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Ultrasonic-assisted extraction of stingless bee (*Trigona* sp.) hive waste: Temporal variation and its potential as a natural sunscreen

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Abstract

This study investigated the use of ultrasonic-assisted extraction (UAE) on Stingless (*Trigona* sp.) beehive waste and evaluated its potential as a sunscreen. Samples were extracted at 30, 60, and 90 minutes using ultrasonic irradiation. The extract was then filtered and concentrated. Phytochemical screening was carried out using color reagents, UV-Vis spectrophotometry, IR spectrophotometry, and GC-MS analysis. The sunscreen activity was determined using a UV-Vis spectrophotometer within the 290-320 nm wavelength range, and SPF values were subsequently calculated according to the Mansur equation. The results showed that UAE extract, when exposed for 60 minutes, produced the highest yield of 17.03%, while 30 and 90 minutes produced yields of 9.03% and 13.50%, respectively. Phytochemicals identification demonstrated the presence of phenolics, flavonoids, tannins, and alkaloids. The UV spectrum exhibited distinct peaks at approximately 230 nm and 250-300 nm. FTIR analysis of the extract detected -OH, aliphatic C-H, C=O, C-OH, and -COOR groups. Furthermore, GC-MS analysis revealed the presence of compounds such as terpenoids, alcohols, fatty acids, and esters. SPF measurements of the 60-minute extract had the highest SPF value of 24.260, thereby classifying it within the ultra-protection category. In summary, ultrasonic extraction provides an effective approach for extracting bioactive compounds from Stingless beehive waste, and the resulting extract exhibits significant potential as a natural sunscreen.

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Keywords

Sunscreen, Stingless bee hive, Ultrasonic-assisted extraction, Trigona sp

Introduction

The UV spectrum is divided into three groups based on wavelength: Ultraviolet C (UVC), ranging from 100 to 290 nm, Ultraviolet B (UVB), between 290 and 320 nm, and Ultraviolet A (UVA), between 320 and 400 nm [1]. UVA is further divided into UVA2 (320 to 340 nm) and UVA1 (340 to 400 nm). Solar UV radiation at the Earth's surface comprises approximately 90 to 99% UVA and 1 to 10% UVB [2]. UVB causes sunburn and

DNA damage. This results in pyrimidine dimer changes associated with non-melanoma skin cancer [3].

Sunscreen is generally used to prevent erythema caused by sun exposure. Sunscreen can also reduce the risk of skin cancer caused by sunlight [4]. Sunscreens containing organic and inorganic ingredients have been proven to protect the skin from UV rays. This protective ability is evident from the mechanism of action of the active compounds in sunscreen. In general, the mechanism of organic compounds involves absorbing high-energy UV radiation at the π bonds of the benzene ring, which are conjugated with carbonyl groups, and releasing low-energy UV radiation [5]. One source of organic compounds that can absorb UV rays is the nest of the Stingless bee.

Honeybees of the Stingless (*Trigona*) species are widely cultivated in the Muntilan district, Magelang. According to information from honey farmers in Muntilan, harvesting honey from Stingless involves squeezing the honey from their nests to obtain both honey and the Stingless' bee nests [6]. The honeybee nests still contain phytochemicals that can be utilised for health benefits. The propolis content within the bee nests offers many advantages, such as in medicines, cosmetics, minerals, enzymes, and vitamins [7]. Previous research has shown that the secondary metabolites present in the ethanol extract of Stingless' nests include flavonoids, alkaloids, tannins, and terpenoids [6]. Previous research also mentioned that the extract of Stingless' honeycomb waste has the potential as a sunscreen with an SPF value of 5,832 at a concentration of 500 ppm [8].

Phenolic compounds such as flavonoids can be used as natural sunscreens. These compounds have aromatic rings in their molecular structure, allowing them to absorb UVA and UVB rays within the wavelength range of 200–400 nm, thus conferring optical protective properties [9]. Various extraction methods, both conventional and non-conventional, are employed to extract bioactive components from plant matrices. Different techniques and solvents can be used to extract phenolics from plant samples, depending on the type and distribution of the phenolics. Due to their high efficiency, low time and solvent requirements, and compatibility with heat-sensitive compounds, ultrasonic-assisted extraction (UAE) is an attractive technique for extracting plant bioactive components [10] .

