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EFFECT OF EXTRACTION METHODS ON YIELD, TOTAL PHENOLIC CONTENT, ANTIOXIDANT, AND ANTI-TYROSINASE PROPERTIES OF *Combretum indicum* LEAVES

(Kesan Kaedah Pengekstrakan Terhadap Hasil, Jumlah Kandungan Fenolik, Sifat Antioksida, dan Anti-Tirosinase Daun *Combretum indicum*)

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Abstract

Plants form a natural source of key bioactive chemicals equipped with excellent antioxidant and anti-tyrosinase properties. The restorative plant *Combretum indicum* (*C. indicum*), which belongs to the *Combretaceae* family, was widely used in customary treatment in the past. The target of this study is to assess the effects of water as a solvent, traditional extraction strategies (maceration and Soxhlet extraction), and extraction time on *C. indicum* yield. The total phenolic content (TPC), as well as antioxidant and tyrosinase inhibitory activities, were observed by utilising an ultraviolet-visible spectrophotometer. DPPH radical scavenging was used to determine the antioxidant property, while a mushroom tyrosinase inhibition assay was utilised to evaluate the anti-tyrosinase activity. Maceration carried out for 72 h showed good outcomes for TPC, antioxidant, and anti-tyrosinase activities. The results showed that TPC, antioxidant assay, and anti-tyrosinase activities had a significant linear correlation (p <0.05). It is evident from the study that *C. indicum* leaves, which contain numerous phytochemicals, are strong antioxidants and tyrosinase inhibitors that can potentially play a significant role in the pharmaceutical and cosmetic industries.

Keywords: antioxidant, anti-tyrosinase, *Combretum indicum*, maceration, Soxhlet

Abstrak

Tumbuh-tumbuhan menghasilkan sumber semula jadi bahan kimia bioaktif utama yang dilengkapi dengan sifat antioksida dan anti-tirosinase yang sangat baik. *Combretum indicum* (*C. indicum*) ialah tumbuhan pemulihan dan ia digunakan secara meluas sebagai rawatan adat pada zaman dahulu. Sasaran kajian ini ialah untuk menilai kesan air sebagai pelarut, strategi pengekstrakan tradisional (maserasi dan Soxhlet), dan masa pengekstrakan ke atas hasil *C. indicum*. Jumlah kandungan fenolik (TPC), aktiviti antioksida, dan aktiviti perencatan tirosinase telah diperhatikan dengan menggunakan spektrofotometer ultraungu tampak. Perencatan radikal DPPH digunakan untuk menentukan sifat antioksida, manakala ujian perencatan tirosinase cendawan telah digunakan untuk mengkaji aktiviti anti-tirosinase. Kaedah maserasi selama 72 jam telah meningkatkan hasil TPC, aktiviti antioksida, dan aktiviti anti-tirosinase. Keputusan menunjukkan bahawa TPC, aktiviti antioksida, dan aktiviti anti-tirosinase mempunyai korelasi linear yang ketara (p <0.05). Ia terbukti daripada kajian bahawa daun *C. indicum* yang mengandungi banyak fitokimia ialah antioksida yang kuat dan perencat tirosinase yang berpotensi memainkan peranan penting dalam industri

farmaseutikal dan kosmetik.

Kata kunci: antioksida, anti-tirosinase, *Combretum indicum*, maserasi, Soxhlet

Introduction

With the developing interest of the public in skin brightening, lightening agents are widely used in cosmetic formulas under dermatological supervision. The dynamic substances that lighten the skin complexion are either natural or synthetic and can act at various degrees of melanogenesis. They are utilised to treat different skin pigmentation issues, or basically, to obtain a lighter complexion because a fairer skin complexion in various societies can be defined as beauty. However, recent studies have shown that some of the synthetic antioxidants and anti-tyrosinases contribute to antagonistic impacts, prompting their prohibition or restricted use under the Food and Drug Administration and other global guidelines. There are concerns about utilising mercury and hydroquinone in skin-brightening products due to their reported adverse effects on human skin wellbeing. The adverse impact of inorganic mercury present in skin cleansers and other skin care products includes damage to the kidney, dermatological skin issues, and reduced skin protection from microbial infections. Other adverse impacts, including anxiety, misery, mental illness, and peripheral neuropathy, can also occur [1].

