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Effect of Different Solvents Extraction on the Total Phenolic Content and Free Radical Scavenging Activity of Sacha Inchi Leaves Oil

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ABSTRACT

Plukenetia volubilis L., also known as Sacha Inchi is a unique commercial crop that has gained in popularity due to its nutrient-rich and functional benefits to human health. There has been significant research on Sacha Inchi seed, only few studies on the leaves have been conducted. Oil extraction from Sacha Inchi leaves is underutilised and mostly unknown to the general public. This study analyzed the effect of pre-treatment which is fresh and dried leaves for solvent extraction on oil yield, Total Phenolic Contents (TPC), and free radical scavenging properties of oil extracted from Sacha Inchi leaves. The dried leaves sample which dried in oven drying at 30°C at 48 hours, was compared to the fresh sample of *Plukenetia volubilis L* leaves. Proximate analysis was carried out on the leaf samples by AOAC method. The nutritional values of Sacha Inchi leave revealing that the dried leaves contained higher composition values of crude fiber (13.43%), protein (18.6%), carbohydrate (47.96%) and ash (3.53%) than Sacha Inchi fresh leaves contained lower contents of protein (6.31%), fibre (3.86%), fat (1.16%), ash (2.47%) and carbohydrate (21.39%), except for moisture content (58.15%). For comparison, the Sacha Inchi leaves were also extracted with Soxhlet extraction, using three different solvents for oil extraction: ethanol, propanol, and hexane from fresh and air-dried leaves yielded the oil yield. Overall, the ethanol extraction for dried leaves produced the highest oil yield (21.35%) than propanol (7.89%) and hexane (5.3%). The greatest TPC values (0.64 and 0.65 mg GAE/g) were found in fresh and air-dried leaves extracted with ethanol extraction, as opposed to leaves extracted with propanol (0.33 and 0.49 mg GAE/g) and hexane (0.45 and 0.59 mg GAE/g). Additionally, the fresh sample with ethanol extract showed a 48.13 ± 29.44% percent inhibition of the free radical-scavenging assay, whereas the air-dried sample showed a higher percentage at 61.18 ± 23.26%. Both analyses produced the same results, with the maximum TPC and free radical scavenging activity reported in air-dried Sacha Inchi leaf samples with ethanol extract versus fresh leaves samples.

Keywords: Sacha inchi oil leaves, soxhlet extraction, phenolic compounds, antioxidant activity.

INTRODUCTION

Sacha Inchi (*Plukenetia volubilis* L.), is an oily plant in the *Euphorbiaceae* family, which originated in Peru's Amazon rainforest. In 2018, the Rubber Industry Smallholders Development Authority (RISDA) introduced the Sacha Inchi plant into Malaysia in order to increase the income of small holders and farmers (Zakaria et al., 2022). Sacha inchi seed oil is recognized as a nutritious supplement because of its high level of polyunsaturated fatty acids, antioxidants, and vitamins A and E. They also provide energy and important fatty acids such as linolenic (Omega-3) and linoleic (Omega-6) acids (Kyaw et al., 2019). Omega-3 and Omega-6 are necessary fatty acids that are beneficial to human health. They have been found to lower the risk of many diseases, including coronary heart disease, diabetes, hypertension, inflammatory skin issues, arthritis, and cancer (Muangrat et al., 2018).

In recent years, there has been a lot of research conducted on the plant components of Sacha Inchi, mainly on its seed and kernel (Kyaw et al, 2019). However, Sacha Inchi leaves are underutilized and neglected due to the lack of studies focused on the leaves and oil derived from Sacha Inchi leaves. Soxhlet extraction is one of the commonly used methods for lipid extraction, especially in food industry. Besides, it is a straightforward and inexpensive method. Rodrigues et al., (2018) investigated the extraction of oil from Eucalyptus leaves using different types of solvent, such as methanol, ethanol and dichloromethane by Soxhlet extraction. Soxhlet extraction method with different solvents (ethanol, propanol and hexane) was being used to extract oil from leaves. The solvents utilised for oil extraction have an impact on the total amount of oil yield as well as the phenolic compounds (Keneni et al., 2021). According to Keneni et al. (2021), oil extraction using n-hexane has the maximum oil yield, making it the most commonly used solvent. It has also been observed that different solvents produce different natural chemicals from a particular substance, and hence the extract composition varies depending on the solvent. As a result, selecting a solvent for oil extraction is one of the most critical processes in chemical oil extraction.

