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The Potential of Temulawak (*Curcuma xanthorrhizaRoxb.*) as a Rejuvenating Agent

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Abstract: This study was conducted to determine the rejuvenating activity of Temulawak (*Curcuma xanthorrhizaRoxb*.)extract cream 10%in the UVB-exposed rats' skin. The efficacy of the Temulawak extract cream was compared with Vitamin C cream 10% as a positive control. The subjects were 30 female Wistar rats, aged 6-8 weeks, and weighing 180-200 grams. The samples were divided randomly and equally into three groups, namely group 1 (control, exposed to UVB only), group 2 (exposed toUVB + treated with Temulawakextract cream 10%) and group 3 (exposed toUVB + treated with Vitamin C cream 10%).Qualitative phytochemical screening of the Temulawak extract revealed the content of alkaloids, saponins, glycosides, flavonoids and terpenoids/steroids, but not tannins. The antioxidant capacity of the extract was found to be 37.32 mg/mL. Before and after treatment for 4 weeks, the skin elasticity, water content and pore size were examined with Skin Analyzer EH 900 U. The results showed that both Temulawak extract and Vitamin C cream improved the skin hydration significantly(from 3 to 15% and 4 to 30%, respectively). Temulawak extract and Vitamin C cream also ameliorated the reduction in skin elasticity (from 50 to 40% and 50 to 50%, respectively) and reduced the diameter of the skin pore (from 0.05 to 0.03 mm and 0.06 to 0.01 mm, respectively). Consistently, the effect of Temulawak extract cream was less than Vitamin C. In general, we reported that both Temulawak extract and Vitamin C cream can be used as a rejuvenating agentwith the efficacy of Temulawak extract cream < Vitamin C cream. Further study is required to determine the potential side effect of both creams. Moreover, clinical study is warranted to prove their efficacy as a rejuvenating agent in humans.
Keywords: Temulawak (*Curcuma xanthorrhizaRoxb*.), Rejuvenating Agent, Phytochemical, UVB, Skin Aging, Rats, Anti-Aging Medicine

I. INTRODUCTION

The aging of an organism begins from the time when one is born. Advancing age is followed by deterioration of the function and the structure of the cells, tissues and organs including the skin. As the most voluminous organ of the body, the skin shows an obvious and visible sign of aging. Skin aging is characterized by features such as wrinkling, loss of elasticity, laxity, and rough-textured appearance. Therefore, for many people, especially females, a considerable amount of daily expense is occupied by cosmetics and pharmaceuticals attempting to prevent or reverse skin aging. Anti-aging medicine is the branch of medicine that aims to reduce the chance of developing aging-related deterioration in skin function and structure [1-4].

Efforts that can be done to improve health and slow down the aging process include improving diet, exercising more and adopting a healthy lifestyle. Recently, antioxidants have become the center of anti-aging research. The free radical theory of aging hypothesizes that free radicals are responsible for the age-related damage at the cellular and tissue levels. In a physiological condition, a balanced equilibrium exists among oxidants, antioxidants and biomolecules. Excess generation of free radicals overwhelms natural cellular antioxidant defenses leading to oxidation and cellular functional impairment. The identification of free radical reactions as promoters of the aging process implies that interventions aimed at limiting or inhibiting them should be able to reduce the rate of aging[5]. Antioxidants are exogenous or endogenous molecules that mitigate any form of oxidative stress or its consequences. They may act from directly scavenging free radicals to increasing antioxidative defenses [6]. Antioxidants are present in natural foods but added amounts beyond the diet may detoxify excess free radicals during oxidative stress. One source of natural antioxidants is the Temulawak(*Curcuma xanthorrhizaRoxb.*)[7].

Curcuma xanthorrhizaRoxb., commonly known as Java turmeric or Temulawak, has been used as a traditional medicinal plant in some tropical countries such as Indonesia and Malaysia for food and medicinal purposes to treat hepatitis, liver disorders, stomach diseases, rheumatism, and skin inflammation [8]. *C. xanthorrhiza* contains bioactive compounds, such as curcuminoids, camphor, geranyl acetate, zerumbone, β-curcumene, zingiberene, ar-curcumene, and xanthorrhizol[9]. Xanthorrhizol, a sesquiterpenoid compound

isolated from the rhizome of C. xanthorrhiza, has been reported to possess a variety of biological properties, including antibacterial, antifungal, anticancer, phytoestrogens, and neuroprotective activities [10–12]; however, its function as a rejuvenating agent has never been investigated. This study was conducted to determine the rejuvenating activity of Temulawak (*Curcuma xanthorrhizaRoxb*.) extract cream 10% in the UVB-exposed rats' skin. The efficacy of the Temulawak extract cream was compared with Vitamin C cream 10% as a positive control.

