### Safety and efficacy assessment of skin gel containing nanoemulsion of Phyllanthus emblica extract: a randomized, double-blind and placebo controlled study

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<th>Journal:</th>
<th>Songklanakarin Journal of Science and Technology</th>
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<td>Manuscript ID</td>
<td>SJST-2018-0333.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Original Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>25-Oct-2018</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
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Safety and efficacy assessment of skin gel containing nanoemulsion of *Phyllanthus emblica* extract: a randomized, double-blind and placebo controlled study

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Abstract

A skin gel containing nanoemulsion of *Phyllanthus emblica* branch extract (emblica nanogel) was tested for its safety and efficacy in a randomized, double-blind and placebo-controlled clinical trial. The patch skin irritation test showed that neither the emblica nanogel nor the placebo nanogel caused skin erythema. The subjects receiving emblica nanogel had a significant lower melanin index of the cheek at weeks 2, 4, 6, and 8; of the forehead at weeks 4, 6, and 8; and of the forearm at week 8 when compared to the placebo group. Skin elasticity was observed to increase for the cheek at week 4, forehead at week 6, and there was no change for the forearm. The levels of skin moisture and erythema of the subjects were not significantly different from base line levels. In conclusion the application of emblica nanogel resulted in significant skin whitening during the 8 weeks of application.

Key words: skin whitening, emblica, nanoemulsion, randomized double-blind, clinical trials, melanin index
1. Introduction

Healthy and youthful skin is desirable for people of all ages, especially those in the growing aged population. With increasing age, skin undergoes alterations characterized by a loss of elasticity and moisture content, uneven tone and tendency to develop wrinkles. Photoaging is very common and causes not only increased melanogenesis but also a reduction in the amount of collagen, eventually giving rise to increased skin pigmentation and wrinkles (Gordon, Mansur, & Gilchrest, 1989; Romero-Graillet et al., 1996; Baroni et al., 2012). Several skin care products have been introduced following the demand for lightening the skin color and looking younger; the most common route of administration is topical application. Many melanogenesis inhibitors such as hydroquinone, arbutin, retinoic acid, kojic acid and α–hydroxy acids have been used as whitening agents in cosmetic products; though there are often some side effects (Haddad et al., 2003; Maeda & Fukuda, 1996; Griffiths et al., 1993; Mishima, Ohyama, Shibata, Seto, & Hatae, 1994; Stiller et al., 1996). During the past several decades, plant extracts have been studied in the search for natural products with anti-tyrosinase activity; these would be a natural bioactive source of a broad spectrum of compounds, including polyphenolics and flavonoids (Chen, Wei, & Marshall, 1991; Kubo et al., 2000; Parvez et al., 2006)

Emblica (Phyllanthus emblica L.), (Euphorbiaceae) is widely distributed in subtropical and tropical areas. The fruit has been used as a major constituent of various traditional and Ayurvedic medicines. Following reports on the pharmacological effects of emblica fruit, which include antioxidant (Liu, Zhao, Wanga, Yangb, & Jiang, 2008), anti-inflammatory (Muthuraman, Sood, & Singla, 2011), and protection from UVB-induced photo-aging (Adil et al., 2010), its demand as an ingredient in cosmetic
products has increased. However, the supply is limited according to the size of the annual crop. The alternative use of other parts of emblica as potential substitutes for the fruit is desirable. Gallic acid is a major phenolic compound in the emblica fruit. Previous reports have demonstrated the potential of the branches and stems of *P. emblica* as alternative sources of phenolic compounds including catechin, epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG) and gallocatechin (GC) (Balasundram, Sundram, & Samman, 2006; Dufresne, & Farnworth, 2003). Our previous data indicated high contents of several phenolic compounds including ascorbic acid, gallic acid, catechin, vanillic acid, vanillin, ferulic acid and ellagic, EGC, EGCG in the alcoholic extract of *P. emblica* branches (Sripanidkulchai & Junlatat, 2014).

