A Split-face Double-blind Randomized Placebo-controlled Trial of the Efficacy of Kakadu plum Extract for Reduction of Periorbital Wrinkles

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ABSTRACT

Background: Periorbital wrinkles result from both intrinsic and extrinsic aging. To combat aging and treat wrinkles, antioxidants play an important role by decreasing oxidative stress. Kakadu plum (*Terminalia ferdinandiana)* extract is well known for its great amount of vitamin C as main ingredient which can scavenge reactive oxygen species (ROS) and give anti-aging effects.

Objective: To study effectiveness and satisfaction of Kakadu plum extract to reduce periorbital wrinkles

Method: 17 volunteers, both male and female of age between 25 and 60 years old who willingly wanted to get rid of their periorbital wrinkles were collected to participate in split-face double-blinded placebo-controlled trial for 8 weeks. 10% *Terminalia ferdinandiana* extract cream and placebo cream were instructed to apply twice daily to periorbital area for 8 weeks. Various parameters such as wrinkle evaluation by dermatologists, elasticity and hydration of the skin around the eyes (crow's feet area and undereye area) were assessed at the start, 4th and 8th week respectively.

Result: Statistically significant results of better skin elasticity (p-value <0.001 at crow's feet, p-value=0.029 at undereye) and better skin hydration (p-value=0.001 at crow's feet, p-value=0.012 at undereye) were shown for *Terminalia ferdinandiana* extract treated side. No side effects were observed.

Conclusion: This study statistically demonstrated that Kakadu plum extract reduced periorbital wrinkles more than placebo cream when it is applied topically with no harmful side effects. So, 10% *Terminalia ferdinandiana* could be safe and effective to reduce periorbital wrinkles.

Keywords: *Terminalia ferdinandiana,* Kakadu, Wrinkles, Elasticity, Hydration

1. Introduction

Nowadays, having flawless and glowing skin without wrinkles, fine lines and dark spots is becoming ultimate beauty goal for almost all people around the world. As the world is developing and becoming modernized, the beauty standard keeps changing and people focus not only on skin beauty but also on having comfortable and healthy life style. This is the reason why anti-aging medicine is becoming important and popular in this era and the researchers keep updating about new anti-aging treatments. They are always searching for treatments with minimal or no side effects to get younger and rejuvenated look especially facial appearance.

Skin being the largest external organ and the most visible area of the body, is aging day by day due to various factors from inside and outside of the body such as hormones, stress, unhealthy foods, lack of enough sleep, smoking, ultraviolet radiation and even environmental pollution. These factors can cause aging of the skin such as sagging, wrinkles, dark spots, decreased skin moisture, lack of skin radiance and age related disorders, importantly, skin cancer. (Farage, 2013)

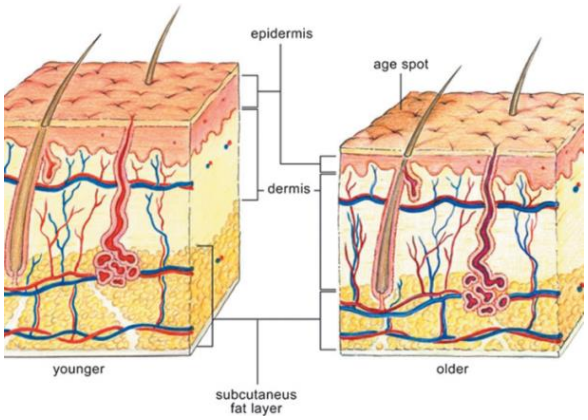

Figure 1.1 Younger skin Vs Older skin (Farage, 2013)

Wrinkles result from internal aging process and also external aging process. Wrinkles around the eyes called crow's feet are the most unwanted part because eyes are regarded as windows to the soul and look is the first thing of communication between people before actual speech. Oxidative damage to cells by producing ROS and free radicals causing inflammation plays an important role for both intrinsic and extrinsic skin aging. (Zhang, 2018)

Topical application of antioxidants can help fighting these free radicals formation because antioxidants are scavengers of those ROS to protect skin from collagen degradation and promote new collagen synthesis resulting in increased skin elasticity and improvement of skin wrinkles providing anti-aging effects.

There are many advantages as well as disadvantages in other anti-wrinkle treatments such as lasers, peeling, fillers, botulinum toxin injection and cosmetic surgery. Topical treatment cream application is non-expensive giving safe and effective result. Hence, it becomes the first thing that people want to try. To get anti-aging effects with minimal or no side effect, we should choose skincare products made with botanical extracts

which work as antioxidants because they are natural, safe and healthy. Examples include grape seed extract, mulberry extract, pomegranate extract and green tea extract which are well-known for their antioxidant properties. Thus, antioxidants from botanical extracts can be used as anti-aging products. (Zhang, 2018)

Among a lot of choices of botanical products, many people are interested in Kakadu plum extract to use in skincare products because of its exceptionally high antioxidant capacity. Kakadu plum is a native Australian fruit, also known as *Terminalia ferdinandiana* or billy goat plum which is regarded as one of the best source of Vitamin C of any plant in the world. (Netzel, 2007)

Figure Error! No text of specified style in document..2 Kakadu plum fruit (Y.Sultanbawa, 2018)

It contains not only ascorbic acid but also phenolic compounds. Kakadu plum was proved of its antioxidant capacities by these following assays. Its oxygen radical-scavenging capacity ORAC-H assay (Oxygen Radical Absorbance Capacity for Hydrophilic compounds) was 4.1fold that of blueberry reference and total reducing capacity TRC, FRAP (Ferric Reducing Antioxidant Power assay) was 13-fold that of blueberry. (Konczak, 2010) *T.ferdinandiana* contains high phenols (gallic acid and ellagic acid). Phenols are also known as antioxidants and scavengers of free radicals. (Wojdylo, 2007) A high level of total phenolic compounds was found in Kakadu plum fruit reaching a level of 160μmol gallic acid equivalence GAE/g enhancing its antioxidant property together with a high level of ascorbic acid proving its high antioxidant capacity. (Konczak, 2010) (Shami, 2013)

Moreover, it is also concluded that Kakadu plum fruit extract has low toxicity and also have anti-microbial bioactivity. (Cock, 2011) It was also proved for its anti-inflammatory property and even also said to have anti-cancer properties because of its rich polyphenols. (Tan, 2011) (Mohanty, 2012)

10% Kakadu plum extract was used to know its efficacy on reducing pigmentation and it was found that it caused skin more oily in two patients and one darker skin in one patient. Other than that, there was no serious side effect. (Setiawan, 2015) So, 10% of extract can be regarded as a safe ingredient to use on the skin.

