A Comparative Study of the Efficacy Between 5% Citrus Grandis Fruit Extract Cream and 2% Hydroquinone Cream For the Treatment of Melasma In Thai People

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ABSTRACT

Melasma is a common, acquired and therapeutically challenging hyperpigmentary disorder of the skin that has significant psychological implications affecting the quality of life. When compare with standard topical medications, natural products are mild, biodegradable and have biological and therapeutic activities with widespread availability. Therefore, the researcher reviews on *Citrus grandis* fruit extract which has anti-tyrosinase activity as a potential alternative. The study was conducted to compare the efficacy between 5% *Citrus grandis* fruit extract cream and 2% hydroquinone cream for the treatment of melasma. Sixteen volunteers depending on the inclusion and exclusion criteria were enrolled in a double-blinded, randomized split face controlled clinical trial. Both creams were randomly applied in designated sides of the face twice daily for 8 weeks. Melasma Area and Severity Index and Mean Melanin Index by using Mexameter® MX18 were assessed with the VISIA® complexion analysis system at the time of their first attendance, 4th and 8th week respectively. The mean of MASI score in 5% *Citrus grandis* showed significant reduction from baseline 8.93±2.01 to 8.18±1.84 and 7.13±1.43, and 2% hydroquinone cream from from baseline 8.99±1.84 to 8.17±1.83 and 7.29±1.56 with p-value <0.001* at week 4 and 8 (with Repeated measure ANOVA) and the mean melanin index of 5% *Citrus grandis* were significantly reduced from baseline 261.19±40.21 to 247.44±37.23 and 232.85±37.09 and hydroquinone cream from baseline 261.30±30.49 to 245.74±28.59 and 228.94±27.22 with p value of <0.001* at week 4 and 8. The mean percentage change of MASI score and Mexameter at each visit between the two groups had no statistical significant differences. No adverse effects were noted throughout the study. Based on these study results, 5% *Citrus grandis* cream can be safely used as an alternative treatment for melasma.

Keywords: *Citrus Grandis*, Hydroquinone, Melasma, MASI score, melanin index value

1. Introduction

Melasma becomes one of the common cosmetic concerns among the population and has delirious impact on the patient's quality of life. Melasma occurs in individuals with Fitzpatrick type III-IV and develops

symmetrically on photo-exposed areas such as face especially on the forehead, nose, cheeks, upper lip and chin (Bagherani, 2015).

Pathogenesis of melasma is not completely understood and complex. The contributing factors that are often implicated in its etiopathogenesis are exposure to intense ultraviolet radiation, genetic predisposition and hormonal influences. Therapeutic interventions include multimodality approach. Moreover, the treatment guidelines are still challenging due to non-persistent treatment results and frequent relapses. To achieve the most suitable treatment and procedure for melasma, different modalities in treatments inhibit the production of melanin or dispersion at different stages through different mechanisms (Bagherani, 2015). Several treatments on the guidelines are from topical, oral medications and chemical peelings, to any types of procedures including non-invasive techniques like lasers to minimally invasive ones (Handel, 2014). (Sarkar, 2014).

Hydroquinone (HQ), hydroxyphenolic compound, is currently the gold standard treatment for melasma which can be enhanced if it is used in combination with other agents or other different treatment modalities. The studies point out that hydroquinone has not only block the melanin synthesis but also interfere with the degradation or formation of melanosomes by inhibiting the RNA and DNA synthesis within melanocytes. The concentration for safety margin ranges from 2-4% and the use of 4% has led to significant improvement in depigmentation statistically. However, hydroquinone preparation more than 5% is not recommended according to its irritation except in the refractory problems (Bagherani, 2015) (Ogbechie-Godec, 2017) (Shankar, 2014).

