การศึกษาแบบสุ่มเปรียบเทียบแบ่งครึ่งใบหน้าถึงประสิทธิผลของการทา ครีม 2%ของสารสกัดเปลือกสนเทียบกับ ยาหลอกในการรักษาฝ้าในคนไทย

A randomized split-face double-blind control trial of 2%Pine Bark Extract topical cream versus placebo on the treatment of melasma in THAIS

ไพลิน โควะวินทวีวัฒน์[1] และสุนิสา ไทยจินดา[2]

[1] นักศึกษาปริญญาโท วิทยาศาสตรมหาบัณฑิต (ตจวิทยา) สำนักวิชาเวชศาสตร์ชะลอวัยและฟื้นฟูสุขภาพ มหาวิทยาลัยแม่ฟ้าหลวง, Phailinko@gmail.com

[2] อาจารย์ที่ปรึกษา วิทยาศาสตรมหาบัณฑิต (ตจวิทยา) สำนักวิชาเวชศาสตร์ชะลอวัยและฟื้นฟูสุขภาพ มหาวิทยาลัยแม่ฟ้าหลวง, sunisa.tha@mfu.ac.th

---

บทคัดย่อ

ฝ้าเป็นเป็นปัญหาความผิดปกติของเม็ดสีบนผิวหนังบริเวณใบหน้าที่พบได้บ่อย ซึ่งมักก่อความกังวลใจในคนมากมายที่มีผิวสีชนิดที่ 3-5 โดยเฉพาะอย่างผิวสีหญิงชาวเอเชีย รวมทั้งหญิงไทย สารสกัดเปลือกสนเป็นสารที่มีคุณสมบัติ ต่อต้านอนุมูลอิสระ ลดการลดการอักเสบ และลดการสร้าง tyrosinase enzyme นอกจากนี้ยังมีคุณสมบัติปกป้องรังสี UV ซึ่งนำมาใช้ในการรักษาฝ้า

วัตถุประสงค์: เพื่อเปรียบเทียบประสิทธิผลของการทา 2% ของสารสกัดเปลือกสน เทียบกับยาหลอก ในการรักษาฝ้าในคนไทย

วิธีการศึกษา: เป็นการศึกษาเชิงทดลองทางคลินิกในอาสาสมัครในรูปแบบ Split- face, Double blind, randomized, controlled clinical trial ในอาสาสมัครคนไทย 18 ท่าน ผู้ซึ่งอายุระหว่าง 30-60 ปี มีสีผิวชนิดที่ 3-5 โดยเทียบประสิทธิภาพ 2% ของสารสกัดเปลือกสน เทียบกับยาหลอก ในแต่ละซีกหน้า อาสาสมัครทุกท่านจะได้รับการทดสอบการแพ้ด้วยวิธี Repeated Open Application Tests ซึ่งให้ผลว่าไม่แพ้ก็จะได้รับการสุ่มให้ใช้ครีมที่กำหนดชื่อ A,Bบนซีกหน้าข้างใด และได้รับครีมกันแดด SPF PA+++ ทาทั่วทั้งใบหน้า ซึ่งครีมทุกชนิดนั้นผ่านการพิจารณาจากคณะกรรมการอาหารและยา อาสาสมัครจะถูกประเมินผลการรักษาในสัปดาห์ที่ 0, 4 และ 8 โดยวัดค่า mean melanin index (MMI) บนแก้ม และทั้งครึ่งซีกหน้า modified MASI (mMASI) score ซึ่งประเมินโดยแพทย์ผิวหนัง 3 ท่าน brown spots, UV spots, ความพึงพอใจของอาสาสมัคร และผลข้างเคียง

ผลการศึกษา: จากอาสาสมัครที่เข้าร่วมวิจัย 18 ท่าน มี 17 ท่านที่เข้าร่วมจนจบโครงการ พบเพียง 1 ท่านที่ไม่สามารถมาตรวจติดตามได้ ในการศึกษาพบ MMI ที่วัดจากแก้ม และครึ่งซีกหน้าที่ใช้ครีม 2% ของสารสกัดเปลือกสนนั้นลดลงอย่างมีนัยสำคัญทางสถิติ เมื่อเทียบกับข้างที่ใช้ครีมยาหลอก ในสัปดาห์ที่ 8 (p = 0.009) แต่ไม่พบความแตกต่างในทางสถิติทั้งสองกลุ่มในการวัดค่า brown spots, UV spots รวมถึง mMASI score และยังพบว่าอาสาสมัครมีความพึงพอใจต่อครึ่งซีกหน้าที่ทา ครีม 2% ของสารสกัดเปลือกสนมากกว่าครีมหลอกอย่างมีนัยสำคัญ ในสัปดาห์ที่ 4