The main reason for this study is to conduct antioxidant properties of Kakadu plum extract would actually be effective for treatment of periorbital wrinkles. Therefore, the main purpose is to study effectiveness of Kakadu plum extract cream for treatment of periorbital wrinkles.

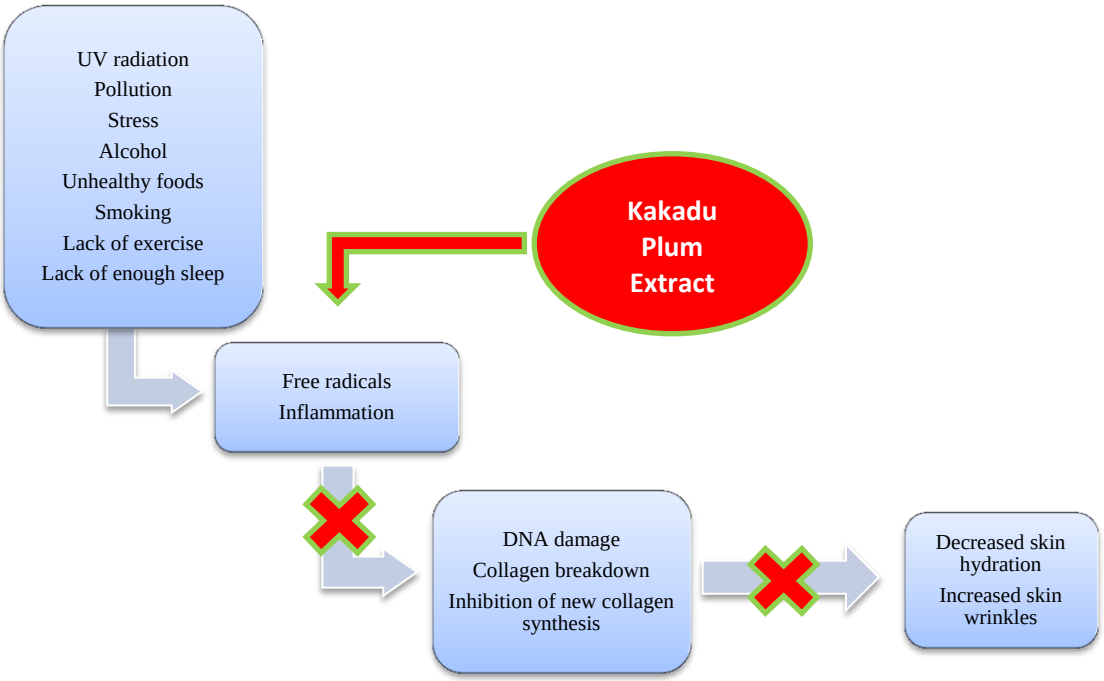

Figure 1.3 Conceptual Framework

2. Objectives of the study

General Objective

To study effectiveness and satisfaction of Kakadu plum extract to reduce periorbital wrinkles

Specific Objective

Primary outcome

To compare anti-wrinkle effect between Kakadu plum extract and placebo cream to decrease periorbital wrinkles

Secondary outcome

To observe adverse effects of Kakadu plum extract

To evaluate participants' satisfaction

3. Materials and methods

Study Design

Comparative, double-blind, randomized, controlled and split face clinical trial

Study Population

25 to 60 years old male and female healthy volunteers who want to get their crow's feet treated

Study Location

Mae Fah Luang University Hospital, Bangkok, Thailand

Sample Size Determination

The efficacy of Kakadu plum extract cream on periorbital wrinkles had never been studied before. The reference data is taken from a similar article which was also a double-blinded split face study between Ginkgo biloba leaf extract serum and standard base cream on the treatment of periorbital wrinkles. Using VISIA® Complexion Analysis System, the results for wrinkle evaluation of skin serum containing Ginkgo biloba leaf extract group showed decrease from 4.55±5.21 at baseline to 3.04±1.21 at 12th week. In standard base serum group, there was slight drop from 4.07±4.51 at baseline to 3.95±1.37 at 12th week. (Tun, 2018)

Different change from baseline of basil extract (μ_1) = 4.55-3.04=1.51

Different change from baseline of placebo (μ_2) = 4.07-3.95 =0.12

Comparing two sample means

Mean difference (μ_d) = μ_1- μ_2 = 1.51 – 0.12 = 1.39

Set α = 0.05 (two-tailed) $Z_{0.025}$ =1.96

Set β = 0.20 $Z_{0.20}$= 0.842

σ_1= 1.21 (Basil)

σ_2 = 1.37 (placebo)

$$n = \frac{(Z_{\alpha/2}+Z\beta)^2(\sigma_1^2+\sigma_2^2)}{\mu_d^2}$$

$$= \frac{(1.96+0.84)^2(1.21^2+1.37^2)}{1.39^2}$$

$$= 13.56 \approx 14$$

Where n = sample size

σ^2= variance

μ_d= mean change between two groups

A dropout rate of 20% was expected. So, 17 volunteers (n=17) were collected

Inclusion Criteria

Male and female healthy volunteers between 25 to 60 years old with periorbital wrinkles (Rao-Goldman's five point Visual Scale Grade 1 to 4: mild to moderately deep wrinkles)

Participants who accept to use Kakadu cream and placebo cream on each side of their faces

Volunteers who do not apply any cream around the eyes at the moment

Subjects who accept to restrict any kind of facial cream including sunscreen and other skincare products particularly around the eyes during study

Accept to restrict facial treatment such as laser treatment and radiofrequency treatment during study

Accept to restrict facial botulinum toxin and filler injection around the eyes

Subjects who want to involve voluntarily in the study and can follow up for 2 consecutive months

