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Antioxidants Activity of Face Toner from Ethyl Acetate Fraction of Okra Seeds

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ABSTRACT

This study explores the potential of okra seeds (Abelmoschus esculentus L.) as a natural face toner, using maceration extraction method. A total of 750 g of Okra was dried for 72 h using methanol solvent showed that okra seeds contain flavonoids with 5% FeCl reagent. FTIR analysis revealed a characteristic functional group profile of flavonoids, including hydroxyl (3400 cm-1), carbonyl (1722 cm-1), and aromatic ring (1591 cm-1 and 1277 cm-1) groups. The antioxidant test was selected from the ethyl acetate fraction at a wavelength of 517 nm with an IC50 value of 44.90 µg/mL, and can be categorized as a very strong antioxidant. DPPH test of toner formula with BHT, ethyl acetate extract, and control produced IC50 values of 85.67 µg/mL, 88.79 µg/mL, 105.69 µg/mL, respectively. The optimal conditions were achieved with 25, 50, and 100 ppm of ethyl acetate fractions. Future studies could expand on these findings by exploring the long-term effects and consumer acceptability of okra seed toners in broader populations.

Keyword: Antioxidant, DPPH Method, Face Toner, Flavonoid, Okra Seeds.

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INTRODUCTION

Indonesia is a growing cosmetics market, contributing significantly to the country's economy. According to the Ministry of Industry (Kemenperin), the Indonesian cosmetics industry is estimated to grow by 5.91% per year in 2021, driven by increasing demand, especially among the middle class [1]. The population of Indonesian women who use cosmetics has reached to 126.8 million, causing skin care products such as facial toners to be widely used to improve skin health and beauty [2], [3]. Face toners, which are used to cleanse and moisturize, often contain alcohol, which helps reduce oil, acts as a preservative, and increases product absorption [4], [5]. However, concerns have arisen because these products contain alcohol and other chemicals.

Excessive use of alcohol in cosmetics can result in adverse health consequences, such as dryness, irritation, and the breakdown of sebum, a vital component for maintaining skin health. Elevated sebum production can lead to the development of blackheads and acne, while prolonged use of alcohol can worsen dryness and irritation [6], [7], [8]. Cosmetic goods containing synthetic chemicals can potentially elicit negative reactions, since individual sensitivities differ. Face toners are specifically formulated to replenish the skin's moisture, regulate pH levels, constrict pores, alleviate inflammation, and function as antibacterial

agents [9], [10]. An optimal toner should possess qualities such as being non-irritating, invigorating, non-adhesive, pleasant in aroma and color, and maintaining a pH 4-7. Typical toner compositions include of solvents, humectants, pH indicators, active components, preservatives, colors, and scents [11].

Cleansing the face with a high pH cleanser can cause skin irritation, leading to the development of toners that balance skin pH. Toners typically contain beta hydroxy acid (BHA) and alpha hydroxy acid (AHA) and are now formulated to include moisturizers that reduce acne and control oiliness [12]. Natural ingredients, such as okra seed extract, are being explored for their potential in skincare. Okra is nutrient-rich and contains bioactive compounds like flavonoids and polysaccharides, which have antioxidant, anti-inflammatory, and anti-cancer properties [13], [14]. Okra seeds are high in flavonoids, offering antioxidant benefits and soothing skin irritation. These qualities make okra an appealing ingredient for the cosmetic, pharmaceutical, and nutraceutical industries.

The study focuses on optimizing the extraction of polyphenol compounds from okra seeds to enhance the quality and bioactivity of the extract. Research shows that okra seeds contain significant amounts of phenolic and flavonoid compounds, making them beneficial for skin health. The antioxidant activity of okra seeds helps combat free radicals and oxidative stress, while their healthy fat content maintains skin moisture. The research aims to utilize flavonoid compounds from okra seeds in face toner formulations.

METHODS

The material used for this study is Okra were purchased from the Berastagi Supermarket, in North Sumatra, Indonesia. The following chemicals were used in the isolation and analysis process such as ethyl acetate, methanol, HCl, H_2SO_4, $FeCl_3$, chloroform, essential oil, phenoxyethanol, pH 5.5 buffer, Mg powder, and DPPH powder.

Sample Preparation

Okra is cut, and the seeds are taken. Then, after the okra seeds are obtained, they are dried in an open room for 72 h and then ground into 1300 g of okra seeds powder (Abelmoschus esculentus L.). The results of drying okra seeds can be seen in **Figure 1.**

Figure 1. Dried okra seeds.