Extraction assisted by ultrasound (UAE) is recognised as an environmentally friendly and highly efficient extraction technology. UAE has the potential to minimise or eliminate the need for organic solvents, thereby reducing its environmental impact. Additionally, UAE has been shown to significantly enhance the production of target bioactive components, making it an attractive method in the industry [11]. Other advantages of UAE include low solvent and energy consumption, as well as reduced extraction temperature and time. UAE can be used for the extraction of heat-sensitive, unstable compounds. The UAE is often employed in the extraction of various types of natural product materials [12]. The UAE method is crucial for improving the extraction of phenolic compounds from plant samples with minimal input, addressing energy and

environmental issues, and facilitating the release of active biological compounds from plant samples during cell disruption [10].

Extraction with UAE within 0-30 minutes results in a very sharp increase in % yield, from 30 to 120 minutes, the rate of yield increase is very slow, and after 120 minutes, the extraction results no longer improve [13]. The extraction times in this study with ultrasonic assistance are 30, 60, and 90 minutes. While previous research has predominantly concentrated on yield behavior within the 30–120 minute UAE range, limited consideration has been given to the impact of extraction time on the photoprotective (SPF) activity of Stingless bee hive waste extracts.

SPF analysis can be performed using several in vitro methods that are efficient and widely used, but they require specialised equipment and materials. Most of these methods are based on spectrophotometric analysis in the 290–400 nm range on a solid artificial substrate where the sunscreen is applied. The most preferred substrate is made from polymethyl methacrylate (PMMA). This method cannot be applied without the substrate and a specific spectrophotometer. Therefore, it is important to rely on simpler methods to measure the photoprotective ability of sunscreens for research, regulatory, or consumer information purposes. One such method was reported by Mansur et al. in 1986, involving a simple UV spectrophotometry test of extracts, and has been used in several studies [14]. The absorbance values are measured using a UV-Vis spectrophotometer within the wavelength range of 290-320 nm, with a 5 nm bandwidth, and the SPF values are computed in accordance with Mansur's equation. According to the United States Food and Drug Administration (FDA), formulations that are considered sunscreens must have an SPF greater than 2. However, to ensure adequate protection and minimise skin damage, the FDA recommends the routine use of sunscreens with an SPF of 15 or higher, along with other protective measures. Based on the above description, this study aims to determine the potential of sunscreens from Stingless' bee hive waste extract using UAE with varying extraction times.

Methods

Instruments and materials

The tools used in this research are common laboratory glassware, such as beakers, measuring flasks, and glass stirrers. An analytical balance for weighing, an ultrasonic probe for extraction (PULSE 650 Ultrasonic Homogenizer from Benchmark), a rotary evaporator (Heidolp), ATR-FTIR Carry 630 by Agilent, a UV-Vis spectrophotometer 1780A, and GC-MS (Agilent).

The materials used in this research are 96% ethanol (technical), ethanol pa (Smart Lab), $FeCl_3 \cdot 6H_2O$ (99% purity Smart Lab), Dragendorf reagent, Mg powder, HCl, and amyl alcohol.

Sample preparation

Honey bee nest waste samples were obtained from a Stingless honey farmer in Gunungpring, Muntilan, Central Java. The samples used were leftover beehives after pressing. Stingless bee hive waste is meticulously cleaned of debris and sliced into thin sections without any drying process.

1. Extraction. A total of 50 g of samples were macerated with 96% ethanol using an ultrasonic probe (650 W, 220 V, 50 Hz, pulse on 5 seconds, pulse off 3 seconds) for varying durations of 30, 60, and 90 minutes, with a sample-to-solvent ratio of 1:10. Additionally, extraction was performed using the maceration method with the same sample-to-solvent ratio as the ultrasonic-assisted extraction (UAE). The extract was filtered, yielding a filtrate and a residue. The extraction was conducted with five replicates. The filtrate was evaporated under vacuum at 50°C until a thick extract was obtained.
2. Phytochemical screening with color reagents. Phytochemical screening was conducted on extracts and esters to identify alkaloids using Dragendorff's reagent, phenolic compounds using FeCl3 reagent, flavonoids with the Shinoda method, and tannins with FeCl3.
3. Identification with a UV-VIS spectrophotometer. The extracts were dissolved in p.a. ethanol to achieve a concentration of 500 ppm. The spectra of the extract solutions were measured across a wavelength range of 200-400 nm. The spectra of the extracts were then compared.
4. Identification of esterification results using FTIR. Each extract was identified by its functional group using Agilent ATR-FTIR within a wavelength range of 4,000-400 cm-1. Each spectrum produced was identified by its functional group [10].
5. Structural elucidation with GC-MS. The extract sample was treated with UAE for 60 minutes, and the ester yield was identified using GC-MS to identify the compounds formed. The GC-MS analysis shown was conducted at a specified temperature, with the column temperature held at 60°C for 2 minutes, then increased to 170°C at 3°C/min. Finally, the temperature will increase to 250°C at 3°C/minute, then stabilize at 250°C for 120 minutes per sample. Injections were performed at 220°C. Carrier gas (helium) at a rate of 10 ml/minute. The peaks formed will be recorded to create a chromatogram [8].
6. SPF value test. The SPF value is calculated using a modified method based on with modifications [6]. UV spectrophotometry measures absorbance at 290-320 nm in 5 nm increments for samples at 2000 ppm in ethanol. The absorbance is determined using the Mansur equation (1) to calculate the SPF value.) to yield the SPF value.

