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Phytochemicals' compound and antioxidant activities in the food of *Pongo tapanuliensis* from Batang Toru Forest, North Sumatra, Indonesia

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Abstract. *Sianipar HF, Widoretno W, Hakim L, Amolia RR, Fatchiyah F. 2024. Phytochemicals' compound and antioxidant activities in the food of* Pongo tapanuliensis *from Batang Toru Forest, North Sumatra, Indonesia. Biodiversitas 25: 3454-3463. Campnosperma auriculatum* (Blume) Hook.fil. and *Agathis borneensis* Warb. are food for *Pongo tapanuliensis* Nurcahyo, Meijaard, Nowak, Fredriksson & Groves, 2017 found in the Batang Toru Forest, Indonesia. Studying the phytochemical properties of both plant species is important part of conservation framework for Tapanuli orangutans because information regarding their nutrient contents and benefits still needs to be investigated. This research examined the closest components, amino acids, and phytochemicals compounds that have antioxidant activity. The *C. auriculatum* and *A. borneensis* fruit were tested for proximate, amino acid, phytochemical, total flavonoid content, LC-MS/MS, and FRAP to determine antioxidant activity. The results of proximate and amino acid tests showed that *C. auriculatum* has higher contents of carbohydrates (74.49%), protein (7.54%), lipids (3.73%), energy from fat (33.57Kcal/100 g), total energy (361.71Kcal /100g), L-Histidine (1704.975ppm), L-Leucine (3759.28ppm), and L-Lysine (2099.038ppm) compared to *A. borneensis*. The phytochemical tests of both samples were positive for containing alkaloids, flavonoids, saponins, steroids, tannins, phenolics, and quinolines. The higher flavonoid content is found in *C. auriculatum* (94.44±2.77mg QE/g). LC-MS/MS results for both samples, *C. auriculatum* that is (3alpha,20R,24Z)-3-Hydroxy-21-oxoeupha-8,24-dien-26-oic acid, terpenoid group, and *A. borneensis*, that is Procyanidin B2, the flavonoid group. The higher antioxidant capacity was found in *C. auriculatum* (4.63±0.111µg/mL). The *C. auriculatum* has excellent potential as a natural source of nutrition and antioxidants for the Tapanuli orangutan; therefore, it is necessary to develop a strategy by cultivating these two plants and using them in pharmacognosy for *P. tapanuliensis*.

Keywords: Antioxidant, food, nutrition, orangutans, phytochemicals

INTRODUCTION

Orangutans, as living creatures, have various activities, such as making nests, socializing, eating, and resting. Among these activities, eating is the activity with the highest frequency (Shariman and Ruppert 2017). Eating foods influences the biological conditions of orangutans in replacing lost energy and the animal's life activities, which will affect its social organization (Preuschoft et al. 2021). Orangutans spend between 50-60% of their life time in the forest for searching for food, 25-35% for resting, and 10-15% for moving (Dalimunthe et al. 2021).

The Tapanuli orangutan is only found in the Batang Toru Forest Ecosystem, North Sumatra, Indonesia, which covers an area of 142,000 hectares of primary forest (Sianipar et al. 2021). Orangutans are a type of animal that does not only depend on one type of food, but most of its food sources come from fruits (Onrizal and Auliah 2019). Based on research, in the Batang Toru Forest North Tapanuli, North Sumatra Province (Khakim 2015), it was recorded that the food eaten by Tapanuli orangutans including fruits (71.06%), shoots (11.72%), flowers (7.85%), leaves (2.82%), bark (2.06%). %), insects (2.79%), and others (1.71%). There are fruits of 2 species with the highest proportion of food consumption in April, May, June, August, September, October, and November, namely *Campnosperma auriculatum* (Blume) Hook.fil. and *Agathis borneensis* Warb. The *C. auriculatum* is a potential food source for key fauna, especially primates in tropical Asian habitats. The *C. auriculatum* can be found in swampy lowland forests that form pure or dominant stands mixed with other species of wood trees, but this tree can also grow well in forests on well-drained soil, especially in valleys or on river banks, up to high altitudes of 1,600 meters above sea level (masl) (Sanusi et al. 2018). The *A. borneensis* is an evergreen tree with a height of up to 55 meters and a diameter of 150 cm or more. The distribution of *A. borneensis* is spread across Indonesia (Kalimantan and Sumatra), Brunei, and Malaysia. The benefits of this plant

for orangutans can treat headaches and muscle aches (Adam et al. 2017).

