ISSN 0974-3618 (Print)
0974-360X (Online)
www.rjptonline.org

RESEARCH ARTICLE

Cream of *Lantana camara* Linn. Enriched with Ascorbic Acid does not Irritate the Skin

David Tjahyadi[1]*, Edy Parwanto [2], Hosea Jaya Edy[3], Joey Joshua Vidova Tjahyadi[4], Laurentia Gabrielle[4], Ashaolu Victoria Oladimeji[5], Seçil Karahüseyin[6]

[1]Department of Histology, Faculty of Medicine, Universitas Trisakti, Indonesia.
[2]Department of Biology, Faculty of Medicine, Universitas Trisakti, Indonesia.
[3]Study Program of Pharmacy, Faculty of Math and Natural Sciences, Universitas Sam Ratulangi, Indonesia.
[4]Medical Education Program, Faculty of Medicine, Universitas Trisakti, Indonesia.
[5]Department of Chemistry, Loyola Institute of Frontier Energy, Loyola College, Chennai, India.
[6]Department of Pharmacognosy, Faculty of Pharmacy, Çukurova Universitesi, Turkey.
*Corresponding Author E-mail: **davesaboch@trisakti.ac.id**

ABSTRACT:

Lantana camara Linn. has potential as an anti-bacterial. The results of our preliminary research show that changes in levels of quercetin, gallic acid, and tannin affect the antibacterial effectiveness of *E. coli, S. aureus*, and *P aeruginosa*. In addition, changes in the levels of Fe, Zn, quercetin, gallic acid and tannin in the preparations mentioned above are caused by free radicals. Free radicals can be counteracted with antioxidants, for example ascorbic acid. The aim of this research is to determine the effect of adding 10% or 15% ascorbic acid to the *L. camara* Linn. leaf extract cream preparation on antioxidant activity. Apart from that, we also carry out cream standardization and irritation tests. In this study there were 6 groups of cream, namely cream 1 to cream 6. Cream 1 is the cream containing 4% LELC. Cream 2 is the cream containing 4% LELC+10% ASC AC. Cream 3 is the cream containing 4% LELC+15% ASC AC. Cream 4 is the cream containing 5% LELC. Cream 5 is the cream containing 5% LELC +10% ASC AC. Cream 6 is the cream containing 5% LELC+15% ASC AC. Organoleptic test results of cream in these studies shows a semi-solid form, has a characteristic odor of LELC, blackish green color, pH around 5, homogeneous, spread ability ranges from 3.8 – 3.9cm. IC_{50} of cream containing 5% LELC+15% ASC AC=2.64ppm. Cream containing 5% LELC+15% ASC AC contains flavonoids, phenolics, and tannins of 0.637±0.018mg/g; 0.487±0.005mg/g; and 0.83±0.06 mg/g, respectively. During the observation, no erythema or edema was found due to exposure to cream containing 5% LELC+15% ASC AC. As a control, the cream base also did not irritate the skin. Thus, the cream containing 5% LELC was proven to be non-irritating to the skin.

KEYWORDS: *Lantana camara* Linn., Cream, Quercetin, Gallic acid, Tannin, Ascorbic acid, Skin irritation.

INTRODUCTION:

Lantana camara Linn. belongs to the Verbenaceae family. *L. camara* Linn., known locally as tembelekan, is a wild plant that grows without any special care.[1] *L. camara* Linn. has potential as an anti-bacterial.[2,3,4] Previous studies have shown that the phytochemical composition of *L. camara* Linn. includes phenols, essential oils, flavonoids, proteins, carbohydrates, and alkaloids. In addition, glycosides iridoid glycosides, phenyl ethanoids, glycosides, oligosaccharides, quinine, saponins, steroids, triterpenes, tannins, and sesquiterpenoids are phytochemical components of *L. camara* Linn.[5] The results of other research show that

Received on 09.11.2024 Revised on 02.05.2025
Accepted on 07.08.2025 Published on 08.11.2025
Available online from November 13, 2025
Research J. Pharmacy and Technology. 2025;18(11):5161-5169.
DOI: 10.52711/0974-360X.2025.00745

LELC contains alkaloids, flavonoids, tannins and triterpenoids.[6]

In addition, it is also shown that the extract of *L. camara* Linn. which is formulated in soap which has an anti-bacterial effect against *S. epidermidis*. Extract of *L. camara* Linn. 4, 6, 8 and 10% showed inhibition against *S. Epidermidis.*[7] Apart from that, tests have also been carried out on the ointment containing leaves extract of *L. camara* Linn. These tests include organoleptic tests, homogeneity tests, and pH tests.[8,9] There has been research showing that *L. camara* Linn. extract ointment 5% more effective than a 10% dose in healing mouse wounds infected with *S. Epidermidis.*[9] In addition to the above research, it is also known that creams containing 3, 4, and 5% LELC showed changes in Fe and Zn levels during storage for 6 months at 45 °C. Based on quercetin levels, cream containing 4% LELC was the most stable for a storage period of 6 months at 45°C, while cream containing 3% of *L. camara* Linn. leaf extract is less stable, and cream containing 5% LELC is unstable.[1]

Changes in the levels of Fe, Zn, quercetin, gallic acid and tannin in the preparations mentioned above are caused by free radicals. Previously, we have standardized cream containing of *L. camara* Linn. leaf extract enriched with ASC AC based on the content of quercetin, gallic acid and tannin. Free radicals are atoms or groups that have 1 or more unpaired electrons. Therefore, free radicals in cream containing LELC above needs to be suppressed so that the levels of the active antibacterial substance do not decrease. Free radicals can be counteracted with antioxidants.[10] ASC AC and vitamin E are examples of antioxidant substances that can be used for this purpose. These antioxidants can provide electrons to free radicals, and can break the chain reaction of free radicals. Antioxidants are important in cellular responses by suppressing oxidative stress.[11]

Antioxidant activity is expressed as a IC_{50} or half-maximal inhibitory concentration against free radicals. IC_{50} is the concentration of the sample that is able to reduce 50% of the free radical DPPH.[12] The antioxidant activity of the extract was tested by the DPPH free radical scavenging test. DPPH is a stable and purple free radical, which can be absorbed at a wavelength of 517 nm. The presence of anti-free radical compounds causes the DPPH free radical to be reduced so that it changes color to yellow. Since the IC_{50} is inversely proportional to the antioxidant potential of the extract, the lower of IC_{50} value, the better of antioxidant power of the extract.[13]

ASC AC has high antioxidant activity. It has been proven that the higher the concentration used, the higher the antioxidant activity. ASC AC is often used to compare how strong the antioxidant potential contained in fruit skin extracts.[14] It should be noted that the use of different solvents in the extraction process affects the results of the antioxidant activity test. Antioxidant activity testing uses the method of measuring free radical capture by DPPH. Measurements have been made on ASC AC which was used as a positive control with an IC_{50} value of 5.63ppm.[15] Based on IC_{50}, antioxidant activity is classified into very strong, strong, medium and weak. Samples have very strong, strong, medium and weak antioxidant activity if they have IC_{50} < 50ppm, 50-100ppm, 100-150 ppm, and 150-200ppm respectively.[16] If the sample has an IC_{50} > 200ppm, then the antioxidant activity is very weak.

