Antioxidant, antimutagenic and antibacterial activities of extracts from *Phyllanthus emblica* branches

Bungorn Sripanidkulchai* and Niramai Fangkrathok1,2

1 Center for Research and Development of Herbal Health Products, Faculty of Pharmaceutical Sciences, Khon Kaen University, Mueang, Khon Kaen, 40002 Thailand.

2 Faculty of Agricultural Technology, Burapha University, Sakaeo Campus, Watthana Nakon, Sakaeo, 27160 Thailand.

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Abstract

*Phyllanthus emblica* is an euphorbiaceous plant that has long been used as an ingredient in traditional medicines and functional foods. Although traditional remedies use several parts including the fruit, leaf, flower, stem and roots, the fruit is the most popular. It has been used for health promotion, anti-aging and also for treatment of wide ranges of symptoms and diseases. Because of the broad spectrum of pharmacological activities and high demand for the *P. emblica* fruit, there is a shortage of raw materials. Furthermore, there is a seasonal limitation in which the fruit comes out once a year. The aim of this study is to investigate the bioactive potential and the possibility to use the plant branch. Alcohol based extracts of *P. emblica* branches were analyzed for total phenolic content, antioxidant, antibacterial and antimutagenic activities. *P. emblica* branches were used to prepare the 50% ethanolic extract by maceration (EBE) and methanolic extract by Soxhlet apparatus (MBE). Total phenolic content was determined by Folin-Ciocalteu method and DPPH was used to analyze the antioxidative activity. Antibacterial activity was evaluated by microdilution method and expressed by minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). Antimutagenicity was performed by preincubation bacterial assay in *Salmonella typhimurium* TA98 and TA100 strains. It was found that both EBE and MBE contained high total phenolic content (643 and 547 TAE mg/g) and strong antioxidative activity (EC50 at 30 and 23 mg/ml). The extracts showed antimutagenicity to both direct and indirect-acting mutagens in TA98 and TA100 strains. Both extracts possess antibacterial activity against *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Salmonella* sp. and *Pseudomonas aeruginosa*. In conclusion, the alcoholic extracts of *P. emblica* branch possess high phenolic content with strong antioxidative, antimutagenic and antibacterial activities as previously reported in *P. emblica* fruit. These findings support the traditional uses and may lead to future utilization of *P. emblica* branch in functional foods and skin products.

Keywords: *Phyllanthus emblica* branch, antioxidant, antibacterial, antimutagenic

1. Introduction

*Phyllanthus emblica* L., emblica or Indian gooseberry or “Makham Pom” in Thai, is an euphorbiaceous plant that is widely distributed in subtropical and tropical areas of China, India, Indonesia, Malaysia and Thailand. The fruit is the most popular part which contains high content of vitamin C and phenolic compounds. It has been used as major constituents for various traditional and Ayurvedic medicines. With previous reports on several pharmacological properties of emblica fruit such as antioxidant (Anila and Vijayalakshmi, 2003; Liu et al., 2008; Khopde et al., 2001), analgesic and anti-pyretic (Perianayagam et al., 2004; Gupta et al., 2008), antimicrobial (Ahmad et al., 1998; Rahman et al., 2009),
antitumor (Jose et al., 2001; Rajeshkumar et al., 2003; Sultana et al., 2008), anti-inflammatory (Dang et al., 2011), immunomodulatory (Liu et al., 2012), hypolipidemic (Anila and Vijayalakshmi, 2002), and hypoglycemic activities (Abesun- 
dara et al., 2004; Akhtar et al., 2011; Nain et al., 2012), and hepatoprotective (Jeena et al., 1999; Achliya et al., 2004; 
Pramyothin et al., 2006), and gastroprotective effects (Bandyopadhyay et al., 2000; Sairam et al., 2002; AL-Rehaily et al., 
2002), protection of ischemia-reperfusion-induced oxidative stress (Bhattacharya et al., 2002), prevention of 
hyperthyroidism (Panda and Kar, 2003) and protection of 
UVB-photodamage (Adil et al., 2010), the demand of the plant’s fruit used for food and medicine, and as an 
ingredient in cosmetics is high. According to its annual crop, the supply of emblica fruits is limited. It is important to search 
for other parts as an alternative substitute for the emblica 
fruit. In the present study, the phenolic content, antioxidant, 
antimutagenic and antibacterial activities of emblica branch 
extracts were investigated.

2. Materials and methods

2.1 Materials

Ascorbic acid was from Caro Erba (Italy), 2, 2-diphe- 

yl-1-picrylhydrazyl (DPPH), α-tocopherol and tannic acid 

were from Sigma (USA). Folin-Ciocalteu reagent was from 

Merck (Germany) and other analytical grade chemicals were 

used.

