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IN-VIVO PRELIMINARY EXAMINATION OF *MORINGA OLEIFERA* LEAVES EXTRACT AS ANTIAGING CANDIDATE IN SWISS WEBSTER MALE MICE (*MUS MUSCULUS*)

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Keywords:

Antioxidant, Antiaging, *Moringa oleifera*, 2, 2-Azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid (ABTS), Tetramethoxy Azobizmethylene Quinon (TMAMQ)

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ABSTRACT: Free radicals are molecules with an unpaired electron that very unstable and react quickly with other compound to gain stability. Once the process is started, it can be a cascade reaction resulting disruption of a living cell. The free radicals formed in our body are combated by antioxidants then interact with free radicals and terminate the chain reaction before vital molecules are damaged. *Moringa oleifera* leaf extract well known can be as a source of antioxidant since it contains flavanoids for reducing free radical. This study was design to produce a crude extract of dark green and bright green of *M. oleifera* leaves using different solvent to determine its antiaging effect in male mice *Mus musculus* Swiss Webster. The antioxidant capacity of the extract were carried out using TMAMQ (Tetramethoxy Azobizmethylene Quinon) and ABTS (2, 2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) method, while the antiaging activity were assessed by histological approach. The results showed that the highest antioxidant capacity was achieved by dark green *M. oleifera* leaves ethanolic extract followed by double-destilated water (ddH_20) and 95% *n*-hexane extracts. The antiaging activity were observed by level of *M. musculus* skin dermis thickness as 516.13 µm and more fibroblast cells 115.67 cells in the visual area of 157.883, 92 μm^2 of skin dermis. The result indicated that the colour of *M. oleifera* leaves and type of extraction solvent play important roles on the antioxidant capacity and antiaging activity.

INTRODUCTION: In recent years, free radicals have been a great deal of attention in human life. The knowledge of free radicals in biology is a high reactivity molecule due to their unpaired electrons in outermost orbital [1]. Free radical molecules are unstable and short-terms, therefore they could easily snatch electrons from other compounds to attain stability. Thus, the attacked molecule loses its electron and becomes a free radical itself, beginning a chain reaction cascade which finally damages the living cell [2, 3].

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In order to terminate or neutralize the free radical and reduce the level of biomolecules damage, some of such low molecular weight molecule known as antioxidant can be attributed to reduce oxidation process [4]. Interaction between antioxidant and free radicals inhibit biomolecules oxidation and stabilizing or deactivating the radicals before attacking healthy cells [4, 5, 6].

Antioxidant activity compounds commonly found in the plants such as essential amino acids, carotenoids, phenols, alkaloids and flavonoids [7, 8, 9]. *Moringa oleifera* is a member of Moringaceae family originated from India, Pakistan, Bangladesh, and Afghanistan. *M. oleifera* is also known as horseradish tree, drumstick tree, benzolive tree, kelor, maronggi, marango, mlonge, moonga or sajna [10]. The *M. oleifera* extract contains vitamin A is ten times higher than carrot, vitamin C is five times than orange, the calcium content is nine times more than whole milk, and the potasium content is three times more than banana [11, 12, 13], besides, *M. oleifera* also contain other secondary metabolites such as phenols, alkaloids and flavonoids that act as antioxidant [13, 14, 15].

Several reports showed that the presence of phenolic compounds and flavonoids in extract of *M. oleifera* leaves is sustainable remedy for anti-cancer, antiinflammation, antiaging, antioxidant, antimicrobial and antiobesity [11, 12, 13, 14, 15, 16]. According to Nirina *et al*., [17] and Abdulkadir *et al*., [18], the maturation stage leaves of *M. oleifera*, which are indicated by difference between the young and the mature leaves was tenderness and color leaves has special characteristic in terms of phennolic compounds. The young leaves were tenderer, bright green color compared to older leaves which were harder and dark green color. Bright green color leaves contains low chlorophyll leaf has higher (%) inhibition of DPPH radicals than dark green color leaves (high chlorophyll leaf), with percentage inhibition of 75.73 ± 1.10% and 58.62 ± 1.13%, respectively.