Consequently, there is a continuous quest for a natural antioxidant and plant-based anti-tyrosinase to replace these synthetic ingredients. Antioxidant activity refers to the ability of a molecule to scavenge free radicals and avert oxidative damage resulting from oxidative stress [2]. Meanwhile, tyrosinase is an enzyme containing copper that is crucial for the formation of melanin [3]. Anti-tyrosinase activity involves the inhibition of tyrosinase function, one of the frequently used techniques to deal with skin pigmentation issues [4]. The phenolic compounds found in plants exhibit strong antioxidant and anti-tyrosinase properties. For instance, extracts from plants like *Ginkgo biloba* have demonstrated antioxidant activity along with an anti-tyrosinase effect [5]. Hence, antioxidant and anti-tyrosinase activities are closely interconnected, and phenolic compounds possess both the ability to scavenge free radicals and inhibit tyrosinase.

Phytochemical extraction is the process of separating bioactive compounds from plant materials using an appropriate solvent and extraction technique [6]. It plays a crucial role in natural product research and drug discovery [7]. The most common extraction methods used are maceration and Soxhlet extraction. The effectiveness of the extraction process is influenced by multiple factors, such as the choice of solvent, extraction time, and temperature [8]. Through the optimisation of these factors, phytochemical-rich extracts can be produced at their highest potential yield and quality, thereby enhancing their applicability in pharmaceutical and cosmetic research.

Combretum indicum (*C. indicum*), a member of the *Combretaceae* family, is a fast-growing woody vine found in various regions, including Malaysia, the Philippines, Thailand, Bangladesh, and India [9]. Other names for the plant include *Quisqualis indica* and Rangoon creeper [10]. A previous study by Barik et al. [11] stated that it possesses various medicinal properties and has therefore been thoroughly used in traditional and folk medicine. This plant possesses a strong potential in the commercial landscape; however, there is minimal research on the benefits and potential of this plant to be used as the base in modern skin care products. This plant is non-seasonal, does not rely on the growing seasons, and grows quicker compared to other plants. Rather than simply overlooking its potential, it would be advantageous to assess this plant as much as possible due to its easy availability for the community and economic purposes. Plants are the prime sources of natural antioxidants. *C. indicum* is a great source of phenols and flavonoids, which play an important role in free radical scavenging [12]. Pharmacological studies have shown various capacities of the plant in antimicrobial, anti-inflammatory, antioxidant, antipyretic, antidiarrheal, and antihyperglycemic activities [13].

The purpose of this research is to identify the best extraction method that gives a high amount of yield and

total phenolic content (TPC), as well as better antioxidant and anti-tyrosinase activities from the leaf extract of *C. indicum*. The study applied maceration and Soxhlet extraction and used ultrapure water as the solvent. Water stands out as the safest and most cost-effective and environmentally friendly solvent that is highly capable of extracting polar compounds, such as polyphenols, flavonoids, and other hydrophilic constituents present in plant materials [14]. The ability of water to form hydrogen bonds enhances the solubilisation and extraction of bioactive molecules.

Materials and Methods

Sample collection

Samples of *C. indicum* leaves were gathered from the Blok W area, Universiti Malaysia Pahang Al-Sultan Abdullah, Gambang, Pahang, Malaysia. The leaves were then cleaned using distilled water and dried in an oven at 70 °C for a day. Later, a blender was used to pulverise the *C. indicum* leaves into powder, which was then stored in a zip-lock bag until further use [15].

Preparation of *C. indicum* leaf extract

The extract was prepared using maceration and Soxhlet extraction with some modifications. First, 40 g of the milled powder was soaked in 400 mL of ultrapure water. The sample was vigorously stirred in an incubator shaker for 12, 24, 48, and 72 h for maceration and 12 h for Soxhlet extraction. Then, Whatman No. 1 filter paper was used to filter the extracts. The filtered extract was centrifuged for 10 min at 2,500 rpm to remove the fine particles of the leaf powder. The extract was then freeze-dried and stored in a chiller until further use [15].