(p = 0.011) นอกจากนี้ยังพบอาการข้างเคียงต่าง ๆ ในสัปดาห์ที่ 8 พบขุยสะเก็ด 5 ราย ในกลุ่มที่ได้รับ ครีม 2% ของสารสกัดเปลือกสน ในขณะที่กลุ่มที่ได้รับครีมยาหลอกนั้นพบขุยสะเก็ด 4 ราย และ1รายที่พบว่าเกิดสิว

สรุป: ครีม 2% ของสารสกัดเปลือกสน มีประสิทธิภาพเหนือกว่าครีมยาหลอก ในการลดรอยดำที่เกิดจากฝ้าอย่างมีนัยสำคัญทางสถิติ ในสัปดาห์ที่ 8 และได้รับความพึงพอใจมากกว่าครีมยาหลอกอย่างมีนัยสำคัญที่สัปดาห์ที่ 4

คำสำคัญ: สารสกัดเปลือกสน, ฝ้า

ABSTRACT

Introduction: Melasma is a commonly acquired disorder of facial hyperpigmentation that is primarily a concern for people with a darker skin type (Fitzpatrick's skin type III-V), especially Asian women, including Thai women. Pine Bark Extract (PBE) has strong antioxidant, anti-inflammatory, anti-tyrosinase properties, as well as UV protection, for the treatment of melasma.

Objectives: To compare the clinical effectiveness of a 2% Pine Bark Extract topical cream and a placebo cream in melasma patients.

Methods: Prospective, split-face, double-blind, randomized, controlled clinical trial studies in 18 Thai subjects who are 30-60 years old with Fitzpatrick's skin type III-V were used to compare the clinical effectiveness of 2% PBE topical cream and a placebo cream to treat melasma. The result of all subjects' Repeated Open Application Tests were negative. Subjects were randomly assigned to use the PBE cream on one side of their face and the placebo cream on the other side of their face for eight weeks. Subjects also had to apply sunscreen SPF 50 PA+++ on their whole face. They were evaluated at the 0^{th}, 4^{th}, and 8^{th} weeks concerning the mean melanin index (MMI) on their cheek and half of their face, as well as the modified MASI (mMASI) score, which is evaluated by three dermatologists, brown spots, UV spots, subject's satisfaction, and adverse effects.

Result: All 17 subjects are completely followed up 3 months, with only one subject did not participate in the follow-up evaluations. Efficacy results demonstrated a statistically significant reduction in MMI on the cheek of the PBE group was significantly less than that of the placebo group (p=0.009) at Week8. And the total MMI on half of the face in the PBE group was significantly less than that of the placebo group (p=0.005) at Week 8. There was no statistically significant reduction in the numbers of brown spots, UV spots, or mMASI score between the groups. The evaluation of subjects' satisfaction with the PBE cream at the 4^{th} week was significantly greater than that of the placebo group (p=0.011). Adverse effects at the 8^{th} week including scaling in 5 subjects of the PBE group, while there were 4 subjects that reported scaling and 1 subject that reported acne as an adverse effect in the placebo group.

Conclusion: The 2% Pine Bark Extract topical cream has higher ability to reduce darkness caused by melasma than the placebo, with significant difference at the 8^{th} week and a higher satisfaction score at the 4^{th} week.

Keywords: Pine Bark Extract, Melasma

1. Introduction

Melasma presents as diffused, light-brown to dark-brown patches, with the areas of pigmentation occurring on the central face, malar, and mandibular regions, forehead, chin, and upper lip. Melasma is a commonly acquired disorder of facial hyperpigmentation that is primarily a concern for people with a darker skin type (Fitzpatrick's skin type III-V), especially Asian women, including Thai women. As melasma is found in countries near tropical zones more than those in other zones, tropical zones are considered as potential sites of prevalence. Such prevalence ranges from 9% in Hispanic populations in the southern United States to 40% in Southeast Asia (Kang S., et al; 2019). In Thailand, melasma is found 40% in of women and 20% of men (Sivayathorn A., 1995).

Pathogenesis of melasma is not fully understood, but the risk factors associated with melisma, such as biologically active melanocyte, genetic and hormonal influences, and exposure to UV light are significant. Some theories can explain parts of the pathogenesis of melasma, such as Melanogenesis in the mechanism of biochemical activation (Basit, 2019). In addition to the epidermal type of melasma, there are also dermal and mixed types. Therefore when pigmentation occurs in the dermis, it is caused from following one of the four pathogenesis pathways, as shown in the heterogeneous histologic findings of melasma: 1) Dermal Extracellular Matrix (ECM) Abnormality (Solar Elastosis); 2) Basement Membrane Disruption; 3) Increased Vascularization; and 4) Increased Number of Mast Cells; this also includes the free radical theory (Soon-Hyo, K et al; 2016).

