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Article

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Evaluation of bioactivities of *Phyllanthus emblica* seed

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Abstract

This research aim to study bioactivity of seed extract compare with fruit extract of Indian gooseberry; *Phyllanthus emblica* that use as medicinal plant widely in Thailand. Analyzed the efficacy of free radical scavenging by using DPPH radical scavenging assay reveal that the highest antioxidant activity found in the highest concentration, 10 mg/ml and both fruit and seed extracts had non-significant percentage of reduction as α -tocopherol. The determination of phytochemical exhibited that seed extract had the higher contents of tannin and flavonoid than fruit extract as 126.71 mg TAE/g extract and 1016.25 mg QE/g extract, respectively. Cytotoxicity studied of extracts against human breast adenocarcinoma cell line by MTT assay at the maximum concentration 10000 μ g/ml found that seed extract had more toxicity than fruit extract at 68.98% and 35.57%. According to the bioactivities results, the seed of *Phyllanthus emblica* has potential to replace the fruit for medical aspects to add value of this plant.

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Keywords: Antioxidant activity; Cytotoxicity; *Phyllanthus emblica*; Phytochemicals; Seed

1. Introduction

Phyllanthus emblica, Indian gooseberry is a tropical and subtropical plant commonly find in Asia. It is a member of Phyllanthaceae family that is a perennials plant high about 8–10 m with bending stem and small white flower [1]. There were evidences that *P. emblica* extracts have a lot of nutritions such as vitamin c, β -carotene, calcium, phosphorus, protein, fat, carbohydrate [2,3] and phytochemicals for example tannins, saponins, flavonoids, alkaloids, steroid, anthraquinone [2,4]. Many parts of *P. emblica* have long term been traditionally use for various diseases such as fruit to meditated diabetes, diarrhea, diuretic and laxative. Leaves treated fever and anti-inflammatory, root use for dental problems and bark is one of remedy for gonorrhea [5]. Furthermore, this with the efficacy of reversal of aging and repair of the damage are associate with aging [6]. There are many products of *P. emblica* for health beneficial in Thailand that made from fruit while seed are eliminate from production process. It waste time to get rid of seed and lost the expense.

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In this study, we searched for the phytochemical contents, antioxidant and cytotoxicity in *Phyllanthus emblica* extracts to find out the potential of seed compared with fruit extract to investigate capability of useless part of this plant for remedy aspects.

2. Materials and methods

2.1. Plant materials and preparation of extract

The samples of *Phyllanthus emblica* was collected from the garden in Chachoengsao province, Thailand. After cleaned, separated seed from fruit then dried in hot air oven at 45 °C for 3 days, after that mashed into powder. Extract seed and fruit powder with ethanol at room temperature by maceration. The extracts were filtered and removed solvent by rotary evaporator to get the extracts and stored in the dark at 4 °C before for activity tests [7].

2.2. Phytochemical contents

Total phenolic constituent of the extracts were determined involving Folin–Ciocalteu reagent and gallic acid as standard by using the colorimetric assay. Dropped 20 µl of extract (0.1 mg/ml) into 96-well plate then mixed with 100 µl Folin–Ciocalteu reagent and 80 µl sodium carbonate solution (7.5%, w/v). The mixture was incubated for 30 min and then absorbance of each well was measured at 765 nm.

Tannin content was examined by Folin–Denis method. Added 50 µl of 0.1 mg/ml extract in 30% MeOH and filled distilled water to final volume 7.5 ml after that mixed Folin–Denis 0.5 ml and 35% NaHCO₃ 1 ml before adjusted final volume to 10 ml then measured the absorbance at 700 nm [8].

Flavonoid content was analyzed. Pipetted 250 µl of extracts (1 mg/ml) in 30% MeOH added distilled water 1.25 ml, 5% NaNO₂ 75 µl and 10% AlCl₃ 150 µl. After incubated the mixture for 6 min then filled 1 M NaOH 500 µl and distilled water 275 µl. The absorbance of the resulting color was measured at 510 nm [9].