Free radicals in food have the potential to trigger oxidation, which causes ageing and disease to humans. Free radicals can be produced by UV light, ionizing radiation, cigarette smoke, pollution, some organic solvents, and industrial waste. Antioxidant compounds are substances that may protect body cells from harm caused by free radicals (Jahan et al., 2015). Natural antioxidants act as nutraceuticals by terminating free radical chain reactions in biological systems, which benefits health (Saravanan et al., 2015). According to Wang et al. (2018), Sacha Inchi leaves have antioxidant and antiproliferative properties against cancer cells. However, antioxidant properties of oil obtained from Sacha Inchi leaves are poorly studied. Phenolic compounds and antioxidants not only have positive effects on health, but they also improve food quality and nutritional value. The leaves contain high phenolic content and antioxidant characteristics, making them useful in the pharmaceutical and food industries (Kittibunchakul et al., 2022). Therefore, the aim of this study was to examine the increased extract yield, total phenolic content, and free radical scavenging assay from extracted Sacha Inchi leaves using a difference of pre-treatments and solvents.

MATERIALS AND METHODS

Preparation of Sacha Inchi leaves powder

The leaves of Sacha Inchi were harvested and collected in Bachok, Kelantan, and packed in permeable plastic bags. Sacha Inchi leaves were transported to the laboratory in less than 24 hours at room temperature. The leaves were categorized based on size and color consistency. The samples were prepared using the procedure described by Rezvankhah et al (2018) with some modifications. The leaves were then cleaned and rinsed. As a pre-treatment, the leaves were divided into fresh and air-dried leaves, which were dried for 48 hours in a hot air oven dryer (Model FDD-720, PROTECH, Selangor, Malaysia) at 30°C. Both fresh and dried leaves were then crushed into a fine powder by mixer grinder (Panasonic Food Processor MX-AC210SW, India) and sieved through a sieve shaker (AG-515 Sieve Shaker, OCTAGON, Thailand) with a particle size of 500μm. The

completed samples were stored in an airtight container until they were used. All of the substances utilized for analysis were analytical grade.

Proximate Composition of Sacha Inchi Leaves

Fresh and air-dried leaves were evaluated for moisture, crude protein, crude fat, crude fibre, and ash content (AOAC, 2005). Sacha Inchi leaves powder samples were weighed in crucibles and dried in an oven at 105°C for 6 hours until a stable weight was attained. The ash content of Sacha Inchi leaves was determined using a muffle furnace at 550°C overnight while the protein content was determined using the Kjeldahl method. The crude fibre content of the samples was evaluated by digestion, and the fat was extracted using the Soxhlet method. All measurements were carried out in triplicate. The carbohydrate percentage was obtained by subtracting the following formulas:

% Percentage of Carbohydrates = 100 - (% moisture + % protein + % fat + % ash + % fibre)

Leaf oil extraction

The Soxhlet extraction method was used according to Rezvankhah et al (2018) with some modification. Ethanol, propanol and hexane were used to compare the oil yield from fresh Sacha Inchi leaves. A cellulose thimble was filled with 20 g of each fresh and air-dried Sacha Inchi leaves powder for the Soxhlet extraction before being transferred to an extractor. The solvent, 150 ml of n-hexane, ethanol and propanol were being put separately into a round-bottom extraction flask and were heated in a water bath. The extraction was performed in triplicate. Each trial run was running continuously for 7 hours. The oil extraction was repeated for air-dried Sacha Inchi leaves sample, and the oil was recovered through solvent evaporation.

Determination of oil yield

After the extraction finished, the solvent was evaporated using a rotary evaporator (Heidolph Vivo RT2, United States) constantly at 60°C, 175 rpm and under vacuum condition to evaporate the hexane (335mbar), propanol (67mbar) and ethanol (175mbar). The oil yield (%) was calculated as follows:

$$\text{Percentage of Oil yield \%} = \frac{W_1\,(g)}{W_2\,(g)} \times 100\% \qquad \textbf{Eqn. 1}$$

Where:
W_1 = Weight of extract oil sample (g)
W_2 = Weight dry sample (g)

The extracted oil obtained was placed in sealed amber glass vials, wrapped with aluminium foil, and chilled at 4°C until further analysis.