Methods of antioxidants extraction plants plays a critical role to the extraction outcomes (*e.g.* yield and phytochemicals contents) [19, 20]. Recently, different studies have shown that selection of the extraction process strongly affect the biological activity [20], namely ethanol extraction methods of *M. oleifera* can restoration of the antioxidant system against oxidative damage in mammalian liver tissue [21]. According to Duraivel *et al*., [22] ethanolic extract of *M oleifera* leaves can reduce sebum content in the skin, and improve water absorption lead to stability of skin moisture.

It also could prolong the shelf life of fibroblast cells or by producing high amounts of collagen [23]. Furthermore, environmentally friendly *M. oleifera* ddH_20 extraction has shown improving healing of cancer line and liver disease [24, 25]. Other studies showed that the highest antioxidant activity was reached by ethanol extract of *Carica papaya* and *Eriobotrya japonica* Lindl. better than aqueous, methanol, and *n*-hexane [26, 27]. Considering the selection of proper solvent extraction of *M. oleifera* which is a critical step in processing antioxidant. This study for the first time used different solvent for extracting *M. oleifera* leaves and determine their antioxidant and anti-aging activity using *in-vivo* method on Swiss Webster male mice (*Mus musculus*).

MATERIALS AND METHODS:

Materials: ABTS or 2, 2'-azino-bis[(3-ethylbenzo-thiazoline-6-sulphonic acid) and TMAMQ stock reagent were prepared through laccase oxidation according to Nugroho Prasetyo *et al*.,[28] Briefly, the ABTS were oxidized then the oxidation was stopped using sodium azide (NaN_3) meanwhile TMAMQ was produced by incubating syring-aldazine (0.17 mM) with 50 µl of laccase (20 nkat/ml) in 50 mM sodium succinate buffer at pH 4.5. ABTS, syringaldazine, sodium succinate and sodium azide (NaN_3) were purchased from Sigma-Aldrich Singapore. All solvents including double-distilled water (ddH_2O), 95% ethanol (C_2H_6O), and n-hexane (C_6H_{14}) were purchased from the Bioanalitik (Indonesia).

The *Trametes hirsuta* laccase was produced and purified as previously described by Nugroho Prasetyo *et al*., [28] The experimental animals in this study were obtained from School of Life Sciences and Technology, Institut Teknologi Bandung (ITB) Indonesia which already had been approved as research center according our Ministry of Health Republic Indonesia under Government Regulation no: 39 Year: 1995 about Research and Development in Health.

Methods:

Plant Materials, Harvesting, and Drying: *M. Oleifera* were grown and harvested at temperature 30 - 33 °C in Poteran Island, District of Sumenep, Indonesia (7°04'01.2 South latitude 113°56'28.1 East longitude) during November 2015 to April 2017. The plant heights at harvest time were 150-200 cm. The fresh leaf materials were collected in fields and determined in Herbarium Bandungnense, School of Life Sciences and Technology, Institut Teknologi Bandung (ITB) Indonesia (Specimen no. 3710). *M. oleifera* bright green leaves samples were collected from the 3 most apical portions of branches and the dark green leaves from the 3 most basic of stems. Dark green leaf has a leaf area approximately ± 296.67 mm^2, while the bright green leaf area is around ± 25 mm^2.

M. oleifera leaves were washed using sterile double - distilled water (ddH_20), and continue rinsed by 1% of salt water for 3-5 min further the leaves were rinsed again using sterile ddH_20. Washed leaves were then dried in an oven at temperature 60 °C until the water content reaches by 10%. The dried leaves were mashed by blender become powder and stored in 4 °C for further step [18]. All preparations were performed in triplicate.

Extracting of *M. oleifera* Leaves: The extraction method of *M. oleifera* leaves was adapted from Rockwood *et al.,* [29] and Charoensin *et al.,* [30] with some modification. Extraction procedure involved macerating individually of *M. oleifera* leaves powder with 50 ml of three different solvents namely ddH_2O, 95% ethanol, and 95% n-hexane in a 250 ml Erlenmeyer flask for 48 h. All macerating process were kept at room temperature except n–hexane was stored at –4°C.

The all mixtures except ddH_20 mixture was then centrifuged at 6000 rpm for 20 min and the supernatant recovered. Meanwhile, the ddH_20 mixtures were filtered using Whatman grade 3 (pore size 6 µm) filter paper. All preparations were performed in triplicate. Furthermore, supernatants were evaporated at 50 °C in oven for 24 h to obtain higher extract concentration. Evaporation processed result in pasta form and weighed using analytical balance (Shimizu Uni Bloc) to measure yield percentage and stored in glass bottles at 4 °C for further analysis.