Determined total phenolic, tannin and flavonoid contents by using a calibration curve prepared with gallic acid, tannic acid and quercetin, respectively. The result of total phenolic, tannin and flavonoid in each extract expressed as milligram of GAE, TAE and QE (gallic acid, tannic acid and quercetin equivalent), respectively per gram extract.

2.3. Antioxidant activity

Free radical scavenging activity on 2,2-diphenyl-2-picrylhydrazyl (DPPH) was evaluated. 100 µM of DPPH solution 190 µl was mixed with the plant extracts 10 µl at the concentration of 0.625, 1.25, 2.5, 5.0 and 10.0 mg/ml into 96-well plate. The mixture was shaken vigorously and incubated in a dark chamber at 37 °C for 30 min and then absorbance of each well was measured at 517 nm. The DPPH solution and α-tocopherol was use as negative control and reference standard, respectively. The activity was expressed as percentage DPPH scavenging relative to control using the following equation:

$$\% \text{ scavenging} = \frac{[A_{\text{control}} - A_{\text{sample}}] \times 100}{A_{\text{control}}} \quad (1)$$

A sample = absorbance of samples; A control = absorbance of control

2.4. Cytotoxicity test

Cell cytotoxicity evaluated by MTT assay which required cellular metabolic activity to convert the colorless tetrazolium to the purple color formazan. Prepared MCF-7 cells at mid-log phase approximately 1.0×10^5 cells/ml in 96-well plates for 24 h after that incubated with the plant extracts (at 0.1, 1, 10, 100, 1000 and 10 000 µg/ml) [7] and without extract as control. Incubated the 96-well plates in 37 °C, 5% CO₂ incubator for 72 h. Added 5 mg/ml of MTT (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) solution 10 µl, left for 4 h. Then, removed extract and replaced with DMSO to dissolve formazan crystal. The level of formazan derivative color was analyzed at 540 nm with the aid of a microplate reader. The 50% cytotoxicity concentration (CC₅₀) was calculated from the

Table 1. Phytochemical contents in *P. emblica* extracts.

Phytochemical	Extract	
	Seed	Fruit
Total phenolic (mg GAE/g extract)	4.53 ± 0.18	6.00 ± 0.18
Tannin (mg TAE/g extract)	126.71 ± 0.92	52.96 ± 1.04
Flavonoid (mg QE/g extract)	1016.25 ± 16.50	865 ± 34.28

calibration curve and activity was expressed as percentage of cell inhibition relative to control using the following equation :

$$\% \text{ cytotoxic} = \frac{[A \text{ control} - A \text{ sample}] \times 100}{A \text{ control}} \quad (2)$$

A sample = absorbance of treated cells; A control = absorbance of control

3. Results and discussion

3.1. Phytochemical contents

There were evidences that *P. emblica* extracts have a lot of phytochemicals for example phenolics, tannins, flavonoids, alkaloids, saponins [10] exhibited potential alternative treatment such as anti-inflammatory, hepatoprotective, effect on serum cholesterol, diabetes, gonorrhoea [5]. Total phenolic compounds have an important role on stabilizing oxidation and deal with antioxidant activity [11]. Total phenolic compounds, tannin and flavonoid content exhibited as mg GAE, mg TAE and mg QE per gram extract as shown in Table 1.

The results analyzing the bioactive substances (total phenolic, tannin and flavonoid) of *P. emblica* extracts exhibited that level of tannin and flavonoid in seed were higher than in fruit extracts. Tannin and flavonoid contents in seed extract were 126.71 mg TAE and 1016.25 mg QE per gram extract, respectively while the higher total phenolic compound in fruit extract was 6000 mg GAE/g extract. Sumalatha reported the presence of major phyto-compounds in *P. emblica* fruit ethanolic extract such as flavonoids, alkaloids, protein, saponin and tannin [2]. Charoentearaboon et al. evaluated the total phenolic contents in *P. emblica* fruit aqueous extract reported that the extracts contained 34.22 ± 1.74 g GAE/100 g extract [12]. There were evidences that *P. emblica* extracts had a lot of phytochemicals for example tannins, gallic acid, ascorbic acid that effected to antioxidant property [4](Eugeny, 2009).

