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# Anti-aging properties of *Curcuma heyneana* Valeton & Zipj: A scientific approach to its use in Javanese tradition

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ABSTRACT

*Ethnopharmacological relevance:* Temu giring, the rhizome of *Curcuma heyneana* Valeton & Zipj (*C. heyneana*), is native to tropical regions, especially in Indonesia. It is traditionally used as a skin care, for cosmetic and body freshness, in Javanese and Balinese women, and has many other bioactivities such as antioxidant, anticancer and antiinflammatory.

*Aim of the study:* The purpose of this study was to determine the antiaging activity of C. heyneana to prove its traditional use.

*Materials and methods:* The antioxidant activity was determined using the DPPH free radical method, and anti-aging activity was examined using in vitro assays such as tyrosinase inhibitor and collagenase inhibitor. In vivo tests were performed by observing histomorphologic changes in rat skin exposed to Ultraviolet (UV) rays. The total curcuminoid contents and chromatographic profiles were determined by Thin Layer Chromatography (TLC) – densitometry.

*Results:* In all in vitro assays, all of the extracts showed a dose-dependent manner in the final concentration range from 62.5 to 500 μg/mL for DPPH assay and 31.25 to 250 μg/mL for tyrosinase inhibition and collagenase inhibition assay. Curcuminoid (CUR), the active principle of *Curcuma* genus shows antioxidant, tyrosinase inhibitor and collagenase inhibitor activity greater than all *C. heyneana* extracts. The in vivo assay results showed that the topical application of the crude extract of *C. heyneana* produced significant improvement effects on the UV-induced skin structure damage. The total CUR content was correlated with the anti-aging activity of *Curcuma heyneana*.

*Conclusions:* The results show that *C. heyneana* contains antioxidant compounds and has potent anti-aging activity, indicating that it can be used as an anti-aging drug candidate or as a phyto-cosmeceutical.

## 1. Introduction

Anti-aging medicine includes observation of visual appearance. Morphological changes to the physical constitution, appearance, and skin are simple indexes to measure aging. Old age is characterized by changes such as decreases in height, increases in the number of wrinkles, and decreases in the size of the face. Study of the mechanisms of aging and anti-aging medicine, in terms of the appearance, involves conducting research on the visual characteristics influenced by the aging process and by anti-aging medicine (Yamada, 2012; Fore, 2006; Fisher et al., 2008)

Physical appearance is strongly influenced by aging of the skin. Changes in skin appearance provide visible signs of aging, including wrinkles, irregular pigmentation, sagging, and elastosis. Skin appearance changes have an important impact on self-esteem and social welfare. Physical appearance can be an indication of health condition,

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and may also be correlated with the risk of death (Cosgrove et al., 2007; Fore, 2006; Sies and Stahl, 2004). There are two types of skin aging. Intrinsic aging is a normal aging process. On the other hand, extrinsic aging or premature aging, also known as photoaging, is an aging process caused by environmental influences, particularly by UV radiation (Kusumawati and Indrayanto, 2013; Saric and Sivamani, 2016). UV exposure is essential for vitamin D activation, but on the skin, UV rays can cause serious problems, such as dermal degradation of collagen and elastic fibers, and can even cause skin cancer (Tu and Tawata, 2015; Sies and Stahl, 2004; Verschooten et al., 2006). UV radiation stimulates melanin production by human epidermal melanocytes in the skin. The content of melanin in the skin determines the color of the skin. Melanogenesis is a process by which the skin produces melanin. Melanin has a photoprotective function; however, accumulation in large quantities reduces the esthetic appearance. Melanogenesis is catalyzed by tyrosinase. Inhibition of tyrosinase inhibits the process of melanogenesis and is expected to prevent abnormal pigmentation of the skin (Lin et al., 2011; Saewan and Jimtaisong, 2013).

Traditionally, the people of Java and Bali use lulur or body scrub to assist in the prevention of the aging process. Lulur is part of beauty care and body freshness in Javanese royal tradition. Since the 17th century, the use of lulur has also been a part of the daily ritual for people of Java and Bali. Lulur has a remarkable softening effect on the skin, making the skin supple and glowing. One of the most well-known ingredients used for lulur in Java and Bali is temu giring (*C. heyneana*). Traditionally, lulur temu giring is used by brides to rejuvenate and soften the skin and eliminate bad odors on the body. Lulur temu giring is usually mixed with other ingredients such as rice flour, turmeric, pandan leaves, and lime peel, to form a natural scrub, and is applied to the skin every day until the wedding day (Ministry of Trade, 2009).