2.2 Preparation of plant extracts

Fresh emblica branches were collected in Khon Kaen 

province in January, 2011. After identification, a voucher 

specimen (KP 021) are deposited in the Faculty of Pharma-

ceutical Sciences, Khon Kaen University herbarium. The 

ethanolic extract of emblica branches (EBE) was produced, 

according to the petty patent (no. 4599, Thailand). The plant 
powder was macerated in 50% ethanol at a ratio of 1:5 for 
7 days then filtered through Whatman® No.1 paper and 
centrifugated at 500 g for 10 min. The supernatant was 
evaporated under rotary evaporator and then freeze-dried in 
lyophilizer (Christ®, German) with %yield of 5.4. In parallel, 
the methanolic extract of emblica branches (MBE) was 
prepared using Soxhlet apparatus at 50°C for 3 h (at a ratio 
of 1:10), then the filtrate was similarly dried with %yield of 
18.83. Both extracts were kept at-20°C until used.

2.3 Determination of total phenolic content

The total phenolic content was determined by the 
Folin-Ciocalteu method (Singleton et al., 1999). The extracts 
were dissolved in methanol at various concentrations (0.1-
5.0 mg/ml), then the extract solution (0.5 ml) was mixed with 
the Folin-Ciocalteu reagent (0.25 ml) and 20% sodium 
carbonate (1.25 ml). After mixing and standing at room 
temperature for 40 min, the absorbance was measured at 725 
nm. The total phenolic content was expressed as mg tannic 
acid equivalent (TAE)/g dried extract.

2.4 Determination of DPPH radical scavenging activity

The free radical scavenging activity was determined by 
the method described by Shimada et al. (1992). The 
extract was dissolved in methanol at various concentrations 
(0.1-5 mg/ml), then 2.8 ml of each extract solution was mixed 
with 0.2 ml of DPPH solution (1 mM in methanol). After incu-
bation at room temperature for 15 min, the absorbance was 
measured at 515 nm. The negative (methanol) and positive 
(vitamin C) controls were run in parallel. The scavenging 
activity was calculated using the formula, % scavenging = 
[(A515_

\text{control} - A515_

\text{sample})/A515_

\text{control}] × 100.

2.5 Antimutagenic test

The preincubation mutation method as originally 
described by Araki et al (1984) and further modified for plant 
extract test by Sripanidkulchai et al. (2002) was carried out in 
both the presence and absence of the rat hepatic microsomal 
fraction (S-9) mixture in order to detect indirect and direct 
antimutagenic, respectively. Two standard test strains, 
Salmonella typhimurium TA98 and TA100 were used. The 
dried plant extract was dissolved in dimethylsulfoxide 
(DMSO) at a concentration of 100 mg/mL. The mixture of 
each extract solution (0-0.1 ml) with 0.5 ml of S-9 mixture or 0.1 M 
phosphate buffer (pH 7.4) and 0.1 ml of the test bacterial 
solution was incubated at 30°C for 30 min in the presence of 
positive mutagens. Three standard positive mutagens were 
used, including two direct-acting (2-aminofluorene, AF-2; and 
4-nitroquinoline-1-oxide, 4-NQO) and one indirect-acting (2- 
aminoanthracene, 2-AA). After incubation, the mixture was 
rapidly mixed with 2 ml of molten top agar containing 0.1 
mmol of histidine and biotin, and poured rapidly into 30-ml 
Vogal-Bonner minimal agar plate and incubated at 37°C for 
48 h. The background (negative) control (using DMSO) and 
positive control (using mutagens) were parallel conducted. 
The revertant colonies were counted, and the toxic effect was 
determined by viewing the background lawn under a stereo 
microscope. The % inhibition of plant extracts on number of 
revertant colonies was calculated.

2.6 Antibacterial test

Clinical isolated strains of five bacteria, including 
Staphylococcus aureus, S. epidermidis, Escherichia coli, 
Salmonella sp and Pseudomonas aeruginosa obtained from 
Srinagarind hospital, Khon Kaen University, Thailand, were 
used. The plant extract was dissolved in DMSO at a concen-
tration of 50 mg/ml, then filtered through a 0.45 μm membrane 
filter and further tested by microbroth dilution method (Buwa 
and Van Staden, 2006). The extract solution was serial diluted 
with Mueller Hilton broth and put into a 96-well microplate
containing 100 µl of test bacteria at concentration of McFarland No 0.5 (1x10^8 cell/ml or 1x10^7 cell/well). DMSO was used as a positive growth control, and standard antibiotics were used as inhibitors of bacterial growth. After incubation at 37°C for 18-24 h, the minimal inhibitory concentration (MIC) was determined as the lowest concentration of each extract that completely inhibited growth of microorganism. Then each 5 µl of the mixture broth was further cultured on an agar plate at 37°C for 18-24 h and the lowest concentration at which no microbial growth was observed as the minimal bactericidal concentrate (MBC) of the extract.