The gold standard treatment for melasma is hydroquinone, the tyrosinase-blocking enzyme of melanogenesis, but it causes several adverse effects such as erythema, skin peeling, irritant contact dermatitis, hypopigmentation of the surrounding skin, development of milia, and exogenous ochronosis. Even after abrupt discontinuation of hydroquinone, rebound hyperpigmentation often occurs rapidly (Polnikorn N, 2014).

Pine bark extract (PBE) provides potent antioxidant, anti-inflammatory, and anticarcinogenic benefits (Sime S and Reeve V; 2004). Moreover, it also acts as a non-competitive tyrosinase inhibitor (Nesterov, et al; 2008). It has been used broadly as an herbal treatment and nutrition supplement for several degenerative diseases. There are several beneficial properties of PBE, including cardio-vascular, lowering cholesterol, significant free radical scavenging activity against reactive oxygen and reactive nitrogen species, recycling vitamin C, and protecting endogenous vitamin E and glutathione from oxidative stress. It also helps to accelerate the wound healing processes, reduce scar formation, inhibit histamine released from mast cells, and increases pro-inflammatory cytokine actions (Iravani S, Zolfaghari; 2011).

PBE contains many **phenolic compounds** (Ni Z., et al, 2002) (another term is polyphenols):

1. **Monomeric phenolic compounds** (catechin, epicatechin, and taxifolin)

2. **Condensed flavonoids** (procyanidins) (major component: 65-75%)

3. **Phenolic acids** (simple phenols): *p*-hydroxy benzoic, protocatechuic, gallic, vanillic, *p*-coumaric, and caffeic and ferulic acids (Rohdewald, 1998)

One major substance in PBE is condensed flavonoids, or condensed tannins or procyanidins, which is a subordinate class of proanthrocyanidins, a compound of oligomers and polymers consisting of (+)-catechin and/ or (–)-epicatechin units (D'Andrea, 2010). A study of animals found that proanthrocyanidins are metabolized in the liver and excreted mainly via urine, with the biliary tract as a secondary excretion method (Stoupi et al., 2010).

Antioxidant and free radical scavenging activities:

The basic chemical structure of PBE components, including phenolic acids, polyphenols, and flavonoids, is ≥1 aromatic rings carrying ≥1 hydroxyl groups and are therefore possibly able to quench free radicals by forming resonance-stabilized phenoxyl radicals (Gabriele D'Andrea, 2010).

Pine bark extract can inactivate the superoxide ($.O_2$-) and hydroxyl radicals (.OH), and can inhibit the formation of singlet oxygen ($^1O_2$) and reactive nitrogen species (RNS) (Packer L., et al; 1999; Blazso G., et al; 1994; Gouchang Z., 1993; Noda Y, 1997). It can also counter lipid peroxidation, generate thiobarbituric acid reactive products, and effectively induce oxidative hemolysis by peroxide hydrogen (Voss P, et al; 2006).

The scavenging activities of polyphenol containing PBE can perform against peroxynitrite ($.ONOO^-$), nitric oxide (.NO), and hydroxyl radicals $(.OH)^-$(Kim YJ, et al; 2008). The other potentialities of polyphenols are regulation of nitric oxide metabolism by blocking both iNOS activity and iNOS mRNA expression (Virgili, et al.; 1998). Comparing the capacity of antioxidants between oligo-polyphenolic compounds and monomeric flavonoids, the oligo-polyphenolic compounds are greater than the monomeric.

Anti-inflammatory effects

Inflammatory response and skin erythema caused by acute exposure to ultraviolet rays (UVR) leads to the expression of many pro-inflammatory genes, such as TNF-α, IL-1α, IL-1β, IL-6, and IL-8. All of these cytokines have NF-κB binding sites in the 5'flanking region of their encoding gene (Gulati OP., 2005).

Orally supplemented PBE had a preventive effect against UV-induced skin erythema, indicating that flavonoid-based dietary supplements have the benefit of photo-protection. In addition to this result, pine bark extract can also reduce UV-induced NF-kB–dependent gene expression, an important step in the inflammatory reaction, in keratinocytes in cell cultures (Saliou C.,et al; 2001).

PBE effect on human skin:

1. The anti-melanogenic effect:

The 2008 in vitro study conducted by Kim YJ supports the previous results. The study showed the anti-melanogenic effects of PBE via its anti-oxidative actions. PBE had the ability to inhibit tyrosinase activity and melanin biosynthesis by suppressing the effects that countered peroxynitrite(.ONOO-), superoxide ($.O_2$-), nitric oxide (.NO), and hydroxyl radical (.OH)-scavenging activities.