Based on the IUCN (2019), Tapanuli orangutans are categorized as Critically Endangered and a new species found in recent years, serves as a priority for conservation (Wich et al. 2019), requiring more intensive treatment to manage their population in Indonesia. In the framework of orangutan management, healthy conditions of orangutans in the forest are the most important thing to avoid extinction. The main information that is very important for orangutans' conservation is the nutritional content of the natural food of orangutans in the forest habitats. According to Onrizal and Auliah's research (2019), the fruits of *Polyalthia lateriflora* (Blume) Kurz, which serves as the food for Sumatran orangutans in Gunung Leuser National Park, North Sumatra, has the best nutrition, with a water content of 62.13%, a fat content of 0.10%, an ash content of 0.21%, protein content of 13 .72% and carbohydrates 23.81%. Besides providing nutrition for orangutans, the food of orangutans can be used as medicine to maintain health conditions (Hamilla 2018). Orangutan food contains different chemical compounds, so it can potentially be a medicine that orangutans naturally use to treat themselves (Laumer et al. 2024). According to research by Atmoko and Ma'ruf (2009), Kalimantan orangutan food taken from the Gunung Beratus Protected Forest in East Kalimantan, such as *Dacryodes rugosa* (Blume) H.J.Lam, *Durio acutifolius* (Mast.) Kosterm., *Madhuca sericea* (Miq.) S.Moore, *Triomma malaccensis* Hook.fil., *Sandoricum koetjape* (Burm.fil.) Merr., and *Scaphium macropodum* (Miq.) Beumée ex K.Heyne, contains alkaloids, steroids and flavonoids, phenolics, and saponins.

The rich profile of phytochemicals such as flavonoids and phenolics is related to plant bioactivity such as natural antioxidants (Tungmunnithum et al. 2018; Kaur et al. 2023), based on research *Ziziphus mauritiana* Lam. leaves have a high phenolic and flavonoid content, the antioxidant activity is much higher than with *Ziziphus spina-christi* (L.) Desf.. Based on its antioxidant composition, *Ziziphus* spp. The leaves can be a natural source of antioxidants that can protect the body from damage caused by free radicals and delay the development of chronic diseases caused by a decrease in Reactive Oxygen Species (ROS), especially hydroxyl and superoxide radicals (Aldhanhani et al. 2022). This research aims to identify the composition of phytochemical profiles, analyze specific phytochemical compounds, and analyze the antioxidant activity of Tapanuli orangutan food. Based on this, research on using Tapanuli orangutan food sources can be used as a good source of nutrition with beneficial antioxidant activity, which is essential consideration for Tapanuli orangutan conservation efforts.

MATERIALS AND METHODS

Study area

The research was conducted between October and December 2023 at the Sustainable Ecosystem Foundation Orangutan Conservation Program Research Station in the Batang Toru Forest Area (Camp Mayang) in North Sumatra, Indonesia (098° 59' 38.21754384" E, 01° 41' 08.71413333" N), at an altitude of 300-1,300 masl, which is considered a tropical forest (Figure 1).