$$SPF = CFx \sum_{290}^{320} EE_{(\lambda)} xIxA_{(\lambda)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where, CF = correction factor (10), EE (λ) = erythmogenic effect of radiation with wavelength λ, Abs (λ) = spectrophotometric absorbance values at wavelength λ [14].

Result and Discussion

Extraction of secondary metabolites from Stingless bee hive waste was carried out using the maceration method and ultrasonic-assisted extraction at 30, 60, and 90 minutes. The use of ultrasound for extraction increased yield [15]. High-frequency ultrasonic waves at 20 kHz or higher can damage cell walls by increasing the solvent's ability to penetrate cells. This causes metabolite compounds to exit the cell walls with high efficiency, thereby increasing yield [16]. The UAE utilizes thermal, mechanical, and cavitation effects to extract bioactive compounds [11].

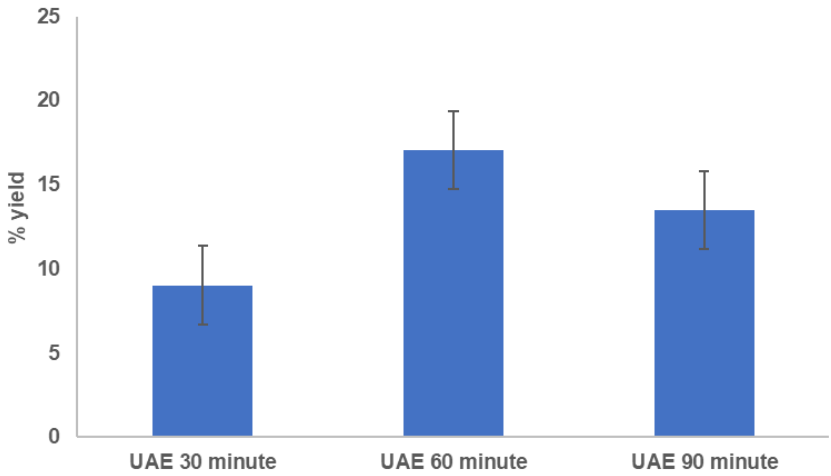

Figure 1. Percentage (%) of extract yield

The ultrasonic thermal effect is the phenomenon in which ultrasonic vibration energy is absorbed by a medium, converting it into heat and increasing the medium's temperature. The ultrasonic mechanical effect is the phenomenon in which the application of ultrasound to a medium causes particle in the medium to vibrate in accordance with the mechanical waves. As a result, particle movement becomes more intense, thereby accelerating mass transfer. Cavitation is considered the most dominant effect. Microscopic bubbles (cavitation nuclei) in a liquid undergo continuous vibration, expansion, and accumulation of energy from the acoustic field. And when the energy exceeds a threshold, cavitation bubbles collapse abruptly. The bubbles in the liquid undergo continuous vibration, expansion, and energy accumulation from the acoustic field. And when the energy exceeds a threshold, the cavitation bubbles collapse abruptly. These microjets have a significant impact force. Upon sudden collapse, cavitation bubbles generate high local temperatures and pressures (5,000 K, 2,000 atm), accompanied by an impressive cooling rate of up to 109 K/s [11]. Ultrasonics can enhance solvent penetration into cells, thereby increasing mass transfer. In addition, ultrasound can damage cell walls, facilitating the release of cell contents. Therefore, effective cell fragmentation and effective mass transfer are considered two factors that cause increased production after the use of UAE. More importantly, ultrasound has an effect on changes in the intracellular matrix, as it has the potential to cause changes in the internal structure of the matrix [17].