Exclusion Criteria

Subjects who are allergic to Kakadu plum extract cream or placebo cream

Subjects with active skin diseases like skin infections and dermatitis

Pregnancy or breast feeding subjects

Subjects with alcohol, smoking or drug abuse

Participants with delayed wound healing or abnormal scaring like keloids

Medical diseases like poorly controlled diabetes mellitus, coagulopathy, photosensitivity or taking immunosuppressant

Subjects who work under strong sun light or who like to expose strong UV light for leisure (e.g. Tanning)

Participants who undergo botulinum toxin injection within 6 months prior to the trial

Subjects who got injected with semi-permanent or non-permanent filler around the eyes within 12 months prior to the trial

Participants who got treated with ablative or non-ablative laser within 6 months, radiofrequency within 1 year, iontophoresis, IPL or microdermabrasion within 3 months prior to study

Discontinuation Criteria

Subjects who cannot continue applying treatment cream and placebo

Volunteers who want to leave the trial for any reasons

Side effects such as severe allergy or skin irritation from treatment cream

Patients who got pregnant during trial

Other medical conditions such as illness or accident

Those who take other treatment options like botulinum toxin injection, filler injection, laser and radiofrequency treatment

Subjects who are not well cooperated or lost follow up during study

Equipments

1. Cutometer® MPA 580

This is the standard equipment to measure skin elasticity and hydration. This device measures the elasticity of upper layer of skin with suction method using negative pressure. It is used for all kinds of cosmetic and skincare formulations especially anti-aging, anti-cellulite or firmness enhancing products. (Tun, 2018)

2. Corneometer®

This device is used to measure hydration of the epidermis especially stratum corneum. It measures water volume by using electric charge which is the transmission of electron from negative electrode to positive electrode. (Tun, 2018) Skin parameters were measured under optimum room temperature approximately 20°C and

approximately 50% air humidity because the higher the air humidity and the room temperature are, the higher is the skin temperature level which can give incorrect results. (www.courage-khazka.de)

Figure 3.1 Cutometer® MPA 580 containing Cutometer and Corneometer probe

3. The VISIA® Complexion Analysis System for Wrinkle Score

VISIA® Complexion Analysis System (Canfield, Fairfield, NJ) is a device used to scan and capture high quality and standardized facial image with multi-spectral imaging system. It detects areas of skin complexion and characteristics such as wrinkles, spots, UV spots, brown spots, textures, pores, red areas, and porphyrins (evidence of bacteria). (Tun, 2018)

Figure 3.2 VISIA® Complexion Analysis System (http://www.beeson.com/skinanalysis.html)

4. 10% Kakadu Plum Extract Cream

Kakadu Plum Extract cream refers to facial cream which contains Aqua, 10% *Teminalia ferdinandiana* fruit extract, Butylene glycol, Cyclopentasiloxane, Glyceryl glucoside, Squalane, Dicaprylyl carbonate, Stearyl alcohol, Cetyl alcohol, Glyceryl monostearate, Dimethicone, Paraffinum liquidum, Acrylate copolymer, Allantoin, Polysorbate 20, Sorbitanmonooleate, DMDM Hydantoin, Tetrasodium EDTA, BHT, Iodopropynyl butylcarbamate

5. Placebo cream

Placebo cream means facial cream which contains Aqua, 10%, Butylene glycol, Cyclopentasiloxane, Glyceryl glucoside, Squalane, Dicaprylyl carbonate, Stearyl alcohol, Cetyl alcohol, Glyceryl monostearate, Dimethicone, Paraffinum liquidum, Acrylate copolymer, Allantoin, Polysorbate 20, Sorbitanmonooleate, DMDM Hydantoin, Tetrasodium EDTA, BHT, Iodopropynyl butylcarbamate

6. Informed consent form and Volunteers' profile documents
7. Adverse effect record form
8. Questionnaires and satisfaction assessment form

Steps of Research

1. Volunteers were selected according to the inclusion and exclusion criteria. The researcher explained details about the aim of research, process steps, expected benefits and possible side effects of the treatment.
2. The subjects signed informed written consent form to participate in this trial.
3. History taking was done.
4. Patch test was performed before cream application.

Under waterproof patch test, the researcher applied Kakadu plum extract to arms of the subjects and then left for 48 hours. The subject had to avoid excessive sweating activities, heavy sunlight during the test and then examined at 48 and 96 hours. According to the international contact dermatitis research group system, any reaction was scored as follows:

+? = any doubtful reaction for example only mild redness

+ = weak positive reaction: appears redness and the skin becomes slightly thickened

++ = strong positive reaction: appears red swollen skin with individual small water blister

+++ = extreme positive reaction: appears intense redness and swelling with coalesced spreading reaction or large blisters

Subjects with positive patch test starting from score ++ were excluded.

5. The researcher took the photograph of each participant by using VISIA® complexion analysis system at baseline, 4^{th}, 8^{th} week respectively.
6. Randomization was done as the followings. Kakadu plum extract cream and placebo cream with similar consistency, color and smell were enclosed in two identical packages and labeled as cream “A” and cream “B”. Dermatologists who evaluated the results and participants were all blinded.

The physician, unrelated with the research, generated randomization sequence by random sequence generator and determined which side of subject’s face had to apply cream A or cream B by using “Block Randomization” and concealed the sequences in opaque envelope. Since this was split face trial, 17 subjects was enrolled so there were total 34 faces. Each block contained two members which were RIGHT (right face) and LEFT (left face).