Citrus fruits, which are the genus of *Rutaceae* family, can be cultivated in the temperate and topical regions and is distributed in Cambodia, India, Japan, Laos, Chile, Philippines, Vietnam, Thailand, Bangladesh, China, India, Nepal and Sri Lanka (Orwa, 2009). They are gaining popularity nowadays because they are rich in bioactive compounds and antioxidants especially ascorbic acid, phenol and flavonoids pectins that are important to human nutrition. Those natural oxidant citrus fruits offer many sources of nutrients, phytoconstituents and dietary ingredients to supply a healthy lifestyle and diet.

Citrus grandis has anti-tyrosinase activity which mechanism of action is to inhibit in the synthesis of melanin by blocking the tyrosinase enzyme. It also has different pharmaceutical effects such as antioxidant and radical scavenging properties, anti-cancer activities, anti-inflammatory properties and anti-microbial properties (Abirami, 2014) (Aung, 2018) (Sawan, 2017).

For its maximum dosage and genotoxity, *Citrus grandis* fruit extracts are used at relatively low concentration in cosmetic formulations. The safety margin and dosage for topical cream ranges from 0.0001% to 15% and the concentration used in this conduct has already approved by Food and Drug Administration of Thailand (Panel, 2016). Therefore, the researcher had conducted the study to compare the efficacy between 5% *Citrus grandis* fruit extract cream and 2% hydroquinone cream for the treatment of melasma in thai people.

2. Objectives of the study

General Objective

To study the efficacy of 5% *Citrus grandis* fruit extract cream for the treatment of melasma in Thai people

Specific Objective

- Primary outcome

To compare the efficacy of 5% *Citrus grandis* fruit extract cream and 2% hydroquinone cream for the treatment of melasma in Thai people

- Secondary outcomes

To observe the adverse effects of 5% *Citrus grandis* fruit extract cream for the treatment of melasma in Thai people

To evaluate each participant's satisfaction between *Citrus grandis* fruit extract cream and 2% hydroquinone cream

3. Materials and methods

Sixteen healthy volunteers with melasma who are between 25 years to 55 years depending on the inclusion and exclusion criteria were enrolled in a double-blinded, randomized split face controlled clinical trial. The researcher explained why and how would the study be performed and then took the history and physical examination of the volunteers for basic information. Informed consent from each volunteer was taken and irritation patch test was performed. After randomization was done by the clinicians, volunteers were instructed to apply 5% *Citrus grandis* fruit extract cream and 2% hydroquinone cream two times daily for 8 weeks consecutively. Volunteers had to apply sunscreen SPF 15, PA +++ (BR derm®, UVA/UVB Defense SPF50) 30 minutes before sunlight exposure during the whole period of the study. Melasma Area and Severity Index (MASI) and Mean Melanin Index by using Mexameter® MX18 together with the VISIA® complexion analysis system were used to assess at the time of their first attendance, 4th week and 8th week respectively.

Sample Size Calculation

The calculation was done by using 2 means and standard deviation (SD) of the measurement from previous research which is the comparative study of the safety and efficacy of 75% mulberry (Morus alba) extract oil versus placebo as a topical treatment for melasma and a randomized, single-blind, placebo-controlled trial. (Alvin, 2011)

Biophysical Techniques

The photos of the volunteers were taken by **VISIA ® Complexion Analysis System** (Canfield, Fairfield, NJ), an equipment which scans the skin and captures the critical visual information by multispectral imaging and analysis of the areas what affects the appearance of skin, UV spot and brown spots etc. The melanin index, the parameter of mexameter, was measured by **Mexameter® MX18** (Courage and Khazaka, Germany). The severity of the melasma was determined by MASI score (Melasma area and severity index).

Statistical Analysis

The data were obtained from patients' files and were analyzed statistically using SPSS 18 software and Microsoft Excel 2010 in Dermatology Clinic, Mae Fah Luang University Hospital.

4. Results and Discussion

According the demonstrated general characteristic of the participants, mean age of the subjects was 46.06±8.63 years. Among them, seven subjects got exposed to sunlight with mean duration of 48.57±33.38 minute. All subjects in this study had Fitzpatrick skin type IV.