Antioxidant Capacity Measurement using ABTS Method: Antioxidant capacity present in *M. oleifera* extracts were determined using ABTS method according to Nugroho Prasetyo *et al*.,[28] and Re *et al*.,[31] with some modifications. The ABTS stock solutions were prepared by dissolving 0.02 g of ABTS in 10 ml of citric buffer pH 4 and 1 ml of Laccase (0.18 % w/v) to a final volume of 100 ml and keep the reaction going for 5 min. The reaction was stopped using 10 µl sodium azide (NaN_3) solution (0.91 % w/v) and subsequently the mixture was stored at room temperature in the dark at vessels for further use. The oxidized ABTS was monitored by measuring its absorbance at λ=436 nm using Hitachi U-2001 UV-Vis spectrophotometer in a 1.5 ml disposable cuvette of 1 cm pathway (Brand GmBH, Germany).

The working solution of the extracts were prepared by diluting using respected solvent in a serial concentration of 0-0.2 mg/ml. The *M. oleifera* leaves extracts of ddH_2O and 95% ethanol was dissolved in ddH_2O while the extracts of 95% n–hexane was dissolved in DMSO 1:4 v/v before diluting. The absorbance of ABTS reagent after mixing with the extract was monitored at 436 nm using Hitachi U-2001 UV-Vis spectrophotometer in 1.5 ml disposable cuvette. The decreasing of the ABTS absorbance indicates the presence of antioxidant quenching capacity of the samples [28].

Antioxidant Capacity Measurement using TMAMQ Method: Antioxidant capacity was assessed using TMAMQ method according Nugroho Prasetyo *et al*., [32] with some modification. TMAMQ stock solutions were prepared initially by dissolving syringaldazine in 20 ml of warm acetone. Syringaldazinequinone (TMAMQ) generation was obtain by incubating syringaldazine (0.17 mM) with 50 µl of laccase (20 nkat/ml) in 50 mM sodium citrate buffer at pH 4.5. The reaction mixture (1 ml) was incubated at 30 °C for 10 min while shaking at 140 rpm in a thermomixer (Eppendorf AG, Germany). The oxidation process was monitored at 530 nm using a Hitachi U–2001 UV-Vis spectrophotometer in 1.5 ml disposable cuvettes of 1 cm pathway (Brand GmBH, Germany). Ethanol was added to a final concentration of 80% (v/v) to stop laccase activity and to stabilize the TMAMQ solution [32]. The absorbance of TMAMQ solution after mixing with

the extracts were monitored at 530 nm. The decreasing of the TMAMQ absorbance indicates the presence of antioxidant quenching capacity of the samples.

Animal and *in-vivo* Antiaging Treatment on *M. musculus*: Male Swiss Webster mice weighing 24-25 g purchased from School of Life Sciences and Technology (SITH–ITB, Bandung Indonesia) were used for all experiments. Mice were farmed four per cage until the beginning of the experiments then during the experiment period were individually housed. In order to keep equal condition, all mice had free access to water and food. The animal experiment was adapted from Zhang *et al*., [33] Taufiquromahman *et al*., [34] and Nurhayati *et al*., [35] with slight modifications. Six Swiss Webster male mice (*M. musculus*) with age of 3 months were selected randomly into 2 groups consisting 3 mice each group. The control group were received sterile aquades and the treated group were daily fed by 95% ethanol extract of *M. oleifera* leaves with a dosage range between 0.27 and 0.28 mg/g of body weight for 30 days.

Sample Collection: Animals were killed by servical dislocation at day 30 of experiment. Skin tissue in intraperitoneal area were collected. Tissue was separated from body and washed two times with sterile distillate water and divided into several parts.

Histological Preparation of *M. musculus* Skin and Calculation of Fibroblast Cell: Analysis of antiaging activity was performed by histological preparations of skin tissue *M. musculus*. The skin tissue (contain epidermis, dermis, and hypodermis) of *M. musculus* was cut into a size of 0.25 cm^2 from the intraperitoneal part, then fixed in 10% of formaldehyde for 24 h. After fixation, specimens were dehydrated in serial ethanol concentration at 10%, 20%, 30%, 50%, 70%, 90%, and 96% v/v for 20 minutes each. Specimens were transferred into xylol-alcohol for 40 min and continued in pure xylol I, 11, III respectively for 20 min. The specimen was embedded in liquid paraffin let it harden at room temperature. The paraffin blocks were cut using a microtome of 4 µm thickness. Furthermore, paraffin was removed by addition of liquid xylol for 10 min. In order to visualize the thickness of dermis layer and fibroblast cells, a combination of Hematoxcylin-eosin (HE) staining technique were implemented.