There were two ways of treatment;

1. (Right, Left) = right face with cream A and left face with cream B
2. (Left, right) = left face with cream A and right face with cream B

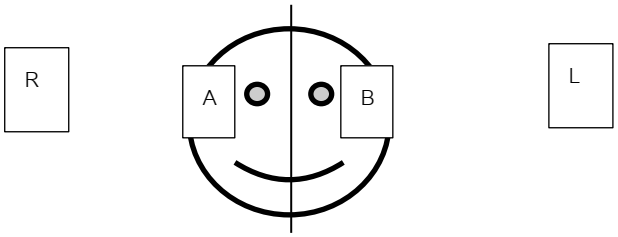

Figure 3.3 Way to apply cream A and B as 1

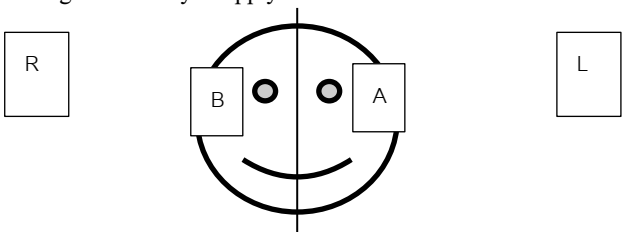

Figure 3.4 Way to apply cream A and B as 2

7. The researcher instructed the subjects to use 0.5 g of Kakadu plum extract cream on one side and placebo 0.5g in other side of the face two times a day after washing face with mild soap.

The researcher explained the participants to restrict using any other creams including sunscreen throughout these 8 weeks of study. If any side effect or reaction occurs, cream should be stopped using and then immediately inform to researcher.

8. Skin elasticity was recorded by Cutometer® MPA 580 and moisture by Corneometer at every follow up and was analyzed after each visit.

9. The landmarks to record data were 1.5cm lateral to lateral canthus and 1.5cm from lower eyelid at mid pupillary line for skin elasticity by Cutometer and skin hydration by Corneometer.

10. Dermatologists' evaluation and patients' assessments were done.

Three different dermatologists evaluated the results from photos taken with VISIA® and gave scoring as 4= excellent improvement, 3= good improvement, 2= moderate improvement, 1= fair improvement, 0= no changes, -1= worse.

Treatment satisfaction score was evaluated by subjects. (0= No satisfaction, 1= Little satisfaction, 2= Average satisfaction, 3= More satisfaction, 4= Most satisfaction).

The adverse reactions were recorded after each follow-up.

4. Results

A. General Characteristics

General demographic data of 17 volunteers were recorded with descriptive statistics as the following.

Table 4.1 Participants' demographic data

Demographic	n=17
Gender n (%)	
Male	3 (17.6)
Female	14 (82.4)
Age	
Mean ±SD	31.29±8.56
Min-Max (years)	26-59
Occupation, n (%)	
Student	7 (41.2)
Government officer	3 (17.6)
Employee	6 (35.3)
Business owner	1 (5.9)
Underlying disease, n (%)	
Yes	0 (0.0)
No	17 (100.0)
Treatment 4 weeks before, n (%)	
Yes	0 (0.0)
No	17 (100.0)
Exposure sunlight, n (%)	
Yes	15 (88.2)
No	2 (11.8)
Skin type, n (%)	
Combination skin	12 (70.6)
Dry skin	2 (11.8)
Oily skin	3 (17.6)

According to Table 4.1, most of subjects were female (82.4%) more than male (17.6%). The mean age of the subject was 31.29±8.56 years, and there were Student (41.2%), Employee (35.3%), Government officer (17.6%) and Business owner (5.9%), respectively. There was no subject with underlying disease and no subject with prior

aesthetic treatment 4 weeks before the study. There were fifteen subjects with routine exposure to sunlight. Majority of the subjects had combination skin (70.6%), oily skin (17.6%) and dry skin (11.8%), respectively.

B. Clinical Evaluation

a) Results of Cutometer for Skin Elasticity

Crow's Feet Area

Table 4.2 Statistical analysis of Cutometer Score compared between *Terminalia ferdinandiana* side and Placebo side at Crow's feet on baseline, follow-up 4th and 8th Week

	Terminalia ferdinandiana side	Placebo side	Paired Difference	p-value(a)
	mean±SD	mean±SD		
Baseline	0.5213±0.1259	0.5655±0.1743	-0.0442±0.1334	0.191
4th week	0.5493±0.1237	0.5728±0.1649	-0.0235±0.1275	0.459
8th week	0.6570±0.1259	0.5758±0.1584	0.0812±0.1563	0.048*
p-value(b)	<0.001*	0.922		

Data were analyzed between groups by Paired t-test (a) and within group by Repeated measure ANOVA (b). * means p-value <0.05 (Sig).

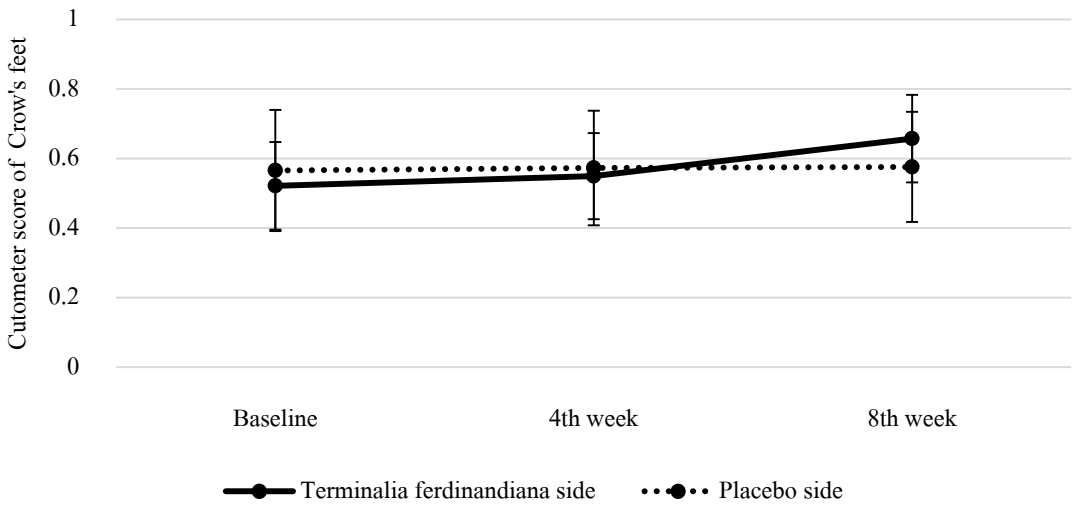

Figure 4.1 Linear graph showing comparison of Cutometer score at Crow's feet in each visit between *Terminalia ferdinandiana* side and Placebo side

According to the statistical analysis results from table 4.2 and figure 4.1, the mean of Cutometer at crow's feet of *Terminalia ferdinandiana* side was statistically significant at the level of 0.05 (p<0.001). For placebo side, the mean was changed, not statistically significant (p=0.922).