Figure 1 shows that extraction with UAE for 30 minutes yields 9.03%, 60 minutes yields 17.03% and 90 minute yields 13.50%. The percentage yield is determined by dividing the weight of the concentrated extract by the initial sample weight used for extraction, then multiplying by 100%, as shown in formula 2.

$$\%\ yields = \frac{weight\ of\ concentrated\ extract\ (g)}{weigth\ of\ sample\ (g)} x100\% \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

These results indicate that 60 minutes of UAE produces the highest yield, with results decreasing at 90 minutes. This suggests that the optimal extraction time for Stingless bee hive waste is 60 minutes. Longer durations lead to the degradation of secondary metabolites, reducing yield. A 60-minute duration allows ultrasonic waves to damage cell walls, increasing the contact between the solvent and the material and promoting the formation of more secondary metabolites on the surface. However, this effect diminishes as the distance from the cell surface increases deeper inside the cell. It is important to note that both processes occur rapidly at the start of extraction. Ultrasonic-assisted extraction significantly enhances yield because ultrasonic waves facilitate mass transfer, particularly during solvent penetration [18]. The UAE is more efficient for extraction because it can produce high yields in a short time [17]. This efficiency reduces processing time, energy use, and solvent consumption, making the UAE an environmentally friendly and cost-effective method [19].

The profiles of compounds contained in extracts can be identified using color reagents, UV-Visible spectrophotometers, ATR-FTIR spectrophotometers, and GC-MS. The results of the phytochemical screening using color reagents are presented in Table 1. These results show that the extract contains phenolic compounds, flavonoids, alkaloids, and tannins. These results are consistent with previous studies that the extract of Stingless bee hive waste contains these metabolite compounds [8].

Table 1. The compounds found in the sample of Stingless Bee hive waste

No	Sample	Test			
		Phenolics	Flavonoids	alkaloids	Tanin
1	Extract the UAE 30	+	+	+	+
2	Extract the UAE 60	+	+	+	+
3	Extract the UAE 90	+	+	+	+

Samples from ultrasonic extraction (UAE) at 30, 60, and 90 minutes showed similar absorption patterns, with λ max values ranging from 285 to 295 nm. This suggests there are no significant structural changes in the main chromophore. The highest absorbance appeared at 30 minutes, then gradually decreased at 60 and 90 minutes, indicating a decline in light-absorbing compounds over time. Since λ max remains unchanged, the structures of the compounds likely remain intact, but their amounts decrease with increasing sonication time. Therefore, 30 minutes is recommended as the optimal extraction time to preserve active compounds.

The UV-Vis spectrum (Figure 2) records the highest absorbance at 30 minutes, yet the maximum yield occurs at 60 minutes. This indicates that an increase in total extract does

not necessarily reflect higher concentrations of UV-absorbing compounds, probably phenolics or aromatics. Extended extraction might dilute these compounds with non-chromophore materials or cause their degradation due to ultrasonic heat and radicals. As a result, the optimal extraction time varies: 60 minutes for maximum yield, 30 minutes for retaining specific compounds. Further experiments are needed to identify the best extraction conditions.

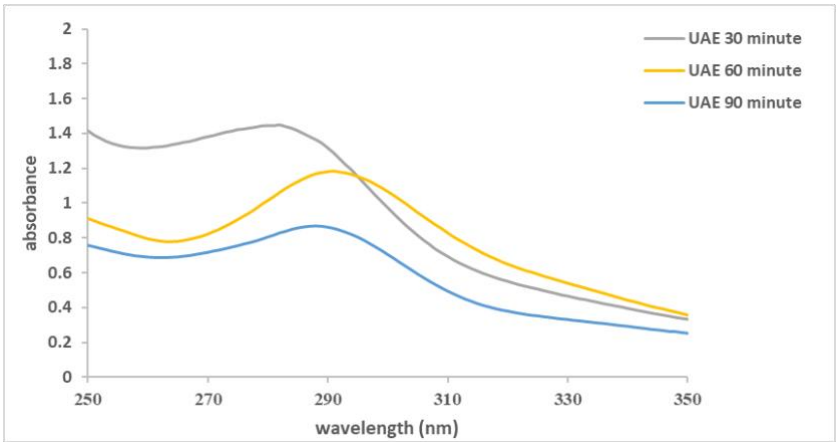

Figure 2. Spectrum of the extract of Stingless bee hive waste (ethanol solvent at a concentration of 500 ppm)