Methanolic extract preparation

The antioxidant levels of the extracted oil were evaluated by re-dissolving it in methanol (MeOH) at a concentration of 1 mg/mL. The mixture was vortexed (IKA MS2 Shaker vortex, German) and centrifuged at 5000 g (SIGMA 3-18KHS, German) for 15 minutes to ensure that it blended well and homogeneously with the solvent. The methanolic phase was collected and then used to assess the quantity of total phenolic compounds and free radical scavenging assay (Muangrat et al., 2018). All experiments were conducted in triplicate. Gallic acid and Trolox were chosen as standards in TPC and DPPH assays. The results were represented as milligrams of gallic acid equivalents (mg GAE/g oil sample) and mol Trolox equivalent (TE)/100 g DW.

Determination of Total Phenolic Compounds

The Total Phenolic Compound (TPC) has been determined using the Folin-Ciocalteu spectrophotometric technique, as modified by Muangrat et al., (2018). A volume of 250 µL of the methanolic extract of the oil sample was diluted with 250 µL of the Folin-Ciocalteu reagent and 4 mL of distilled water. After 3 minutes of agitation, 500 µL of 1N sodium carbonate (Na_2CO_3) was added. After allowing the solution to stand for 1 hour in the dark condition at room temperature (20°C - 22°C), the absorbance at 765 nm on the UV-Vis spectrophotometer (Shizmadzu UV Mini-1240, Japan) was measured against a blank.

TPC was calculated by comparing it to a standard curve of gallic acid solutions and was represented as milligrams of gallic acid equivalents per gram of oil sample (mg GAE/g oil sample). Gallic acid standard solutions were tested at wavelengths of 765 nm at concentrations of 50, 150, 200, 250, and 500 g/ml. The absorbance of the standard gallic acid solution was then calculated at each concentration. The total phenolic content of ethanol, propanol, and hexane extracts of Sacha Inchi leaves oil was determined using a straight-line equation ($y= 0.009x + 0.5578$, $R^2 = 0.9972$).

Determination of Free Radical Scavenging Assay

The oil recovered with ethanol was used to conduct the (DPPH) radical scavenging test since it extracted the most oil and phenolic content. The free radical scavenging activity of methanolic leaf oil extract was evaluated using a modified version of the Lee et al. (2015). The 500 µl of methanolic DPPH solution (0.2 mM) was being mixed with 500 µl of extracted oil in a series of concentrations ranging from 10-50 mg/ml. After that, the mixtures were wrapped with aluminium foil and incubated in a dark place such as a lab cabinet at 20-22°C for 60 minutes. At 517 nm, the absorbance was measured (Perkin-Elmer 45 UV-Vis Spectrometer). Trolox was utilized as the standard, while DPPH mixture without sample was used as the blank. Each sample was tested in triplicate. The following equation was used to calculate DPPH's radical scavenging activity:

$$\text{DPPH scavenging effect (\%)} = \frac{(A_o - A_1)}{A_o} \times 100 \qquad \textbf{Eqn. 2}$$

where, A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of methanolic leaf extract. A graph between scavenging percentage and extract concentration were being created, and the exact concentration that provided 50% scavenging (IC_{50}) was derived from this.

Statistical Analysis

All of the experiments have been performed in triplicate. Two-way ANOVA and Independent sample T-test were used to analyse the data. Duncan's test was used to identify the differences in mean values. The findings were presented as mean values with standard deviations. All statistical analyses were carried out using SPSS 20.0 with a significance level of 5%.

RESULTS AND DISCUSSION

Proximate composition of Sacha Inchi leaves

Table 1 shows the composition of fresh Sacha Inchi leaves and air-dried Sacha Inchi leaves in terms of moisture content, total crude protein, fat, crude fibre, ash and carbohydrate content.

Table 1: Proximate composition (%) of Sacha Inchi leaves

Composition (%)	Sacha Inchi leaves samples	
	Fresh leaves	**Dried leaves**
Moisture	58.15 ± 0.11^{a}	10.37 ± 0.14^{b}
Crude Protein	6.31 ± 0.27^{b}	18.6 ± 0.76^{a}
Crude Fibre	3.86 ± 0.18^{b}	13.43 ± 0.43^{a}
Crude Fat	1.16 ± 0.09^{b}	6.11 ± 0.10^{a}
Ash	2.47 ± 0.15^{b}	3.53 ± 0.01^{a}
Total Carbohydrate	21.39 ± 0.31^{b}	47.96 ± 0.48^{a}

Mean values ± standard deviation; means values with different superscript letters in the same row demonstrate significant differences, ($p<0.05$).