Comparing Cutometer score at Crow's feet between *Terminalia ferdinandiana* and placebo side; it is found that mean of Cutometer score at Crow's feet of *Terminalia ferdinandiana* was significantly higher than Cutometer score of placebo side at 8th week (p=0.048).

Table 4.3 Multiple comparison analysis (Post-hoc) of Cutometer Score at Crow's feet

	Terminalia ferdinandiana side	Placebo side
	p-value	p-value
Baseline vs 4th week	0.737	1.000
Baseline vs 8th week	<0.001*	1.000
4th week vs 8th week	<0.001*	1.000

Multiple comparisons were done by Bonferroni method. * means p value <0.05 (Sig).

According to the Multiple comparisons results from table 4.3, Cutometer Score at Crow's feet in *Terminalia ferdinandiana* side showed statistically significant difference between baseline vs. follow-up 8th week (p<0.001) and follow-up 4th week vs. 8th week (p<0.001). Placebo side was not significant at all.

Undereye Area

Table 4.4 Statistical analysis of Cutometer Score compared between *Terminalia ferdinandiana* side and Placebo side under the eye on baseline, follow-up 4th and 8th week

	Terminalia ferdinandiana side	Placebo side	Paired Difference	p-value (a)
	mean±SD	mean±SD		
Baseline	0.5160±0.1752	0.5472±0.1682	-0.0311±0.1491	0.402
4th week	0.5861±0.1346	0.5911±0.1222	-0.0050±0.1183	0.864
8th week	0.6093±0.1323	0.5653±0.1134	0.0440±0.1379	0.207
p-value (b)	0.029*	0.403		

Data were analyzed between groups by Paired t-test (a) and within group by Repeated measure ANOVA (b). * means p-value <0.05 (Sig).

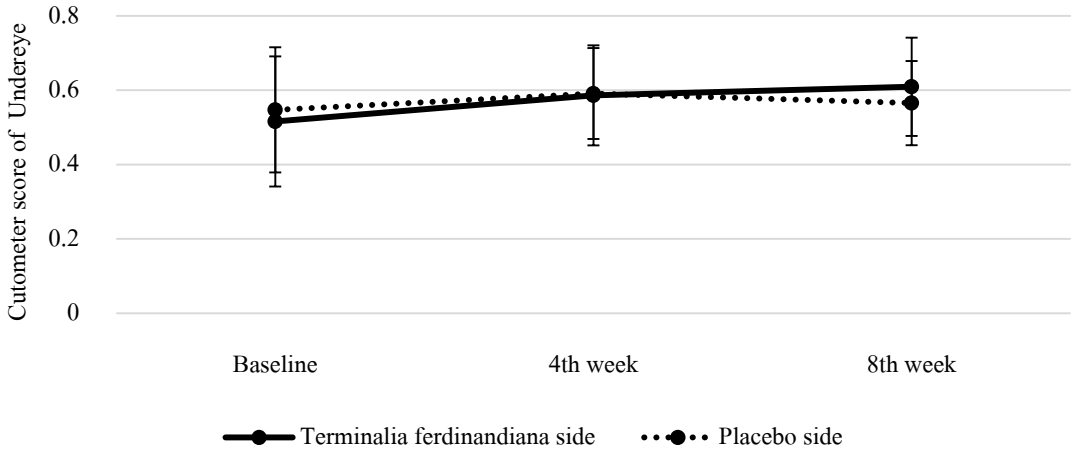

Figure 4.2 Linear graph showing comparison of Cutometer score under the eye in each visit between *Terminalia ferdinandiana* side and Placebo side

According to the statistical analysis results from table 4.4 and figure 4.2, the mean of Cutometer at undereye of *Terminalia ferdinandiana* side was statistically significant at the level of 0.05 (p=0.029). For placebo side, the mean of Cutometer was changed, not statistically significant (p=0.403).

The comparison of Cutometer score at undereye between *Terminalia ferdinandiana* and placebo side; mean of Cutometer score at undereye of *Terminalia ferdinandiana* was not significantly different with Cutometer score of placebo side at all visits.

Table 4.5 Multiple comparison analysis (Post-hoc) of Cutometer Score under the eye

	Terminalia ferdinandiana side	Placebo side
	p-value	p-value
Baseline vs 4th week	0.296	0.671
Baseline vs 8th week	0.017*	1.000
4th week vs 8th week	1.000	0.967

Multiple comparisons were done by Bonferroni method. * means p value <0.05 (Sig).

According to the Multiple comparisons results from table 4.5, Cutometer Score at undereye in *Terminalia ferdinandiana* side showed statistically significant difference between baseline vs. follow-up 8th week (p=0.017). Placebo side was not significant at all visits.

b) Results of Corneometer for Skin Hydration

Crow's Feet Area

Table 4.6 Statistical analysis of Corneometer score compared between *Terminalia ferdinandiana* side and Placebo side at Crow's feet on baseline, follow-up 4th and 8th week

	Terminalia ferdinandiana side	Placebo side	Paired Difference	p-value (a)
	mean±SD	mean±SD		
Baseline	55.78±12.10	62.00±12.61	-6.22±8.84	0.010
4th week	64.66±13.45	61.68±9.23	2.98±9.45	0.212
8th week	64.80±10.97	60.33±14.09	4.47±11.54	0.130
p-value (b)	0.001*	0.840		

Data were analyzed between groups by Paired t-test (a) and within group by Repeated measure ANOVA (b). * means p-value <0.05 (Sig).