Extract Yield Percentage

The percentage of yield (%) of *C. indicum* extract was calculated using Equation 1:

$$\text{Percentage Yield} = \frac{\text{Weight of } C.\ indicum \text{ extract gained (g)}}{\text{Initial weight of powder extract (g)}} \times 100\% \quad (1)$$

Total phenolic content

For each extract, the TPC was determined using the Folin-Ciocalteu (FC) method [16] with minor modifications. The sample was diluted in 1:10 mg/mL, and gallic acid calibration solutions (50–300 µg/mL) were prepared. Then, 1 mL of extract or a standard solution of gallic acid was added to a 25 mL volumetric flask containing 10 mL of distilled water. Subsequently, 1 mL of FC reagent was added to the mixture. After 5 min, 10 mL of 7.5% w/v sodium carbonate was added. To prepare a precise 25 mL solution, 3 mL of distilled water was added, and the mixture was incubated for 45 min at 25 °C. The absorbance of the sample was determined using an ultraviolet-visible (UV-Vis) spectrophotometer at 765 nm. Following that, a gallic acid calibration curve was constructed. Using the regression equation, the concentration of gallic acid in each extract was estimated. Finally, the results were evaluated as TPC in milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g) using Equation 2:

$$\text{TPC} = \frac{\text{Concentration of sample (mg/mL)} \times \text{Volume used during the assay (mL)}}{\text{Mass used during the assay (g)}} \times 100\% \quad (2)$$

DPPH assay

The free radical scavenging activity of the prepared extracts was quantified in vitro by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals using the method applied by [17]. The samples were diluted with water (1 mg/mL), and ascorbic acid (1–0.062 µg/mL) was used as a standard. The DPPH stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol. Then, 6 mL of DPPH was mixed with 0.2 mL of plant extract or the standard solution in a test tube, followed by incubation for 30 min. The absorbance was measured using a UV-Vis spectrophotometer at 517 nm. The control sample was prepared by substituting 0.2 mL of the test sample with 0.2 mL of methanol. The experiment was conducted in triplicate, and the radical scavenging activity was calculated using Equation 3:

$$\text{DPPH Radical Scavenging Activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\% \quad (3)$$

Where $A_{control}$ is the absorbance of the control sample and A_{sample} is the absorbance of the test sample.

Mushroom tyrosinase inhibition assay

As previously reported by [18], inhibition tests with certain modifications were carried out in this study, where L-3,4-dihydroxyphenylalanine (L-DOPA) was used as a substrate to evaluate the inhibitory effect of *C. indicum* leaf extract on tyrosinase activity. The response combination consisted of 6.85 mL of phosphate buffer (0.05 M, pH 6.5), 0.15 mL of mushroom tyrosinase (2,500 units/mL), 2 mL of plant extract solution, and 1 mL of 5 Mm L-DOPA. After L-DOPA was added, the dopachrome formation of the reaction mixture was monitored at 492 nm instantaneously. Kojic acid (200, 250, 300, 350, and 400 µg/mL) was used as a standard. The control sample was prepared by substituting 2 mL of the test sample with 2 mL of phosphate buffer (0.05 M, pH 6.5). The experiment was conducted in triplicate, and the inhibition activity of tyrosinase was calculated using Equation 4:

$$\text{Anti-Tyrosinase Activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\% \quad (4)$$

Where $A_{control}$ is the absorbance of the control sample and A_{sample} is the absorbance of the test sample.