Based on Table 1, there was a significant difference ($p<0.05$) in moisture content, crude protein, crude fibre, crude fat, ash and carbohydrate content between fresh and dried Sacha Inchi leaves. Fresh Sacha Inchi leaves contained lower contents of protein, fibre, fat, ash and carbohydrate than air-dried leaves samples, except for moisture content (58.15%). According to Muangrat et al. (2018), the drying temperature has an effect on the moisture content of the sample. The drying process allowed the water in the leaves to move diffusely to their surface and then the water was evaporated (Setiyoningrum et al., 2018). Thus, foods with low moisture content may have a high storage value. The lower the moisture content, the less microbial activity and reduces the spoilage (Agomuo et al., 2017). As a result, the excessive moisture level in the sample significantly reduced the nutritious contents ($p<0.05$). The nutritional value of dried Sacha Inchi leaves is increased due to their low moisture content.

The percentage of crude fat content of Sacha Inchi air-dried leaves (6.11%) was higher than that of fresh leaves (1.16%). The amount of crude fat in both leaves appears to be moderate and may be sufficient for ingestion without posing a health risk (Sultana, 2020). Percentage of crude protein was significantly higher ($p<0.05$) in dried leaves than fresh Sacha Inchi leaves. In terms of crude fibre, there was a significant difference ($p<0.05$) between fresh and air-dried samples, with fresh leaves having the lowest fibre content (3.43% and 13.43%). Crude fibre is largely composed of cellulose with small amounts of lignin, which enhances digestibility for humans. Through data tabulated in Table 1, it could be clearly concluded that fresh and dried Sacha Inchi leaves powder were rich to a great extent in many significant components such as protein, fibre and carbohydrates. This result was in agreement with the findings of Rajput et al. (2017) on *Moringa oleifera* leaves, which reported that dried leaves contained higher composition of crude protein, carbohydrates and crude fibre than fresh leaves.

Solvent extraction of oil from Sacha Inchi leaves

In this study, different solvents such as hexane, ethanol and propanol were used to extract oil from fresh and dried leaves, by Soxhlet extraction for seven (7) hours as the method was modified from Rezvankhah et al (2018). Table 2 showed the percentage of oil yield on different solvent of the sample.

Table 2: Percentage of Oil yield extracted from Sacha Inchi leaves using different solvents.

Type of Solvents	Oil yield (% w/w)	
	Fresh leaves	Air-dried leaves
Ethanol	8.67 ± 0.02^{aB}	21.35 ± 0.15^{aA}
Propanol	5.45 ± 0.06^{cB}	7.89 ± 0.12^{bA}
Hexane	7.78 ± 0.11^{bA}	5.3 ± 0.16^{cB}

Mean values ± SD; Mean value followed by different lowercase letters in the same column demonstrate significant differences ($p<0.05$).
Mean values followed by different uppercase letters in the same row demonstrate significant differences ($p<0.05$).

Table 2 represents the oil yield derived from air-dried Sacha Inchi leaf samples which were significantly ($p<0.05$) higher than fresh leaves. The highest oil yield was obtained from air-dried leaves that had been pre-treated in an oven dryer for 36 hours. Damaged cells or oil glands caused by the process may increase the rate of oil evaporation in powder form materials. Therefore, dried leaves samples increase in oil yield extraction. In contrast to Kumar et al. (2021), this study found that samples treated to high temperatures yielded less oil. Based on the result shown in Table 2, air-dried leaves sample extracted by hexane obtained lower oil yield than fresh leaves. Similar findings were also reported by Setiyonningrum et al., (2018), stating that the drying sample caused a decrease in the oil output of kaffir lime leaves. This also may be due to changes in biological structure of the plant cells which resulted in reduced essential oil yield (Azhari et al., 2020). Drying process was suspected of causing damage to tissue of plants, resulting in the release of volatile chemicals. As a result of the drying process of Sacha Inchi leaves, oil production of hexane extract is reduced.