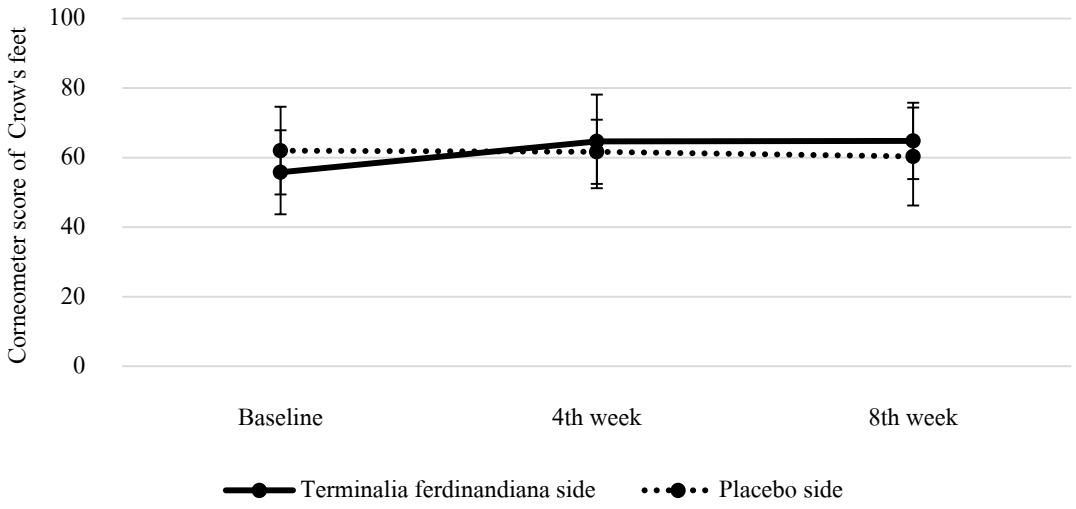

Figure 4.3 Linear graph showing comparison of Corneometer score at Crow's feet in each visit between *Terminalia ferdinandiana* side and Placebo side

According to the statistical analysis results from table 4.6 and figure 4.3, mean of Corneometer score at Crow's feet in *Terminalia ferdinandiana* side showed statistically significant difference at the level of 0.05 (p=0.001). For placebo side, the mean of Corneometer score was changed not statistically significant (p=0.840).

The comparison of Corneometer score at Crow's feet between *Terminalia ferdinandiana* and placebo side; it is found that mean of Corneometer score at Crow's feet of *Terminalia ferdinandiana* was not significantly different with Corneometer score of placebo side at all visits.

Table 4.7 Multiple comparison analysis (Post-hoc) of Corneometer score at Crow's feet

	Terminalia ferdinandiana side	Placebo side
	p-value	p-value
Baseline vs 4th week	0.018*	1.000
Baseline vs 8th week	0.005*	1.000
4th week vs 8th week	1.000	1.000

Multiple comparisons were done by Bonferroni method. * means p value <0.05 (Sig).

According to the Multiple comparisons results from table 4.7, Corneometer score at Crow's feet in *Terminalia ferdinandiana* side was statistically significant difference between baseline vs. follow-up 4th (p=0.018) and baseline vs. follow-up 8th (p=0.005). Placebo side was not statistically significant at all visits.

Undereye Area

Table 4.8 Statistical analysis of Corneometer score compared between *Terminalia ferdinandiana* side and Placebo side under the eye on baseline, follow-up 4th and 8th week

	Terminalia ferdinandiana side	Placebo side	Paired Difference	p-value (a)
	mean±SD	mean±SD		
Baseline	58.85±17.07	61.31±16.29	-2.46±6.18	0.120
4th week	62.19±14.52	63.28±13.62	-1.09±11.2	0.694
8th week	67.52±11.19	62.07±11.49	5.45±7.10	0.006*
p-value (b)	0.012*	0.695		

Data were analyzed between groups by Paired t-test (a) and within group by Repeated measure ANOVA (b). * means p-value <0.05 (Sig).

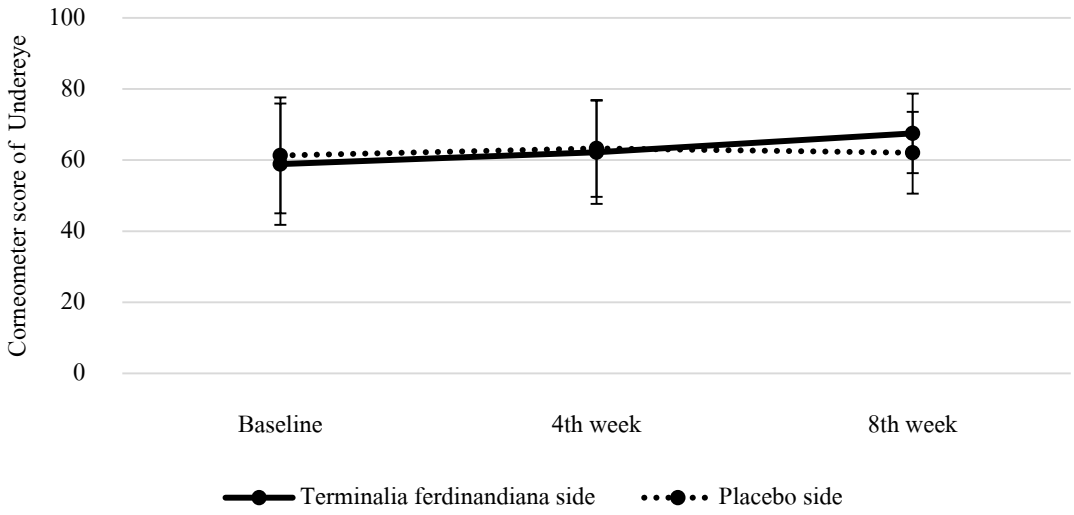

Figure 4.4 Linear graph showing comparison of Corneometer score under the eye in each visit between *Terminalia ferdinandiana* side and Placebo side

According to the statistical analysis results from table 4.8 and figure 4.4, mean of Corneometer score at undereye in *Terminalia ferdinandiana* side was statistically significant at the level of 0.05 (p=0.012). For placebo side, the results was not statistically significant (p=0.695).

The comparison of Corneometer score at undereye between *Terminalia ferdinandiana* and placebo side; it is found that mean of Corneometer score at undereye of *Terminalia ferdinandiana* was significantly higher than Corneometer score at undereye of placebo side at 8th week (p=0.006).

Table 4.9 Multiple comparison analysis (Post-hoc) of Corneometer score under the eye

	Terminalia ferdinandiana side	Placebo side
	p-value	p-value
Baseline vs 4th week	0.634	1.000
Baseline vs 8th week	0.033*	1.000
4th week vs 8th week	0.119	1.000

Multiple comparisons were done by Bonferroni method. * means p value <0.05 (Sig).