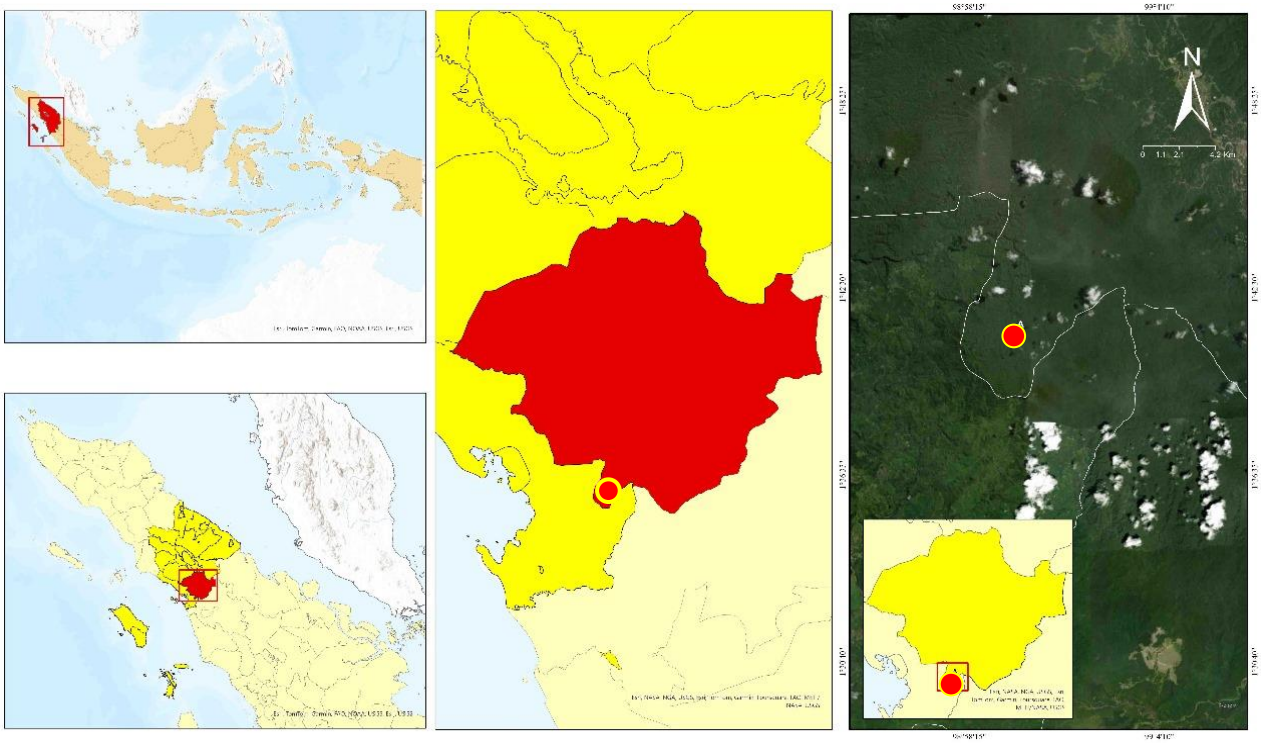


Figure 1. Map of research station of the Sustainable Ecosystem Foundation Orangutan for Conservation Program in Batang Toru Forest, North Sumatra, Indonesia. Red dot is Camp Mayang

Species identification

Plant samples of *C. auriculatum* and *A. borneensis* plant samples were collected from Batang Toru Forest, North Sumatra, Indonesia. Plant identification using dried leaves was carried out at the UPT Herbal Materia Medica Batu Laboratory, East Java Province (No: 0009.3/137/102.20/2024 for *C. auriculatum* and 0009.3/134/102.20/2024 for *A. borneensis*).

Sample preparation

The ripe fruits of *C. auriculatum* and *A. borneensis* collected from several plants in the same population were washed using clean water then were put in the oven at 60°C until dry for 36 hours. Therefore, they were grounded with a blender until they became simplicia powder (Hustiany 2024).

Extraction of *C. auriculatum* and *A. borneensis*

Ten grams of simplicia powder was extracted with a ratio of 96% ethanol solvent (1:10), and the extraction results were filtered. The extract was concentrated using a rotary evaporator at a temperature of 40°C and 90 rpm (Wardani et al. 2019).

Proximate analysis

Proximate analyzes such as water, ash content, protein, lipids, carbohydrates, and energy from fat are carried out at PT. Saraswanti Indo Genetech following the Indonesian National Standard method (SNI 01-2891-1992). The analysis was carried out in triplicate, and the results obtained were average values. Proximate analysis begins with approximately 30 g of powder weighed in a hot air oven at 105°C to constant weight, and the difference in weight is recorded as water content. Next, 3 g of powder was put into a porcelain crucible previously weighed and ignited in a furnace at a temperature of 550°C. The ash content is determined immediately after obtaining white ash, keeping the weight constant. Measurement of total fat content was carried out using the soxhletation method and total protein content was carried out using the Kjehdahl method (Eden and Rumambarsari 2020).

Amino acid content analysis

Amino acid content analysis was carried out in triplicate at PT. Saraswanti Indo Genetech, Bogor, Indonesia. L-histidine, L-leucine, L-isoleucine, and L-lysine were analyzed using UPLC. Tag Ultra C18 1.7 µm (2.1 × 100 mm) column was used for UPLC; amino Acid Analysis of Eluent A AccQ.Tag Ultra concentrate forms the mobile phase, while Amino Acid Analysis of Eluent B forms the second mobile phase. Samples are injected into the system for amino acid Analysis of Eluent B AccQ. Ultra Tags; C: Aquabides; D: AccQ.Tag Ultra 10% in water. At 0.5 mL/min, the flow rate was set. The detector used in this study is a Photometric Diode Array (PDA) operating at a wavelength of 260 nm (Fatchiyah et al. 2020).

Phytochemicals screening

Phytochemical tests have been carried out at the Bioscience Laboratory Center, Brawijaya University. Extraction of *C. auriculatum* and *A. borneensis* were filtered using Whatman paper No. 1. The filtrate was filtered for phytochemical content, including alkaloids, flavonoids, saponins, steroids, tannins, phenolics, and quinolines in triplicate. Alkaloid test one ml of extract was stirred with 5 ml of 1% hydrochloric acid in a steam bath (60°C) for 15 minutes and filtered. Wagner's reagent was applied to 1 ml of filtrate. The presence of alkaloids is indicated by the observation of a foggy brown tint. Test for flavonoids 1 ml of 10% NaOH was combined with 1 ml of extract. It was given a little shake. The presence of flavonoids is indicated by a murky yellow precipitate. Test for saponins. 5 ml of distilled water was combined with around 1 ml of extract. It was violently shaken for five minutes. The main indication for foams was their persistence. Test for the presence of steroids 1 ml of extract was combined with 1 ml of saturated H_2SO_4. A steroidal ring was revealed by a crimson precipitate. Test for tannins. 3% $FeCl_3$ was mixed with one ml. If tannins are present, the precipitate will be greenish-black in color. Test for phenols, 45-50°C was reached by heating 1 ml of the extract in water. 1 ml of 3% $FeCl_3$ was then added. The development of a blue or green tint will signal the existence of phenols. Test for quinoline 1 ml of extract was mixed with 1 ml of 10% KOH (Maimulyanti et al. 2016).