Furthermore, the significant difference ($p<0.05$) in oil yield between fresh and dried leaves is due to moisture content. According to Table 1, the greater moisture content of fresh leaves resulted in a lower amount of oil generated when compared to air-dried leaves. This occurrence could have been caused by the oil's hydrolysis process, as well as water and lipase enzymes (Fotsing et al., 2021). Heat evaporates the essential oils which flow onto the leaf surface with water (Setiyonningrum et al, 2018). The lower the moisture level of the sample, the higher the oil production. In addition, the presence of extractable components with different polarities may be correlated to the yield of extractable oil in different solvents. As demonstrated in Table 2, the oil yield using ethanol (21.35%) was significantly ($p<0.05$) higher than that of propanol (7.89%) and hexane (5.3%). According to Keneni et al. (2021), the oil yield increases as the polarity of the solvent utilised in extraction increases. This is strengthened by the finding of Alrashidi et al. (2022), who discovered that the ethanol solvent yielded the maximum *Nigella sativa* oil output. Thus, polar solvents such as ethanol and propanol enhanced oil yield extraction.

However, hexane as a non-polar solvent extracts higher oil yield in fresh Sacha Inchi leaves. The proportion of fresh leaves oil with hexane significantly (7.78%) higher than oil extracted from air-dried leaves (5.53%). Keneni et al. (2021) demonstrated in their comprehensive research on oil extraction that using n-hexane results in the highest oil yield, making it the best widely used solvent. Hexane is soluble in most oils, which contributes to its greater potential to result in higher yield. Moreover, using ethanol as a solvent is better for the environment because it is less toxic. Therefore, temperature increases both the diffusion coefficient and the solubility of the oil in the solvent, improving the extraction rate (Abed et al., 2015).

Total Phenolic Content

Table 3 shows the total phenolic content (TPC) of extracted oil from fresh and air-dried Sacha Inchi leaves by ethanol, propanol and hexane.

Table 3: Total phenolic content of Sacha Inchi oil leaves extracted with different solvents.

Type of Solvents	Total Phenolic Content (mg GAE/g)	
	Fresh leaves	**Air-dried leaves**
Ethanol	0.64 ± 0.04^{a}	0.65 ± 0.08^{a}
Propanol	0.33 ± 0.03^{b}	0.49 ± 0.09^{b}
Hexane	0.45 ± 0.01^{b}	0.59 ± 0.01^{ab}

Mean values ± SD; means followed by different superscripts letters in the same column demonstrate significant differences ($p<0.05$).

Table 3 shows that oil extracted from air-dried Sacha Inchi leaves has a higher value of TPC when compared to fresh leaves extracted. During the drying process, the cell membrane of the leaves was destroyed, allowing all of the bioactive components to be released (Mustafa & Chin, 2023). As a result, the TPC of oil produced from dried Sacha Inchi leaves increased. This finding has been supported by Kittibunchakul et al., (2020), who found that roasting Sacha inchi leaves at 60°C increased their antioxidant capacity. Rajput et al. (2017) also found that dried Moringa leaves have higher TPC values than fresh Moringa leaves. Thus, higher the drying temperature of the sample, the higher the phenolic content.

The recovery, yield, and type of phenolics in an extract are affected by the type and polarity of the extracting solvents, the time and temperature of the extractions, and the physical properties of the materials (Jahromi et al., 2017). Therefore, different types of solvent were used to determine which solvent could obtain the highest extraction yield, and indicate the relationship between solvent polarity and extraction yield. Based on the result, the phenolic content of oil found from ethanol extract in fresh leaves and dried leaves of Sacha Inchi were significantly higher ($p<0.05$) than propanol and hexane. Phenolic compounds are influenced by changes in solvent polarity. According to Johari and Khong (2019), the bioactivity of the ethanol extract can be related to its higher phenolic content. Figure 1 showed phenolic concentration increased with the polarity of the solvent. Afshari and Sayyed-Alangi (2016) found that the greatest total phenol concentration was obtained after 18 hours using an ethanol extract. Therefore, selecting the appropriate solvent type is an essential step when determining a sample's total phenolic content.

In this study, gallic acid (GAE) was utilized as a reference solution to measure the total phenol components in samples. It is because of renewable and stable phenol that is also reasonably inexpensive in comparison to others. The findings of standard gallic acid calibration curve in the form of a concentration versus absorption graph (Fig. 1). According to the standard graph, the calibration curve with an equation for regression for gallic acid absorption at concentrations of 50, 150, 200, 250, and 500 g/mL is $y = 0.009x + 0.5578$. In a standard solution of phenol compounds, a linear relationship between absorbance and concentration is achieved. The correlation coefficient (R^2) of 0.9972, which is close to 1 in absorbance measurement, shows that the regression model is linear. Therefore, the oil extracted from air-dried leaves has higher TPC compared to oil extracted from fresh leaves.