Toxicological tests are generally carried out on animals, for example mice and Wistar rats.[17] Of course, the toxicological test data are extrapolated to humans.[18] Beside that, irritation properties testing refers to: Regulation of the Head of the Food and Drug Supervisory Agency regarding Guidelines for In Vivo Non-Clinical Toxicity Testing in 2014. Irritation activity testing is carried out on preparations with the optimum formula. The test uses a method of observing the appearance of edema and erythema on the skin of test animals.[19] If the results of these observations show that no mice died, it is concluded that the test material is safe for use on the skin, does not cause side effects or toxicity reactions.[20]

Based on the description above, we tested the effect of adding ASC AC to the cream containing of LELC on IC_{50} which can assess antioxidant activity. Apart from that, we also carried out skin irritation tests on rabbits to assess the safety aspects for the skin.

METHODS:

Time and Place of Research:

Preparation and manufacture of cream, and standardization of the LELC which includes measurements of pH, spread ability and organoleptics was carried out at the Biomedical Laboratory, Faculty of Medicine, Universitas Trisakti, Jakarta. Preparation, manufacture of cream, and standardization have been done in March 2024. Measurement of flavonoid, tannin, phenolic, and antioxidant activity test of cream containing of LELC or in combination with ASC AC were carried out at the Pharmacy Laboratory, Pharmacy Study Program, Faculty of Mathematics and Natural Sciences, Universitas Sam Ratulangi, Manado. These measurements were carried out in April - May 2024.

Cream manufacture:

The basic ingredients for the cream are prepared with the following ingredients: stearic acid 16 g, cetyl alcohol 2 g, liquid paraffin 10 mL, methyl paraben 0.2 grams, TEA 7 drops, glycerol 8.5 mL, aquadest Ad 100 g.

Making cream is done by mixing stearic acid, cetyl alcohol and liquid paraffin into porcelain cup 1, while the other substances are in porcelain cup 2. Both are heated at 70°C until completely melted without stirring. After melting, mix the two ingredients (cups 1 and 2) into the hot mortar, and stir quickly using a hot stamper. Slowly add the hot aquabidestilata at 70°C, stirring continuously until a creamy base is formed. After it is ready, wait until it cools, then add the LELC (4% and 5%), and ASC AC (10% and 15%) according to the formula. It should be noted that to obtain a homogeneous cream preparation, stirring must be carried out slowly and continuously. Cream formulations containing 4%, and 5% LELC were made by adding 4, and 5 grams LELC, respectively, to 100 grams of cream base.

In this study there were 6 groups of cream, namely cream 1 to cream 6. Cream 1 is the cream containing 4% LELC. Cream 2 is the cream containing 4% LELC + 10% ASC AC. Cream 3 is the cream containing 4% LELC + 15% ASC AC. Cream 4 is the cream containing 5% LELC. Cream 5 is the cream containing 5% LELC + 10% ASC AC. Cream 6 is the cream containing 5% LELC + 15% ASC AC.

Cream standardization:
Each time the study used leaf extract as an active ingredient in cream, it is necessary to standardize the preparations. Cream standardization is carried out by carrying out pH tests, organoleptic tests, homogeneity tests, and spreadability.[21]

Cream phytochemical levels:
Measurements of flavonoid, phenolic, and tannin levels were carried out on the 6 groups of creams mentioned above. The tool used to measure phytochemical levels is AAS.[21]

Antioxidant activity of cream:
The antioxidant activity of cream containing LELC or combined with ASC AC was conducted using the DPPH method.

Irritation test:
Irritation properties testing refers to: Regulation of the Head of the Food and Drug Supervisory Agency regarding Guidelines for In Vivo Non-Clinical Toxicity Testing in 2014 (Peraturan Kepala Badan Pengawas Obat dan Makanan tentang Pedoman Uji Toksisitas Nonklinik Secara In Vivo tahun 2014). Irritation activity testing is carried out on preparations with the optimum formula. The test uses a method of observing the appearance of edema and erythema on the skin of test animals. The test animals used were New Zealand male albino rabbits with healthy skin, weighing around 1.5 - 2 kg. The rabbits were placed in individual cages and acclimatized for five days while still being given enough food and drink.

The hair on the rabbit's back is shaved with scissors carefully to avoid wounds on the skin. Parts of the rabbit skin were cleaned, and left for 1 day, so as not to interfere with the testing process. Cream weighing 0.5 grams is smeared on the rabbit's shaved back. The next step is to cover it with a sterile bandage, and attach it with plaster. The exposure time for the cream is 4 hours. After the bandage is removed, the back skin is cleaned with water. Observation of the test response assessed whether there was erythema and edema on the skin of the rabbit's back exposed to the cream. After the covering bandage is removed, observations are made for the next 1 hour. Follow-up observations were carried out at 24, 48 and 72 hours after removing the bandage.

Observation and assessment of the emergence of erythema and edema is expressed with scores of 0, 1, 2, 3, and 4. A score of 0 means that no erythema or edema occurs. A score of 1 means there is very mild erythema or edema (almost invisible). A score of 2 means there is mild erythema (starting to become red), and there is mild edema (red spots starting to appear). A score of 3 means there is moderate erythema (red), and there is moderate edema (reddish spots measuring 1 mm). A score of 4 means there is strong erythema (very reddish = purplish red), and there is strong edema (red spots measuring > 1mm).

Erythema and edema guidelines use scores of 0, 1, 2, 3, and 4. A score of 0 means no erythema or edema occurs. A score of 1 means there is very mild (almost invisible) erythema or edema. A score of 2 means there is mild erythema (starting to turn red), and there is mild edema (red spots starting to appear). A score of 3 means there is moderate erythema (red), and there is moderate edema (reddish spots measuring 1 mm). A score of 4 means there is strong erythema (very reddish = purplish red), and there is strong edema (red spots measuring > 1 mm).