Measurement of total flavonoid content

A colorimetric approach was used to measure the total flavonoid content of the extract in triplicate. 1.5 mL of methanol, 0.1 mL of 5% NaNO2 solution, and 0.5 mL of extract were combined. Thus, after 5 minutes, 0.1 mL of 10% $AlCl_3$ H_2O solution was added, followed by 2.8 mL of distilled water and 0.1 mL of 1M NaOH. After the solution was thoroughly mixed, the absorbance was measured at 700 nm (Phuyal et al. 2020).

LC-MS/MS (Liquid Chromatography-Mass Spectrometry/Mass Spectrometry)

To perform LC-MS/MS analysis, 10 mg of extract was mixed with 10 mL of ethanol. Five (5) µL of solution was taken with a microsyringe and injected into the sample area and UPLC column. This is a liquid chromatography analysis carried out with a mobile water phase. Ammonium with 5 mM formate (A) and acetonitrile with 0.05% formate acid (B) were used. The flow rate was maintained at 0.2 mL/min for 23 min, with an injection volume of 5 µL. To analyze the mass spectrum, an electrospray ionization (ESI) source was used, operating in positive ionization mode in the mass range 50-1200 m/z. The source and desolvation process temperatures were set at 100°C and 350°C, respectively. Chromatograms and mass spectra resulting from UPLC-MS/MS were used using MassLynx Version 4.1 software. Data were recorded, including each detected peak peak area and m/z value. Then, the data was cross-referenced and interpreted using various chemical databases such as PubChem, HMDB, ChemSpider and

CFM-ID to find the chemical contents in the extract (Bakar et al. 2020).

Antioxidant activity with FRAP (Ferric Reducing Antioxidant Potential) method

Samples with a quantity of 100 µL as well as positive controls (0, 2, 4, 6, 8, 10 µg/mL), were then added to 2.5 mL phosphate buffer pH 6.6 and 2.5 mL 1% potassium ferricyanide in each sample and control. Therefore, dark incubation was conducted for the sample extract for 20 minutes at 50°C. Finally, 2.5 mL of 10% TCA was added to the sample extract *C. auriculatum* and *A. borneensis*. From each solution, 5 mL distilled water and 1 mL FeCl3 0.1%. UV-Vis spectrophotometer set at 700 nm to measure absorbance. Ascorbic acid and quercetin were used as a comparison with the sample extract (Stavova et al. 2017).

$$\% \text{ Antioxidant} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \text{ x } 100$$

Data analysis

Statistical analyses were conducted to investigate the differences in Tapanuli orangutan food content, including proximate, amino acids, and antioxidants, between *C. auriculatum* and *A. borneensis*. The t-test was carried out using SPSS ver 16.0 software at a confidence level of 95%. To analyze variations in the food content of Tapanuli orangutans between 2 plant species, an additional Tukey Post-Test was carried out. A One-way ANOVA was carried out for proximate, amino acid, and antioxidant results at a confidence level of 95%. The test was carried out using SPSS ver. 16.0 and GraphPad Prism 8 software (Dwiwibangga et al. 2022).

RESULTS AND DISCUSSION

Proximate content

Proximate content (Table 1) such as carbohydrates (74.49%), protein (7.54%), lipid (3.73%), energy from fat (33.57 Kcal/100g), total energy (361.71 Kcal/100g) is higher in *C. auriculatum*. In comparison, ash (5.32 %) and water (15.98%) were higher in *A. borneensis*. These findings have practical implications for nutritionists and individuals interested in fruit nutrition. While there were significant differences between *C. auriculatum* and *A. borneensis* in carbohydrates, fat, energy from fat, total energy, and water, the fact that there were no significant differences between protein and ash is a key finding. This indicates that the protein and ash content of the two fruits are not significantly different, is a crucial insight for our understanding of their nutritional profiles. Orangutans' optimum needs for proximates are protein (6.1-26.0%), fat (2.9-9.8%), and energy (3.2-4.3 Kcal/100 g) (Dierenfeld 1997). From this information, *C. auriculatum* fruit meets orangutans' requirements of protein, fat and energy. Meanwhile, *A. borneensis* only fulfills the orangutan's requirements of protein and energy because the fat content in the fruit is <2.9% (0.745-1.89%).