3.2. Antioxidant activity

The potentiality of both extracts to scavenge DPPH free radical was examined by the degree of discoloration indicated the scavenging potential [13]. DPPH radical scavenging activity of extracts was investigated as shown in Table 2.

Table 2. DPPH scavenging activity of seed and fruit of *P. emblica* extracts.

Concentration (mg/ml)	% DPPH reduction	
	Seed	Fruit
0.625	86.86 ^c ± 1.17	43.37 ^c ± 2.01
1.25	89.37 ^b ± 0.21	72.64 ^b ± 0.55
2.5	91.32 ^a ± 0.11	84.39 ^a ± 0.35
5.0	91.76 ^a ± 0.02	83.04 ^a ± 1.50
10.0	91.88 ^a ± 0.07	83.23 ^a ± 2.40

Values are mean ± SD. Different superscript letters within the same column indicate significant ($P < 0.05$).

The result revealed that the scavenging effect and the concentration increased simultaneously. The percentages of DPPH reduction in both extracts were inflated from the lowest (0.625 mg/ml) to the highest concentration (10 mg/ml). Similar to Yadav and Bhatnagar reported that fruit extract showed high scavenging activity with 75.75% inhibition [14]. Charoenteeraboon et al. have evaluated the antioxidant activity of aqueous extract of fruit reported that the extract exhibited $0.182 \pm 0.07 \mu\text{g GAE}/1 \text{ ml extract}$ [12] (see Fig. 1).

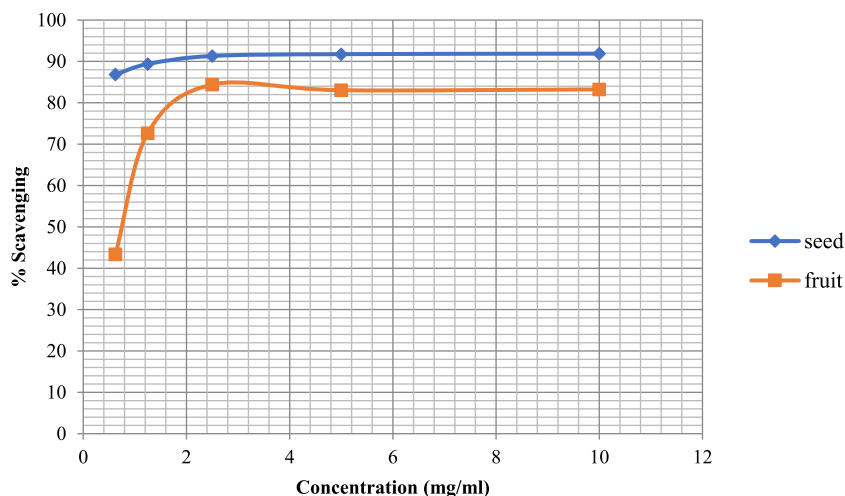


Fig. 1. Scavenging activity of seed and fruit of *P. emblica* extracts.

Phenolics are a big group of plant secondary metabolites for example anthocyanin, flavonol, vanillin, ferulic acid, caffeic acid, and gallic acid. Phenolics had many functional properties, mainly due to their antioxidant capacity [15]. The consequences of phytochemical contents and scavenging activity revealed that seed extract have potential to use as antioxidant substances.

3.3. Cytotoxicity assay

To search for cytotoxicity of *P. emblica* extracts against MCF-7 cell lines by MTT assay and the result was demonstrated in Table 3 (see Fig. 2).

Table 3. Inhibitory response of MCF-7 cells to *P. emblica* extracts.