The assessment of the potential for irritation is calculated based on the average value of the emergence of erythema plus the average value of the emergence of edema and then divided by the time period required for the appearance of these two parameters (erythema and edema). A value of 0 - 0.4 means that the preparation has no potential for irritation. A value of 0.5 - 1.9 means that the preparation has little potential for irritation or has the potential for mild irritation. A value of 2 - 4.9 means the preparation has the potential for mild or moderate irritation. A value of 5 - 8 means the preparation has the potential for strong or severe irritation.[19]

Table 1. Cream composition

Cream Compound	Cream 1	Cream 2	Cream 3	Cream 4	Cream 5	Cream 6
Stearic acid	16g	16g	16g	16g	16g	16g
Cetyl alcohol	2g	2g	2g	2g	2g	2g
Liquid paraffin	10mL	10mL	10mL	10mL	10mL	10mL
Methyl paraben	0.2g	0.2g	0.2g	0.2g	0.2g	0.2g
TEA	7 drops	7 drops	7 drops	7 drops	7 drops	7 drops
Glycerol	8.5mL	8.5mL	8.5mL	8.5mL	8.5mL	8.5mL
Thick extract	4g	4g	4g	5g	5g	5g
Ascorbic acid	0g	10g	15g	0g	10 g	15g
Water	add 100 g	add 100g	add 100g	add 100 g	add 100g	add 100 g

Abbreviations: Cream 1 = the cream containing 4% LELC; Cream 2 = the cream containing 4% LELC + 10% ASC AC; Cream 3 = the cream containing 4% LELC + 15% ASC AC; Cream 4 = the cream containing 5% LELC; Cream 5 = the cream containing 5% LELC + 10% ASC AC; Cream 6 = the cream containing 5% LELC + 15% ASC AC.

Research Ethics:

This research has passed the ethical review number 065/KER/FK/V/2023 from the Research Ethics Commission of the Faculty of Medicine, Trisakti University.

Analysis Method:

The data obtained included pH, spread ability, organoleptics, levels of flavonoid, phenolic, tannin, antioxidant activity, and irritations parameters. To determine changes in levels of flavonoid, phenolic, and tannin due to the addition of ASC AC to cream containing LELC, a linear regression was used. To determine changes in antioxidant activity in cream containing LELC or in combined with ASC AC, a linear regression test was used. The irritation criteria for cream preparations in this study are based on BPOM RI 2014.[19]

RESULTS:

Cream composition:

The composition of the cream contains LELC in combination with ASC AC is presented in Table 1.

Standardization of cream containing LELC:

Each time the study used cream containing LELC it is necessary to standardize the preparation. Cream standardization is carried out by conducting organoleptic tests, pH tests, homogeneity tests, spread ability tests and phytochemical content.

Organoleptic test (shape, smell, and color):

The results of organoleptic tests of cream containing of LELC is presented in Table 2.

Based on the Table 2, the organoleptic test of the cream shows that it is semi-solid, the cream has the characteristic odor of LELC, and is colored like the extract. The cream base has an average pH of 6, while the cream containing LELC or combined with ASC AC has an average pH of 5. Cream base preparations, cream containing LELC or those combined with ASC AC are homogeneous. The cream base preparation has a higher spread ability compared to cream containing of LELC, or those combined with ASC AC.

Flavonoid, phenolic and tannin levels of cream:

Measurement results of flavonoid, phenolic, and tannin levels of cream containing LELC or in combination with ASC AC is presented in Table 3.

Table 2. Organoleptic test results of cream containing of LELC or in combination with ASC AC

Test	Cream base	Cream 1	Cream 2	Cream 3	Cream 4	Cream 5	Cream 6
Oeganoleptic test (shape)	ss	ss	ss	ss	ss	ss	ss
Oeganoleptic test (smell)	-	Ldo	Ldo	Ldo	Ldo	Ldo	Ldo
Oeganoleptic test (colour)	yw	sgb	sgb	sgb	sgb	sgb	sgb
pH	6 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0
Homogeneity	hmg	hmg	hmg	hmg	hmg	hmg	hmg
Spreadability (100 gr)	5.21 ± 0.41	3.8 ± 0.28	3.9 ± 0.31	3.9 ± 0.32	3.8 ± 0.29	3.9 ± 0.29	3.9 ± 0.28

Abbreviations: Cream 1 = the cream containing 4% LELC; Cream 2 = the cream containing 4% LELC + 10% ASC AC; Cream 3 = the cream containing 4% LELC + 15% ASC AC; Cream 4 = the cream containing 5% LELC; Cream 5 = the cream containing 5% LELC + 10% ASC AC; Cream 6 = the cream containing 5% LELC + 15% ASC AC; ss=semi-solid; Ldo = LELC's distinctive odor; yw = yellowish white; sgb=slightly greenish black; hmg = homogenous.

Tabel 3. Measurement results of flavonoid, phenolic, and tannin levels of cream

Measurement results	Cream 1 (mean ± SD)	Cream 2 (mean ± SD)	Cream 3 (mean ± SD)	Cream 4 (mean ± SD)	Cream 5 (mean ± SD)	Cream 6 (mean ± SD)
Flavonoid (mg/g)	0.627 ± 0.031	0.615 ± 0.013	0.637 ± 0.018	0.615 ± 0.026	0.601 ± 0.01	0.637 ± 0.018
Phenolic (mg/g)	0.454 ± 0.040	0.481 ± 0.010	0.478 ± 0.010	0.487 ± 0.006	0.485 ± 0.013	0.487 ± 0.006
Tannin (mg/g)	0.870 ± 0.080	0.820 ± 0.070	0.800 ± 0.060	0.860 ± 0.050	0.830 ± 0.050	0.830 ± 0.006

Abbreviation: Cream 1 = the cream containing 4% LELC. Cream 2 = the cream containing 4% LELC + 10% ASC AC. Cream 3 = the cream containing 4% LELC + 15% ASC AC. Cream 4 = the cream containing 5% LELC. Cream 5 = the cream containing 5% LELC + 10% ASC AC. Cream 6 = the cream containing 5% LELC + 15% ASC AC.