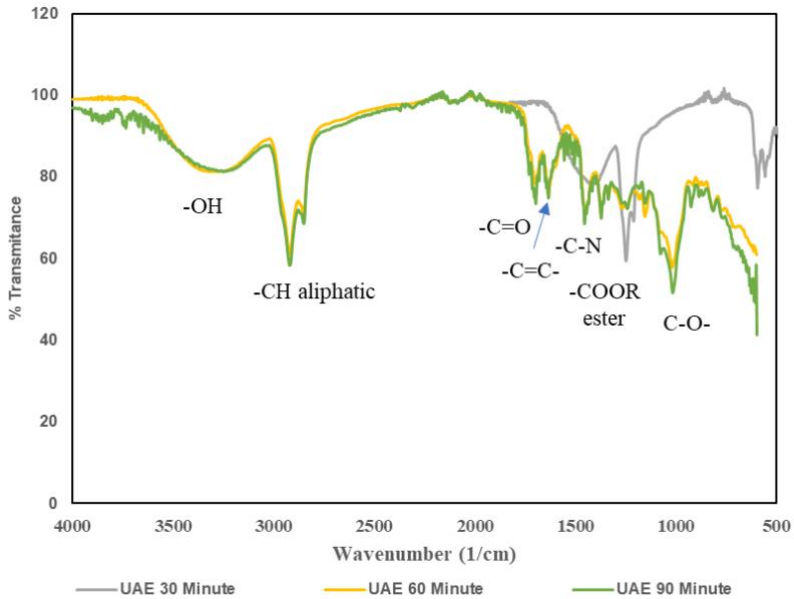

Figure 3. Infrared spectrum of the extract sample and etcher of Stingless' honeycomb waste

The functional groups of the compounds contained in the extract were identified using ATR-FTIR spectrophotometry. The spectrum results (Figure 3) show that the extract contains -OH functional groups at a wavelength of 3,200-3,500 cm-1, aliphatic -CH groups at 2,800-2,900 cm -1, C=O groups at 1600-1700 cm-1, C-OH groups at 100-1100 cm-1, -COOR ester groups at 1,200-1,250 cm-1, and -CH3 groups at 1,300-1,400 cm-1. In addition, there are C-N groups in the 1,448 cm^{-1} region and C-O alcohol groups at 1,019 cm^{-1}. The formation of -OH groups indicates that the sample contains compounds such as alcohols, polyphenols, or free fatty acids. The -CH group indicates the presence of symmetrical and asymmetrical C-H bonds from long hydrocarbon chains, such as fatty

acid chains in oils or esters. The C=O group indicates the presence of carbonyl bonds, typical of esters, aldehydes, ketones, or carboxylic acids (free fatty acids) [6], [8]. The -C-N group indicates the presence of nitrogen-based compounds such as alkaloids, amides, or even proteins. All extract spectra show relatively similar profiles, with the most noticeable difference occurring in the 30-minute UAE spectrum. This curve has a very different profile, with many sharp peaks and very high transmittance in most of the fingerprint region (1,200-1,300 cm-1), which is very flat. This may indicate a significant difference in chemical composition in the 30-minute treatment.

Table 2. Compounds in Stingless bee hive waste extract via GC-MS.

No	Tr	extract		Molecular Formula	Name of the compound
		% area	% SI		
1	15,826	0.41	92	$C_{18}H_{36}O_2$	Ethyl Cetylate/palmitate
2	17,554	2.96	90	$C_{20}H_{38}O_2$	Ethyl Octadec-9-Enoate
3	18,929	0.73	89	$C_{21}H_{38}O_2$	11,14-Eicosadienoic acid, methyl ester
4	19,485	0.73	89	$C_{18}H_{32}O_2$	Linoleic acid
5	23,079	0.52	93	$C_{30}H_{50}$	squalene
6	23,515	0.68	96	$C_{28}H_{58}$	Octacosane
7	25,410	0.28	87		n-Hexatriacontane
8	28,834	9.83	80	$C_{32}H_{52}O_2$	Lanosterol
9	30,406	16.50	83	$C_{30}H_{50}O$	Lanost-7-en-3-one
10	30,942	42.95	86	$C_{28}H_{48}O_4$	Ergost-25-ene-3,5,6,12-tetrol
11	31,245	18.71	88	$C_{30}H_{50}O$	Cycloeucalenol
12	31,559	4.45	86	$C_{18}H_{30}O_2$	15,16-Dinorlabdane, 8,13:13,20-diepoxy-, (13S)-

The identification of compounds in the extract via Gas Chromatography-Mass Spectrometry (Table 2) revealed twelve constituents, comprising fatty acid esters, terpenoids, long-chain alkanes, and steroids. This finding is consistent with results obtained from infrared spectrophotometry and colorimetric analyses. Additionally, it corroborates previous research outcomes. According to some studies, a beehive contains various compounds, including flavonoids, biflavonoids, triterpenoids, fatty acids, alcohols, aromatic aldehydes, and sterols. Some of the literature reiterates the outcome of this investigation [8].