Extraction, Partition and Phytochemical Screening of Okra Seeds (Abelmoschus esculentus L.)

Extraction and Partition

The extraction process was carried out by weighing 1300 g of okra seeds (Abelmoschus esculentus L.), which were then maceration with technical methanol for 24 h at room temperature. The maceration was repeated until a clear extract was obtained, which was then evaporated using a rotary evaporator to produce a dry methanol extract. This extract was dissolved with ethyl acetate, filtered, and the filtrate was evaporated again to obtain ethyl acetate extract. The methanol residue was partitioned with n-hexane until the n-hexane layer was clear, then both layers (methanol and n-hexane) were evaporated to produce methanol extract and n-hexane extract.

Phytochemical Screening of Okra Seeds (Abelmoschus esculentus L.)

Methanol, ethyl acetate, and n-hexane extracts of okra seeds *(Abelmoschus esculentus L.)* were tested for alkaloids, flavonoids, tannins, steroids, triterpenoids, and saponins [15].

Alkaloid

4 mg of sample was dissolved with methanol and ammonia, filtered, added 2M HCl, then mixed with Mayer, Wagner, and Dragendorff reagents. Positive results are indicated by white, brown, or orange precipitate.

Flavonoid

1 mg of methanol extract is dissolved with methanol, Mg ribbon and concentrated HCl were added. Yellow, blue, orange, or red color indicates a positive result.

Tannin

1 mg sample was dissolved in methanol, boiled, filtered, and 1% FeCl3 was added. Dark blue or greenish black color indicates a positive result.

Steroids and Triterpenoids (Liebermann-Burchard Test)

1 mg sample was dissolved with anhydrous acetic acid and concentrated H_2SO_4. Purple to orange color indicates triterpenoids, while blue or green indicates steroids.

Saponin

1 mg of ethanol extract was dissolved in distilled water, shaken, and 1M HCl was added. Stable foam for 10 min indicated a positive result.

Antioxidant Test and Okra Seeds Face Toner

Antioxidant Test

Antioxidant testing follows previous research conducted Sembiring et al [16]. The variation of methanol extract solution concentration was made by first making a stock solution of 1000 mg/mL, namely by dissolving 10 mg of methanol extract into methanol p.a in a 100 mL measuring flask. The stock solution, variations of solution concentrations of 10, 20, 30 and 40 mg/mL were made. Then, a 0.3 mM DPPH solution was made by dissolving 11.83 mg of DPPH powder in methanol p.a in a 100 mL measuring flask and covered with aluminum foil and 1 mL of 0.3 mM DPPH solution was added to the 2.5 mL methanol extract solution in a test tube, homogenized and incubated for 30 min at 37°C. Then the absorbance was measured with a maximum wavelength of 517 nm. Ascorbic acid was used as a comparison. A similar procedure was carried out on ethyl acetate extract and n-hexane extract.

Face Toner Test

The formulation of facial tonic is based on the basic formulation and pre-formulation study of face tonic by dissolving okra seed extract in glycerin, phenoxy ethanol, hydrosol rose (water fraction), and stirred evenly. Furthermore, essential oil and BHT that have been dissolved in polysorbate 20 (oil fraction) are added, and the water phase is added to the oil phase. Then the mixture is added with pH 5.5 buffer solution ad 100 ml and stirred until homogeneous.

Calculation of DPPH radical scavenging activity was carried out by the percentage difference (%) between sample and control absorption. Percent increase (%) of inhibitor indicates an increase in antioxidant activity [17]. Percent (%) of inhibitor was calculated using Eq. (1):

$$\%\ \text{Inhibitor} = \frac{Absorbance\ Control - Absorbance\ Sample}{Absorbance\ Control} \quad (1)$$

Characterization

The Fourier Transform Infrared (FTIR) spectra of the okra seeds were obtained using the IR Prestige-21 Shimadzu spectrometer to identify functional groups present in okra seeds. Data were collected within the spectral range of 4000 to 400 cm^{-1}.

RESULTS AND DISCUSSION

Extraction and Partition of Okra Seeds (Abelmoschus esculentus L.)

