol crude extracts of longan peels and seeds were in the range of 0.68-1.73 μg/mL nearly to ascorbic acid (1.37 μg/mL). Reversed- phase TLC profile of the crude extracts showed main spots equivalent to the authentic standards of polyphenolic compounds such as gallic acid, ellagic acid and flavonoids compound such as quercitin. The amounts of gallic acid, corilagin and ellagic acid ranged from 1.20 to 23.86 mg/g of the dried crude extracts.
Conclusion: From this study, the crude ethanolic extracts of longan peels and seeds exhibit good antioxidant activity. The obtained extracts were consisted of polyphenolic compounds, namely gallic acid, corilagin and ellagic acid. Further investigations on insomnia and immunomodulation are needed to confirm the biological activities of the extracts that can be applied to food supplement purpose.

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Introduction

Longan (*Dimocarpus longan* Lour, syn. *Euphoria longan* Lam.) is one of the major economic fruit in Thailand. Longan fruits are commonly consumed fresh or commercially prepared to dried and canned products. During the processing, longan peels and seeds are generally discarded as waste. Previous phytochemical studies have reported polyphenolic compounds in longan seeds including gallic acid, ellagic acid, ellagitanninis corilagin, chebulagic acid, ellagic acid, 4-O-α-L-arabinofuranoside, isomallotinic acid, geranin [1], ethyl galate, 1-β-O-galloyl-D-glucopyranose, methyl brevifolin carboxylate, brevifolin [2]. It has been shown that polyphenolc extracts of longan pericarps display anti-inflammatory [3], anti-hyperglycaemic [4], anti-oxidant and anticancer activities [5]. In this work, the crude ethanolic extracts of longan seeds and peels were subjected to determination of antioxidant activity and contents of major polyphenolic compounds.

Methods

***Preparation of crude ethanolic extracts*:** The powdered longan peels and seeds were defatted using hexane as solvent. The dried defatted samples were extracted 10 times using 20% and 95% ethanol at room temperature. The combined filtrates of ethanol solution were evaporated under reduced pressure at room temperature.
Scavenging of diphenyl-picrylhydrazyl (DPPH) radicals assay: The free radical scavenging activity of crude ethanolic extracts was analyzed by the DPPH assay [6]. The amount of 100 µL of various concentrations sample were reacted with 100 µL of $6x10^{-3}$ M DPPH ethanolic solution in a 96-well plate, incubated at 37 °C for 30 min. The absorbance was measured at 517 nm using a UV–VIS microplate reader. All experiments were carried out in triplicates.
***Phytochemical screening*:** Phytochemical screening was investigated using reversed-phase TLC technique. Crude ethanolic extract (10 mg) was dissolved in 1 ml of 50 % ethanol and partition with 1 ml of ethyl acetate. The amount

of 15 µl from ethyl acetate fraction was applied to RP18 W/UV254 coated on aluminium foil TLC plates, film thickness 150 micron and developed in 0.1% trifluoro acetic acid (TFA) in water : 0.1% trifluoroacetic acid (TFA) in acetonitrile (ACN) (10:90), identified by co-TLC with authentic standards (gallic acid, ellagic acid and quercitin). Visualization of the compounds was achieved by spraying the sheets with 1% methanolic diphenylboryl-oxyethylamine (NP), followed by 5% ethanolic polyethylene glycol 4000 (PEG). The chroma-tograms were evaluated with 254 and 366 nm UV light.

HPLC analysis: Exactly 1 mg of gallic acid, ellagic acid and corilagin authentic standard were dissolved by methanol in 5 ml volumetric flask. The accurately weighed 20 mg of crude ethanolic extracts and dissolved in methanol and transferred to a 5-ml volumetric flask, sonicated for 20 min. The solution was filtered through 0.45 µm pore size filter units before use. Ten microliters of vary concentration of authentic standards and crude ethanolic extract solution were analyzed using a Waters e2695 separation module equipped with waters 2998 photodiode array detector. The chemical compounds were separated on a pHedure C-18 column; 250 mm x 4.6 mm with 5 µm (Vertisep™). The mobile phase was a gradient elution system consisting of solvent A (0.005 % trifluoroacetic acid/H_2O) and solvent B (0.001 % trifluoroacetic acid/acetonitrile). The elution was as follows: 0 to 10 min, 14 % solvent B; 10 to 30 min, 18 % solvent B; 30 to 50 min, 25 % solvent B; 50 to 60 min, 35 % solvent B; 60 to 70 min, 70 % solvent B; 70 to 80 min, 100 % solvent B; 80 to 90 min, 100 % solvent B, flow rate 1.0 ml/min at 30 °C. The wavelength was monitored at 254 nm.

Results

Radical scavenging activity: Table 1 presents the IC_{50} values for the investigated extracts of longan peels and seeds. The values of IC_{50} are in the range of 0.68-1.73 µg/mL, nearly to ascorbic acid (1.37 µg/mL) and gallic acid (0.98 µg/mL).

Table1. Antioxidant activity (IC_{50}) of ethanolic extracts of longan peels and seeds compared with authentic standards.