Melasma area and severity index (MASI)

Table 4.1 Statistical analysis of MASI between 5% *Citrus grandis* fruit extract cream and 2% Hydroquinone cream on 0th, 4th and 8th week

	5% *Citrus grandis* fruit extract cream	**2% Hydroquinone cream**	**Mean difference**	**p-value (a)**
	Mean±SD	**Mean±SD**		
Week 0	8.93±2.01	8.99±1.84	0.06	0.842
Week 4	8.18±1.84	8.17±1.83	0.01	0.979
Week 8	7.13±1.43	7.29±1.56	0.16	0.406
p-value (b)	<0.001*	<0.001*		

Data were analyzed between group with Independent t-test (a), within group with Repeated measure ANOVA (b)

* p<0.05

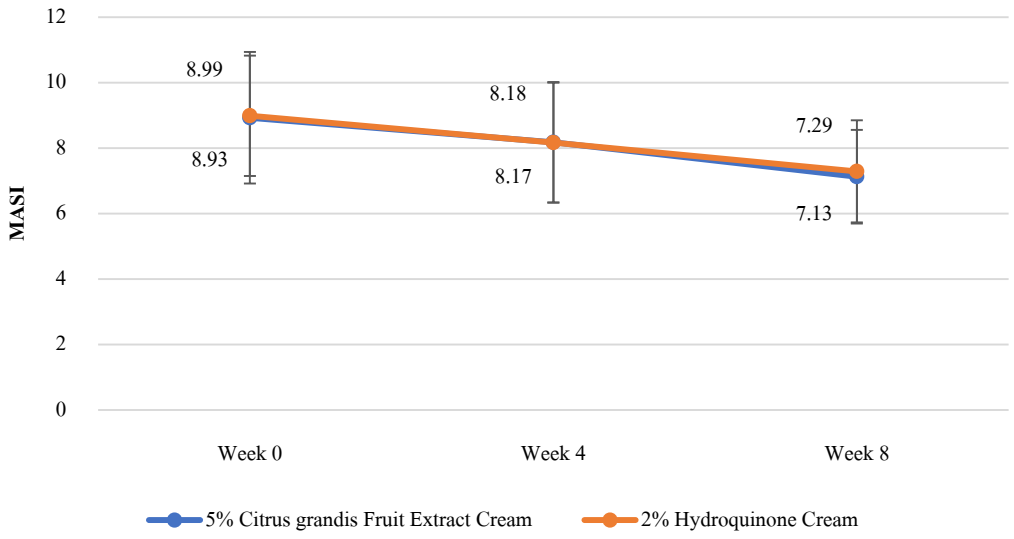

Figure 4.1 Linear graph showing comparison of MASI at each visit between 5% *Citrus grandis* fruit extract cream and 2% Hydroquinone cream

According to the statistical analysis results from table 4.1 and figure 4.1, the mean of MASI score in 5% *Citrus grandis* fruit extract cream on 0 week was 8.93±2.01, 4th week 8.18±1.84 and 8th week 7.13±1.43. The mean of MASI in each visit decreases significantly and statistically at the level of 0.05 ($p<0.001$). For 2% Hydroquinone cream, the results showed 8.99±1.84 at week 0, 8.17±1.83 at week 4 and 7.29±1.56 at week 8. The mean of MASI in each visit showed statistical significant reduction at the level of 0.05 ($p<0.001$). The comparison of MASI between 5% *Citrus grandis* fruit extract cream and 2% Hydroquinone showed that mean of MASI between two treatment groups did not have any statistical significant difference at each visit.