The number of fibroblasts cells in *M. musculus* skin was performed using manual quantitative method in the area of 457, 7 µm x 344,4 µm [34]. Qualitative analysis of skin tissue was performed to differentiate the control and treatment group by level of dermis thickness. Fibroblast cells in the specimen were indicated by dark blue colour showed by respective staining with a very large in size. Images were captured by a light Microscope (Zeiss, Germany) with Optilab application (Phoenix, AZ 85012 USA) by 400 times magnification.

Data Analysis: Antioxidant capacity of *M. oleifera* leaves extracts were obtained from decreasing of absorbance values, then calculated using Microsoft Excel 2010 program (Microsoft, Rochester, NY, USA) and calibrate with standard curve of Trolox (for measurement using ABTS reagents) to determine the Trolox Equivalent Antioxidant Activity (TEAC) value. The degree of decoloration of TMAMQ solution indicates the scavenging efficiency of the added antioxidant sample. The stoichiometrical reduction of TMAMQ (1 µM of TMAMQ reduced by 1 µM antioxidant) was then calculated from a dose-response curve of added antioxidant [28]. The results of thick dermis calculations and the number of fibroblast cells in histology preparations were analyzed by the T-Independence Test method using Minitab v.16 software (Minitab Ltd., Coventry, UK) with $p < 0.05$.

RESULTS AND DISCUSSION:

Antioxidant Capacity of *M. oleifera* Leaves Extract: ABTS and TMAMQ method were widely used to evaluate antioxidant activities within a relatively short time compared with other methods. Both methods have their respective advantages and disadvantages. The ABTS method is the production of $ABTS^{\bullet+}$ directly with the addition of laccase, where ABTS acts as a substrate to produced bluish green color radical $ABTS^{\bullet+}$ otherwise it can also use potassium persulfate ($K_2S_2O_8$) to produce $ABTS^{++}$ blue chromophore [31].

ABTS was water-soluble compound that can be used directly to the water-soluble antioxidant such

as pure compounds and food extracts [26, 31]. However, the use of laccase in the formation of ABTS radicals will be less stable without the presence of such inhibitors like sodium azide (NaN_3). Therefore, it was a fact that even though TMAMQ as well used laccase as oxidant agent however, the laccase was removed using molecular precipitation and purified by filtration.

Accordingly, TMAMQ relatively more stable than ABTS radical further it was able to measure all kind samples and can be stored for a longer time [27, 28, 32]. The antioxidant capacity of *M. oleifera* leaves extracts (1 mg/ml) **Fig. 1** showed that among the three different solvents and two type of leaves colour, the highest antioxidant capacity was achieved by *M. oleifera* dark green leaves extracts.