Results and Discussion

Extraction Yield

Figure 1 shows the impact of extraction time on the yield of *C. indicum* leaf extract. The extract yield of the plant was observed to gradually increase from 4.19% at 12 h to 6.96% at 24 h, 8.88% at 48 h, and 9.28% at 72 h using maceration. The increasing trend in the extract yield over the extraction period shows a time-dependent extraction process. The highest extract yield of 9.28% obtained at 72 h using maceration implies that the increase in retention time extends the interaction between the solvent and the matrix and, therefore, improves the extraction efficiency. The swelling of the cell wall increases the solvent's penetration into the plant material, thus increasing the leaf extract. Moreover, the maceration technique naturally swells plant matrices over an extended period of time by achieving solute-solvent diffusivity through osmotic pressure [19]. Apart from maceration, Soxhlet extraction is an alternative for herb extraction in a shorter time. The Soxhlet extraction technique showed better recovery of 10.08% at 12 h compared to maceration. This could be due to the heat used by the Soxhlet device to continuously vaporise and condense the solvent. The heating involved in the extraction process enhances the efficiency of the plant cell wall disruption and increases the solubility of the compounds in the solvent, leading to higher yields. Research studies have indicated that Soxhlet extraction is capable of breaking down cell walls more effectively compared to other extraction techniques, including maceration and ultrasound [20]. However, it should be noted that exposing plant materials that are thermolabile to high temperatures may cause herbal degradation [21]. Besides, Soxhlet extraction involves higher solvent consumption and energy requirements [22]. On the other hand, maceration is a gentle process and applies a milder temperature, which does not destroy bioactive compounds. Furthermore, it is simple, more convenient, and cost-effective [23]. Factors such as the preservation of compounds, cost, sustainability, and equipment complexity are important to consider when selecting the most suitable extraction method. Although the yield of Soxhlet extraction was slightly higher (10.08%) at 12 h compared to maceration at 72 h (9.28%), the difference in yield is relatively small. Hence, maceration at 72 h emerges as the optimal extraction method to yield *C. indicum* extract due to its efficiency, cost-effectiveness, sustainability, and preservation of compounds.

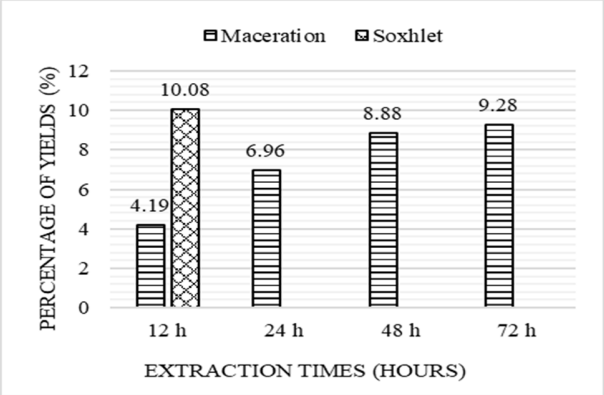

Figure 1. The extract yield of *C. indicum* leaves obtained using maceration and Soxhlet extraction

Total phenolic content

The TPC of each extract with a concentration of 0.1 mg/mL was determined by the FC method using gallic acid as the standard. A calibration curve with the regression equation of $y = 0.0027x + 0.0987$ and $R^2 = 0.9923$ was constructed using the absorbance values obtained at different concentrations of gallic acid. Figure 2 shows the TPC for different extraction times with the use of maceration and Soxhlet techniques. The lowest TPC of 64 ± 0.1275 mg GAE g^{-1} was observed at 12 h, and the highest TPC of 136 ± 0.3003 mg GAE g^{-1} was observed at 72 h using maceration. The results show that the TPC of the extract increases as the extraction time increases from 12 to 72 h. This is because more phenolic compounds are gradually released from the *C. indicum* leaves into the solvent over time. Increasing the extraction duration enhances the extraction of polyphenolic compounds [24].

The TPC of the *C. indicum* leaves extracted at 12 h using Soxhlet extraction was 135 ± 0.1342 mg GAE g^{-1}. The result obtained shows that Soxhlet extraction is able to give a comparable result to maceration at 72 h in a shorter time. Similar to the extracts derived from the parts of *Calophyllum incrassatum*, the barks of *Calophyllum rubiginosum*, and the leaves and barks of *Calophyllum canum*, the technique of Soxhlet extraction showed a high phenol extraction rate in a short time [25]. However, the high temperature used in Soxhlet extraction might contribute to the degradation of the heat-sensitive properties of some phenolic compounds [26]. A study observed the degradation of catechins in tea due to the high temperature used in Soxhlet extraction. The total polyphenol concentrations from Soxhlet extraction at 70 °C were lower than those from maceration at 40 °C [27]. Although Soxhlet extraction has a time-efficient nature, the increased temperature implied during extraction may impair the integrity of certain phenolic compounds. It is important to consider both extraction efficiency and the preservation of phenolic compounds when selecting the optimal extraction method [28]. Thus, maceration at 72 h is the optimal method for the extraction of *C. indicum* leaves due to its ability to extract phenolic compounds while maintaining its integrity.