The comparison of UAE results reveals a unique pattern: the SPF value increases from UAE 30 (14,625) to UAE 60 (24,260), then drops sharply from UAE 60 (24,260) to UAE 90 (12,041). This pattern suggests that excessively long ultrasonic exposure (90 minutes) may actually degrade or damage the compounds responsible for UV protection. A duration of 60 minutes yields the best results within the ultra category shown in Figure 4 and Figure 5.

The ability of Stingless bee hive waste extract to act as a sunscreen is due to compounds in the extract that can absorb UV rays. Several studies have reported that tannins and flavonoids contain aromatic rings that function as chromophores and can absorb UV radiation. When UV rays interact with aromatic compounds or compounds containing conjugated chromophores, resonance occurs through electron transfer [20], [21].

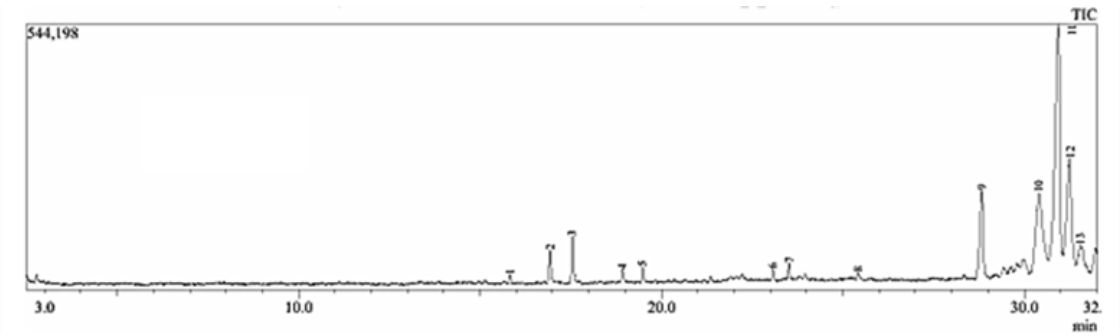

Figure 4. GC chromatogram of the Stingless bee hive waste extract (UAE 60 minutes)

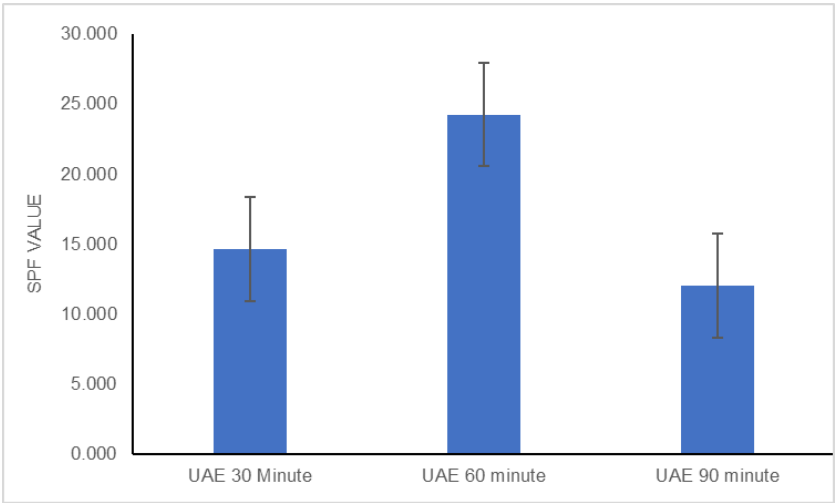

Figure 5. Sunscreen activity from Stingless' hive waste extract

Conclusion

Ultrasonics can be used to extract Stingless (*Trigona* sp) bee hive waste. The optimal extraction time for secondary metabolites in Stingless bee hive waste is 60 minutes. Ultrasonic waves improve extraction results effectively. The extract shows potential as a sunscreen.

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