Cream containing 4% LELC or combined with ASC AC reduced tannic acid levels (R^2= 0.9423). Regarding the treatment of adding 10% or 15% ASC AC to cream containing 4% LELC did not change the quercetin levels (R^2 = 0.206), but was unable to change the gallic acid levels (R^2 = 0.6575). Cream containing 5% LELC combined with ASC AC is not too strong in reducing tannins (R^2 = 0.75). Regarding the treatment of adding 10% or 15% ASC AC to cream containing 5% LELC did not change the flavonoids levels (R^2 = 0.3674), but was unable to change the phenolic levels (R^2 = 0).

Antioxidant activity of cream containing 4% LELC:
Determination of IC_{50} in the cream containing 4% LELC as follows: % DPPH radical inhibition = [(Absorbance control - Absorbance of test material): Absorbance control] x 100 %. Absorbance control (absorbance of test material) = 0.7757; absorbance of test material for 10 , 20, 40, 60, and 80 ppm are 0.4184, 0.3693, 0.3391, 0.3318, and 0,2568 respectively. Result of % inhibition of DPPH radical 10, 20, 40, 60, and 80 ppm are 46.06%, 52.39%, 56.28%, 57.22%, and 66.89% respectively.

The sample concentration and percentage inhibition are plotted on the x and y axes respectively in the linear regression equation. This equation is used to determine the IC_{50} value of each sample expressed by a y value of 50 and the x value that will be obtained as IC_{50}. Regression equation to calculate IC_{50} in cream containing 4% LELC is y = 4.649x + 41.821. The y value is replaced with 50 (determination from IC_{50}) → 50 = 4.649x + 41.821 → x = 1.759303ppm. The x value = is the IC_{50} value = 1.759303 ppm. IC_{50} of cream containing 4 % LELC = 1.759303 ppm.

Antioxidant activity of cream containing 4% LELC + 10% ASC AC;
Determination of IC_{50} in the cream containing 4% LELC + 10% ASC AC as follows: % DPPH radical inhibition = [(Absorbance control - Absorbance of test material) : Absorbance control] x 100 %. Absorbance control (absorbance of test material) = 0.7757; absorbance of test material for 2, 4, 6, 8, and 10 ppm are 0.4131, 0.3640, 0.3179, 0.3076, and 0,2488 respectively. Result of % inhibition of DPPH radical 2, 4, 6, 8, and 10 ppm are 46.75%, 53.07%, 59.02%, 60.34%, and 67.97% respectively. Linear regression equation → y = 4.971x + 42.517. The y value is replaced with 50 (determination from IC_{50}) → 50 = 4.971x + 42,517 → x= 1.505330 ppm. The x value = the IC_{50} value = 1.505330ppm. IC_{50} of cream containing 4% LELC + 10% ASC AC = 1.505330ppm.

Antioxidant activity of cream containing 4% LELC+ 15% ASC AC:
Determination of IC_{50} in the cream containing 4% LELC+ 15% ASC AC as follows: %DPPH radical inhibition = [(Absorbance control - Absorbance of test material): Absorbance control] x 100%. Absorbance control (absorbance of test material) = 0.7757; absorbance of test material for 2, 4, 6, 8, and 10 ppm are 0.3949, 0.3518, 0.3459, 0.3019, and 0,2468 respectively. Result of % inhibition of DPPH radical 2, 4, 6, 8, and 10 ppm are 49.09%, 54.65%, 55.41%, 61.18%, and 68.18% respectively. Linear regression equation → y = 4.461x + 44.299. The y value is replaced with 50 (determination from IC_{50}) → 50 = 4.461x + 44,299 → x = 1.277964 ppm. The x value = is the IC_{50} value: = 1.277964 ppm. IC_{50} of cream containing 4% LELC + 15% ASC AC = 1.277964 ppm.

Antioxidant activity of cream containing 5% LELC:
Determination of IC_{50} in the cream containing 5% LELC as follows: % DPPH radical inhibition = [(Absorbance control - Absorbance of test material): Absorbance control] x 100 %. Absorbance control (absorbance of test material) = 0.7757; absorbance of test material for 2, 4, 6, 8, and 10 ppm are 0.4315, 0.3556, 0.3279, 0.3070, and 0,2575 respectively. Result of % inhibition of DPPH radical 2, 4, 6, 8, and 10 ppm are 44.37%, 54.16%, 57.73%, 60.43%, and 66.81% respectively. Linear regression equation → y = 5.115 x + 41.355. The y value is replaced with 50 (determination from IC_{50}) → 50 = 5.115 x + 41.355 → x = 1.690127 ppm. The x value = the IC_{50} value = 3.365619 ppm. IC_{50} of cream containing 5% LELC= 1.690127 ppm.

Antioxidant activity of cream containing 5% LELC + 10% ASC AC:
Determination of IC_{50} in the cream containing 5% LELC + 10% ASC AC as follows: % DPPH radical inhibition = [(Absorbance control - Absorbance of test material) : Absorbance control] x 100 %. Absorbance control (absorbance of test material) = 0.7757; absorbance of test material for 2, 4, 6, 8, and 10 ppm are 0.4021, 0.3782, 0.3197, 0.3078, and 0,2628 respectively. Result of % inhibition of DPPH radical 2, 4, 6, 8, and 10 ppm are 48.17%, 51.24%, 58.78%, 60.31%, and 66.12% respectively. Linear regression equation → y = 4.497 x + 43.433. The y value is replaced with 50 (determination from IC_{50}) → 50 = 4.497 x + 43,433 → x = 1.460306ppm. The x value = the IC_{50} value = 1.460306 ppm. IC_{50} of cream containing 5% LELC + 10% ASC AC = 1.460306 ppm.

Antioxidant activity of cream containing 5% LELC + 15% ASC AC:
Determination of IC_{50} in the cream containing 5% LELC + 15% ASC AC as follows: % DPPH radical inhibition = [(Absorbance control - Absorbance of test material) : Absorbance control] x 100 %. Absorbance control (absorbance of test material) = 0.7757; absorbance of test material for 2, 4, 6, 8, and 10 ppm are 0.4148,

0.3447, 0.3052, 0.2805, and 0,2310 respectively. Result of % inhibition of DPPH radical 2, 4, 6, 8, and 10 ppm are 46.52%, 55.56%, 60.65%, 63.83%, and 70.22% respectively. Linear regression equation → y = 5.567 x + 42.655. The y value is replaced with 50 (determination from IC_{50}) → 50 = 5.567 x + 42,655 → x = 1.319382 ppm. The x value = the IC_{50} value = 1.319382 ppm. IC_{50} of cream containing 5% LELC + 15% ASC AC = 1.319382 ppm.