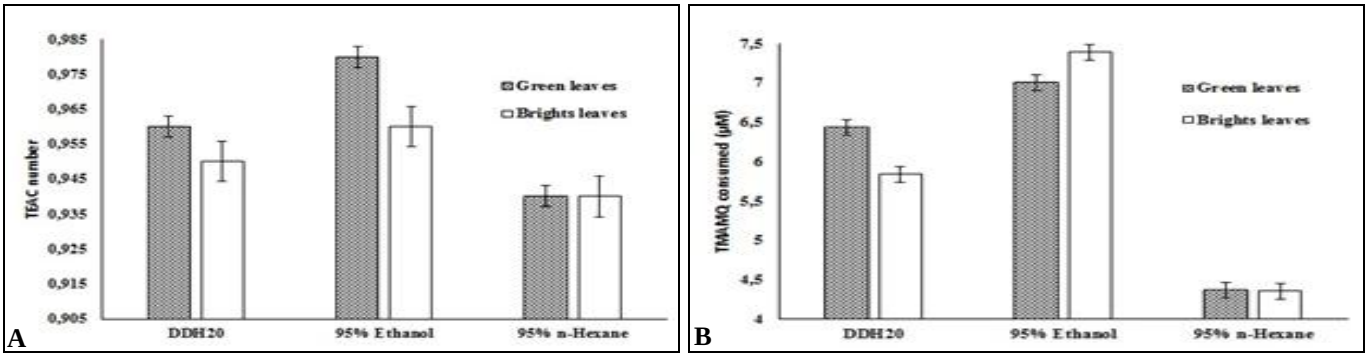

FIG. 1: ANTIOXIDANT CAPACITY OF DARK AND BRIGHT GREEN *M. OLEIFERA* LEAVES EXTRACT EXTRACTS (1 mg/ml) IN mm TEAC (A) AND µm TMAMQ (B). EACH TYPE OF SOLVENTS AND COLOUR LEAVES WERE TESTED IN TRIPLICATE. DATA ABOVE IS LISTED IN AVERAGE VALUE FOR EACH TREATMENT

There is a significantly differences between the TEAC and TMAMQ values among the origin of solvents, but there are no significant differences between different leaves colour. The results showed that 1 mg/ml of 95% ethanol extracts of *M. oleifera* dark green leaves could reduce 0.98 mM of ABTS radical and 7.01 µM of TMAMQ, **Fig. 1**. TEAC value of dark green *M. oleifera* leaves showed 45.71% antioxidant capacity smaller than TEAC standard [29] that were 2.10 mM and 11.71% lower than α-Tocopherol (vitamin E) (1 mg/ml can reduce 1.11 mM of Trolox). The extracts also could reduce 7.01 µM of TMAMQ, so that antioxidant capacity of extracts were 3.5 times better than TMAMQ in vitamin C that was 2.3 µM, and 3 times higher than α–Tocopherol (1 mg/ml can reduce 2.29 µM of TMAMQ) [32].

Similar results were obtained in antioxidant capacity of *M. oleifera* bright green leaves extracts. The highest antioxidant capacity value was found in 95% ethanol extracts (1 mg/ml of extract (0.96 mM/mg/ml). Successively lowest values were obtained in 95% n-hexane extracts before the ddH_2O extracts.

The antioxidant capacity of 95% ethanol extracts of bright green leaves were smaller than dark green leaves, it was 45.71% smaller than TEAC standard, and 13.51% lower than in vitamin C 28, that was 1 mg/ml ascorbic acid (vitamin C) could reduce 7.39 µM of TMAMQ (3.2 times greater than the TMAMQ standard) and 3.97 times higher than in vitamin C [32], which was 1 mg/ml ascorbic acid could reduce 1.86 µM of TMAMQ.

At the same taxonomic order Brassicales, the antioxidant capacity of *M. oleifera* leaves extracts were also higher than Broccoli (*Brassica oleracea*) and Papaya (*Carica papaya*), it was 1 mg/ml of *B. oleracea* extracts could reduce 0.01 mM of Trolox, and 1 mg/ml of *C. papaya* extracts could reduce 0.71 mM of Trolox. Those facts above can be explained because the total flavonoid content in *M. oleifera* leaves extracts were 3.74 times higher than *B. oleracea*, it was 58.7 mg QE/g extract (mg quercetin equivalent per g of extract) compared to 15.7 mg QE/g extract, and 3.69 times higher than *C. papaya*, it was 15.9 mg QE/g extract [36, 37, 38, 39, 40].

The solubility of antioxidant compounds in solvent has a significant effect at the time of extraction process. Thus, the polarity of solvents has an indirect function in the extraction process, lead to higher solubility of antioxidant compounds [31, 40]. **Fig. 1** shows that ethanolic extraction has highest antioxidant capacity in both type of leaves since ethanol is class of semi-polar solvent that is more power in dissolving for antioxidant compounds.

In accordance of Roopalatha *et al*., [40] and Masfria *et al*., [41] that semi-polar compounds in *M. oleifera* could be alkaloids, flavonoids, and glycosides. Fig. 1 also shows information that dark green green M. oleifera leaves extracts has a little higher antioxidant capacity than bright green leaves. It was possible because of higher content of chlorophyll in the dark green *M. oleifera* leaves than the bright one, so the amount of Mg^{2+} ions is also higher. This is in accordance with Han *et al*., [42] the rate of free radical reduction in flavonoid compounds increased in the presence of Mg^{2+} ions to stabilize one electron from the radical. The electron transfer process was followed by the transfer of protons from antioxidant cation radicals to G• (galvinoxyl radicals) to produce neutral antioxidants and GH. Flavonoids were stable antioxidants because flavonoids were not pro–oxidants, which were could turn into radicals in certain circumstances or while donated the electrons to free radicals, like carotenoids [43].