According to the Multiple comparisons results from table 4.9, Corneometer score at undereye in *Terminalia ferdinandiana* side showed statistically significant difference between baseline vs. follow-up 8th week (p=0.033). For placebo side was not statistically significant difference at all visits.

c) Results of dermatologists' evaluation score

Table 4.10 Dermatologists' evaluation score

Improvement	*Terminalia ferdinandiana* cream		Placebo cream	
	Week 4 (n)	Week 8 (n)	Week 4 (n)	Week 8 (n)
4= excellent improvement	-	-	-	-
3= good improvement	-	2	-	-
2= moderate improvement	2	9	-	2
1= fair improvement	9	6	5	11
0= no changes	6	-	12	4
-1= worse	-	-	-	-

Table 4.11 Statistical analysis of wrinkle score by dermatologists

	Terminalia ferdinandiana side	Placebo side	p-value
	median (IQR)	median (IQR)	
4th week	1 (0 - 1)	0 (0 - 1)	0.033*
8th week	2 (1 - 2)	1 (0.5 - 1)	0.002*
p-value	<0.001*	0.008*	

Data were analyzed by Wilcoxon Signed Ranks test. * means p-value <0.05.

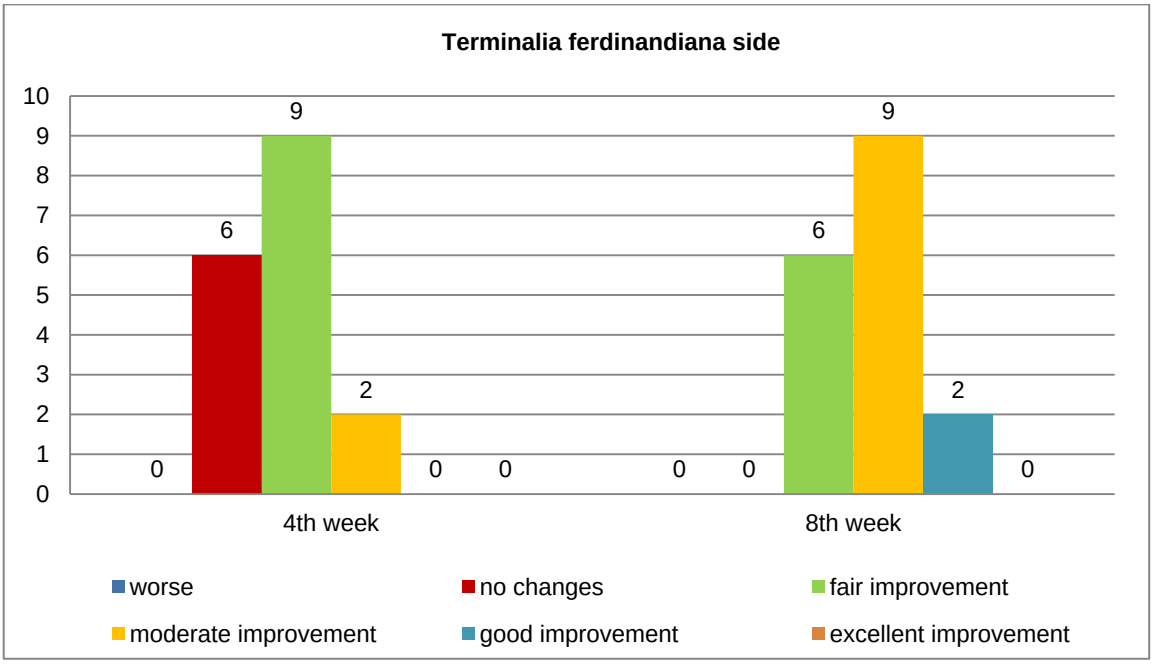

Figure 4.5 Column chart showing wrinkle score by 3 dermatologists on *Terminalia ferdinandiana* extract treated side

Figure 4.6 Column chart showing wrinkle score by 3 dermatologists on placebo treated side

Above table 4.10, 4.11 and Figure 4.5, 4.6 showed three Dermatologists' wrinkle evaluation score, the median of Dermatologists' evaluation score in *Terminalia ferdinandiana* side was statistically significant at the level of 0.05 ($p<0.001$). For placebo side, the median of Dermatologists' evaluation score was statistically significant at the level of 0.05 ($p=0.008$).

The comparison of Dermatologists' evaluation score between *Terminalia ferdinandiana* and placebo side; it is found that median of Dermatologists' evaluation score of *Terminalia ferdinandiana* was significantly higher than Dermatologists' evaluation score of placebo side at both 4th week (p=0.033) and 8th week (p=0.002) follow-up.

d) Patients' Satisfaction Score

Table 4.12 The frequencies of patient's satisfaction score

Satisfaction score n (%)	*Terminalia ferdinandiana* side	Placebo side
No satisfaction (0)	-	1 (5.9)
Little satisfaction (1)	-	9 (52.9)
Average satisfaction (2)	3 (17.6)	7 (41.2)
More satisfaction (3)	12 (70.6)	-
Most satisfaction (4)	2 (11.8)	-