Based on the phytochemical profile data of cream containing LELC or a combination with ASC AC, it shows that the addition of ASC AC to cream containing 4% or 5% LELC decreases IC_{50}, meaning it increases antioxidant activity. Because cream containing 4% or 5% LELC combined with ASC AC has an IC_{50} value <50 ppm, its antioxidant activity is strong. The above data show that the addition of ASC AC to the cream containing 4% or 5% LELC decreases IC50, meaning it increases antioxidant activity. Since the cream has an IC_{50} value < 50 ppm, it can be stated that its antioxidant activity is strong.

Iritation test:

The results of shaving, and testing preparation for cream containing 5% LELC + 15% ASC AC which was exposed for 4 hours, and covered with a bandage is presented in Figure 1.

The results of the irritation test of cream containing 5% LELC + 15% ASC AC are presented in Figure 2.

The results of this study show that no rabbits died due to skin irritation tests.

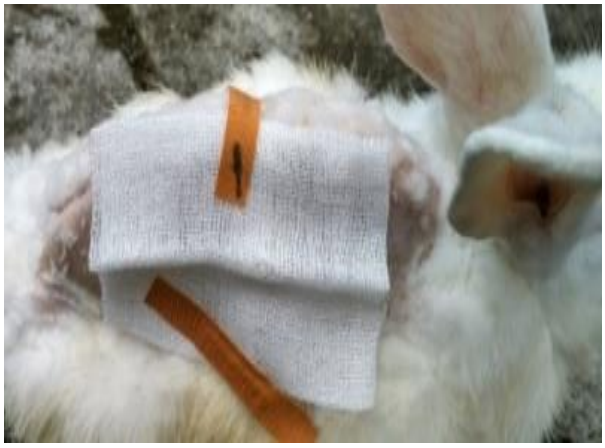

Figure 1. The results of shaving, and testing preparation for cream containing 5% LELC + 15% ASC AC which was exposed for 4 hours, and covered with a bandage.

DISCUSSION:

Organoleptic test results of cream containing 4% and 5% LELC and the combination with 10% or 15% ASC AC shows a semi-solid form, has a characteristic odor of LELC, blackish green color, pH is around 5, homogeneous, ability to spread ranges from 3.8 – 3.9 cm. The organoleptic test results in this study demonstrated that the results were the same as the results of our previous study.[1,21] This is due to the cream component of LELC similar or alike. This is because the components of cream containing LELC are similar or identical.

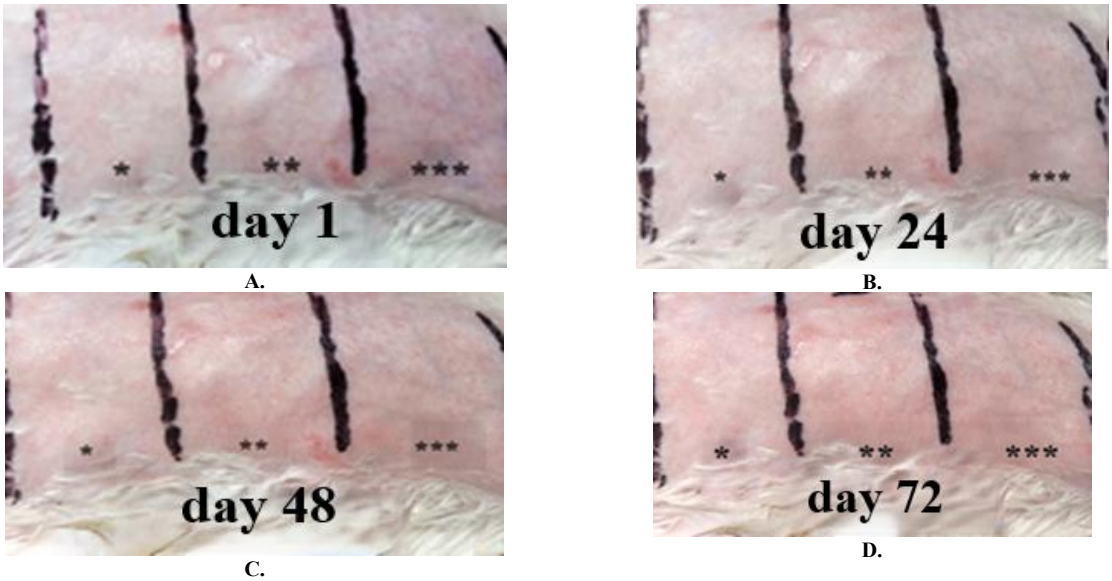

Figure 2. Irritation test results. A=After 4 hours of exposure to the tested cream, it was then washed, then observed 1 hour after washing. B=After 4 hours of exposure to the tested cream, it was then washed, then observed 24 hours after washing. C=After 4 hours of exposure to the tested cream, it was then washed, then observed 48 hours after washing. D= After 4 hours of exposure to the tested cream, it was then washed, then observed 72 hours after washing. *=Application site of cream base. **=Negative control without smearing. ***=Application site of cream (5% LELC + 15% ASC AC).

Previous research results have demonstrated that *L. camara* Linn. is a wild plant that can be used as a source of bioactive substance,[22,23,24] such as quercetin, gallic acid and tanic acid.[25] A previous study showed that levels of flavonoids in the LELC was 243.89±1.30mg Quercetin Equivalent/gram.[26] In addition to bioactive substances, it should be noted that *L. camara* Linn. contains heavy metals resulting from the accumulation of absorbed nutrients.[27] There are several factors that influence flavonoid levels, including temperature. Cream storage temperature has been demonstrated to change quercetin levels, thereby affecting antibacterial activity.[21] Based on the facts in this study that ASC AC combined with 4% LELC in cream reduced tannin levels (R^2= 0.9423). The addition of ASC AC did not change flavonoid levels (R^2 = 0.206), but was not able to change phenolic levels (R^2 = 0.6575). The research results can be applied to maintain levels of quercetin, gallic acid and tannic acid in preparations, especially cream preparations. The results of this study are important and in accordance with previous studies that demonstrated the importance of measuring ascorbic acid levels in herbal preparations.[28,29,30]

Our suggestion above is based on the fact that cream preparations that are stored for a certain period of time can experience changes in flavonoid (quercetin as a flavonoids equivalent) levels or in other words they are unstable.[1] Significant changes in flavonoid levels in preparations due to the influence of storage temperature have also been demonstrated by previous studies.[21] The stability of preparations containing LELC needs to be maintained so that the flavonoid, phenolic and tannin contents remain stable. It is hoped that preparations containing flavonoids, phenolics, and tannins will be stable, so that they function optimally as antibacterials and skin wound healers. This is in accordance with the results of previous research on the importance of extract optimization.[31]