*Curcuma* (Zingiberaceae) is a rhizomatous species that has various ethnomedicinal uses. There are about 50 *Curcuma* species in the tropics, the most studied being turmeric (*C. longa*) (Dutta, 2015; Srivastava et al., 2009). Chemical content that has certain pharmacological activity is the key of a medicinal plant. These compounds become an important basis that guides its potential as a source of new drugs. The major bioactive compounds in the *Curcuma* genus is CUR that consisted curcumin (C) and its derivatives, demethoxy curcumin (DMC), and bisdemethoxy curcumin (BDMC); these have been shown to have a broad spectrum of biological action (Dutta, 2015; Jayaprakasha et al., 2006). C has strong antioxidant properties, and thus, it is a choice for cancer prevention, liver protection, and premature aging (Anand et al., 2008; Gul and Basheer, 2016; Jayaprakasha et al., 2006; Mohiuddin et al., 2010).

*C. heyneana*, one of the species of the *Curcuma* genus, is a plant that is widely found in Java, Indonesia. It is used traditionally for the treatment of diseases such as for cancer and inflammatory conditions, and also for skin appearance. The chemical content of *C heyneana* is known to include germacrone, dehydrocurdione, isocurcumenol, curcumenol, curcumanolides A and B, zerumbone, oxycurcumenol, and zedoarondiol (Cho et al., 2009).

Based on its CUR content and antioxidant activity, in the current research, we performed in vitro and in vivo bioassays to investigate the anti-aging properties of this plant, in order to provide a scientific basis for its development as an anti-aging medicine or as a phytocosmeceutical.

## 2. Methods and materials

### 2.1. Chemicals and solvents

The following reagents were analytical quality: curcumin (Sigma-Aldrich, Singapore), mushroom tyrosinase, a collagenase from ChC, DPPH (Sigma-Aldrich, Japan), and ((7-methoxycoumarin-4-yl)acetyl-L-prolyl-L-leucylglycyl-L-leucyl-[Nβ-(2,4-dinitrophenyl)-L-2,3-diaminopropionyl]-L-alanyl-L-arginine amide) (Peptide Institute, Osaka, Japan). Water was purified in a Millipore Milli-Q system (Bedford, MA). Solvents and reagents were purchased from TCI (Tokyo, Japan), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Nacalai Tesque, Merck (Indonesia), unless otherwise specified. All chemicals and solvents were of analytical grade.

### 2.2. Plant material and extract preparation

*C. heyneana* rhizomes were obtained from a cultivated area in Pucangan hill, Jombang, East Java, Indonesia, in March 2015 (coordinates 112°17′7″ E and 7°23′10″ S). A voucher specimen was identified and deposited in the Herbarium of Airlangga University (KLN CH032015). The rhizomes were washed, cut into pieces, and dried using an oven at 40 °C for three days. Dry rhizomes were ground into powder and stored in a sealed container until use. Ethanolic extract was obtained by extracting the plant material (100 g of rhizomes) using 70% ethanol (plant:solvent, 1:10, w/v), in a microwave (30% generator power), for one min. Dry rhizome powder (100 g) was also extracted, separately, with the same conditions, using n-hexane, ethyl acetate, and methanol, respectively. The extracts were dried by evaporating the solvent under reduced pressure to obtain the crude ethanol extract (CE), n-hexane extract (HEX), ethyl acetate extract (EA), and methanol extract (MEOH).

### 2.3. Simultaneous determination of C, DMC and BDMC content

Curcuminoid standard was dissolved in methanol at 1.0 mg/mL to obtain a stock solution. Five concentrations were derived from the stock solution in order to determine the calibration curve of Curcuminoid, and were applied on the TLC plate to obtain the final amounts of 65–130 ng/spot. The mobile phase condition was optimized to chloroform:methanol (40:1) through our study.