Carbohydrates, protein, and fat are the main energy sources for orangutans; carbohydrates function as energy reserves (Galdikas 1988), and protein has a role in replacing damaged body tissue that needs to be overhauled. The main function of protein for the body is to form and maintain existing tissue and new tissue (Wang et al. 2022), and fat has the benefit of protecting organs in the body (Rafeeq et al. 2020). Fruit is the main food source that orangutans usually consume, containing complete nutrients in the form of water, carbohydrates, and more than 60% of the energy needed by the body. Fruit can be a good choice to fulfill orangutan nutrition, especially for adult orangutans who have high mobility. They require more energy and, therefore, more carbohydrates than orangutans infant (Mahyana et al. 2023).

Amino acid content

Organic compounds called Amino Acids (AA) consist of both acid groups and amino groups. The method for measuring amino acids uses HPLC originates from breaking hydrogen bonds in proteins through acid hydrolysis. According to Alagawany et al. (2021), primates need amino acids. Amino acids have a number of purposes, including healing damaged tissue following an injury, shielding the liver from harmful chemicals, controlling cholesterol metabolism, decreasing blood pressure, lowering blood ammonia levels, and promoting the release of growth hormone. Based on test results, the amino acid content in both fruits, such as L-Histidine (1704.975 ppm), L-Leucine (3759.28 ppm), and L-Lysine (2099.038 ppm), is higher in *C. auriculatum* and L-Isoleucine is higher in *A. borneensis* (Table 2).

Orangutans are the only large ape mammals that live in trees, belonging to the Homonidae family (Nater et al. 2017). According to Herring et al. (2021), The type of feed is related to the content of essential and non-essential amino acids. High activity in orangutans requires adequate amino acid intake to support tissue repair and new protein synthesis. The recommended requirements of mammalian omnivores in zoos for dietary amino acids are for the amino acid histidine (38.6-48.8 ppm), isoleucine (65.4-82.5 ppm), leucine (132-176 ppm), and lysine (100 ppm). Therefore, *C. auriculatum* and *A. borneensis* fulfill the amino acid needs of orangutans.

The amino acid histidine functions in protein biosynthesis that one-carbon unit metabolism, the formation of major dipeptides in the brain and muscle, such as carnosine, and the conversion of histamine via decarboxylation. Like most AAs, histidine can cross the blood-brain barrier. However, increased levels of homocarnosine and histidine were found in the brains of mice, guinea pigs, and baby monkeys experiencing protein malnutrition. This may be due to higher hydrolysis of intramuscular proteins and peptides (Wu 2013).

Decreased levels of isoleucine, leucine, and lysine in the plasma of protein-deficient monkeys were accompanied by increased levels of histidine in the brain, which inhibits histidine from crossing the blood-brain barrier. In primates, protein deficiency and specific AA deficiency can cause impaired thermoregulation, increased cortisol levels, decreased growth hormone levels, edema, and psychomotor dysregulation (Enwonwu 1987).

Phytochemical profile

Differences in food types greatly influence the phytochemical content, which are natural compounds that play a role in health and have bioactive effects. The higher the phytochemical content, the better the feed is for the orangutans immune system. The highest phytochemical content in *C. auriculatum* is found in tannins and phenolics (Table 3); tannin and phenolic testing with the addition of $FeCl_3$ condensed to blackish blue. This color change occurs when $FeCl_3$ reacts with one of the hydroxyl groups present in tannin and phenolic compounds (Mahayani et al. 2013). Phenolic compounds have a role in forming stable phenoxy radicals in oxidation reactions, and tannin has a function as a binding agent that can stabilize the lipid fraction, thereby preventing oxidation, which can damage fat so that *C. auriculatum* fruit has great potential as an antioxidant (Shahidi and Ambigaipalan 2015; Nazaruddin et al. 2024).

A. borneensis is a tree that can reach a height of 55 m and a diameter of 100 cm. This species is useful as a medicinal plant, where the wood powder is used to treat headaches and myalgia, and the leaf extract has antiplasmodial and cytotoxic activity (Adam et al. 2017). The highest phytochemical content with three positive values was found in flavonoids, tannins, and phenolics, which are included in the polyphenol group (Table 3). Compounds with hydroxyl groups directly bonded to aromatic hydrocarbon ring groups are known as polyphenolic compounds (Minatel et al. 2017).

This research shows that *A. borneensis* has the highest phytochemical content compared to *C. auriculatum*, therefore this feed has the potential to maintain the health of Tapanuli orangutans.

Total Flavonoids Content (TFC)

Flavonoids also have a carbonyl system conjugated with an aromatic ring so that flavonoid characterization can be carried out using spectrophotometry (Huynh et al. 2024). Therefore, UV-Vis spectrophotometry was used to determine flavonoid levels in *C. auriculatum* and *A. borneensis*, where the standard solutions used were quercetin and ascorbic acid because they are often found in the form of glycosides (Harborne 1984). Flavonoids are one of the most common types of phenolic compounds in plants (Tungmunnithum et al. 2018). Flavonoids can be found in almost all parts of plants, especially in photosynthetic plant cells, which contain chlorophyll (Agati et al. 2020). Table 4 shows that *C. auriculatum* has the highest total flavonoid content (94.44 mg QE/g) and the antioxidant activity increases with TFC level.