We demonstrated that ASC AC added to cream containing 4% LELC had the effect of changing quercetin levels, but had less effect on changing levels of gallic acid, and tannic acid. Therefore, we recommend that to maintain the levels of quercetin, gallic acid, and tannic acid in the preparation it is necessary to combine it with other compounds besides ASC AC. ASC AC combined with 5% LELC in the cream is not too strong in reducing tannic acid levels (R^2= 0.75). Beside that, the addition of ASC AC did not change quercetin levels (R^2 = 0.3674), but was not able to change gallic acid levels (R^2 = 0). Based on this data, we decided to choose cream containing 5% LELC combined with ASC AC compared to cream containing 4% LELC which is being developed as an antibacterial and skin wound healer. Our choice of cream containing leaf extract of *L. camara* Linn. in combination with ASC AC is in line with the statement that ASC AC has the potential to fight various types of diseases in humans. It was further stated that ASC AC acts as an enzyme cofactor that is needed in various physiological functions.[32,33]

The data above shows that the addition of ASC AC to cream containing 4% and 5% of LELC reduces IC_{50}, meaning it increases antioxidant activity. Because 4% and 5% of LELC or combined with ASC AC, has an IC_{50} value of < 50ppm, its antioxidant activity is strong. The results of this study are in line with the concept that IC_{50} is related to antioxidant activity.[34] The results of other studies demonstrated that the IC_{50} of the methanol extract of *L. camara* Linn. 204.3µg/mL,[35] while *C. racemosa* extract has an IC_{50} value of 159.8 ppm.[36] As a comparison, the IC_{50} for *D. longan* seeds extract is 32.13 ug/mL, while *D. longan* peels extract is 23.50ug/mL.[37] These results are certainly different from the results of our research, because the samples measured by IC_{50} were different. Different results regarding antioxidant activity have been demonstrated in previous studies.[38,39, 40]

Skin irritation test in the development of cream containing LELCcombinet with ASC AC is still an area of intensive research. The irritating effect of the preparation can be evaluated not only on the skin of experimental animals, but can also be carried out on reconstructions of human epidermis and corneal epithelium.[41] Applying the cream to the mice's backs was carried out in a closed manner using sterile gauze, bandages and non-irritating plaster, thus ensuring and helping absorption of the test material, as well as avoiding environmental influences.[42] The bandage we used in this study is easy to buy on the market, but can be used well. The choice of bandage is in accordance with the conclusions of previous research.[43] During the observation in this research, no erythema or edema was found due to exposure of cream containing 5% LELC+ 15% ASC AC, and it was also shown that the cream base was not irritating to the skin.

In this study it was demonstrated that cream containing 5% leaf extract of *L. camara* Linn. combined with 15% ASC AC did not irritate the skin. These data make it clear that the components in the cream are not irritating to the skin. Based on the results of the irritation test, it seems that cream containing LELC+ 15% ASC AC could be an alternative to be developed as a topical preparation for skin wounds. We hope that other research will be produced so that it can be used as a basis for future research regarding the development of cream containing LELC.

LIMITATIONS:

Although we have demonstrated standardization of cream containing LELCcombined with ASC AC, assessed IC_{50}, and skin irritation tests in rabbits, we have not been able to test its antibacterial activity. Testing the antibacterial activity of cream containing LELCcombined with ASC AC still needs to be carried out. The basis for developing cream containing LELCrequires data on levels of quercetin, gallic acid, tanic acid, IC_{50}, and antibacterial activity.

CONCLUSION:

The results of organoleptic tests on creams containing LELCor combined with 10% or 15% ASC AC showed a semi-solid form, a distinctive odor leaf extract of *L. camara* Linn., blackish green color, pH around 5, homogeneous, spreadability ranging from 3.8 - 3.9cm. IC_{50} of cream 1 = 1.759ppm, cream 2 = 1.5053ppm, cream 3 = 1.278ppm, cream 4 = 1.690ppm, and cream 5 = 1.460ppm, and cream 6 = 1.319ppm.

During the observation, no erythema or edema to the skin was found due to exposure of cream containing 5% LELC+ 15% ASC AC. IC_{50} of cream containing 5% LELC+ ASC AC 15% = 1.319 ppm, does not irritate the skin. Based on the results of the irritation test, it seems that cream containing 5% LELC+ 15% ASC AC could be an alternative to be developed as a topical preparation for skin wounds. We hope that other research will be produced so that it can be used as a basis for future research regarding the development of cream containing LELC.

ORCID ID:

David Tjahyadi 0000-0003-1154-193x; Edy Parwanto=0000-0002-0797-6925; Hosea Jaya Edy=0000-0003-1522-3437; Joey Joshua Vidova Tjahyadi=0000-0002-6764-1123; Laurentia Gabrielle=0000-0002-1310-6395; Ashaolu Victoria Oladimeji=0000-0001-8022-6533; Seçil Karahüseyin=0000-0002-3515-2974

CONFLICT OF INTERESTS:

All authors declare that no conflict of interest.

FUNDING SOURCE:

Publication of this manuscript is supported by funding number 0802/PUF/FK/2023-2024 Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia.

ETHICAL STATEMENT:

The ethical clearance number 058/KER/FK/IU2024 for this research was obatained from the Research Ethics Commission, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia.

ACKNOWLEDGMENTS:

We would like to thank the Leaders and Staff of the Pharmacy Laboratory, Pharmacy study program, Faculty of Mathematics and Natural Sciences, Universitas Sam Ratulangi, Manado for the opportunity given to us so that this research could be carried out.

AUTHORS CONTRIBUTIONS:

EP and DT=Schemed and designed experiment. EP, HJE, DV, SK, AVO=data collecting, analysis, and interpretation of the results. EP, HJE, JJNT, LG=images review and processing. EP, DV, SK, HJE=writing of the manuscript. All author's=reviewing and approved the final manuscript.

ABBREVIATION:

LELC = leaf extract of *Lantana camara* Linn.; ASC AC = ascorbic acid; IC_{50} = half-maximal inhibitory concentration; DPPH = 2,2-Diphenyl-1-picrylhydrazyl; TEA = triethanolamine; pH = potential of hydrogen; AAS = atomic absorbance spectrophometric; ppm = parts per million; λ = wave length; nm = nano meter; R = replican; ss = semi -solid; Ldo = LELC's distinctive odor; yw = yellowish white; sgb = slightly greenish black; hmg = homogeneous; SD = standard deviation; g = gram; mg/g = milligram per gram; µg/mL = microgram per milli liter.