Antioxidant activity

The DPPH test is a reliable and widely used method to assess the ability of an extract to scavenge free radicals [29]. The results of DPPH inhibition for different extraction times and methods are shown in Figure 3. The antioxidant activity of the *C. indicum* leaves extracted using maceration was reported to be 11.098% at 12 h, 29.016% at 24 h, 39.306% at 48 h, and 83.398% at 72 h. The results indicate a clear trend of increasing antioxidant activity with prolonged extraction time. An increased extraction time allows the extraction of a wider range of bioactive compounds, including phenolic compounds, which are well-known for their antioxidant capability [24]. Overall, the maceration technique for 72 h exhibited the highest antioxidant activity at 83.398%, surpassing other extraction times, including Soxhlet extraction, which showed 79.809% of antioxidant activity. Hence, maceration demonstrated 4% greater antioxidant efficacy compared to Soxhlet extraction. The elevated TPC values observed at longer extraction

times signify a higher concentration of antioxidant compounds within the extract. From the past outcome, it can be derived that the highest number of antioxidants corresponds to the highest TPC in the extracts, which reveals the positive correlation between TPC and antioxidant activity [30].

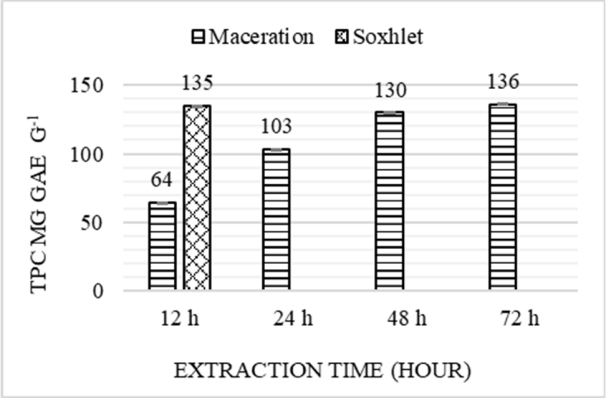

Figure 2. The TPC of *C. indicum* leaves extracted using maceration and Soxhlet extraction. The values are the means ± standard deviation of triplicate analyses. Columns labelled with different extraction times are significantly different (p <0.05) by the ANOVA single factor test

A significant contribution to antioxidant ability is made by phenolic and flavonoid molecules [31]. A study by [32] deduced that all parts of *C. indicum* possess strong antioxidant potential. Recent evidence indicates that diets high in polyphenolic compounds play an important role in combating oxidative stress disorder due to their antioxidant behaviour [33]. Hence, the polyphenolic compounds found in *C. indicum* leaves may potentially prevent oxidative diseases related to stress.

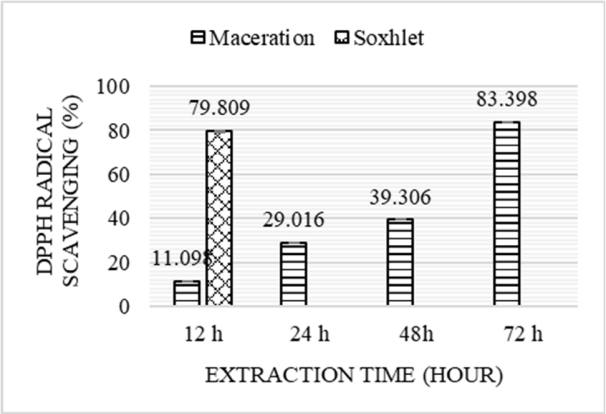

Figure 3. Antioxidant activity of *C. indicum* leaves extracted using maceration and Soxhlet extraction. The values are means ± standard deviation of triplicate analyses. Columns labelled with different extraction times are significantly different (p <0.05) by the ANOVA single factor test