3. Results

3.1 Phenolic content and antioxidative activity of P. emblica branch extracts

Total phenolic content and DPPH radical scavenging activity of alcoholic extracts of P. emblica branches are shown in Table 1. Both 50% ethanolic extract of emblica branches (EBE) and methanolic extract of emblica branches (MBE) possess similar high phenolic contents (643.07±24.34 and 547.41±20.46 TAE mg/g dried extract) and strong antioxidative activity (EC_{50} of DPPH at 29.93±2.15 and 22.89±3.60 mg/ml, respectively.

3.2 Antimutagenicity of P. emblica branch extracts

Using bacterial mutation assay, neither EBE and MBE up to 100 mg/plate showed mutagenicity to TA98 and TA100 strains of Salmonella typhimurium in the presence and absence of S-9 activation (data not shown), suggesting the safety of these P. emblica branch extracts. For antimutagenic test in the absence of S-9 activation, EBE and MBE showed antimutagenicity to AF-2 with IC_{50} at 9.2±0.3 and 6.2±0.05; >10 and 4.7±0.05 mg/plate for TA98 and TA100, respectively. Similar effect was also observed when testing with another direct mutagen, 4-NQO, that gave the IC_{50} at 6.1±0.2 and 9.7±0.2; >10 and 8.7±0.2 mg/plate for TA98 and TA100, respectively. The results on indirect mutagen 2AA revealed the strong antimutagenicity of both EBE and MBE, giving the IC_{50} at 0.7±0.1 and 0.9±0.1 for TA98 and 0.6±0.1 and 0.6±0.1 mg/plate for TA100, respectively (Figure 1).

3.3 Antibacterial activity of P. emblica branch extracts

The antibacterial activity of alcoholic extracts of P. emblica branch on five clinical strains was assessed by MIC and MBC values. Both EBE and MBE similarly inhibited S. epidermidis, E. coli, Salmonella sp and P. aeruginosa. However, EBE inhibited S. aureus slightly more than MBE did. Among these five bacteria tested, S. epidermidis was the most sensitive to EBE and MBE (Table 2).

4. Discussion and Conclusions

According to traditional uses of all parts of the plant including fruits, seeds, leaves, branches, bark, flowers and roots (Habib-ur-Rehman et al., 2007) and to encounter the fruit’s seasonal limitation, this study selected the branch as a substitute candidate with the reason that branches can be obtained at anytime of the year. To our knowledge, besides the fruit, there are some phytochemical and pharmacological studies of the plant leaves (Srirama et al., 2012; Jeyasankar et al., 2012), but there are very few reports on the plant’s...
branches. Our results revealed antioxidant, antimutagenic and antibacterial activities of *P. emblica* branch extracts. The plant extraction process included the cold extraction with 50% ethanol and Soxhlet extraction with methanol to obtain two crude alcoholic extracts namely EBE and MBE, respectively. Both plant extracts showed high phenolic contents with strong antioxidative activity as observed in the fruit part (Sripanidkulchai and Junlatat, 2014). The finding that EBE and MBE possess high antioxidative activity, suggests that alcoholic extracts of *P. emblica* branches has potential to be used. Moreover, the absence of mutagenicity of the plant branch extracts suggests the safety of the branch part. However, acute and chronic toxicities of the branch extract should be further confirmed in animal model. The antimutagenesis of both extracts support the anticancer effect of *P. emblica* fruit (Jose et al., 2001). The antibacterial activity of the alcoholic extracts of the plant branch was found to be similar to that previously reported in the plant fruit (Ahmed et al., 1998). Moreover, our recent study also demonstrated anti-inflammatory and antimelanogenesis effects of the plant branch extract, which contained at least seven phenolic compounds, i.e., gallic acid, vanillic acid, epigallocatechin gallate, vanillin, coumaric acid and ellagic acid, as identified by HPLC analysis (Sripanidkulchai and Junlatat, 2014). Based on the clinical efficacy of the fruit part of *P. emblica* to promote health and longevity, anti-aging and revitalizing, the plant alone or used as an ingredient of polyherbal formulations has been used in skin and beauty products. The fruit extract was also reported to promote collagen synthesis, inhibit the collagen degradation (Fuji et al., 2008; Chanvorachote et al., 2009) and act against UVB-induced photo-aging in human skin fibroblasts (Adil et al., 2010). Therefore further studies on these activities of the branch extracts should be done.

In conclusion, the present study demonstrates high phenolic content and strong antioxidative activity of ethanolic and methanolic extracts of *P. emblica* branches as previously observed in the plant fruit. The studies on selected pharmacological activities showed the potential effects of the alcoholic extracts of *P. emblica* branch as antibacterial and antimutagenic activities. These results suggested that *P. emblica* branch can be a substitute of *P. emblica* fruit, which is highly demanded for the utilization as an ingredient in traditional medicine and cosmetics.

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