Each sample was prepared in methanol at 2.5 mg/mL, and was spotted 1.0 µL with a 7.0 mm bandwidth on an aluminum TLC plate 60F254 (20 × 10 cm; E. Merck, Germany) using a Camag Linomat 5 syringe, in triplicate, under the following conditions: application rate, 150 nL/s; space between each band, 7.0 mm; slit dimension, 5.00 mm × 0.45 mm; and scanning speed, 20 mm/s. The TLC plate was eluted for 8 cm using chloroform:methanol (40:1) as mobile phase, in the automated developing chamber version 2 (Camag, Muttenz, Switzerland). The plates were inspected using a CAMAG visualizer under 365 nm, and were photographed and uploaded to the computer software (WinCats). Densitometric scanning was carried out and operated by Camag Videoscan software. The C, DMC and BDMC content in the sample was determined by the peak area using linear regression, and was expressed in mg/g of the extract.

### 2.4. Antioxidant assay

The antioxidant activity was determined using a DPPH assay (Matsunami et al., 2006). The extract solution was prepared in DMSO at 10 mg/mL, and serially diluted into different concentrations. One microliter of each different concentrations of extract solution, in triplicate, was mixed with 100 µL freshly prepared DPPH methanolic solution (250 mM), on a 96-well microplate; and the final concentrations of the extracts were 100, 50, 25, 12.5, and 6.25 µg/mL. After incubation in the dark for 30 min, at room temperature, DPPH level of each well was evaluated by detection of the absorbance at 515 nm, using a Multiscan Go Thermo Scientific microplate reader. DMSO was used as a negative control and trolox as a positive control. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

$$\text{Inhibition ratio (\%)} = [(A_{control} - A_{test})/A_{test}] \times 100$$

where $A_{control}$ is the absorbance of the control (DMSO) and $A_{test}$ is the absorbance of the extracts. The IC50 of the inhibition ratio was

determined graphically.

### 2.5. Tyrosinase inhibitor

Tyrosinase inhibitory activity was determined using the dopachrome method with L-DOPA as the substrate (Widyowati et al., 2016). The sample solution (10 µL) was mixed with 40 µL of 2.5 mM L-tyrosine solution in 0.05 M phosphate buffer (pH 6.80), and 50 µL of mushroom tyrosinase (100 U/mL) in the same buffer, in a 96-well microplate, and the final concentrations of the extracts were 100, 50, 25, 12.5, and 6.25 µg/mL, in triplicate. After incubation at 25 °C for 5 min, the amount of dopachrome was determined by measuring the optical density (OD) at 475 nm using a Multiscan Go Thermo Scientific microplate reader. Arbutin was used as a positive control. The inhibition of tyrosinase was determined using the following formula:

$$\text{Inhibition ratio } (\%) = [1 - (A_{sample} - A_{blank})/(A_{control} - A_{blank})] \times 100$$

where $A_{control}$ is without the test sample and $A_{blank}$ is without both the test sample and tyrosinase. The IC50 of the inhibition ratio was determined graphically.

### 2.6. Collagenase inhibitor

Collagenase inhibitory activity was measured using the modified method described by Teramachi et al. (2005). The sample mixture (10 µL), 10 µg/mL of the enzyme (collagenase derived from *Clostridium histolyticum*) and 50 mM Tricine buffer (pH 7.5), was added to a 96-well microtiter plate, the final concentrations of the extracts were 100, 50, 25, 12.5, and 6.25 µg/mL in triplicate. After 10 min incubation at 37 °C, the substrate solution ((7-methoxycoumarin-4-yl)acetyl-L-prolyl-L-leucylglycyl-L-leucyl-[Nβ-(2,4-dinitrophenyl)-L-2,3-diaminopropionyl]-L-alanyl-L-arginine amide) was added, to obtain a final concentration of 10 µM, and was allowed to react. The fluorescence values were measured after 0 and 30 min incubation at 37 °C, at an excitation of 320 nm and an emission of 405 nm, using a fluorescence plate reader (EnSpire, Perkin Elmer, Inc., Japan). Caffeic acid was used as a positive control, and the IC50 of the activity was determined graphically

### 2.7. Animals and histomorphologic analysis of UV exposure on rat skin

The research was designed based on the ethical standards for animal use, and was approved by the Airlangga University Ethical Committee of Animal Experimentation (protocol number 1146/10). UV model of animal experiment was done using the modified method described by Saric and Sivamani (2016). Forty male rats (ICR), aged 3 months and weighing 150–200 g, were used in this study. They were obtained from the laboratory animal center.

The back of each rat was shaved every three days; the area shaved measured 3 × 3 cm. Rats were randomly grouped into four groups: normal, vehicle, crude extract, and retinoic acid as positive control (Kong et al., 2015).