*P. emblica* branch extract also exhibited potent inhibitory effects on mushroom tyrosinase activity and down regulation of tyrosinase related proteins genes in B16 murine melanoma cells (Sripanidkulchai & Junlatat, 2014). With the advantage of enhanced of solubility, bioavailability and stability of nanocarrier dosage forms, the nanoemulsion of *P. emblica* branch extract has been successfully developed with sustainable releases of EGC and EGCG (Chaiittianan & Sripanidkulchai, 2014). Therefore, in this study the nanoemulsion of emblica branch extract was further developed as a skin gel and tested for its safety and efficacy.

2. Materials and methods

2.1 Preparation of skin gel products

*Phyllanthus emblica* collected from Amphur Muang, Khon Kaen Province, Thailand was dried at 50°C and ground to a powder, then extracted with 50% ethanol.
and filtered through Whatman® No.1 paper. The filtrate was dried under a rotary evaporator at 45°C, and then freeze-dried in lyophilizer with yield of 5.4%.

Under HPLC analysis, the extract contained several phenolic compounds, including three major compounds, i.e., vanillic acid, gallic acid and epigallocatechin at concentrations of 6.9, 6.3 and 1.9 mg/g, respectively. A nanoemulsion containing of ethanolic extract of *Phyllanthus emblica* branch (0.15%), isopropyl myristate (0.6%), Brij® (0.35%) and distilled water (98.9%) was prepared by modified microemulsion technic with hot high pressure homogenization as previously described by Chaiittianan and Sripanidkulchai (2013). Then the skin gel (so called emblica nanogel) was formulated to compose of nanoemulsion (80%), hydroxyethylcellulose base (2%), glycerine (3%), propylene glycol (0.2%), methylparaben (0.04%), propyl paraben (1.76%) and distilled water (13%). Finally, each gram of emblica nanogel contained 1.2 mg of *P. emblica* extract. The placebo gel (so called placebo nanogel) contained the same pharmaceutical ingredients, except for the ethanolic extract of *P. emblica*.

2.2 Clinical studies

The randomized, double blind and placebo controlled protocol was approved by Khon Kaen University Ethical Committees on clinical trials (HE532270). The demographic information of participants was shown in Table 1.

2.3 Skin irritation test

Prior to product application, a patch skin irritation test was conducted with 50 healthy Thais residing in Khon Kaen province. They had been informed about the study conditions and each subject signed an informed consent form. Apart for the absence of
All subjects were applied 0.5 ml each of tested materials in an area measuring 1 x 1 cm on the upper right arm. Our tested products included (1) distilled water (negative control), (2) 1% sodium lauryl sulfate (positive control), (3) emblica nanogel and (4) placebo nanogel. There were two rows of treated areas, the first starting 4 cm from the shoulder, and the second spaced 0.5 cm from the first. After the product application, clean cotton patches and clear plastic tape were used to cover each area. After 24 h, signs of skin irritation were assessed by evaluating the degree of skin redness and swelling with the naked eye, giving scores at 5 levels as 0 (no erythema), 1 (very slight erythema), 2 (well-defined erythema), 3 (moderate to severe erythema) and 4 (injury in depth) (North American Science Association, nd). In parallel, the erythema index was also measured colorimetrically using a Mexameter® (MX 18, Courage & Khazaka, Germany).

2.4 Efficacy test

1. Subjects and treatment

Forty healthy volunteers were separately included in the study after their informed consent was obtained. The exclusion criteria were: having a skin disease, taking medication or food supplements, using whitening products, allergy to facial products, and pregnancy or lactation. In this double-blind trial with a placebo control, the subjects were randomly assigned into two groups by a blind investigator. Two sets of products with identical packages were blindly labeled to be number one and number two. Each subject received two identical packages and was instructed to daily and separately apply these two products (either emblica nanogel or placebo nanogel) on the assigned side of face (0.3 g of gel on half face) and forearm (0.15 g of gel on the area of
in the morning and the evening. The trial lasted for 8 weeks. The subject and product codes were opened after complete the experiment.

2. Assessment of product efficacy

The subjects were evaluated for the product efficacy by assessing three different areas of the skin, which were check, forehead and forearm for 4 items, including melanin index and erythema (Maxamer® MX18, Courage & Khazaka, Germany), moisture and elasticity (Multi dermascope® MDS800, Courage & Khazaka, Germany) at 0, 2, 4, 6, and 8 weeks after the product application. The measurements of each point were conducted by blind investigator for three times, and the average value was used for further analysis.