In an ex vivo study evaluating the whitening activity of PBE after exposure to ultraviolet and infrared radiation and visible light, it was found that PBE can reduce melanin deposition in skin cultures treated with PBE after irradiation. Thus, it can be concluded that PBE may have lightening properties (Ayres EL, et al; 2015).

2. Treated melasma by oral PBE:

There are several studies that have found that PBE possesses strong antioxidant and anti-inflammatory properties. For example, in a 2002 study, 30 women diagnosed with melasma were prescribed to take a 25mg dose of oral PBE three times per day (Z. Ni, et al; 2002). After thirty days of treatment, the average melasma area and pigmentary intensity was reduced. The general rate of effectiveness was around 80%. No side effects were observed.

Percutaneous absorption of Pine Bark Extract in human skin

A 2004 study demonstrated that PBE can penetrate through human skin. A 5% (W/V) pine bark solution was applied on a sample of cadaver skin for 24 hours and measured at 0.5, 1, 2, 4, 6, 8, 10, and 12 hours. The main substances of PBE were detected in one half hour. As a result of the percutaneous absorption analysis, it can be concluded that PBE can be used for topical application on human skin (Sarikaki V., et al; 2004).

There were no serious adverse effects from the topical application of PBE. Common adverse effects reported by healthy persons across 70 clinical trials (n=5723) included gastrointestinal discomfort, dizziness, headaches, and nausea. No alarming symptoms, even at high dosages, have been reported to date. Thus, a dosage of 20–100 mg of PBE per day for long periods (months) and 100–300 mg for shorter periods were determined as nontoxic (Oliff, 2010).

This study aims to create a new treatment choice for melasma, by advocating for the strong antioxidant, anti-inflammatory, and anti-tyrosinase properties of pine bark extract and using the scientific method to prove PBE's efficacy in the treatment of melasma. Moreover, there are no previous studies of topical PBE treatments for melasma.

2. Objectives of the study

To compare the clinical effectiveness of the 2% Pine Bark Extract (PBE) topical cream and the placebo cream in melasma patients.

3. Materials and methods

This study was designed as a split-face, double blind, randomized, controlled clinical trial of 18 Thai subjects who are 30-60 years old, have Fitzpatrick's skin type III-V diagnosed by a dermatologist, and who have been diagnosed as having epidermal, dermal, or mixed type melasma on the facial area. Subjects excluded were those who had history of skin disorders, including malignant or premalignant lesions, infected skin lesions, or dermatitis in the treatment area, and those who had a history of hypersensitivity to cosmetic products, are taking oral pills, hormone replacement therapy, topical bleaching agents, chemical peeling, or have had phototherapy or laser within the previous eight weeks, and those who are pregnant and lactating. The experimental study was conducted at Mae Fah Luang University, Bangkok, Thailand after the approval of the Ethics Review Board.

All subjects were required to sign an informed consent form explaining the benefits, risks, and possible complications of the treatment. A Repeated Open Application Test (ROAT)is using a small amount 0.1 ml of the product apply on patients 'skin located on left flexor forearm area 5x5 cm^2, it was applied two times a day for seven days(Hannuksela, M., & Salo, H.;1986). If the result presented any rash including redness, wheal, flare, soreness or other irritation occur on the spot, gently wash the product off and stop using, the patient will be treated appropriately. A randomization program by using https://www.randomizer.org/ was used to assign which half of the patient's face would be applied with either PBE cream or the placebo cream into pattern 1 or 2. Pattern 1 is applying cream A on right face and cream B on left face. Pattern 2 applying cream A on left face and cream B on right face, when researcher set a code cream A is 2%Pine Bark Extract cream and cream B is placebo.

The 2% Pine Bark Extract cream contained 2 gm of PBE in 100 gm of cream and was given to subjects to in the unit of one fingertip to apply on half of their face, and the placebo cream was used on the other half of their face for eight weeks. Subjects additionally had to apply SPF 50 PA+++ sunscreen to their entire face. All products were approved by the THAI FDA. Subjects were followed up with and evaluated at Weeks 0, 4, and 8, measuring the MMI (by Mexameter®), mMASI score, brown spots, UV spots (by using VISIA®), and the subjects' satisfaction (using the four point Likert scale) and adverse effects.