REFERENCES:

1. Parwanto MLE, Tjahyadi D, Edy HJ, Wratsangka R, Guyansyah A. Stability of Lantana camara Linn. leaf extract cream base on the level of Fe, Mg, Zn and quercetin equivalent of flavonoid. IJPR. 2021; 13(1): 3069-3086. https://doi.org/10.31838/ijpr/2021.13.01.441
2. Ganjewala D, Sam S, Khan KH. Biochemical compositions and antibacterial activities of Lantana camara plants with yellow, lavender, red and white flowers. Eur Asian J BioSci. 2009; 3: 69-77.
3. Barreto FS, Sousa EO, Campos AR, Costa JGM, Rodrigues FFG. Antibacterial activity of Lantana camara Linn and Lantana montevidensis brig extracts from Cariri-Ceará, Brazil. J Young Pharm. 2010; 2 (1): 42- 44.
4. Badakhshan MP, Sasidharan S, Rameshwar NJ, Ramanathan S. A comparative study: antimicrobial activity of methanol extracts of Lantana camara various parts. Pharmacog Resch. 2009; 1 (6): 348-351.
5. Suryati, Santoni A, Arifin B, Ferdinal N, Salim E, Amelia A, et al., Analysis of chemical content, cytotoxic and anti-bacterial activity essential oil of Lantana Camara Linn leaves from various regions. Molekul. 2022; 17(2): 156-164. https://doi.org/10.20884/1.jm.2022.17.2.5143. Available from: http://jos.unsoed.ac.id/index.php/jm/article/view/5143
6. Murugesan S, Senthilkumar N, Suresh Babu D, Rajasugunasekar D. Chemical constituents andtoxicity assessment of the leaf oil of Lantana camara Linn from Tamilnadu regions. Asian J Plant Sci Resch. 2016; 6(3): 32-42.
7. Edy HJ, 2012. Formula sabun opaque anti–bakteri ekstrak daun tembelekan (Lantana camara L.) terhadap Staphylococcus epidermidis. FMIPA, Progdi Farmasi UNSRAT, Manado, Sulawesi Utara, Indonesia.
8. Parwanto EML, Senjaya H, Edy HJ. Formulasi salep antibakteri ekstrak etanol daun L camara (Lantana camara L). Pharmacon. 2013; 2 (3): 104 – 108.
9. Parwanto MLE. Efficcacy of Lantana camara Linn. leaf extracts ointment on dermal wound healing were infected with Staphylococcus epidermidis. Int J Basic Clin Pharmacol. 2017; 6: 503- 510. DOI: http://dx.doi.org/10.18203/2319-2003.ijbcp20170457
10. Martemucci G, Costagliola C, Mariano M, D'andrea L, Napolitano P,