II. MATERIALS AND METHODS

2.1 Animals

Thirty6 to 8-week-old experimentally naïve female Wistar albino rats with an average initial body weight of 180 – 200 g (Universitas Prima Indonesia, Medan, North Sumatra, Indonesia) were used. Animals were housed under environmentally controlled conditions, food and water were available ad libitum throughout the experiment. Animals were allowed to adjust to a new condition for seven days. The protocols used conformed to guidelines of animal studies and were approved by the committee on the ethics of animal experiments in the Faculty of Medicine, Universitas Prima Indonesia (No. 004/KEPK/UNPRI/VIII/2020).

2.2Preparation of the extract and phytochemical analysis

Temulawak (*Curcuma xanthorrhizaRoxb*.) extract was prepared according to the previously described method [13]. In brief, plant materials were first chopped, then washed with fresh water to remove dirt and other contaminants. They were shade-dried for several days with occasional sun drying. The dried materials were pulverized into a coarse powder, then macerated with 70% ethanol in the Soxhlet apparatusfor 10 days with frequent agitation. The crude extract from the previous step was filtered by using Whatman paper and the solvent was dried by vacuum rotary evaporator under reduced pressure at a maximum temperature of 40 °C. The final fraction wasdesignated as crude extract and stored at -20 °C until further use. The crude extracts were tested for the presence of alkaloids, phenolic, flavonoids, steroids, tannins, saponins and triterpenoid glycosides were performed using a previously described method [14]. The qualitative results are expressed as (+) for the presence and (−) for the absence of phytochemicals.The free radical-scavenging activity was measured in terms of hydrogen donating or radicalscavenging ability using the stable radical 1-1-diphenyl 2-picryl hydrazyl (DPPH) as previously described[13].

2.3Experimental design

The samples were divided randomly and equally into three groups, namely group 1 (control, exposed to UVB only), group 2 (exposed to UVB + treated with Temulawak extract cream 10%), and group 3 (exposed to UVB + treated with Vitamin C cream 10%).All groups were irradiated with UVB light three times a week (on Monday, Wednesday, and Friday), starting from 50 mJ/cm2 in the first week, 70 mJ/cm2 in the second week, and 80 mJ/cm2 for the last two weeks, resulting in a sum of 840 mJ/cm2 of UVB received in four weeks[15].The irradiation distance was 3 cm from the skin for 45 minutes. The measurement of the exposure dose was carried out using a UV meter (Beltron, Germany). The creams were applied on the rats' skin twice a day: 20 minutes before UV irradiation to allow absorption of cream, and 4 hours after irradiation (reactive oxygen species or ROS formation starts 4 hours after UV exposure). Creams were also applied on days when no irradiation was performed. To avoid the acute effects of irradiation, the skin hydration, elasticity and pore diameter were examined 48 hours after the last irradiation.

2.4Examination of Skin Aging Parameter

The anti-aging study was conducted using non-invasive biophysical methods. The anti-aging parameters include moisture content, elasticity, and pore size were measured by Skin Analyzer EH 900 U.

III. RESULTS AND DISCUSSION

3.1 Phytochemical screening of Temulawak (*Curcuma xanthorrhizaRoxb*.)extract

Phytochemical analysis and antioxidant activity of Temulawak (*Curcuma xanthorrhizaRoxb*.)extract were carried out in this research. The preliminary phytochemical screening revealed the presence of alkaloids, saponins, glycosides, flavonoids, and terpenoids/steroids. The tannins were not detected in the Temulawak (*Curcuma xanthorrhizaRoxb*.) ethanolic extract (Table 1). The results of this study were supported by the results of research conducted by Prasetyorini*et al.*[16] which showed qualitatively that the extract of Temulawak contains alkaloid, flavonoid, steroid, quinone and triterpenoid compounds, and does not contain tannins. Slightly different from the results of this study, the results of screening by other researchers showed several secondary metabolites in the Temulawak extract including phenols, terpenoids, flavonoids, saponins, alkaloids and contain tannins[17]. Furthermore, Putri *et al.*[18] also showed that the ethanol extract of Temulawak contains flavonoids, saponins, and tannins. These differences can be caused by different plant sources between studies. Secondary