2.5 Statistical analysis

Data are expressed as mean ± SD. Significance of differences were examined using one-way analysis of variance (ANOVA) and Duncan’s multiple range test. Significant differences were set at p-values of less than 0.05.

3. Results

3.1 Product safety

With one subject dropping out, the skin irritation test included 49 healthy volunteers (10 males and 39 females) with mean age of 28.54 (20-52) years, of which 92% had never been previously reported to be allergic to any substances. After 24 h application and using naked eye evaluation, the nano gel products caused negligible irritation to the skin, as 93.9% of subjects had no erythema at the applied area of the upper arm and only 6.1% of subjects showed very slight erythema. The positive control
(1% sodium lauryl sulfate) caused very slight erythema (8.2%) and well-defined erythema (91.8%) (Figure 1). This was confirmed in measurements with the Mexameter; 1% sodium lauryl sulfate caused significant skin erythema (Figure 2). The results suggested that the nano gel products were safe to use in further studies.

3.2 Product efficacy

Forty subjects were separately recruited into the study and were subsequently randomized into two different treatments on their body. 20 subjects were asked to blindly apply product number one (emblica nanogel) on right half of face and forearm, and product number two (placebo nanogel) on left half of face and forearm, and vice versa for another 20 subjects. With one subject dropping out, 39 subjects (5 males and 34 females) with mean age at 36.8 (22-52) years completed the study on product efficacy without adverse effects.

Compared to the placebo nanogel, the emblica nanogel significantly decreased melanin index at weeks 2, 4, 6, and 8 after application to the cheek ($P < 0.001$). In parallel, the melanin indices of forehead of emblica nanogel treated subjects were significantly decreased at weeks 4, 6 and 8 ($P < 0.001$), whereas the melanin indices of the forearm were significantly decreased only at week 8 ($P < 0.001$) (Figure 3).

However, the melanin index of emblica nanogel treatments at the cheek, forehead and forearm showed significant and gradual lower values than the baseline at all time points of this study ($P < 0.05$).

Emblica nanogel significantly increased skin elasticity of the cheek at week 4, forehead at week 6. However, the skin elasticity of the forearm did not change (Figure 4). When compared to the baseline data, emblica nanogel decreased elasticity of the forehead at week 8 and increased elasticity of the forearm at weeks 2, 4, 6 of the study.
There were no significant differences in skin moisture between test and placebo groups (Figure 5). However, when compared to the baseline data, both emblica nanogel and placebo nanogel tended to increase skin moisture. In terms of erythema index, which reflects the degree of skin irritation, both test and placebo products tended to decrease the erythema index (Figure 6). Finally, the whitening effect of the emblica nanogel was demonstrated (Figure 7).

4. Discussion and conclusion

Measuring skin irritation potential is necessary as part of toxicological evaluation of cosmetic products, and in this study it was conducted prior to the efficacy test. The emblica nanogel containing emblica branch extract as the main ingredient and placebo nanogel containing other ingredients of gel base caused no skin irritation. There were no adverse effects such as burning or pruritis. Therefore emblica and placebo nanogels were considered safe for use in subjects for the efficacy test.

In this study we have demonstrated the potential of a nanoemulsion containing *P. emblica* branch extract, as formulated in nanogel form, as a skin whitening agent with additional benefits. The emblica nanogel displayed skin whitening effects from the second week of product application and throughout the 8 weeks of the study period. Based on the different degree of pigmentation of the skin, therefore, three areas were included in this study. The melanin index of emblica nanogel treated cheek skin was most sensitive and lower than those of the placebo nanogel treated since the second week, whereas the melanin indices of emblica nanogel applied to the forehead and forearm were affected at week 8. In contrast, the melanin indices of placebo nanogel treatments of these three areas were unchanged throughout the 8 week period. Our results confirm previous reports on the antioxidant and anti tyrosinase activities of *P.*
emblica branch extract as an active ingredient in the emblica nanogel (Sripanidkulchai & Junlatat, 2014). In term of elasticity index, when compared to the placebo group, emblica nanogel significantly increased the elasticity at the cheek only at 4 weeks of treatment, suggesting a mild effect of the emblica nanogels on skin elasticity. Both of the emblica and placebo nanogels showed a tendency to increase the moisture index of cheek, forehead and forearm from the baseline values, probably indicating the moisturizing effect of the gel base of these products. The erythema index that reflects skin irritation demonstrated that both emblica and placebo nanogels did not irritate the applied skin of subjects throughout 8 weeks of application. Moreover, when compared to baseline values (at week 0), a significant decrease in erythema index was observed (at 2 and 8 weeks for the cheek, at 4 and 8 weeks for the forehead and at 8 weeks for the forearm), indicating the skin lightening effect of the products.