Okra seeds are extracted by maceration using methanol as the solvent. Maceration is a basic extraction technique characterized by its extended extraction time and limited extraction effectiveness. It has potential use in the extraction of thermolabile components [18], [19]. Methanol can dissolve almost every variety of secondary metabolites, such alkaloids obtained from plants, steroids, saponins, and flavonoids [20], [21]. Additionally, n-hexane is non-polar and ethyl acetate is semi-polar, making both of these solvents highly suitable for partitioning. The extraction and partitioning processes yielded three extracts from okra seeds, namely, ethyl acetate, methanol, and n-hexane in **Figure 2**.

Figure 2. Extract (a) ethyl acetate; (b) methanol, and (c) n-hexane of okra seeds.

The better results of okra seed extract in ethyl acetate over the extract in n-hexane can be attributed to the significant disparity in polarity between the two solvents. Given that the acquisition of chemicals relies on the uniformity of solvents, it may be inferred that the compounds present in Okra seeds extract possess polarities that are almost equivalent to those of ethyl acetate. Compounds exhibiting solvent-like properties will selectively dissolve in other compounds with comparable properties. A phytochemical screening was conducted on each extract to identify the presence of secondary metabolite chemicals.

Table 1. Phytochemical screening of ethyl acetate, methanol, n-hexane of okra seeds

Extract	Weight (g)	Content (%)	Flavonoid
Ethyl acetate	84.696	40	+
Methanol	116.457	55	-
n-hexane	10.587	5	+

(+) = contain compound, (-) = do not contain compound

In Table 1, the findings of phytochemical screening of the methanol and ethyl acetate extract of okra seeds containing steroids, triterpenoids, flavonoids, saponin and tannins were presented. The black colour observed with the addition of $FeCl_3$ 5% is not attributed to the tannins present in the ethyl acetate extract. Tannins are intrinsically insoluble in ethyl acetate [22].

Antioxidant Test with DPPH method

The IC_{50} value serves as an indicator of antioxidant activity and is derived from the regression line equation, where concentration is the independent variable and percent silencing is the dependent variable. The antioxidant activity of the methanol extract, ethyl acetate extract, and n-hexane extract of okra seeds was assessed by DPPH free radical scavenging employing UV-visible spectroscopy at a maximum wavelength of 517 nm with absorbance's control 0.887 in **Table 2**.

Table 2. Absorbance, and Percent inhibition of Ascorbic Acid with three times repetition.

Concentration (ppm)	Absorbance (Repetition)			Mean	% Inhibition	IC_{50}
	1	2	3			
3	0.409	0.410	0.410	0.410	55.68	
6	0.298	0.298	0.298	0.298	67.78	
9	0.177	0.178	0.177	0.177	80.86	**1.381**
12	0.089	0.089	0.088	0.089	90.38	
15	0.042	0.040	0.041	0.041	95.57	

Table 2 shown the linear regression equation yielded an inhibition concentration 50% (IC50) value. The percentage of inhibition increases proportionally with the concentration of the sample, while the concentration of DPPH decreases significantly. The abstraction of hydrogen radicals from antioxidant compounds by DPPH free radicals' results in the formation of DPPH (1,1-diphenyl-2-picrylhydrazine). The reduction of free radicals is further evidenced by a shift in the solution's colour from purple to a faint yellow colour. A higher silencing percentage indicates a greater level of antioxidant activity in the sample in Figure 3. IC_{50} is the concentration of an antioxidant chemical needed to abolish 50% of DPPH free radicals within 15-30 minutes.

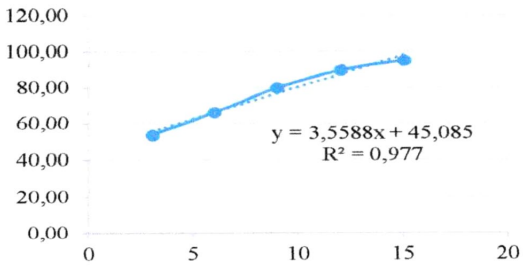

Figure 3. Linear regression equation of ascorbic acid.

Furthermore, ethyl acetate fraction has special types compared to methanol and n-hexane. Ethyl acetate can extract a unique range of antioxidant compounds that might not be as effectively extracted by methanol or n-hexane. This includes certain phenolic acids, flavonoids, and other semi-polar antioxidants that contribute significantly to the overall antioxidant activity. The antioxidants extracted by ethyl acetate might work synergistically with each other or with those from other fractions to provide a more potent antioxidant effect. Testing the ethyl acetate fraction helps determine if these specific compounds or combinations have higher antioxidant activity. The antioxidant activity of the ethyl acetate fraction to the methanol and n-hexane fractions, researchers can identify which solvent extracts the most potent antioxidants from a particular material [23], [24]. The absorbance, percent inhibition, and IC_{50} shown in **Table 3**.