metabolites are synthesized as an adaptation response in plants to the environment which includes factors such as temperature, humidity, soil pH, wind, environmental toxicity, and others. The number and functional groups present in secondary metabolite compounds can vary from one place to another[19]. Other researchers also mentioned that the synthesis and accumulation of secondary metabolites in plants are very complex, influenced by many factors including internal developmental genetic factors (genes, enzymes) and by external environmental factors (light, temperature, water, salinity) [20].

Table 1:Phytochemical screening and DPPH radical-scavenging activitiesof Temulawak(*Curcuma xanthorrhizaRoxb*.)ethanolic extract

Screening	Test	Presence or Amount
Alkaloids	Dragendorff's Test	(+)
Flavonoids	Kokate's method	(+)
Tannins	Kokate's method	(-)
Saponins	Kokate's method	(+)
Triterpenoid/steroids	Salkowski test	(+)
Glycosides	Keller-Killiani test	(+)
Antioxidant activity	DPPH radical-scavenging test	37.32 mg/mL

Next, the DPPH radical-scavenging activity revealed that the IC50 value of Temulawak (*Curcuma xanthorrhizaRoxb*.)ethanolic extract was 37.32 mg/mL (Table 1).According to Phongpaichit*et al*.[21] extracts with IC50 values ranging from 10 to 50 mg/mL are considered to have strong antioxidant activity, extracts that have IC50 values ranging from 50 to 100 mg/mL are considered to show moderate antioxidant activity, while extracts with IC50>100 mg/mL are considered to have weak antioxidant activity. This indicates that the Temulawak (*Curcuma xanthorrhizaRoxb*.)extract cream in this study had a very strong antioxidant activity.The results of this study are supported by many previous studies. Kuntorini*et al*.[22]found Temulawak extract has strong antioxidant activity with an IC50 range of 17.70 to 55.22 mg/ml.Different from the results of this study, both Rosidi*et al*.[23]and Septyanti*et al*.[24] reportedthat Temulawak extract was categorized as a compound with intermediate antioxidant activity.

3.2 Anti-Aging Activity of Temulawak (*Curcuma xanthorrhizaRoxb*.)extract

The anti-aging activity of Temulawak (*Curcuma xanthorrhizaRoxb*.)extract was examined using the Skin Analyzer EH 900 U, where the test parameters include: water content (skin moisture), elasticity, and pore size. Measuring anti-aging activity begins by measuring the initial skin condition before treatment was carried out and after 4 weeks of treatment, in order to determine the effect of the cream used.

3.2.1 Water content

The results showed that the water content of the rats' skin in the group given the cream base (Agroup) in the first week was 3% which was categorized as dry and after 4 weeks of treatment the water content increased to 4% but still included in the dry category. In the Bgroup treated with Temulawak (*Curcuma xanthorrhizaRoxb*.) extract cream 10% for 4 weeks, there was an improvement in the water content of the skin, from 3% (dry) to 15% (normal). In the C group treated with vitamin C cream 10%, there was a more significant improvement in skin moisture content, from 4% (dry) to 30% (higher) (Table 2). The results of this examination indicated that administration of both Temulawak extract cream and vitamin C cream improves the skin water content in the UVB-exposed rats' skin. The effect of Temulawak extract cream was less thanvitamin C cream in this regard.

3.2.2 Elasticity

Results showed that there was a significant decrease in skin elasticity in group A treated with a cream base, which was from 55% (normal) to 30% (loosen skin). Treatment of Temulawak extract cream 10% in group B reduced the decrease in skin elasticity as observed in group A. In group B the decrease in skin elasticity only occurred from 50% (normal category) to 40% (aging category). Furthermore, in group C given vitamin C cream10%, there was no change in skin elasticity, which was from 50% (normal) and maintained to 50% (normal) after 4 weeks of the study (Table 2). These results indicated the anti-aging activity of bothTemulawak extract cream and vitamin C cream, with Temulawak extract cream being less effective thanvitamin C cream.