Table 1. Proximate content of *C. auriculatum* and *Agathis borneensis* fruit

Sample	Component proximate						
	Carbohydrate (%)	Protein (%)	Lipid (%)	Energy from fat (Kcal/100g)	Energy total (Kcal/100g)	Ash (%)	Water (%)
C. auriculatum	74.49[a]	7.54[a]	3.73[a]	33.57[a]	361.71[a]	4.7[a]	9.52[a]
A. borneensis	70.94[b]	6.65[a]	1.09[b]	9.84[b]	320.22[b]	5.32[a]	15.98[b]

Note: Different letters in one column showed significant differences, while similar letters showed no significant differences in food content between the two species. (Tukey's HSD; P<0.05)

Table 2. The amino acid content of *C. auriculatum* and *Agathis borneensis* fruits

Sample	Concentration (ppm)			
	L-Histidine	L-Isoleucine	L-Leucine	L-Lysine
C. auriculatum	1704.975[a]	1799.747[a]	3759.28[a]	2099.038[a]
A. borneensis	1308.22[b]	2210.008[b]	3380.372[b]	1201.42[b]

Note: Different letters in one column showed significant differences, while similar letters showed no significant differences in food content between the two species (Tukey's HSD; P<0.05)

Table 3. Phytochemical screening using color reactions

Sample	Alkaloids	Flavonoids	Saponin	Steroids	Tannin	Phenolics	Quinolines
Campnosperma auriculatum	++	++	+	+	+++	+++	++
Agathis borneensis	++	+++	+	+	+++	+++	++

Note: -: Targeted compound absence; +: Low target compound intensity, ++: Moderate target compound intensity; and +++: High target compound intensity

Table 4. Total flavonoids content

Sample	Concentration (mg QE/g)
Campnosperma auriculatum	94.44± 2.77 [a]
Agathis borneensis	61.98± 3.56 [a]

LC-MS/MS analysis of *C. auriculatum* fruit extract and *A. borneensis* fruit extract

The chromatogram of the ethanol extract of *C. auriculatum* using LC-MS is shown in Figure 2. The components that are bioactive in the ethanol extract of *C. auriculatum* fruit that can be identified using LC-MS/MS are listed in Table 5. Nine chemical compounds were identified the most, namely Caffeine (99.88%), [3- Amino-5-[hydroxy(oxo)azaniumyl]-1,3,5-triazinan-1-yl]-hydroxy-oxoazanium, L-trans-5-Hydroxy-2-piperidinecarboxylic acid, 5-(Cycloocten-1-yl)-5-ethyl-barbituric acid, Methysergide, Kaempferol, Davallialactone, N-(3beta-Hydroxylup-20(29) -en-28-oyl)-5-aminopentanoic acid, (3alpha,20R,24Z)-3-Hydroxy-21-oxoeupha-8,24-dien-26-oic acid. The caffeine content has a useful effect on relaxing smooth muscles, especially bronchial smooth muscles and cardiac stimulation, and stimulation of the nervous system (Rodak et al. 2021); kaempferol is useful in inhibiting the inflammatory process (Alam et al. 2020).

The chromatogram of the ethanol extract of *A. borneensis* using LC-MS is shown in Figure 3. The components that are bioactive in the ethanol extract of *C. auriculatum* fruit that can be identified using LS are listed in Table 5. Nine chemical compounds were identified the most, namely Lyngbyacarbonate, Procyanidin B2, Methylsciadopitysin Catechin, Eugenol Acetate, Tatarinoid B, Combretastatin A4**,** Apigenin, Methyl Angolensate, 7"-O-. The most abundant bioactive compound of *C. auriculatum* is (3alpha,20R,24Z)-3-Hydroxy-21-oxoeupha-8,24-dien-26-oic acid terpenoids group because its content is 99.93%, which has anti-inflammatory, antioxidant, health benefits. metabolic, antimicrobial, and *A. borneensis* Procyanidin B2 flavonoids group of 99.75% which has the benefits of supporting cardiovascular health, anti-inflammatory, promoting cognitive function and protecting brain cells, helps in regulating blood sugar levels (Evard et al. 2016; He et al. 2023).

The eugenol compound has pharmacological activity as an analgesic, antiemetic, antimicrobial, antidiabetic, antiviral, antifungal, anti-inflammatory, antiseptic, antispasmodic, stimulant, and local anesthetic (Batiha et al. 2020). Catechins are recognized for their anti-inflammatory, antioxidant, antiviral, antitumor, antiobesity, and hepatoprotective activity (Sen et al. 2022).