Data was analyzed using IBM SPSS Statistics, Version 25.0 (Armonk, NewYork: IBM Corp; 2015). Normal distributive data were analyzed by repeated ANOVA measures and non-normal distributive data were analyzed by The Friedman Test

4. Results

Table 1 General Characteristics of the Sample

	Number (n)
Sex	
Male	3
Female	14
Age (years), mean±SD (Min - Max)	45.35±9.03 (32 – 59)
Fitpatrick skin type, n(%)	
Type 3	2
Type 4	10
Type 5	5
Melasma type, n(%)	
Mixed	16
Dermal	1
Aggravation factors	
Sunlight	8
Hormones	0
Pregnancy	0
Smoking	1
Onset of melasma (years), mean±SD (Min - Max)	39.59±7.84(28 - 55)

Onset after Pregnancy	2

Table 1 demonstrates the general characteristics of the subjects; most are female (14 subjects) with a mean age of 45.35±9.03 years old. There are ten subjects who have Fitzpatrick skin type IV, five who have Fitzpatrick skin type V, and two who have Fitzpatrick skin type III. The majority (16 subjects) have mixed type melasma, and the most common aggravation factor is sunlight (8 subjects). The age range of the onset of melasma is 39.59±7.84 years old, and there are only two subjects whose melasma was onset after pregnancy.

Table 2 Comparison between the mean of MMI on the cheek in the Pine Bark Extract and placebo groups, including Total Mean Melanin Index on half of the face, brown spots, UV spots, and mMASI.

	Pine Bark Extract	Placebo	p-value (a)
	mean±SD	mean±SD	
Mean Melanin Index on Cheek			
Baseline	257.37±74.61[a]	259.63±65.29	0.742
4th week	233.20±57.61	241.45±62.12	0.218
8th week	223.08±48.94[a,b]	238.83±53.33	0.009*
p-value (b)	0.002*	0.110	
Total Mean Melanin Index on half face			
Baseline	292.15±83.01[a,b]	296.03±74.31[a,b]	0.335
4th week	277.23±73.91[a]	279.11±73.43[a]	0.626
8th week	267.08±70.14[b,c]	279.30±68.49[b]	0.005*
p-value (b)	<0.001*	0.029*	
brown spot on cheek			
Baseline	228.82±83.21	225.94±75.65	0.671
4th week	228.12±79.59	222.47±73.89	0.872
8th week	226.53±92.90	203.71±77.45	0.034*
p-value (b)	0.407	0.060	
UV spot			
Baseline	218.47±85.87	219.47±83.70	0.849
4th week	228.00±92.36	225.24±91.10	0.693
8th week	220.82±84.63	224.47±81.49	0.527
p-value (b)	0.515	0.933	
mMASI			
Baseline	1.96±0.89[a,b]	1.93±0.93[a]	0.697
4th week	1.72±0.8[c]	1.82±0.92	0.452
8th week	1.56±0.78[a]	1.83±0.80	0.073
p-value (b)	<0.001*	0.007*	

Analyzed by using Paired t-test (a), Repeated measure ANOVA (b)

* p<0.05

Table 3 Comparison between mean of satisfactory SCORE (by 4 point Likert scale) in Pine Bark Extract and placebo group

	PINE BARK EXTRACT	PLACEBO	P-VALUE (A)
	MEDIAN (IQR)	MEDIAN (IQR)	
Baseline	0 (0, 0)^{A,B,C}	0 (0, 0)^{A,B,C}	1.000
4th week	2 (2, 3)^{A}	2 (1.5, 2)^{A}	0.011*
8th week	3 (2, 3)^{B}	2 (1.5, 2.5)^{B}	0.097
P-VALUE (B)	<0.001*	<0.001*	

Analyzed by using Wilcoxon Signed Ranks Test (a), Friedman test (b)

* p<0.05

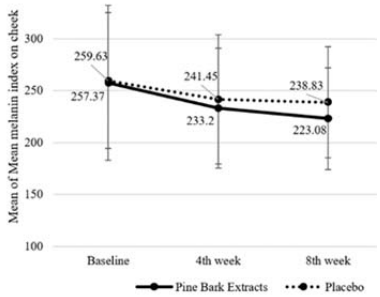


Fig. 1. Comparison of the mean of MMI on the cheeks of the PBE and placebo groups.