3.2.3 Pore size

Next, we showed that there was no change observed in the skin pore diameter skin elasticity in group A treated with a cream base, which was from 0.06 mm (normal)to0.05 mm (normal). Treatment of Temulawak extract cream 10% in group B reducedpore size from 0.05 mm (normal)to0.03 mm (small). Furthermore, in group C given vitamin C cream10%, this decrease was more evident which was from 0.06 mm (normal) to 0.01 mm (smooth)after 4 weeks of the study (Table 2). These results indicated the anti-aging activity of bothTemulawak extract cream and vitamin C cream, with Temulawak extract cream being less effective thanvitamin C cream.

Table 2:Anti-Aging activitiesofTemulawak(*Curcuma xanthorrhizaRoxb.*)extract

Screening	Group	Week	
		I	IV
Water content	**A**	Dry (3%)	Dry (4%)
	B	Dry (3%)	Normal (15%)
	C	Dry (4%)	Higher (30%)
Elasticity	**A**	55% (Normal)	30% (Loose Skin)
	B	50% (Normal)	40% (Ageing)
	C	50% (Normal)	50% (Normal)
Pore size	**A**	0.06 mm (Normal)	0.05 mm (Normal)
	B	0.05 mm (Normal)	0.03 mm (Small)
	C	0.06 mm (Normal)	0.01 mm (Smooth)

A: Group of rats treated with a cream base + UVB exposure for 4 weeks
B: Group of rats treated with Temulawak extract cream 10%+ UVB exposure for 4 weeks
C: Group of rats treated with Vitamin C cream 10% + UVB exposure for 4 weeks

The use of creams that contain rhizome extract as a rejuvenating agent has previously been reported. Ginger (*Zingiber officinale*) has been reported both in animal and clinical trials in humans to possess anti-aging activity. Tsukahara *et al*.[25] proved that ginger extract cream inhibited elastase and prevented wrinkle formation in mice exposed to UVB radiation. Furthermore, this ginger extract cream was also shown to increase skin elasticity and reduce the wrinkle but did not affect water content in 20 middle-aged Japanese men after treatment for 1 year[26]. The result of this study was similar to the results of those previously reported, which used extract cream containing rhizome as a rejuvenation agent.

Oxidative stress induced by reactive oxygen species can accelerate skin aging, which is characterized by wrinkles and atypical pigmentation. Because UVB increases the formation of ROS in cells, skin aging in this study was induced experimentally by exposing rats to UVB. The use of antioxidants is an effective approach to prevent symptoms associated with skin aging[27]. To date,an antioxidant is considered the most widely used anti-aging agents[28]. The potential of Temulawak as an antioxidant has been widely researched as described above, and in this study, it has been proven that the potential ofa cream containingTemulawakextract as a rejuvenation agent (anti-aging agent) through preclinical tests on animals in this case rats. Administration of Temulawak extract cream in this study can improve all anti-aging parameters including water content, elasticity and pore size.

However, when compared to vitamin C, the results of this study showed that Temulawakextract cream has relatively lower anti-aging activity. Vitamin C for the skin can act as a powerful antioxidant that protects the skin against the negative influence of external factors such as pollution, UV rays, climate, air conditioning, cigarette smoke, etc. In addition, vitamin C stimulates the formation of skin collagen which will maintain skin elasticity, flexibility, and smoothness. Vitamin C can also brighten the skin, without any adverse side effects[29].

Vitamin C works as an antioxidant, which prevents the oxidation of the skin's constituent fibers, namely collagen, and elastin, at the expense of being oxidized by free radicals. Vitamin C also functions as a co-factor, accelerates the formation of collagen. Vitamin C stimulates and increases skin collagen production by increasing the reproductive ability of old dermal fibroblast cells. Fibroblasts are cells of the connective tissue that produce collagen and elastin fibers and are found in the dermis. Another advantage of vitamin C is that it can brighten the skin by emphasizing the skin pigmentation process[30].

IV. CONCLUSION

In general, we found that Temulawak extract contains alkaloids, saponins, glycosides, flavonoids, and terpenoids/steroids, but not tanninswith high antioxidant activity with a low IC_{50}. Next, we found that both Temulawak extract cream 10% and Vitamin C cream 10% can be used as a rejuvenating agentwith the efficacy of Temulawak extract cream < Vitamin C cream. Further study is required to determine the potential side effect of both creams. Moreover, clinical study is warranted to prove their efficacy as a rejuvenating agent in humans.

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