Concentration ($\mu\text{g}/\text{ml}$)	% cytotoxic	
	Seed	Fruit
0.1	$33.48^b \pm 8.44$	$5.68^b \pm 2.88$
1.0	$35.57^b \pm 6.84$	$7.38^b \pm 1.29$
10	$43.05^{ab} \pm 5.33$	$10.84^b \pm 3.74$
100	$51.62^{ab} \pm 13.78$	$11.07^b \pm 3.45$
1000	$59.63^{ab} \pm 12.21$	$13.39^b \pm 5.86$
10000	$68.98^a \pm 2.92$	$39.07^a \pm 2.85$

Values are mean \pm SD. Different superscript letters within the same column indicate significant ($P < 0.05$).

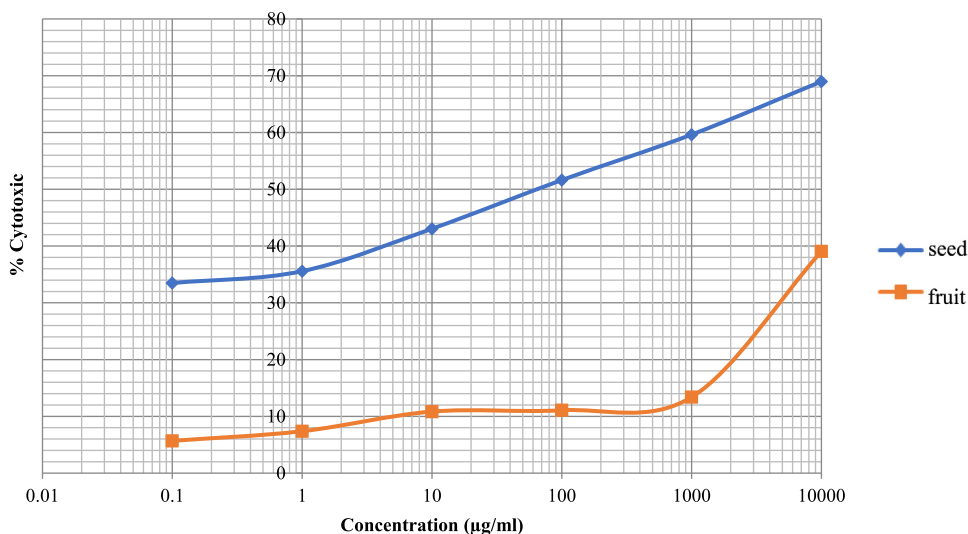


Fig. 2. Effect of *P. emblica* seed and fruit extract on the growth of MCF-7 breast cancer cell.

The cytotoxicity against MCF-7 cells depends on extracts concentration, the more concentration of extract treated on MCF-7 cells, the more toxic on cells expressed. Both extracts showed the highest toxic against MCF-7 at 10 000 µg/ml. Cytotoxic percentage of the seed and fruit extracts were 68.98 and 39.07 while CC_{50} were 2295.93 and 13 677.43 µg/ml, respectively. Similar to Sumalatha reported that *P. emblica* fruit ethanolic extract possessed more potential cytotoxic activity against HT-29 cell lines [2]. Wei et al. were tested proliferation of MCF-7 breast cancer with purified phenolics from *P. emblica* fruit and found that phenolics yield effected the survival on MCF-7 cell line [10].

From the results of phytochemical contents and antiproliferative activity of seed extract revealed that seed extract are possibility to suppress activities of MCF-7 cells [10].

4. Conclusion

In this study, the bioactivities (phytochemical contents, antioxidant activity, and cytotoxicity assay) from seed and fruit extract of *P. emblica* were determined. The results exhibited that seed extracts had high level of tannin and flavonoid at 126.71 mg TAE and 1016.25 mg QE per gram extract, respectively. Fruit extract had high content of total phenolic by 6.0 mg GAE/g extract. Seed extract has potential antioxidant activities and cytotoxicity against MCF-7 cell lines. This study provides the informations for the uses of *P. emblica* seed become a part of products such as supplementary food, herbal medicine or cosmetic aspects to add value. Therefore, the further study aims to investigate the more qualitative and quantitative of phytochemicals in seed of *P. emblica*.

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