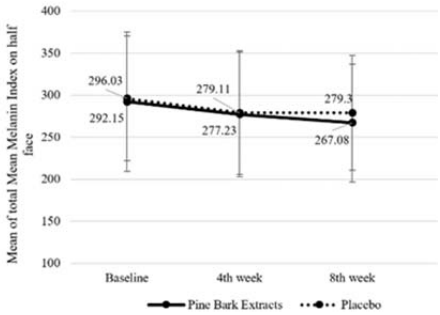
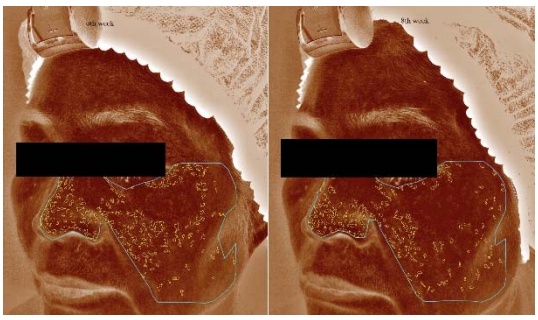
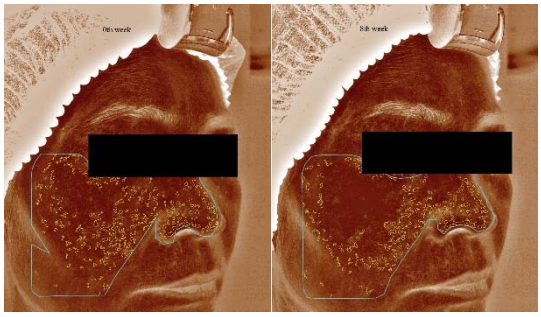


Fig. 2. Comparison of the mean of total Mean Melanin Index on half of the face in the Pine Bark Extract and placebo groups





(Left) Fig. 3. Comparison **of** brown spots in the PBE group at Weeks 0 and 8.

(Right) Fig. 4. Comparison of brown spots in the placebo group at Weeks 0 and 8.

Mean Melanin Index on Cheek

The results at Week 8 show the mean of MMI on the cheek in the pine bark extract (PBE) group was significantly less than the placebo group (p=0.009).

Total Mean Melanin Index on half face

The results at Week 8 shows the mean of the total MMI on half of the face in the PBE group was significantly less than that of the placebo group (p=0.005).

Both the MMI on the cheek and on half of the face in the PBE group had similar statistically significant outcomes, meaning that PBE had the ability to decrease the darkness of melasma lesions on both the cheek and on half of the face.

Brown spots on the cheek

The mean of brown spots in the PBE group had no statistically significant differences when compared to the placebo group (p>0.05).

UV spot

The mean measurement of UV spots across all follow up visits did not show a statistically significant difference between both groups (p>0.05).

Modified MASI

The mean measurement of mMASI across all follow up visits did not show a statistically significant difference between both groups (p>0.05).

Satisfaction score

Comparison of the satisfaction scores between the PBE group and the placebo group shows that the median and interquartile range of satisfaction of the PBE group at Week 4 was significantly greater than that of the placebo group (p=0.011).

Adverse effects

From the clinical trial, adverse effects observed by patients and doctors included:

- Proportion at Week 0 (before treatment):
  - scaling in PBE group: 5/34; scaling in placebo group: 8/34
- Proportion at Week 4:
  - scaling in PBE group: 5/34; scaling in placebo group: 5/34
  - dryness in PBE group: 1/34; dryness in placebo group: 1/34
- Proportion at Week 8:
  - scaling in PBE group: 5/34; scaling in placebo group: 4/34
  - acne (2 papules) in placebo group: 1/34

5. Discussion

Mean Melanin Index on Cheek and half face

According to the ex vivo study evaluating the whitening activity of PBE after exposure to ultraviolet radiation, infrared radiation, and visible light, it was found that PBE can reduce the deposition of melanin in skin cultures treated with PBE after irradiation. Thus, it can be concluded that PBE may have lightening properties (Ayres EL, et al; 2015).

It was proven that PBE is a lightening agent, as the outcome measured by the Mexameter absorption principle contributed to markedly higher reduction of darkness in the PBE group than in the placebo group.

One limitation of the Mexameter is that within a small area, the degree of darkness can be measured by specific points, but cannot cover the total number spots.

Finally, the study demonstrates that PBE does not have a rebound hyperpigmentation effect.

Therefore, this study can conclude that 2% PBE cream has the ability to decrease the darkness of melasma significantly more than a placebo cream.

Brown spots on the cheek

There are various reasons for this, as follows: Firstly, the causes of increasing darkness in the PBE group included some confounding factors such as exposure to strong sunlight and that the amount of cream was less than 1FTU (due to the low viscosity of the cream and the small amount distributed across a large area).Secondly, as most subjects had mixed type melasma, the PBE may have lightened the melasma in the epidermal portion, but not in the dermal portions, which maintained the base line measurement. Thirdly, pine bark extract is a hydrosoluble flavonoid. While it is able to penetrate the epidermis, PBE cream is an emulsion that contains both hydrosoluble and non-hydrosoluble aspects. Considering that the basement membrane of the skin is semipermeable barrier, some ingredients may have diffused through the basement membrane in just a small amount. Finally, the concentration of the PBE cream is not strong enough to inhibit the tyrosinase enzyme, nor to act as an antioxidant. Therefore, 2%

PBE cream does not have the ability to decrease the number of melasma spots when measured only by the presence of brown spots.