Table 3. Absorbance, and Percent inhibition of Ethyl Acetate extract with three times repetition.

Concentration (ppm)	Absorbance (Repetition)			Mean	% Inhibition	IC_{50}
	1	2	3			
6.25	0.713	0.714	0.714	0.714	19.50	
12.25	0.685	0.688	0.690	0.688	22.44	
25	0.523	0.520	0.526	0.523	41.04	4,90
50	0.337	0.338	0.337	0.337	62.01	
100	0.145	0.145	0.145	0.145	83.65	

In Figure 4, as the concentration of the ethyl acetate extract increases, there is a corresponding increase in the percentage inhibition. This suggests a dose-dependent response, where higher concentrations of the extract lead to greater antioxidant activity [25], [26]. At lower concentrations, the percentage inhibition might show a gradual increase, indicating that even at minimal doses, the extract exhibits some level of antioxidant activity. This can be attributed to the presence of potent antioxidant compounds in the ethyl acetate fraction.

The concentration increases, the graph might display a more pronounced rise in percentage inhibition. This phase indicates that the antioxidant compounds are becoming more effective as their concentration in the solution increases. The concentration-dependent increase in percentage inhibition confirms the presence of bioactive compounds with antioxidant properties in the ethyl acetate extract [27], [28]. The plateau effect at higher concentrations indicates an optimal range for maximum efficacy without wasting excess material.

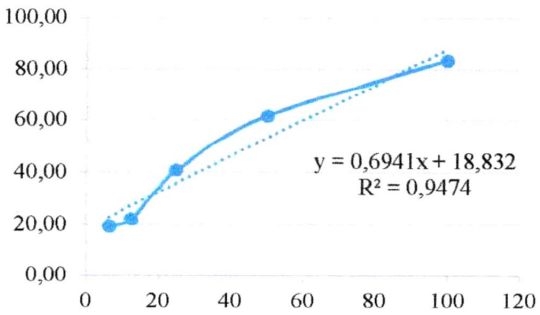

Figure 4. Linear regression equation of ethyl acetate extract.

Face Toner

The formulation of the face toner preparation involved the use of a controlled concentration of BHT, ethyl extract, and a control. This particular concentration was selected due to its nature as a cosmetic formulation designed for skin care. An extract antioxidant activity test was conducted to verify the presence of antioxidant activity in cucumber fruit extract. This is supported by the IC_{50} value of 21.22 µg/ml obtained from cucumber fruit extract. This IC_{50} value falls under the category of potent antioxidants [29].

The formulation was derived by incrementally raising the concentration of the extract, beginning with concentrations of 6.25, 12.5, 25, 50, and 100 ppm in Table 4. As the concentration of the extract in the recipe increases, the percentage of inhibition achieved also increases.

Table 4. Absorbance, and Percent inhibition of Toner + BHT with three times repetition.

Concentration (ppm)	Absorbance (Repetition)			Mean	% Inhibition	IC_{50}
	1	2	3			
6.25	0.980	0.979	0.982	0.980	19.50	
12.25	0.920	0.915	0.916	0.917	7.56	
25	0.755	0.752	0.756	0.754	23.99	**85.67**
50	0.549	0.548	0.552	0.550	44.56	
100	0.494	0.494	0.494	0.491	50.50	

Furthermore, the face toner formulation, the ethyl acetate fraction of okra seeds *(Abelmoschus esculentus L.)* was made with concentrations of 6.25, 12.5, 25, 50, and 100 ppm in **Table 5**. The objective of the work was to assess if there were any alterations in the physical and chemical composition of the preparation, as well as its capacity to suppress the antioxidant activity derived from the ethyl acetate fraction of okra seeds *(Abelmoschus esculentus L)*.