Antiaging *in-vivo* on *M. musculus*: Aging is a process of decreasing the ability of tissues or cells to repair themselves, maintaining normal structure and function [43]. The visible changes in skin aging such as dry, coarse, sagging, and wrinkled skin are accompanied by visible facial expressions [44]. UV exposure affects the structure and function of the human skin. Ultra violet rays (UVA and UVB) produce free radicals that cause damage to DNA can affect or accelerate the aging process [45].

Free radicals cause damage to the skin, such as lowering the performance of protective enzymes causing collagen damage and elastin tissue, skin blood vessels and disrupting the distribution of melanin. These damages cause the skin to thicken, stiff, and not elastic, wrinkled, pale and dry, and the appearance of blackish or brownish spots [44, 45]. The treatment of *M. oleifera* leaves extract which has the highest antioxidant capacity (dark green leaves in 95% ethanol solvents) in *M. musculus* gives an effect as shown by the average dermal skin thicker than the control **Table 1** and **Fig. 2**.

TABLE 1: DERMIS THICKNESS AND NUMBER OF FIBROBLAST CELLS OF *M. MUSCULUS* SKIN AFTER *M. OLEIFERA* EXTRACT TREATMENT

Group of treatment	Dermis thickness (µm)	Number of fibroblast cell
No treatment	325.25±83.76[a]	82.33±18.90[c]
Moringa leaves extract treatment	516.13±51.21[b]	115.67±8.15[d]

*Different letter shows significant different p<0.05

Table 1 and **Fig. 2** showed that the dermis thickness of treated *M. musculus* by *M. oleifera* leaves extract was higher than untreated one of 513.16 µm and 325.25 µm respectively. The fibroblast cell number increased after treating with *M. oleifera* extracts as 115 cells per 157,631.88 μm^2 dermis visual area compared to the control groups of 82 cells per 157,631.88 μm^2 visual area. It shows that *M. oleifera* leaves extract treatment could improve fibroblast cells proliferation and lead to increasing amount of collagen and other extracellular matrix compounds such as hyaluronan acid therefore the dermis thickness increases and the firmness of the skin can be maintained. This is in accordance with the report stated that addition of antioxidant would improve skin cells protection and prevent collagen degradation [13, 46, 47].

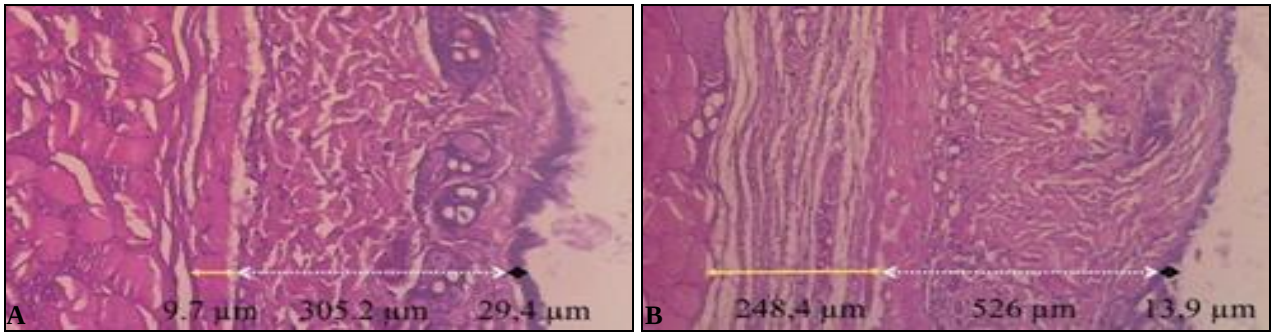

FIG. 2: DIFFERENTIATION OF SKIN THICKNESS IN *M. MUSCULUS*. (A) CONTROL, AND (B) TREATMENT WITH DARK GREEN *M. OLEIFERA* LEAVES EXTRACTED WITH 95% ETHANOL IN 30 DAYS; (1) EPIDERMIS (BLACK LINE), (2) DERMIS (WHITE LINE), (3) HYPODERMIS (YELLOW LINE) IN MAGNIFICATION 100X

The fibroblast cells aging status was more prevalent in the histological skin specimen of *M. musculus* in control groups compared to those treated one **Table 1**.