UV spot

The mean measurement of UV spots across all follow up visits did not show a statistically significant difference between both groups ($p>0.05$). This is due to the concentration of the PBE cream, as it is not strong enough to provide protection against UV radiation. Therefore, 2% PBE cream does not have ability to decrease the number of UV spots when measured only by the presence of UV spots.

Modified MASI

As mMASI score is a subjective measurement designed to evaluate area and darkness, it cannot measure darkness to the same degree as the Mexameter. This study shows a statistically significant decrease in darkness as measured by the Mexameter, which is an objective measurement. Thus, it can be concluded that 2% PBE cream has the ability to decrease the darkness of melasma spots significantly more than the placebo when measured objectively, but such differences are not shown in subjective measurements.

Satisfaction score

Subjects pay attention on their lesion, and use as a rough scale to grading satisfaction. But dermatologists look for whole face, and use equations to calculate including entire area and darkness into the best outcome that relate to result of brown spot and UV spot. However, most subjects have maximum appreciated with treatment at Week 4.

By the part of darkness, it decreases in objective measurement level by Mexameter, but it does not change in subjective level (observation by dermatologists), while number of spots is stable. Basically, equation of mMASI consists of Area (number of melasma spots) and Darkness (grading by dermatologists), these are subjective level. So, change of only one factor such as darkness does not have an enough power to demonstrate all difference.

Adverse effects

There are some mild adverse effects that occur in research period such as scaling, dryness and acne. These problems were treated appropriately by physician.

6. Conclusion

The 2% Pine Bark Extract topical cream has a higher ability to reduce the darkness of melasma than the placebo, demonstrating a significant difference at Week 8 (p=0.009) and a higher satisfaction score at Week 4 (p=0.011). However, there was not a significant difference in the reduction of the mMASI score, the number of brown spots, or the number of UV spots between the two groups. Mild adverse effects, such as scaling, dryness, and acne were reported by both groups.

Despite the small sample size, a significant improvement in the MMI and satisfaction were measured, and adverse effects were minimal in both groups. These indicate that the 2% Pine Bark Extract topical cream had, to some degree, decreased the darkness of melasma and acted as a whitening agent. The authors recommend a larger sample size to more accurately document the efficacy of Pine Bark Extract, increasing the frequency of application to three times per day, increasing the concentration of Pine Bark Extract topical cream to 4%, and increasing the study's duration to three months.

Acknowledgements

Firstly, I would like to thank my supervisor, Dr. Sunisa Thaichinda. Secondly, I would like to thank Assoc. Prof. Wongdyan Pandii very much for providing great suggestions for the statistical analysis. Thirdly, I would like to thank Prof. Thamthiwat Nararatwanchai, M.D., Ph.D., who gave me skillful comments on how to perform my research.

References

Ayres, E. L., Costa A., Eberlin S., & Clerici S. P. (2015). Ex vivo study for evaluating the whitening activity of Pycnogenol® after exposure to ultraviolet and infrared radiations, and visible light. *Surgical & Cosmetic Dermatology*, 7(4). doi: 10.5935/scd1984-8773.201574736.

Basit, H., Godse, K. V., Al Aboud A. M. Melasma. [Updated 2019 May 6]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019 Jan.Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK459271/

Blazso, G., Gabor, M., Sibbel, R., Rohdewald, P. (1994). An anti-inflammatory and superoxide radical scavenging activities of a procyanidins containing extract from the bark of *Pinus pinaster* sol. and its fractions. *Pharm Parmacol Lett*, 3,217-220.

D'Andrea, G. (2010). Pycnogenol: A blend of procyanidins with multifaceted therapeutic applications? *Fitoterapia, 81*(7), 724-736. doi:10.1016/j.fitote.2010.06.011

Gulati, O. P. (2005). The nutraceutical Pycnogenol: its role in cardiovascular health and blood glucose control. doi:10.14748/bmr.v16.94

Gouchang, Z. (1993). Ultraviolet radiation-induced oxidative stress in cultured human skin fibroblasts and antioxidant protection. *Biol Res Rep Univ,*33:1-86.

Hannuksela, M., & Salo, H. (1986). The repeated open application test (ROAT). *Contact Dermatitis (01051873), 14*(4), 221.

Iravani, S., & Zolfaghari, B. (2011). Pharmaceutical and nutraceutical effects of Pinus pinaster bark extract.