The observation time was six weeks. In the normal group (n = 10), animals did not receive UV ray exposure. In the vehicle group (n = 10), all animals received UV ray exposure for 21 days, followed by application of the vehicle (Natrium CMC 0.05%) topically for a 21 day period. In the crude ethanolic extract group (n = 10), all animals received UV ray exposure for 21 days, followed by application of the crude ethanolic extract (10% in the vehicle) topically for a 21 day period. In the retinoic acid group (n = 10), all animals received UV ray exposure for 21 days, followed by application of retinoic acid (0.05% in the vehicle) topically for a 21 day period.

#### 2.7.1. Ultraviolet radiation (UVR)

UV light exposure was performed using a wooden box, which at the top of the box was installed a TL20W/12RS UV lamp. Animals are placed inside the wooden box, where the distance of the UV lamp is 20 cm from the back of the animals. All animals from all groups, except animal of control group, were exposed for 60 s every day (exposure was increased 60 s every two days) for 21 days. The exposure time was optimized based on the strength of the lamp, according to the dose of erythematous on the skin of the animal.

#### 2.7.2. Biopsy procedures and histologic analysis

Rat skin histology samples were obtained by fixing rat skin biopsies into 10% formalin solution, and were then embedded into paraffin blocks for sectioning and staining using a combination of hematoxylin–eosin. All of the sample histology slides were observed by one pathologist using an optical microscope (Olympus BX50, USA), and were captured by Software Cell D (Olympus).

### 2.8. Statistical analysis

The results are expressed as means ± SEM (standart error of the mean). Statistical differences between groups were estimated using one-way analysis of variance (ANOVA) with Duncan's test, and were considered statistically significant at $p < 0.05$.

## 3. Results

The extraction yield of CE obtained with the extraction process using 70% ethanol was 9.69 g. The extraction processes using n-hexane, ethyl acetate, and methanol, respectively, produced 6.4956 g HEX, 7.5991 g EA, and 7.7003 g MEOH (Table 1).

### 3.1. Determination of C, DMC and BDMC content

CUR, consisting of C, DMC, and BDMC, is the principal active compound that contributes to the activity of plants in the genus *Curcuma* (Anand et al., 2008; Dutta, 2015; Gonçalves et al., 2014; Gul and Basheer, 2016; Jadoon et al., 2015; Sarvalkar et al., 2011). In Fig. 1A shows that DMC has a larger spot than C and BDMC in all extracts.

TLC plate was developed in chloroform:methanol (40:1) and was visualized as the spots with fluorescence color under 365 nm.

C, DMC and BDMC content was quantified using the TLC densitometric method, (Fig. 1B). The results showed that the DMC has a larger peak area and content than C and BDMC in all extracts (Table 1).

### 3.2. Antioxidant, tyrosinase inhibitor, and collagenase inhibitor activity

In all assays, all of the extracts showed dose-dependent activity in the final concentration range from 62.5 to 500 µg/mL for the DPPH assay and 31.25 to 250 µg/mL for the tyrosinase inhibitor and collagenase inhibitor assays (Fig. 2).

The IC50 calculation for each assay used the curves for each standard and the samples of *C. heyneana*. The curves were calculated using

**Table 1**
The extraction yield of extract and the C, DMC and BDMC content in extracts of *C. heyneana* rhizome.

| Samples | Extraction yield (g) | C (mg/g extract) | DMC (mg/g extract) | BDMC (mg/g extract) |
|---|---|---|---|---|
| CE | 9.6900 | 3.11 ± 0.110a | 15.97 ± 0.059a | 3.25 ± 0.004a |
| HEX | 6.4956 | 0.11 ± 0.091d | 0.07 ± 0.011d | NA |
| EA | 7.5991 | 0.73 ± 0.056b | 3.62 ± 0.017b | 2.40 ± 0.088b |
| MEOH | 7.7003 | 0.27 ± 0.120c | 1.77 ± 0.103c | 0.93 ± 0.106c |

Values are expressed as mean ± SEM (n = 3); means in the same column followed by different letters are significantly different at $p < 0.05$ using Duncan's tests. CE, crude ethanolic extract; HEX, n-hexane extract; EA, ethyl acetate extract; MEOH, methanol extract; C, Curcumin; DMC, demethoxy curcumin; BDMC, bis-demethoxy curcumin.