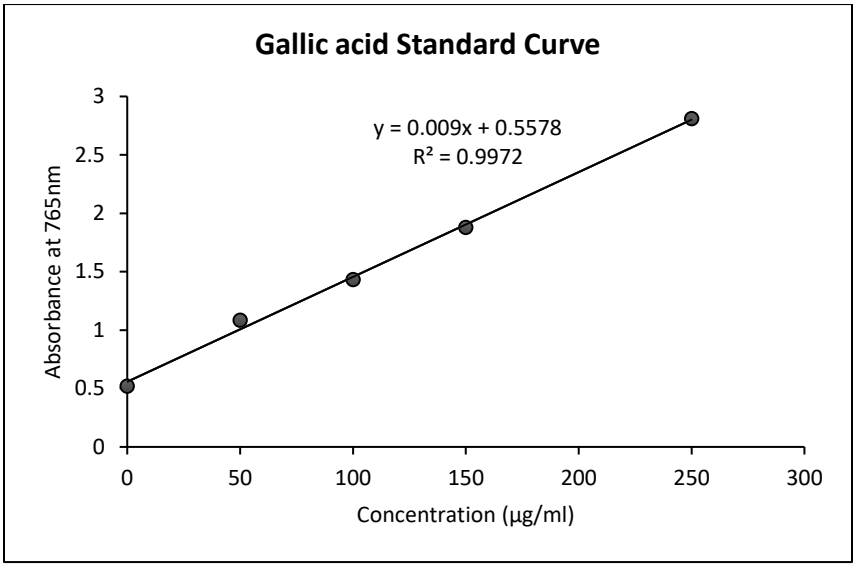

Fig. 1: Calibration curve for standard Gallic acid

Free Radical Scavenging Assay

The free radical scavenging activities of oil extracted by ethanol from fresh and air-dried Sacha Inchi leaves were investigated using the DPPH scavenging assay. The oil recovered with ethanol was used to conduct the (DPPH) radical scavenging test since it extracted the most oil and phenolic content. The more polar the solvent, the more phenolic chemicals and antioxidants are present in the sample.

Table 4: Free radical scavenging activities of fresh and air- dried Sacha Inchi leaves oil

Oil extracted sample	Percentage Inhibition of DPPH (%)	IC_{50} DPPH (µg/ml)
Fresh leaves	48.13 ± 29.44^{b}	36.61
Air-dried leaves	61.18 ± 23.26^{a}	9.42

Mean values ± standard deviation; means followed by different superscript letters in the same column demonstrate significant differences ($p<0.05$).

Table 4 showed that the free radical scavenging activity of air-dried leaves oil (61.18%) was significantly higher than fresh leaves oil (48.13%). The higher antioxidant activity observed in samples due to exposure to higher drying temperature may be caused by releasing the phytochemical compounds that are bound before, which degrade the components of the cell and new compounds with enhanced antioxidant properties are formed (Meng et al., 2018). This study was correlated with Rajput et al. (2017), as the percentage of radical scavenging rate of dried moringa leaf (9489.8%) sample increased significantly ($p<0.05$) compared to fresh moringa leaves (6207.8%). Thus, an increase of drying rate, increases the radical scavenging activity of the sample.

Furthermore, the solvent extract has an effect on the sample's radical scavenging activities. According to the results in Table 4, oil from air-dried Sacha Inchi leaves extracted with ethanol had lower IC_{50} values than fresh leaves. This means that an ethanolic extract of air-dried leaves is more antiradical than fresh leaves. Due to the different solvents used and the amounts of their polyphenolic compounds and other phytochemical ingredients,

different extracts exhibit varying antiradical activity. Afshari and Sayyed-Alangi (2016) discovered similar results while evaluating the DPPH scavenging activity of *Cressa cretica*, which discovered that the ethanol extract showed higher DPPH scavenging than the aqueous extracts and methanol. Furthermore, phytoconstituents, antioxidant activity, and other biological activities varied significantly depending on the solvent extraction methods (Serafini et al., 2020). Therefore, the higher the polarity of the solvent, the greater radical scavenging of samples.