Based on GCMS analysis on *A. borneensis*, leaves hexane extract contains three main compounds: 8-trimethyl-2-vinyl- (20%) 1, 4-pentandien-3-ol (38%), bicyclo [5.2.0] nonane, 4-methylene-2,8, and 8a(2H)-phenanthrenol (13.3%). Meanwhile, stem bark extract contains two main compounds: 2-hexene, 4-4,5-trimethyl- (14.4%), and (Z, E)-farnesol (11.4%) (Adam et al. 2017).

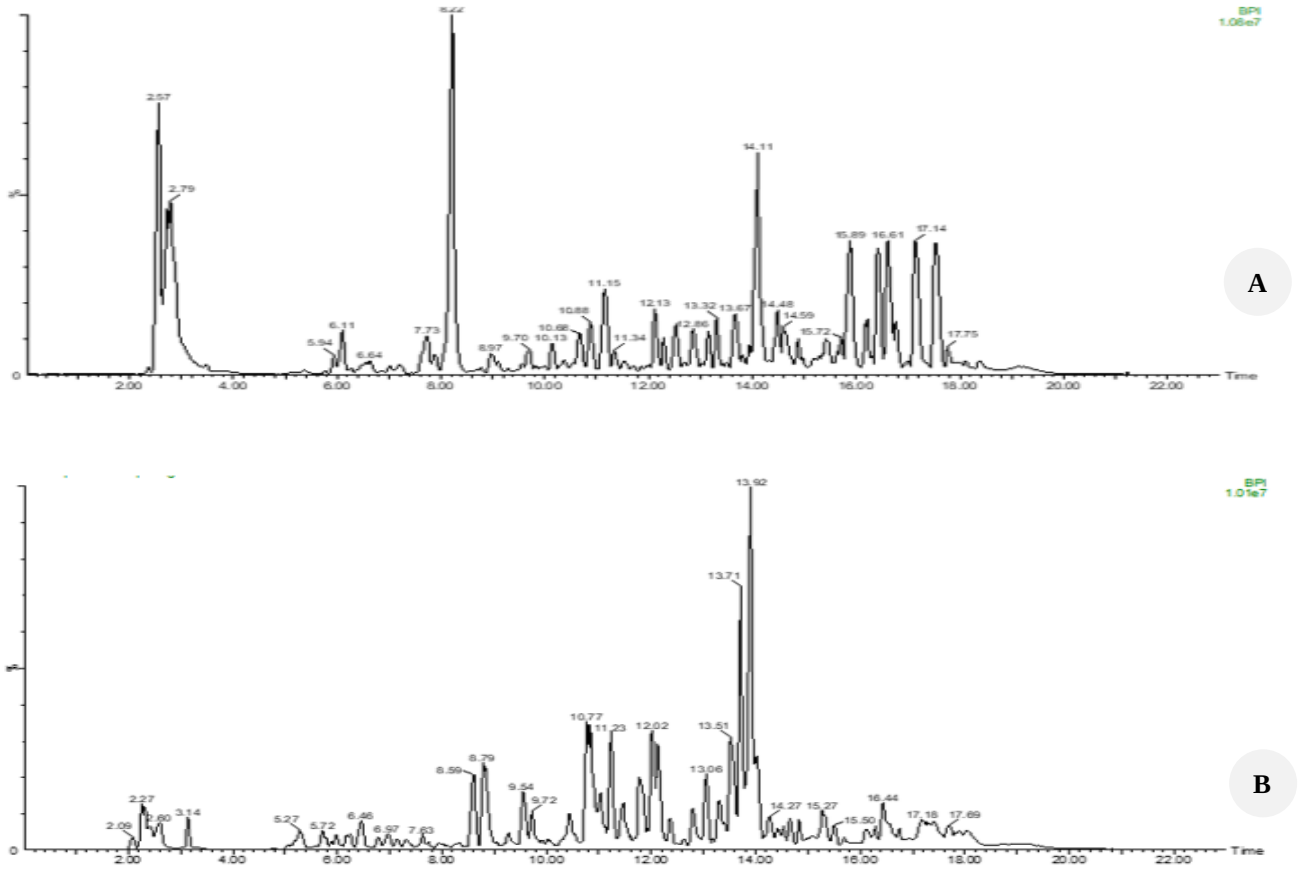


Figure 2. LC-MS/MS chromatogram profile of: A. *Campnosperma auriculatum*; and B. *Agathis borneensis* fruit extract

Meanwhile, three main compounds were found in the bark: formaldehyde (11.7%), thiophene (16.5%), and methyl (10.5%). One compound, bicyclo [5.2.0] nonane, 4-methylene-2,8,8-trimethyl-2-vinyl, was found in both hexane with methanol leaf extracts, and germacrene D and cubebene were found in the same two extracts from stem bark (Sanusi et al. 2018). Based on this research, the bioactive compounds found in *C. auriculatum* and *A. borneensis* are different, this is because previous research used samples of *A. borneensis* leaves and bark from *C. auriculatum*, but this research used *C. auriculatum* and *A. borneensis* fruit.