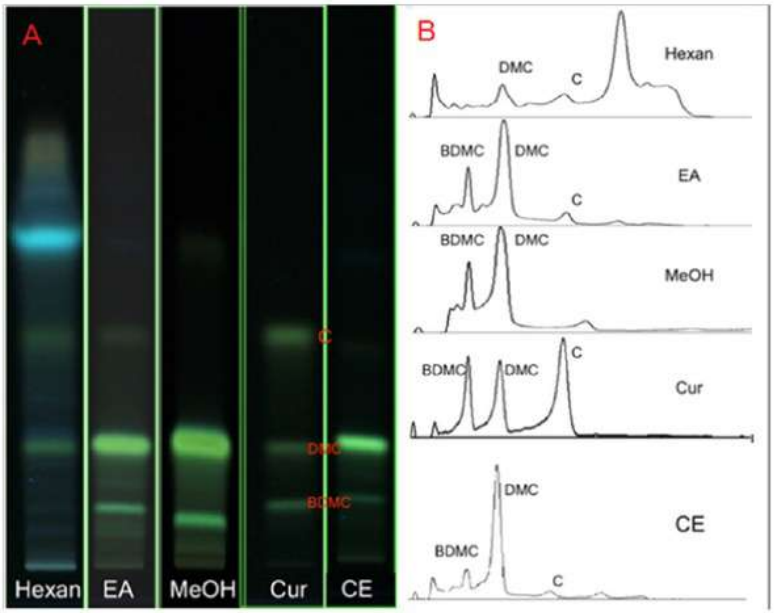


**Fig. 1.** TLC profiles of HEX, EA, MEOH, CE and CUR (color online only).

five concentration in the ranges described in Fig. 2 and showed the linear relationships and strong correlation coefficients ($r > 0.95$). CUR, the principal active constituent of the *Curcuma* genus, showed antioxidant, tyrosinase inhibitor, and collagenase inhibitor activity greater than all *C. heyneana* extracts. All of the extracts had an IC50 greater than 300 μg/mL, and n-hexane and methanol extract had an IC50 greater than 500 μg/mL. The IC50 of tyrosinase inhibitor activity for all the extracts exhibited a wide range, from 155.50 to 403.99 μg/mL, while the collagenase inhibitor activity IC50 ranged from 204.80 to 454.75 μg/mL (Table 2).

### 3.3. Histomorphologic effects of UV exposure on rat skin

Crude ethanolic extracts was selected for further rat in vivo study, because the activity of antioxidant, tyrosinase inhibitor and collagenase inhibitor of CE was higher than the hexane, ethyl acetate and methanol fractions. Histology analysis showed changes caused by UV exposure and changes after treatment with the samples. In Fig. 3, after UV exposure epidermal thickness (ET) of vehicle group increased three-fold from 4.07 ± 0.27 μm (normal skin) to 12.21 ± 0.41 μm. Topical application of the extract for 21 days decreased the ET to 8.09 ± 0.10 μm, while topical application of retinoic acid as a reference drug decreased the ET to 4.14 ± 0.27 μm, which was not significantly different to the normal group. UV ray exposure caused pyknotic nuclei of epidermal cells, considered to be sunburn cells (SBCs). SBCs incidence was calculated per linear millimeter of the epidermis for each animal. There was only 4.40 ± 0.28 SBC/mm$^2$ in the epidermis of normal rats. UV exposure increased the SBCs in vehicle group to 60.80 ± 2.56 SBC/mm$^2$. Topical application of the extract decreased six-fold in the number of SBC. Normal dermis showed orderly arranged collagen, abundant elastic fibers, and extrafibrillar matrix. In vehicle group, UV exposure damaged the arrangement of collagen so that the distance between collagen increased by approximately five times, as compared to normal rat skin. Topical application of the extract decreased the distance between collagen by 22% compared to normal group, while application of retinoic acid regenerated the distance between collagen to a normal group.

Normal skin had 41.53 ± 0.60 fibroblasts/10,000 μm$^2$. In vehicle group, after UV exposure, the number of fibroblast decreased to 9.74 ± 0.26 fibroblasts/10,000 μm$^2$. After topical application of the crude extract, the number of fibroblast increased to 16.60 ± 0.33 fibroblasts/10,000 μm$^2$; but the application of retinoid acid increased the number of fibroblast larger. In addition, in the normal dermal layer, the elastic fibers were neatly arranged, but in the vehicle group, after UV exposure, the number of elastic fibers reduced, and the structure became irregular. Topical application of crude extract increased the number of elastic fibers so that the arrangement was tightly and regularly (Table 3).