D'Alessandro AG. Free radical properties, source and targets, antioxidant consumption and health. Oxygen 2022; 2: 48-78. https://doi.org/10.3390/ oxygen2020006. Available from: https://www.mdpi.com/2673- 9801/2/2/6
11. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr J 2015, 15, 71. https://doi.org/10.1186/s12937- 016-0186-5
12. Ghozaly MR, Safitri EB. Uji aktivitas antioksidan ekstrak N-heksan, etil asetat dan metanol dari varietas umbi wortel (Daucus Carota L.) Dengan Metode DPPH (1,1-Difenil-2-Pikrilhidrazil). Sainstech Farma 2016; 9 (2): 13-18.
13. Klotoe JR, Fanou BA, Agbodjento E, Houehou A, Fah L, Dougnon V, et al. Antifungal activity of Ocimum gratissimum L., Lantana camara L. & Pteleopsis suberosa Engl. & Dies used in the treatment of vulvovaginal candidiasis in Benin. Future J Pharm Sci 2021; 7: 237: 1-11. https://doi.org/10.1186/s43094-021-00383-4
14. Sari FN, Sari Y. Antioxidant activity test on Indonesian typical fruit peel waste uji aktivitas antioksidan pada limbah kulit buah-buahan khas indonesia. JAF 2023; 8(1): 123 – 131
15. Prasetyo E, Kharomah NZW, Rahayu TP. Uji aktivitas antioksidan menggunakan metode DPPH (2,2-difenil-1-pikrilhidrazil) terhadap ekstrak etanol kulit buah durian (Durio zibethinnus L.) dari Desa Alasmalang Kabupaten Banyumas. Jurnal Pharmascience. 2021; 08 (01): 75-82
16. Li Z, Teng J, Lyu Y, Hu X, Zhao Y, Wang M. Enhanced antioxidant activity for apple juice fermented with Lactobacillus plantarum Atcc14917. Molecules 2019; 24(1): 1–12. doi: 10.3390/molecules24010051.
17. Makiyah A, Tresnayanti S. Uji toksisitas akut yang diukur dengan penentuan ld50 ekstrak etanol umbi iles-iles (amorphophallus variabilis bl.) pada tikus putih strain wistar. Majalah Kedokteran Bandung 2017; 49 (3): 145–155. DOI: https://doi.org/10.15395/mkb.v49n3.1130. http://journal.fk.unpad.ac.id/index.php/mkb/article/view/1130
18. Reddy N, Lynch B, Gujral J, Karnik K. Alternatives to animal testing in toxicity testing: Current status and future perspectives in food safety assessments. Food Chem Toxicol. 2023; 179: 113944. doi: 10.1016/j.fct.2023.113944. Epub 2023 Jul 14. PMID: 37453475].
19. BPOM RI (Badan Pengawasan Obat dan Makanan, Republik Indonesia), 2014. Pedoman uji toksisitas nonklinik secara in vivo, Peraturan Kepala Badan Pengawas Obat dan Makanan Republik Indonesia No 7 Tahun 2014.
20. Samirana PO, Pratiwi DMN, Musdwiyuni NW, Andhini DAA, Mahendra AN, Yadnya-Putra AAGR. Uji pendahuluan toksisitas akut dermal sediaan salep ekstrak etanol 70% daun binahong (Anredera scandens (l.) moq.) terstandar. Jurnal Kimia. 2018; 12 (2): 180-186. DOI: 10.24843/JCHEM.2018.v12.i02.p14
21. Parwanto E, Amalia H, Tjahyadi D, Edy HJ, Oladimeji AV, Tjahyadi JJV, et al. Effect of extreme temperature storage on flavonoids levels and antibacterial activity of lantana camara linn. leaf extract cream. Res J Pharm Technol. 2023; 16(5): 2419-6. doi: 10.52711/0974-360X.2023.00399
22. Ingawale GS, Goswami-Giri AS. Isolation and characterization of bioactive molecule from Lantana camara. Asian J Research Chem 2014; 7(3): 339-344.
23. Punasiya R, Choudhary J, Pillai S. Analgesic activity of ethanolic extract of flower of Lantana camara. Res. J. Pharm. Dosage Form. & Tech. 2017; 9(4): 180-182. doi: 10.5958/0975-4377.2017.00029.5
24. Pradhan S, Pattnaik S. Phytochemical screening of components present floral essential oil of an indigenous variety of Lantana camara, Linn (Verbenaceae). Res. J. Pharmacognosy and Phytochem. 2017; 9(4): 203-209. doi: 10.5958/0975-4385.2017.00037.1
25. Parwanto E, Tjahyadi D, Amalia H, Edy HJ, Oladimeji AV, Tjahyadi JJV, et al. The potential of lantana camara linn. as a source of quercetin, gallic acid, and tannic acid. J Hunan University (Natural Sciences) 2023; 50 (5): 112-123. https://doi.org/10.55463/issn.1674-2974.50.5.12.
26. Mansoori A, Singh N, Dubey SK, Thakur TK, Alkan N, Das SN, et al. Phytochemical characterization and assessment of crude extracts from lantana camara l. for antioxidant and antimicrobial activity. Front Agron 2020; 2(582268): 1-14. doi: 10.3389/fagro.2020.582268
27. Awasthi A. Determination of bio-accumulated Cd, Cr, Cu, Ni and Pb in a wild plant Lantana camara L. Verbenaceae, grown on waste dump site and assessment of its phyto-extraction potential for studied metals. AJRC. 2023; 16(4): 271-6. doi: 10.52711/0974-4150.2023.00045
28. Hima V, Rubesh Kumar S, Duganath N, Devanna N. A novel validated stability indicating chromatographic method for the simultaneous estimation of ascorbic acid and gallic acid in the ayurvedic capsule dosage form of amla by UFLC. Asian J. Research Chem. 2013; 6(9): 826-831.
29. Diab DA, Asaad NA. Comparative analysis of ascorbic acid content and antioxidant activity of some fruit juices in Syria. Research J. Pharm. and Tech. 2018; 11(2): 515-520. doi: 10.5958/0974-360X.2018.00095.1
30. Atul S. Gurav, Ajit S. Kulkarni. Efavirenz cocrystals with Ascorbic acid: A strategy for polymorphic modification and improvement of dissolution properties. Research J Pharm and Tech. 2024; 17(1): 213-1. doi: 10.52711/0974-360X.2024.00034
31. Amina BB, Roukia H, Mahfoud HA, Ahlem T, Sabrina B, Chahrazed B, et al. Optimization of Extraction conditions of the Polyphenols, Flavonoids and the Antioxidant activity of the plant Ammosperma cinereum (Brassicaceae) through the Response Surface Methodology (RSM). Asian J. Research Chem. 2020; 13(1): 01-06. doi: 10.5958/0974-4150.2020.00001.2
32. Ali A, Riaz S, Khalid W, Fatima M, Mubeen U, Babar Q, et al. Potential of ascorbic acid in human health against different diseases: an updated narrative review. Int J Food Properties 2024; 27(1): 493–515. https://doi.org/10.1080/10942912.2024.2327335
33. Rajaram S, Natham R. Enhancement of rifampicin bioavailability by immune enhancing nutrient (ascorbic acid) as chitosan nanoparticles for tuberculosis therapy. Research J Pharm and Tech 2020; 13(12): 5924-5928. doi: 10.5958/0974-360X.2020.01034.3
34. Caldwell GW, Yan Z, Lang W, Masucci JA. The IC (50) concept revisited. Curr Top Med Chem. 2012; 12(11): 1282-90. doi: 10.2174/156802612800672844.
35. Boddupally M, Rani SS. In vitro antioxidant and in vivo hepatoprotective activity of lantana camara in paracetamol-induced liver injury in experimental rats. IJCBS 2023; 24(5): 656-663.
36. Sirait SM, Rosita T, Rahmatia L. Formulation and evaluation of sea grape (Caulerpa racemose) extract as hand cream and its antioxidant activity test. JKR 2022; 7(1): 47 – 56.
37. Muthukumarasamy R, Ilyana A, Fithriyaani NA, Najihah NA, Asyiqin N, Sekar M. Formulation and evaluation of natural antioxidant cream comprising methanolic peel extract of Dimocarpus longan. IJPCR. 2016; 8(9): 1305-1309.
38. Jenifer P, Balakrishnan CP, Pillai SC. In-vitro antioxidant activity of marine red algae Gracilaria foliifera. Asian J Pharm Tech. 2017; 7(2): 105-108. doi: 10.5958/2231-5713.2017.00018.6
39. Hasni S, Khelil A, Habita S, Bireche K, Mahcene Z, Boual Z, et al. The effect of the Feeding system on the Antioxidant activity of Camel urine. Asian Journal of Pharmaceutical Research 2022; 12(4): 261-6. doi: 10.52711/2231-5691.2022.00042
40. Ashfaq MH, Siddique A, Shahid S. Antioxidant activity of Cinnamon zeylanicum: (A Review). Asian J Pharmaceutic Resch. 2021; 11(2): 106-6. doi: 10.52711/2231-5691.2021.00021
41. Zerbinati N, Sommatis S, Maccario C, Di Francesco S, Capillo MC, Grimaldi G, et al. A practical approach for the in vitro safety and efficacy assessment of an anti-ageing cosmetic cream enriched with functional compounds. Molecules 2021; 26(24); 7592: 1-12. doi: 10.3390/molecules26247592.
42. Kick BL, Gumber S, Wang H, Moore RH, Taylor DK. Evaluation of 4 presurgical skin preparation methods in mice. J Am Assoc Lab Anim Sci 2019; 58(1): 71-77. doi: 10.30802/AALAS-JAALAS-18-000047.
43. Bhoyar SD, Malhotra K, Madke B. Dressing materials: a comprehensive review. J Cutan Aesthet Surg. 2023; 16(2): 81-89. doi: 10.4103/JCAS.JCAS_163_22.