Melanin Index Value Measured by Mexameter® MX18

Table 4.2 Statistical analysis of Mexameter between 5% *Citrus grandis* fruit extract cream and 2% Hydroquinone cream on 0th, 4th and 8th week

	5% *Citrus grandis* fruit extract cream	**2% Hydroquinone cream**	**Mean difference**	**p-value (a)**
	Mean±SD	**Mean±SD**		
Week 0	261.19±40.21	261.30±30.49	0.11	0.991
Week 4	247.44±37.23	245.74±28.59	1.70	0.869
Week 8	232.85±37.09	228.94±27.22	3.92	0.715
p-value (b)	<0.001*	<0.001*		

Data were analyzed between group with Independent t-test (a), within group with Repeated measure ANOVA (b)

* $p<0.05$

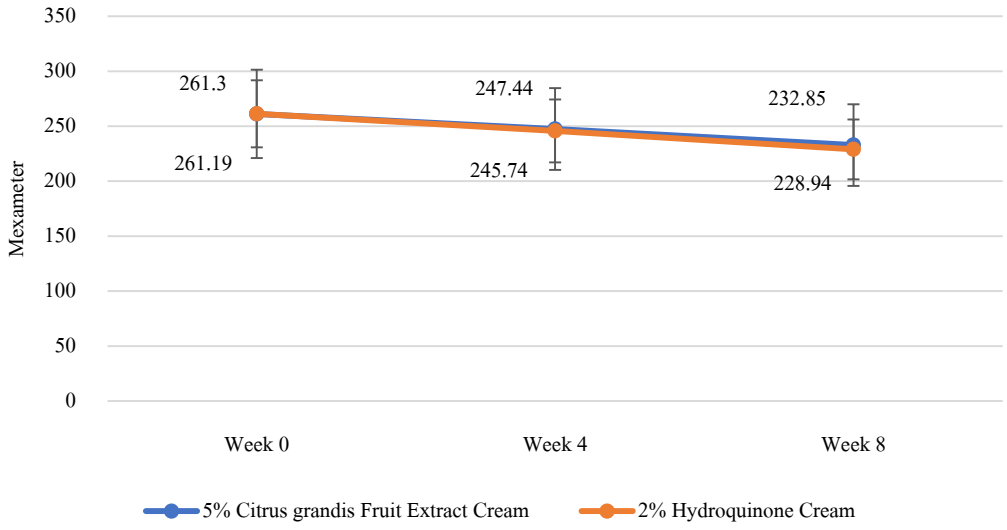

Figure 4.2 Linear graph showing comparison of Mexameter at each visit between 5% *Citrus grandis* fruit extract cream and 2% Hydroquinone cream

According to the statistical analysis results from table 4.2 and figure 4.2, mean melanin index value measured by Mexameter in 5% *Citrus grandis* fruit extract cream on 0th week was 261.19±40.21, 4th week 247.44±37.23 and 8th week 232.85±37.09. The mean of Mexameter in each visit showed statistically significant reduction at the level of 0.05 ($p<0.001$). For 2% Hydroquinone cream, the result at 0th week was 261.30±30.49, 4th week 245.74±28.59 and 8th week 228.94±27.22. The mean of Mexameter in each visit 5% *Citrus grandis* cream statistically significant decreased at the level of 0.05 ($p<0.001$). The comparison of Mexameter between 5% *Citrus grandis* fruit extract cream and 2% Hydroquinone cream found that mean of Mexameter between both treatment groups did not show any statistically significant differences at each visit.

Dermatologist's satisfaction

Table 4.3 Dermatologists satisfaction score

Week 0 compare with	**5% *Citrus grandis* fruit extract cream**	**2% Hydroquinone cream**	**p-value**
	n(%)	n(%)	
Week 4			
No satisfaction (0)	4 (25.0)	5 (31.3)	1.000
Little satisfaction (1)	12 (75.0)	11 (68.8)	
Week 8			
No satisfaction (0)	2 (12.5)	0 (0.0)	
Little satisfaction (1) and Average satisfactions (2)	14 (87.5)	16 (100.0)	0.144

Data were analyzed with Chi-square test, *$p<0.05$

Above table 4.3 showed dermatologist's satisfaction score between 5% *Citrus grandis* fruit extract cream and 2% hydroquinone cream. It was found that the majority of dermatologist's satisfaction score of 5% *Citrus grandis* fruit extract cream showed no satisfaction (25.0%), while 2% hydroquinone cream showed no satisfaction (31.3%) in week 4. There was no statistical significant difference between group ($p=1.000$).