The results showed that the topical application of the crude extract of *C. heyneana* produced significant improvement in the UV-induced damage to the skin structure.

## 4. Discussion

The chromatographic profile of the extract indicates the pattern of biological activity or the chemical content present in the extract (Bhise and Salunkhe, 2009; Sanja et al., 2009). All compounds, not only active compounds, has a contribution to pharmacological activity of the extract so that chromatographic profile analysis is a comprehensive approach to the quality consistency and stability of the extract. In addition, other approaches are also made by selecting one or more compounds used for herbal product quality assurance. This selected compound is usually known as an active principle of a medicinal plant (Xie et al., 2006). In the *Curcuma* genus, CUR have been known as the principal active constituents that play a role in the various biological activities; these consist of C, DMC, and BDMC (Amalraj et al., 2017; Sasikumar, 2005). The total curcuminoid content found in the *C. heyneana* samples was lower than other *Curcuma* species reported in the literature (Dutta, 2015; Nahak and Rajani, 2011). The CUR content in turmeric comprises ≈ 77% C, ≈ 17% DMC, and ≈ 3% BDMC (Goel and Aggarwal, 2010). All extracts of *C. heyneana* exhibited higher DMC than C (Fig. 1). These differences result in lower antioxidant activity of the *C. heyneana* extract, compared to C. CE had higher total CUR content than HEX, EA and MEOH. CE also exhibited higher antioxidant activity, tyrosinase inhibitor activity and collagenase inhibitor activity than HEX, EA and MEOH. This suggests that differences in total CUR content

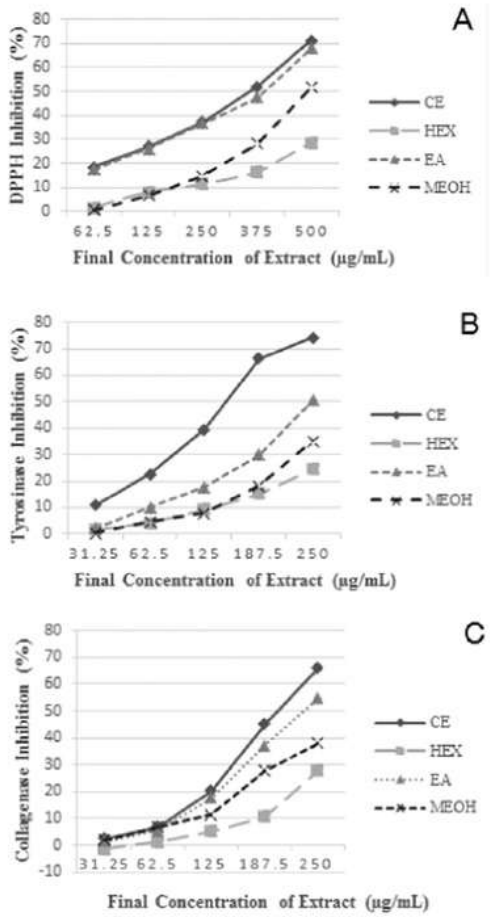


**Fig. 2.** Dose-dependent antioxidant (A), tyrosinase inhibitor (B), and collagenase inhibitor (C) activity evaluated with in vitro assays. The tests were performed in triplicate. CE, crude ethanolic extract; HEX, n-hexane extract; EA, ethyl acetate extract; MEOH, methanol extract.

in the extracts affect their biological activity.

Photoaging is caused by chronic exposure to UV light. This leads to incomplete repair of damage, which ultimately results in abnormal structures and functions; this is termed premature aging (Chiu et al., 2017; Fore, 2006). UV exposure on rat skin for 21 days, according to erythema dose, caused changes in the structure and affected the function of the skin. Epidermal thickness increased three-fold compared to normal conditions. This is due to the regeneration of cells in basal keratinocytes. Mature keratinocytes develop into stratum spinosum, stratum granulosum, and stratum corneum. This is reflected by an increase in epidermal thickness, acting as a protective function of the epidermis against UV exposure (Fore, 2006). The thickness of rat skin epidermis treated with topical CE and retinoic acid was lower than that treated with the vehicle only. Retinoic acid has been used for skin aging therapy. The use of topical retinoic acid shows histologic improvements such as increased collagen synthesis through blocking collagenase activity which is the basis of skin aging therapy (Kong et al., 2015). The ability of retinoic acid to suppress the increase in ET was greater than that of the CE. Both retinoic acid and the CE induced intrinsic skin protection, in order to restore normal skin conditions.