Kang, S. e., Amagai, M. e., Bruckner, A. L. e., Enk, A. H. e., Margolis, D. J. e., McMichael, A. J. e., & Orringer, J. S. e. (2019). *Fitzpatrick's dermatology in general medicine, 9e*. New York, N.Y.: McGraw-Hill Education LLC.

Kim, Y. J., Kang, K. S., & Yokozawa, T. (2008). The anti-melanogenic effect of pycnogenol by its anti-oxidative actions. *Food and Chemical Toxicology, 46*(7), 2466-2471. doi:10.1016/j.fct.2008.04.002

McLeod, S. D., Ranson, M., & Mason, R. S. (1994, 1994). *Effects of estrogens on human melanocytes in vitro*, Great Britain.

Nesterov A, Zhao J, Jia Q. (2008). Natural tyrosinase inhibitors for skin hyperpigmentation[Internet]. Retrieved Sep 10, 2019, from https://www.slideshare.net/AlexandreSashaNester/drug-fut

Ni, Z., Mu, Y., & Gulati, O. (2002). Treatment of melasma with Pycnogenol®. *Phytotherapy Research, 16*(6), 567.

Noda, Y., Anzai, K., Mori, A., Kohno, M., Shinmie, M.,Packer, L.(1997). Hydroxyl and superoxide radical scavenging activities of natural source antioxidants using the computerized JES FR30 ESR spectrometer system. *Biochem Mol Biol Int*, 42, 35-44.

Oliff, H. (2010). Scientific and Clinical Monograph for PYCNOGENOL. Retrieved Sep 10, 2019, from https://www.pycnogenol.com/fileadmin/pdf/Consumers/Pycnog_FullMono120809_LOW.pdf

Packer, L., Rimbach, G., Virgili, F. (1999). Antioxidant activity and biologic properties of a procyanidin-rich extract from the pine (*Pinus maritima*) bark, Pycnogenol.*Free Rad Biol Med*; 27, 704-724.

Polnikorn, N. (2014). New Approach for Laser Treatment of Melasma and Hyperpigmented Lesions. Pigmentary Disorders 1:128. doi:10.4172/ JPD.1000128

Rohdewald, P. (1998). Pycnogenol. ln Flavonoids in Health and Disease Rice Evans CA, Packer L.(eds). *Dekker lnc*: 405-419.

Saliou, C., Rimbach, G., Moini, H., McLaughlin, L., Hosseini, S., Lee, J., . . . Packer, L. (2001). Solar ultraviolet-induced erythema in human skin and nuclear factor-kappa-B–dependent gene expression in keratinocytes are modulated by a French maritime pine bark extract. *Free Radical Biology and Medicine, 30*(2), 154-160. doi:10.1016/S0891-5849(00)00445-7

Sarikaki, V., Rallis, M., Tanojo, H., Panteri, I., Dotsikas, Y., Loukas, Y. L., . . . Packer, L. (2004). In Vitro Percutaneous Absorption of Pine Bark Extract (Pycnogenol) in Human Skin. *Journal of Toxicology -- Cutaneous & Ocular Toxicology, 23*(3), 149-158. doi:10.1081/CUS-200035353

Sime, S., & Reeve, V. E. (2004). Protection from inflammation, immunosuppression and carcinogenesis induced by UV radiation in mice by topical Pycnogenol®*(2),* 193. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=edsbl&AN=RN155779207&site=eds-live&authtype=ip,uid

Sivayathorn, A. (1995, 1995). *Melasma in Orientals*, Great Britain.

Soon-Hyo, K., Young-Ji, H., Soo-Keun, L., & Kyoung-Chan, P. (2016). Heterogeneous Pathology of Melasma and Its Clinical Implications. doi:10.3390/ijms17060824

Stoupi, S., Williamson, G., Viton, F., Barron, D., King, L. J., Brown, J. E., & Clifford, M. N. (2010). In Vivo Bioavailability, Absorption, Excretion, and Pharmacokinetics of [^1^4C]Procyanidin B2 in Male Rats*(2),* 287. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=edsbl&AN=RN264819787&site=eds live&authtype=ip,uid

Virgili, F., Kim, D., Packer, L. (1998). Procyanidins extracted from pine bark protect alpha-tocopherol in ECV 304 endothelial cells challenged by activated RAW 264 7 macrophages: role of nitric oxide and peroxynitrite. *FEBS Lett*. 431,315–318

Voss, P., Horakova, L., Jakstadt, M., Kiekebusch, D., & Grune, T. (2006). Ferritin oxidation and proteasomal degradation: Protection by antioxidants. *Free Radical Research, 40*(7), 673-683. doi:10.1080/10715760500419357