Aging of fibroblast cells was characterized by a larger size and irregular ends shape, whereas health fibroblast cells have a thinner and unspread form **Fig. 3**. Further, the thickness of collagen after treating with the extract as well thicker and denser than the skin of *M. musculus* without treatment.

Inhibition of aging in fibroblast cells were influenced by the content of antioxidants in the *M. oleifera* leaves extracts. One of the highest content in *M. oleifera* were flavonoids. Flavonoids could improve the synthesis of hyaluronan synthase-3 (HAS3) and hyaluronan synthase-2 (HAS2) acids in fibroblast cells [48]. Hyaluronan is one of the simplest glycosaminaminoglycans (GAGs) forming an extracellular matrix in the skin that acts as a filler in the dermis and binds water in the skin [49].

The increasing of hyaluronan synthase amount for the formation of hyaluronan could prevent apoptosis in fibroblast cells, and cell damage by environmental stress such as free radicals can be inhibited [50, 51]. High production of extracellular matrix can maintain firmness on the skin as well aging can be inhibited.

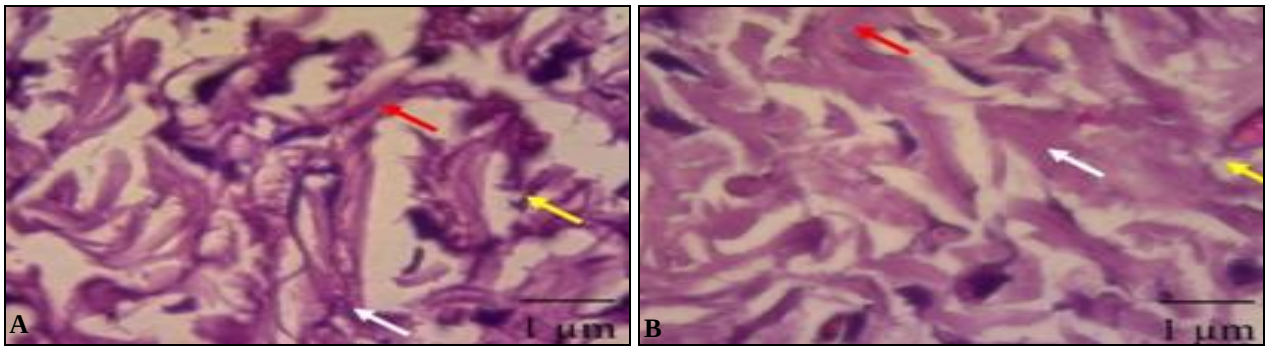

FIG. 3: FIBROBLAST CELLS ON *M. MUSCULUS* DERMIS. (A) CONTROL, AND (B) TREATMENT WITH DARK GREEN *M. OLEIFERA* LEAVES EXTRACTED WITH 95% ETHANOL IN 30 DAYS (RED ARROWS SHOWED AGING CELLS, WHITE ARROWS SHOWED NON–AGING CELLS, AND YELLOW ARROWS SHOWED COLLAGEN) (MAGNIFICATION 1000X)

Flavonoids could also inhibit UV rays from producing ROS in the skin to induce MAP kinase expression that can be inhibited the production of MMP (Matrix Metallo Proteinase) to degrade collagen, gelatin, and proteoglycans in the skin [52, 53]. Flavonoids could also inhibit signal transduction from NF-κB in stress and apoptotic stress pathways then collagen damage, aging and apoptosis in fibroblast cells may be inhibited [54, 55].

CONCLUSION: According to this study, the addition of *M. oleifera* leaves extract can show an effect of higher fibroblast cell amount than controls. This result suggests that *M. oleifera* leaves extract may act as an antiaging agent *in-vivo* in the skin of *M. musculus* because they have a high antioxidant capacity, especially in 95% ethanol extracts.

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CONFLICT OF INTEREST: The authors declare no conflict of interest

Author Contributions: MI performed extraction leaves, antioxidant capacity and antiaging experiment. I performed *M. oleifera* selection and guidance. MI and AWPDN performed and analyzed histological preparation. MPK and ENP conceived the study. MI, MPK, and ENP wrote the manuscript.

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