The histological appearance of UV exposed rat skin revealed a large number of SBCs. UV exposure is believed to cause direct injury to nuclear DNA in keratinocytes, thus stimulating the formation of SBCs.

**Table 2**
IC50 values of antioxidant, tyrosinase inhibitor, and collagenase inhibitor activity, for all extracts.

| Samples | IC50 (µg/mL) | | |
|---|---|---|---|
| | Antioxidant | Tyrosinase inhibitor | Collagenase inhibitor |
| CUR | 60.08 ± 1.17a | 59.65 ± 1.19b | 140.33 ± 2.01b |
| CE | 338.18 ± 8.17b | 155.50 ± 3.99c | 204.80 ± 2.12c |
| HEX | more than 500 | 403.99 ± 0.41f | 454.75 ± 5.52f |
| EA | 363.26 ± 7.21b | 265.44 ± 1.70d | 238.58 ± 1.07d |
| MEOH | more than 500 | 359.04 ± 12.15e | 323.44 ± 5.61e |
| T | 28.97 ± 0.41a | – | – |
| AR | – | 38.02 ± 0.25a | – |
| CA | – | – | 30.16 ± 0.55a |

Values are expressed as mean ± SEM (n = 3); means in the same column followed by different letters are significantly different at $p < 0.05$ using Duncan's tests. CUR, curcuminoid; CE, crude ethanolic extract; HEX, n-hexane extract; EA, ethyl acetate extract; MEOH, methanol extract; T, trolox; AR, arbutin; CA, caffeic acid.
Linear range (µg/mL) for antioxidant assay: T = 6.25–50; C = 12.50–100; samples = 62.50–500.
Linear range (µg/mL) for tyrosinase inhibitor assay: AR = 6.25–50; C = 12.5–100; samples = 62.5–500.
Linear range (µg/mL) for collagenase inhibitor assay: CA = 6.25–50; C = 31.25–250; samples = 62.5–500.

SBCs are easily recognized by their pyknotic nuclei and eosinophilic cytoplasm. Ultrastructurally, SBCs show the characteristics of apoptotic keratinocytes, with the appearance of nuclear chromatin condensation and dark cytoplasm tonofilament due to densely packed bundles. The decrease in the number of SBCs suggests a mechanism of defense or repair of DNA damage so there is no stimulation of apoptotic keratinocyte (Okada et al., 2003).

UV exposure causes dermal changes in both cellular and cell matrix components. The dermal layer contains connective tissue composed of various types of collagens, elastin, fibronectin, proteoglycan, and other extracellular matrix molecules. UV exposure causes an imbalance between the synthesis and degradation of the extracellular components. This imbalance caused the amount of fibroblasts decreases and thus affects its functional ability. The synthesis of new collagen decreases, collagen fibers become messy, also collagen fragmentation that causes disorganization of the whole skin. Structure and composition Elastic fibers undergo irreversible changes so elastin becomes amorphous and functional activity is poor. The structure and composition of elastic fibers changes, causing fibers with amorphous elastin, which have poor functional activity. Collagen fibers become disorganized, randomly oriented, and fragmented (Ghersetich et al., 1994; Hwang et al., 2012; Pasquali-Ronchetti and Baccaeani-Contri, 1997; Zouboulis and Boschnakow, 2001).

Chronic UV skin exposure causes photoaging which is characterized by thickening, roughness, coarse wrinkles, mottled pigmentation, and histologic changes, including impairment of collagen fibers, extreme accumulation of abnormal elastic fibers, and an enhancement of glycosaminoglycans (Serafini et al., 2014). Synthetic or natural substances that can inhibit, delay, or repair damage caused by UV exposure are referred to as photo-chemoprevention.

The results of this study indicated that the antioxidant activity of crude extract *C. heyneana* was smaller than curcuminoid. This was related to the content of demethoxy curcumin greater than curcumin in *C. heyneana*. This research also showed that curcuminoid levels in crude *C. heyneana* extract plays a significant role in antioxidant activity. Crude extract C. heyneana also had activity of tyrosinase inhibitor and collagenase inhibitor. This corresponded to improvements in the appearance of UV induced-rat skin histomorphometry with parameters such as epidermal thickness, number of sunburn cells, collagen tissue, fibroblasts and elastin.