With respect to the chemical constitutes of emblica branch extract, several phenolic compounds have been reported, including gallic acid, vanillic acid, epigallocatechin, epigallocatechin gallate and ellagic acid (Chaiittianan & Sripanidkulchai, 2014). These naturally occurring polyphenolic compounds have previously been found to inhibit melanogenesis. Gallic acid significantly inhibited melanin synthesis and tyrosinase activity and decreased the expression of melanogenesis-related proteins, such as microphthalmia-associated transcription factor, tyrosinase, tyrosinase-related protein-1 and dopachrome tautomerase via several markers of the signal cascade pathway, including the activation of the MEK/ERK, PI3k/Akt (Su et al., 2013; Kumar et al., 2013). Ellagic acid was reported to inhibit melanogenesis (Phrutivorapongkul et al., 2013; Shimogaki, Tanaka, Tamai, & Masuda, 2000). Extracts from several parts of plants containing these phenolic compounds have...
been shown to have potential as whitening cosmetic products based on their anti-tyrosinase activities; these include grape seeds and peels (Hsu et al., 2012), peaches (Kim, Kim, Yu, & Yook, 2012), fruit pericarp of *Litchi chinensis* (Kanlayavattanakul, Ospodpant, Ruktanonchai, & Lourith, 2012), pomegranate fruit peel (Fawole, Makunga, & Opara, 2012), mushrooms (Alam, Yoon, Lee, Lee, & Lee, 2011; Yoon et al., 2011) and green tea (No et al., 1999). Therefore, *P. emblica* branch extract can be an alternative source of these phenolic compounds. Taken together the data from this study suggest that nano-emblica gels have skin lightening effects without side effects of skin irritation.

**Acknowledgement**

This work was granted by the National Nanotechnology Center (NANOTEC) of Thailand. The authors would like to thank the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand for facility support.
References


Figure 1 Evaluation of skin appearance (by naked eye) after 24 h application of emblica nanogel. Data obtained from 49 volunteers (10 males and 39 females) with average age of 28.5 (20-52) years. (0 = no erythema, 1 = very slight erythema, 2 = well defined erythema, 3 = moderate to severe erythema, 4 = injury in depth)
Figure 2 Erythema indices of 49 volunteers’ arms after 24 h application of emblica nano products. *significant difference from baseline at p < 0.05.
Figure 3 Melanin indices of volunteers’ skin treated with emblica nanogel (A = cheek, C = forehead, E = forearm) and placebo nanogel (B = cheek, D = forehead, F = forearm), a significant difference from placebo during the same week at p < 0.05, b significant difference from week 0 within the same group at p < 0.05
Figure 4 Elasticity indices of volunteers’ skin treated with emblica nanogel (A = cheek, C = forehead, E = forearm) and placebo nanogel (B = cheek, D = forehead, F = forearm), a
significant difference from placebo during the same week at $p < 0.05$, b significant difference from week 0 within the same group at $p < 0.05$. 

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

![Graph D](image4)

![Graph E](image5)

![Graph F](image6)
**Figure 5** Moisture indices of volunteers’ skin treated with emblica nanogel (A = cheek, C = forehead, E = forearm) and placebo nanogel (B = cheek, D = forehead, F = forearm), \(^b\) significant difference from week 0 within the same group at \(p < 0.05\).
Figure 6 Erythema indices of volunteers treated with emblica nanogel (A = cheek, C = forehead, E = forearm) and placebo nanogel (B = cheek, D = forehead, F = forearm). a
significant difference from placebo during the same week at p < 0.05, significant difference from week 0 within the same group at p < 0.05.

Figure 7 Whitening effect of the skin gel product on the face of a representative volunteer.
### Table 1 Demographic information of participants

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<td>Female</td>
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