Radical scavenging activity of an ethanol extract from Sacha Inchi leaves increased with concentration between 10 mg/ml to 50 mg/ml (Fig. 2). The IC_{50} of a substance has an opposite correlation to its antioxidant capability since it is necessary to reduce the DPPH concentration by 50%, according to a linear regression analysis (Nur-Hadirah et al., 2021). A lower IC_{50} indicates a higher antioxidant activity of a compound. According on Fig. 2, the antioxidant capacity values of fresh and dried Sacha Inchi oil leaves in the DPPH radical scavenging assay were lower than standard antioxidants (Trolox). However, the percentage of inhibition has achieved 50% (IC_{50}) for both of the oils extracted. The IC_{50} of oil extracted from fresh and air-dried Sacha Inchi leaves were found to be 36.61 and 9.42 μg/ml respectively. The oil extracted from air-dried leaves had lower IC_{50} values than fresh leaves oil.

The DPPH values rose after drying the Sacha Inchi leaves sample in comparison to the fresh sample. Therefore, oil extracted from air-dried leaves contain higher antioxidants as compared with extracted oil from fresh leaves. This finding was supported by Agomuo et al. (2017), who discovered that the IC_{50} values of guava essential oil samples decreased as the DPPH radical scavenging activities increased. Hence, the antioxidant capacity of oil extracts increases with lowering IC_{50} values (Wuttisin et al., 2021).

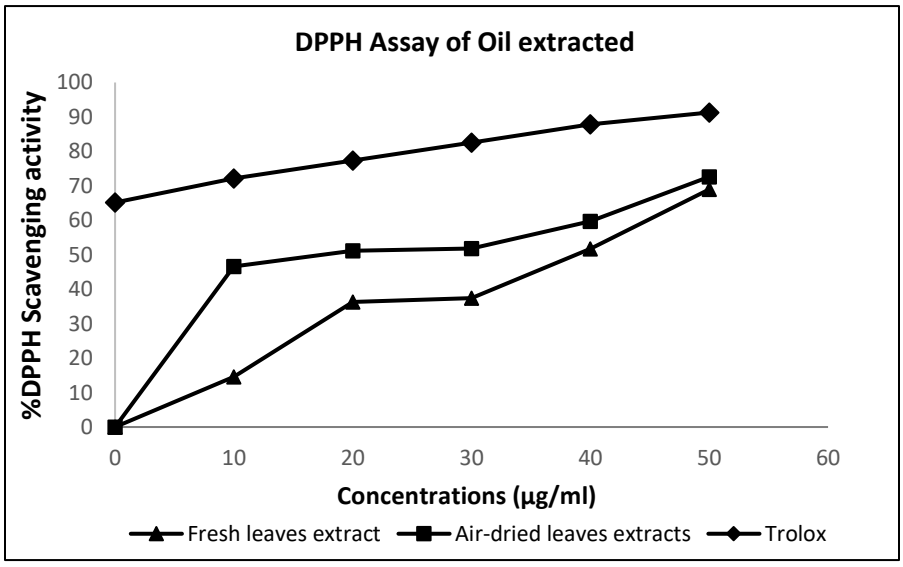

Fig. 2: Percentage of DPPH inhibition of oil extracted from Sacha Inchi leaves

CONCLUSION

The proximate results expressed in this study might be considered as initial information on the nutritional components of *Plukenetia volubilis* L leaves. The dried Sacha Inchi leaves contain higher protein, fibre and carbohydrate content than fresh leaves. Soxhlet extraction was used to extract oil from Sacha Inchi leaves, and the effects of different types of solvent (ethanol, propanol and hexane) and pre-treatment (fresh and air-dried leaves) on oil extraction yield, phenolic components, and antioxidant properties were studied. Oil extraction by using ethanol from air-dried leaves provided greater results in terms of oil yield (21.35%), phenolic compound (0.65%) and free radical scavenging activities (61.18%) than oil extracted from fresh leaves. Due to the synthesis

of phenolic compounds during the drying process, Sacha Inchi oil derived from air-dried leaves showed higher oxidation resistance. As a result, to maximise the antioxidant activity of Sacha Inchi leaves products, proper pre-treatment sample conditions and processing methods should be applied. All of these analyses are necessary in order to generate higher quality and quantity of oil. Sacha Inchi leaves oil may have a high natural antioxidant content and potential to be commercialised in the related food industries. The findings of this study are also important in the future research on the benefits of Sacha Inchi products, especially to the manufacturer of oil products derived from Sacha Inchi plants.

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