The majority of dermatologist's satisfaction score at 8th week showed no satisfaction (12.5%) and little and average satisfaction (87.5%) in 5% *Citrus grandis* fruit extract cream, while 2% hydroquinone cream showed no satisfaction (0%), little satisfaction and average satisfaction (100.0%). There was no statistical significant difference between group ($p=0.144$).

Patient satisfaction scores

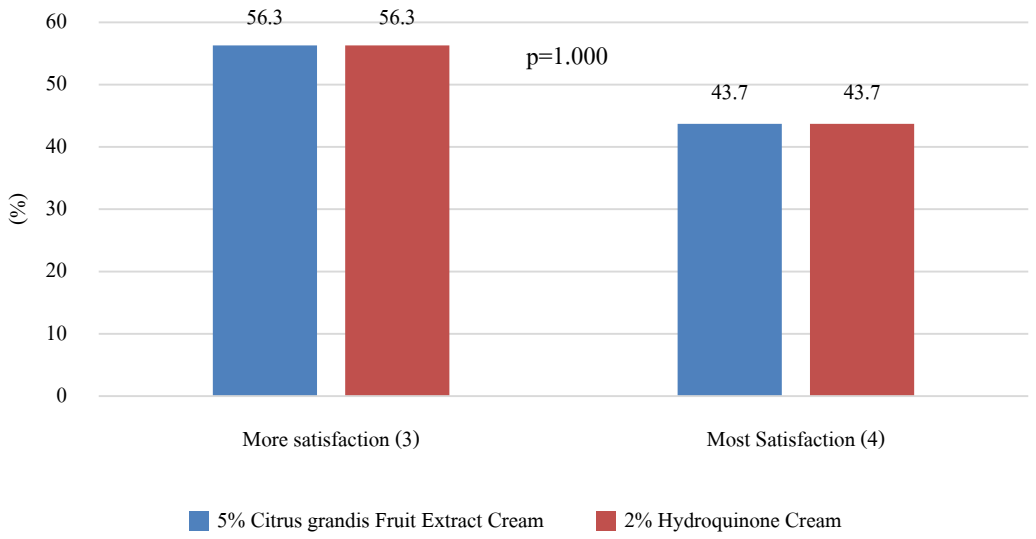

Figure 4.3 Bar chart reveals the percentage of patient's satisfaction score between 5% *Citrus grandis* fruit extract cream and 2% Hydroquinone cream at 8th week

Above figure 4.3 of patient's satisfaction score at 8th week between 5% *Citrus grandis* fruit extract cream and 2% Hydroquinone cream, most of subjects rated more satisfaction (56.3%) and most satisfaction (43.7%) for 5% *Citrus grandis* fruit extract cream and subjects rated more satisfaction (56.3%) and most satisfaction (43.7%) for 2% Hydroquinone cream. The patient's satisfaction score at 8th week between two group did not have any statistical significant difference (p=1.000)

5. Discussion and Conclusion

Both 5% *Citrus grandis* fruit extract cream and hydroquinone cream have the efficacy in reduction of the melanin index over the 8-week period study. Since both creams showed no statistical significant difference, they revealed the same effectiveness in the treatment of melasma. No adverse effects from both creams were recorded throughout the period of the study.

To sum up, the study has proved that 5% *Citrus grandis* cream has anti-tyrosinase activity to a certain extent and hence it can be used as effective, safe and alternative agent in the treatment for melasma in both clinical and aesthetic fields. The reason for this observation and conduct is to be explored with further researches. However, the conduct period was limited to access further complications and unwanted outcomes which are likely to occur in long-term use. Due to small sample size of the study, the researcher had difficulties in interpretation of its exact effectiveness on the treatment of melasma. Therefore, higher concentration of *Citrus grandis* and larger sample size of the study with long term follow-up period are recommended for further researches.

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