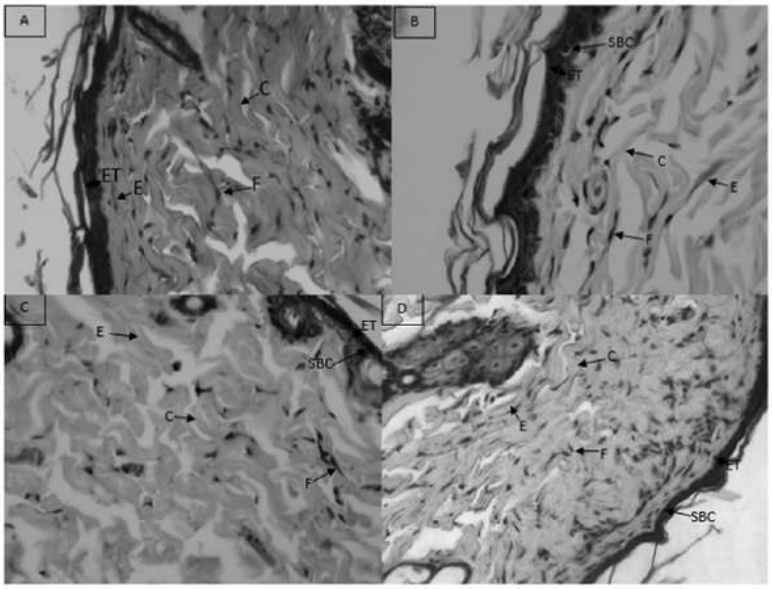


**Fig. 3.** Histologic analysis of rat skin specimen. Histologic rat skin specimens was performed using H & E staining. (A) Normal group, no UV exposure + no treatment; (B) vehicle group, UV exposure + vehicle treatment; (C) crude extract group, UV exposure + crude extract treatment; (D) retinoic acid group, UV exposure + retinoic acid treatment. Cellular morphology was viewed at 40× magnification. ET, epidermal thickness; SBC, sunburn cell; C, collagen fiber; F, fibroblast; E, elastic fiber.

**Table 3**
Histologic analysis of UV exposed rat skin specimens.

| Groups | Epidermal thickness (μm) | Number of SBC/mm$^2$ in epidermis | Number of fibroblasts/10,000 μm$^2$ | Distance between collagen fibers (μm) | Elastic fibers |
|---|---|---|---|---|---|
| Normal | 4.07 ± 0.27b | 4.40 ± 0.28b | 41.53 ± 0.60b | 3.09 ± 0.10b | ++++ |
| Vehicle | 12.21 ± 0.41a | 60.80 ± 2.56a | 9.74 ± 0.26a | 14.70 ± 0.40a | + |
| CE | 8.09 ± 0.10a,b | 11.80 ± 0.95ab | 16.60 ± 0.33a,b | 11.60 ± 0.42ab | ++ |
| RA | 4.14 ± 0.27b | 28.00 ± 1.76ab | 40.86 ± 0.30b | 3.02 ± 0.16ab | ++++ |

Values are expressed as mean ± SEM (n = 10); means in the same column followed by different letters are significantly different at $p < 0.05$ using Duncan's tests. (a) Significantly different from the normal group at $p < 0.05$. (b) Significantly different from the vehicle group at $p < 0.05$. Normal group, no UV exposure + no treatment; vehicle group, UV exposure + vehicle treatment; CE group, UV exposure + crude extract treatment; RA group, UV exposure + retinoic acid treatment.

## 5. Conclusions

*C. heyneana* known as Temu giring has been used traditionally as skin care. The present study showed that crude extract of *C.heyneana* has the potential to be used as an anti-aging compound, and may be developed into phytocosmeceutical products.

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## Glossary

*AR:* Arbutin
*BDMC:* Bis-demethoxy Curcumin
*C:* Cucumin
*CA:* Caffeic Acid
*CE:* Crude Ethanol Extract
*CUR:* Curcuminoid
*DMC:* Demethoxy Curcumin
*EA:* Ethyl Acetate Extract
*ET:* Epidermis Thickness
*HEX:* n-Hexane Extract
*MEOH:* Methano Extract
*RA:* Retinoic Acid
*SBC:* Sunburn Cell
*SEM:* Standart Error of Mean
*T:* Trolox
*TLC:* Thin Layer Chromatography
*UV:* Ultra Violet

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