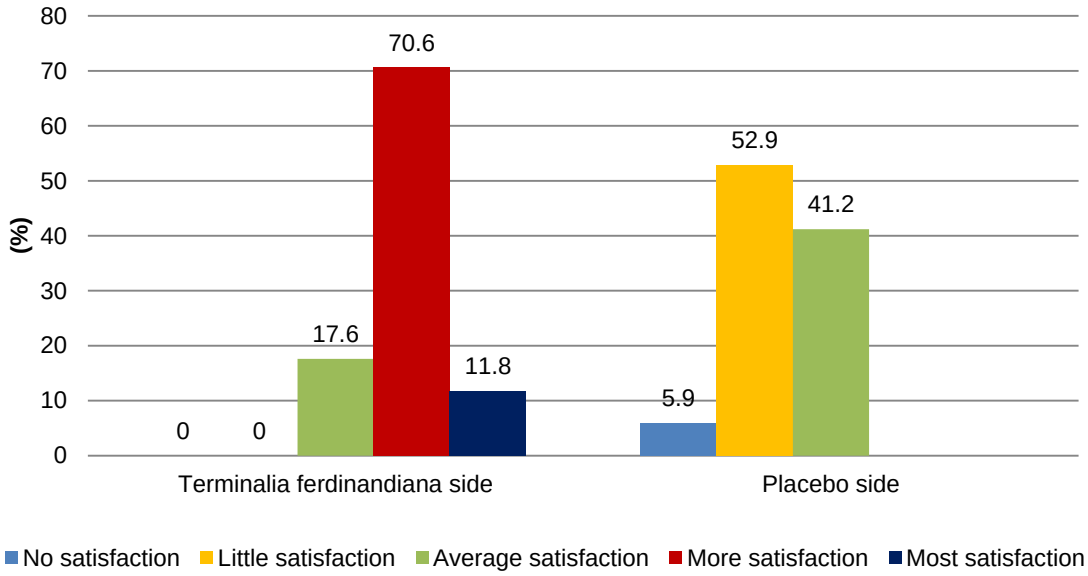

Figure 4.7 Bar chart reveals the frequencies of patient's satisfaction score at 8th week

Above table 4.12 and figure 4.7 showed patients' satisfaction score. At *Terminalia ferdinandiana* treated side, most of subjects rated more satisfaction (70.6%), average satisfaction (17.6%) and most satisfaction (11.8%).

For placebo treated side, most of the subject rated little satisfaction (52.9%), average satisfaction (41.2%). There was one subject with no satisfaction on placebo side.

e) Side effects

There were no side effects in any of the volunteers being treated with 10% *Terminalia ferdinandiana* extract cream.

5. Discussion

Signs of aging such as facial lines and wrinkles are likely to develop from a lot of factors like genetics, hormones, photo damage, smoking, diet, repeated facial expression etc. Underlying main mechanism is oxidative stress and damage to cells by reactive oxygen species and free radicals. (Zhang, 2018) Kakadu plum (*Terminalia ferdinandiana*) is from tropical tree of Combretaceae family in Australia. Its fruits are proved to have antioxidant properties. (Netzel, 2007) Vitamin C which is a potent antioxidant is the main ingredient of Kakadu plum. It is also composed of other antioxidants like Vitamin E and other phenolic compounds like gallic acid, ellagic acid. (Mohanty, 2012) It also helps other great antioxidants like vitamin E restored and keeps glutathione, L-cysteine and N-acetyl cysteine in its reduced from to get higher antioxidant effects and slow down sign of aging. (Mohanty, 2012)

This study is a double-blinded, randomized, placebo-controlled and split-face clinical trial. Seventeen volunteers (3 males and 14 females) with periorbital wrinkles were recruited. Elasticity and hydration of the skin were studied for 8 weeks with baseline, 4^{th}, 8^{th}week follow-up and dermatologists' wrinkle score and patients' satisfactory score were taken at 8^{th} week of study.

The cutometer which measures elasticity of the skin showed significantly better result in Kakadu plum treated side. When comparing cutometer result of two sides at crow's feet area, skin elasticity of Kakadu side statistically increased in 8^{th} week follow-up phase. Crow's feet area of extract cream side was significantly different between baseline and 8^{th} week, 4^{th} and 8^{th} week, respectively. Undereye area of extract side was statistically significant between baseline and 8^{th} week but placebo side did not show any significant change at all.

Another parameter is corneometer which is a measurement for hydration of stratum corneum. Skin hydration of periorbital area increased significantly in *Terminalia ferdinandiana* side but, placebo side stayed statistically insignificant. Skin hydration of crow's feet area was significantly different between baseline vs 4^{th} and baseline vs 8^{th} at extract cream side. Concerning undereye area, there was significant increase in hydration of Kakadu plum treated side at 8^{th} week compared to placebo side. Comparing follow up phases, significant different result was seen between baseline vs 8^{th} week visits at extract cream side. Hydration of placebo side did not show significant increase in hydration.

Facial wrinkle assessment was done by three physicians by using photos taken with VISIA® and wrinkle score for both sides of treatment was analyzed. Both extract cream side and placebo side showed significant result. However, *Terminalia ferdinandiana* side showed much improvement statistically and better wrinkle assessment score than placebo side with each follow-up visit.

Additionally, 12 out of 17 volunteers rated as more satisfied, 3 rated average satisfied and 2 gave very satisfied as satisfaction score assessment on extract cream side. None of them was unsatisfactory. All volunteers tolerated the treatment very well. There were no irritation and side effects such as allergy and hypersensitivity

reaction. It is found to be relevant with a prior study which concluded 10% *Terminalia ferdinandiana* extract as a safe topical treatment.

6. Conclusion

In this study, *Terminalia ferdinandiana* extract topical cream statistically reduced periorbital wrinkles at 8th week follow-up phase compared with placebo cream. In addition, it was also shown to have increased skin moisturization effect. Importantly, there were no harmful side effects during and after treatment. The main conclusion that can be drawn is that this research provides a safe and effective alternative treatment for wrinkles.

Limitation

This is a study of only 8 weeks duration based on small sample size (17 participants). Therefore, better results and effects will be more visible with longer duration of treatment time and larger sample size.

Although the author advised the volunteers to strict sun exposure time, avoid alcohol drinking and smoking, some factors that affect wrinkles such as eating and sleeping pattern couldn't be controlled.

Nevertheless, this study showed efficacy of 10% *Terminalia ferdinandiana* extract on improving wrinkles.

Recommendation

This study may be useful as a database for further research about skin rejuvenation, skin hydration, and skin smoothness.

This research may be used as an alternative of anti-wrinkle treatment to compare with other anti-oxidant products.

Effect of Kakadu plum extract should be tested compared with active vitamin C products.

Efficacy and safety of Kakadu plum extract cream for other conditions like eczema, psoriasis, post-inflammatory hyperpigmentation, melasma, acne should be done with 10% or higher concentration of *Terminalia ferdinandiana*.

Further study should be done to know whitening and cosmetic effects of Kakadu plum in cosmetic products like hand cream, body lotion, sunscreen, etc.

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