Table 5. Absorbance, and Percent inhibition of Toner + ethyl acetate extract with three times repetition

Concentration (ppm)	Absorbance (Repetition)			Mean	% Inhibition	IC_{50}
	1	2	3			
6.25	0.986	0.993	0.987	0.989	0.30	
12.25	0.891	0.890	0.891	0.891	10.18	
25	0.774	0.770	0.775	0.773	22.08	88,79
50	0.557	0.552	0.555	0.555	44.05	
100	0.501	0.515	0.510	0.509	48.69	

In **Table 6**, the absorbance and percent inhibition of the control sample were analyzed to provide a baseline comparison for evaluating the effectiveness of BHT (Butylated Hydroxytoluene) and the ethyl acetate extract. By comparing these values, it was observed that both BHT and the ethyl acetate extract exhibited significant antioxidant activity. The percent inhibition values indicate that the ethyl acetate extract, like BHT, effectively reduces oxidative stress, which is crucial for its potential use in skincare formulations such as face toners. This comparison highlights the efficacy of the ethyl acetate extract as a natural antioxidant source, offering a promising alternative to synthetic antioxidants like BHT [30], [31].

Table 6. Absorbance, and Percent inhibition of control with three times repetition

Concentration (ppm)	Absorbance (Repetition)			Mean	% Inhibition	IC_{50}
	1	2	3			
6.25	0.990	0.993	0.990	0.991	0.10	
12.25	0.985	0.984	0.983	0.984	0.81	
25	0.883	0.881	0.880	0.881	11.19	105,69
50	0.601	0.605	0.610	0.605	39.01	
100	0.588	0.585	0.593	0.589	40.63	

The regression equation and IC_{50} values of face toner with BHT, ethyl acetate extract, and control as a comparison can be seen in **Table 7**.

In face toner formulations using okra seeds, BHT, ethyl acetate extract, and control. The concentration of okra seeds (expressed in ppm) is closely related to antioxidant activity or other biological activities, as measured by percent inhibition

Table 7. Regression equation and IC50 values of face toner with BHT, ethyl acetate extract, and control

Sample	Regression Equation	IC_{50} values (µg/mL)
BHT	y = 0.5208x + 5.3847	85.67
Ethyl acetate extract	y = 0.4984x + 5.746	88.79
Control	y = 0.4729x + 0.021	105.69

and IC_{50}. This concentration indicates the amount of okra seeds used in the face toner formulation. The higher the concentration, the greater the amount of active ingredient available to have an effect on the skin. Percent inhibition is a measure of how effective the formulation is in inhibiting a particular activity, such as free radicals in an antioxidant test [32], [33].

Analysis FTIR

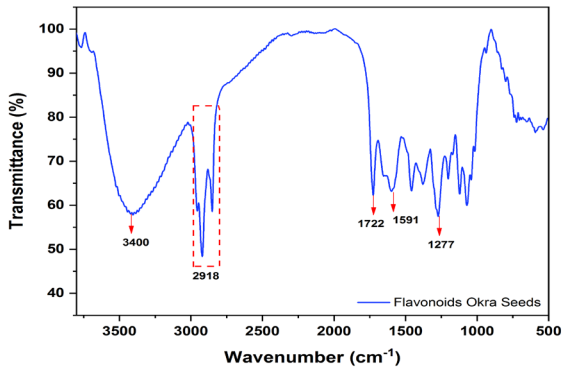

Figure 5. FTIR Spectrum of Okra Seed Flavonoids

The results of the FTIR analysis of okra seed flavonoids are presented in Figure 4.1. The broad stretching vibration at a wavenumber of 3400 cm^{-1} is associated with the hydroxyl (OH) group of alcohols. Furthermore, the wavenumber of 2918 cm-1 indicates the C-H group of hydrocarbons. The wavenumber of 1722 cm-1 corresponds to the carbonyl (C=O) group with bending vibration. The absorption at 1591 cm-1 indicates the C=C group of aromatic compounds in ring B of the flavonoid structure with bending vibration. Then, the absorption at 1277 cm-1 is associated with the aromatic C-O group in the phenolic bond connected to the aromatic ring with bending vibration. The results of this FTIR analysis are presented in **Table 8**.

Table 8. FTIR Analysis of Okra Seed Flavonoids

Wavenumber (cm^{-1})	Functional group	Vibration	Intensity
3400	O-H	Streaching	Strong
2918	C-H	Streaching	Medium
1722	C=O	Bending	Strong
1591	C=C	Bending	Medium to strong
1277	C-O	Bending	strong

CONCLUSION

The ethyl acetate fraction from okra seeds showed potential for facial toner production at 25, 50, and 100 ppm. The formulated toner exhibited strong antioxidant activity due to flavonoids, as confirmed by FTIR analysis revealing characteristic hydroxyl, carbonyl, and aromatic ring groups. Further studies are needed to explore higher concentrations and alternative methods for assessing antioxidant efficacy, thus enhancing our knowledge of okra's potential in skincare.

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