Anti-tyrosinase activity

The relationship between the inhibitory action of tyrosinase and the antioxidant activity of plant extracts is closely linked to their phenolic content [34]. In this study, a direct correlation between the anti-tyrosinase activity and the TPC was observed from 12 to 72 h extraction periods (20.16 ± 0.0040% to 46.37 ± 0.0029%, respectively), as depicted in Figure 4. The

anti-tyrosinase activity of the extract demonstrates a progressive increase over time. Meanwhile, the 12 h extraction using Soxhlet extraction exhibits the lowest tyrosinase inhibition activity, registering at 13.31 ± 0.0037%. This observation is expected to be the aftereffect of bioactive material deterioration at high temperatures during Soxhlet extraction. Besides, high temperatures can also accelerate chemical reactions, including oxidation and hydrolysis, which may contribute further to the degradation of bioactive compounds present in the plant material [35]. Notably, the 72 h maceration technique demonstrated 33.06% greater tyrosinase inhibition activity compared to the 12 h Soxhlet method, highlighting the better efficacy of prolonged maceration in extracting phenolic compounds with anti-tyrosinase properties.

Tyrosinase is a pivotal enzyme in melanin synthesis [36]. The inhibition of its activity garners considerable attention in the skin care and cosmetic industries for its role in treating hyperpigmentation [37]. Additionally, the anti-tyrosinase activity of the *C. indicum* leaf extract obtained through maceration at 72 h further underscores its potential relevance in addressing hyperpigmentation concerns.

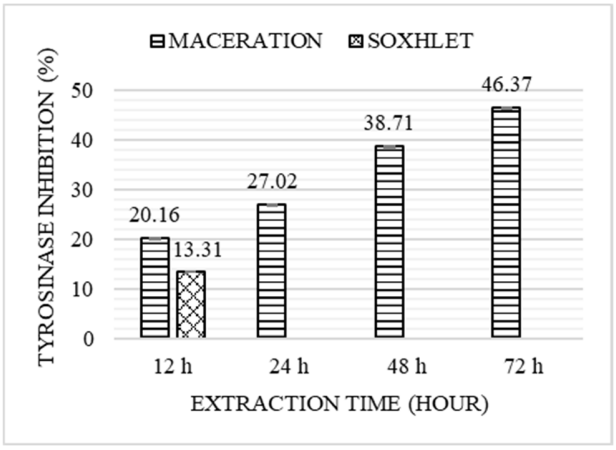

Figure 4. Anti-tyrosinase activity of *C. indicum* leaves extracted using maceration and Soxhlet extraction. The values are means ± standard deviation of triplicate analyses. Columns labelled with different extraction times are significantly different (p <0.05) by the ANOVA single factor test

Conclusions

In conclusion, this study revealed that extraction time and extraction methods have a significant impact on the yield, TPC, antioxidant, and anti-tyrosinase properties of the extracts obtained. The findings show that 72 h of maceration is the most effective method to extract *C. indicum* leaves for an optimum yield. As Soxhlet extraction requires a shorter time and negligible energy consumption to finish the extraction cycle of the plant, it remains viable for use. However, in order to obtain extracts with good antioxidant and anti-tyrosinase properties without any possible thermal degradation of the compounds, maceration for 72 h was determined to be the best technique to extract *C. indicum* leaves. Furthermore, the maceration strategy used in this study showed significant results, where the antioxidant activity was 29.02% and the anti-tyrosinase activity was 20.16%. This shows that the target of this study is effectively accomplished. Therefore, the extraction method of *C. indicum* leaves is a success. Overall, it can be said that *C. indicum* leaves could act as a potential source of natural antioxidants to be utilised for nutraceuticals and utilitarian food applications, as well as in the pharmaceutical and cosmetic industries concerning their viability as skin-brightening or lightening agents for their excellent anti-tyrosinase properties.

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