Sample	Anti-oxidant activity (µg/mL)
20 % ethanolic longan peels extract	1.19
95 % ethanolic longan peels extract	0.68
20 % ethanolic longan seeds extract	1.73
95 % ethanolic longan seeds extract	0.82
gallic acid	0.98
ascorbic acid	1.37

Phytochemical analysis: In Figure. 1, Reverse phase TLC profil of the 20 % and 95 % ethanol extracts of longon peels showed some spots and same color as flavonoids compounds (quercitin) and phenolic compounds (gallic acid and ellagic acid) when visualised under 366 nm UV light. It is observed that the 20% and 95% ethanol extracts of longon seeds showed only phenolic compounds as gallic acid and ellagic acid.

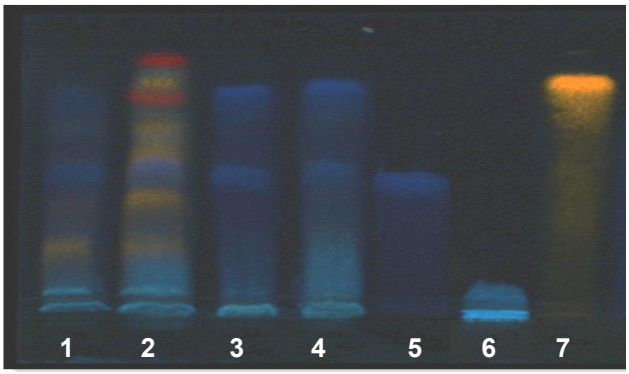

Figure 1: Reversed-phase TLC profiles of crude ethanolic extracts developed using the solvent system 0.1% TFA/H2O : 0.1% TFA/ACN (10:90), band 1: 20 % ethanolic extract of longan peels, band 2: 95 % ethanolic extract of longan peels, band 3: 20 % ethanolic extract of longan seeds, band 4: 95 % ethanolic extract of longan seeds, band 5 :gallic acid, band 6: ellagic acid and band 7:quercitin. The visualizing reagent was1% NP/PEG and detection under 366 nm UV light.

The chemical constituents of longan peels and seeds were analyzed using HPLC technique as shown in Figure. 2. The chromatogram results showed the peaks of gallic acid (RT:9.27), corilagin (RT :18.26) and ellagic acid (RT:38.45).

The linear regression of calibration curves of authentic standards showed a linear relationship in the concentration range of 1-60 µg/ml. The contents of gallic acid, corilagin and ellagic acid in the crude extracts of longan peels and seeds are presented in Table 2.

Table 2 The content of phenolic compounds in ethanolic extract of longan peels and seeds

Sample	content (mg/g of dried crude extracts)		
	gallic acid	corilagin	ellagic acid
20 % ethanolic longan peels extract	4.16	1.2	5.2
95 % ethanolic longan peels extract	5.78	1.10	9.15
20 % ethanolic longan seeds extract	8.24	5.8	8.21
95 % ethanolic longan seeds extract	5.97	10.1	23.86

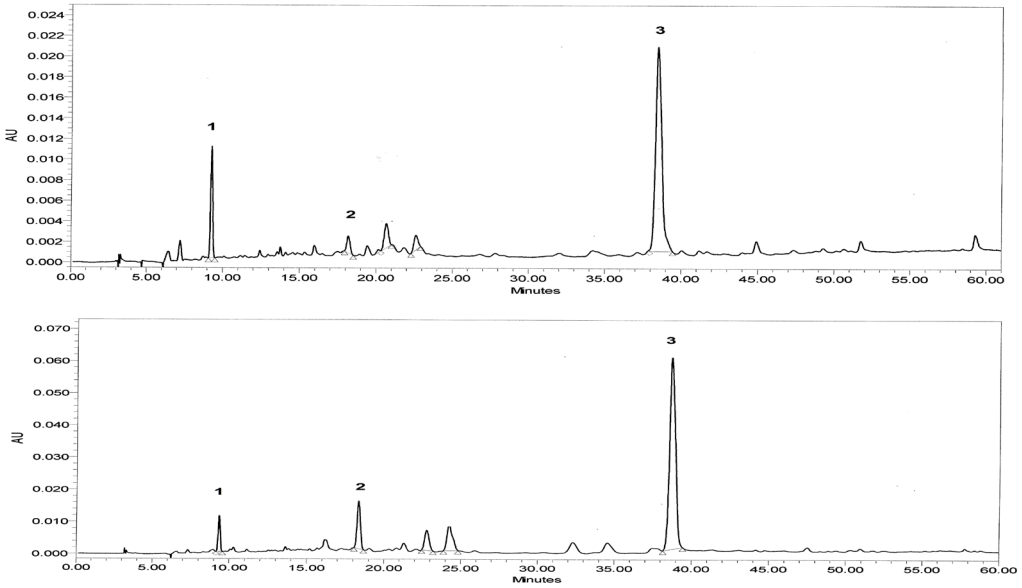

Figure 2: HPLC profile of phenolic compounds from 95 % ethanol extract of longan peels [A] and seeds [B] at 254 nm, 1:gallic acid, 2:corilagin and 3:ellagic acid

Discussion

The obtained extracts exhibit good antioxidant activities, indicating that they have their potential use. The HPLC analysis showed that 95% ethanol is a suitable solvent in obtaining polyphenolic compounds from peels and seeds. The total content of polyphenolic compounds in the peel extracts appears to be lower than that of the seed extracts. However, the peel extracts showed higher flavonoids content. The corilagin content was greater in the seed extracts than in the peel extracts. Some unindentified compounds on the TLC profile may justify further study.

Conclusion

This study showed that the extracts of longan peels and seeds using 95% ethanol contain high poly-phenolic compounds and have their potential use.

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