Antioxidant activity

The FRAP test, a key method in measuring antioxidant activity, was conducted using quercetin and ascorbic acid standards, which played a crucial role in the process. The addition of TCA precipitates the potassium ferrocyanide complex (Dontha 2016), while the introduction of FeCl3 creates a distinct green-blue complex (Berlin blue). The decrease in power serves as a reliable indicator of the potential for antioxidant compounds (Nurhayati et al. 2024).

In the FRAP antioxidant test, polyphenolic compounds are chelating agents against Fe metal (Santos et al. 2017). Chelating agents prevent the formation of oxidative molecules by blocking the catalytic activity of metals and preventing the initiation of oxidant formation (Zeng et al. 2023). The FRAP antioxidant test reaction involves a reduction reaction in the Fe^{3+} complex to Fe^{2+} by the O-H group in polyphenols because they can stabilize radicals by giving them electrons or hydrogen atoms. Chemicals with reduced power can function as antioxidants. This makes radical molecules more stable (Moukette et al. 2015; Gulcin 2020).

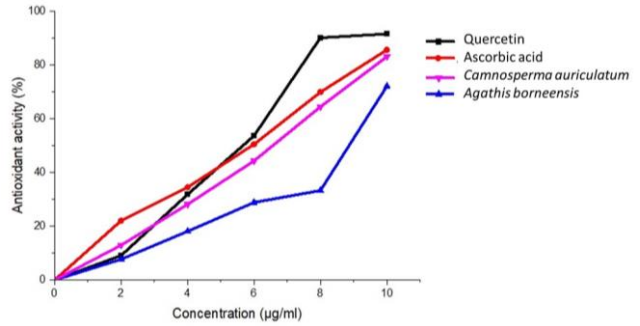


Figure 3. Percentage of antioxidant activity of *Campnosperma auriculatum* and *Agathis borneensis*

Table 5. Data from LCMS/MS chromatogram interpretation of *Campnosperma auriculatum* and *Agathis borneensis* fruit extract

Sample	RT	Measured mass(m/z)	Formula	% Fit conf	Bioactive compound	Group	Structure
C. auriculatum	0.27	195.0881	$C_8H_{11}N_4O_2$	99.88	Caffeine	Alkaloids	
	0.44	195.0885	$C_3H_{11}N_6O_4$	98.23	[3-Amino-5-[hydroxy(oxo)azaniumyl]-1,3,5-triazinan-1-yl]-hydroxy-oxoazanium	Alkaloids	
	6.11	265.1554	$C_{14}H_{21}N_2O_3$	99.79	5-(Cycloocten-1-yl)-5-ethyl-barbituric acid	Diazine	
	8.22	354.2182	$C_{21}H_{28}N_3O_2$	99.77	Methysergide	Alkaloids	
	8.97	287.0558	$C_{15}H_{11}O_6$	99.74	Kaempferol	Flavonoids	

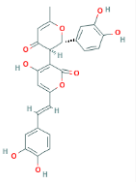
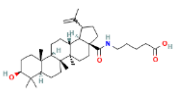
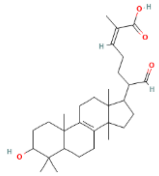
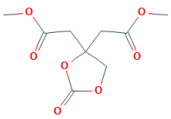
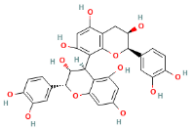
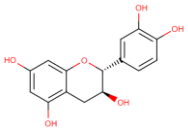
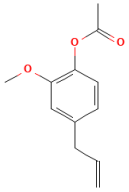
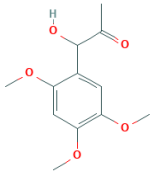
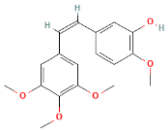
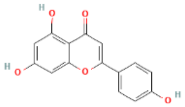
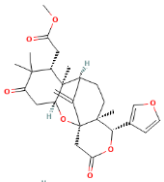
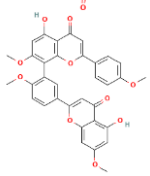
	10.68	465.1187	$C_{25}H_{21}O_9$	99.71	Davallialactone	Terpenoids	
	15.43	556.4370	$C_{35}H_{58}NO_4$	99.56	N-(3beta-Hydroxylup-20(29)-en-28-oyl)-5-aminopentanoic acid	Steroids	
	16.42	471.3481	$C_{30}H_{47}O_4$	99.93	(3alpha,20R,24Z)-3-Hydroxy-21-oxoeupha-8,24-dien-26-oic acid	Terpenoids	
A. borneensis	2.27	233.0666	$C_9H_{13}O_7$	98.28	Lyngbyacarbonate	Polyketides	
	5.72	579.1498	$C_{30}H_{27}O_{12}$	99.75	Procyanidin B2	Flavonoids	
	5.98	291.0875	$C_{15}H_{15}O_6$	99.02	Catechin	Flavonoids	
	6.24	207.1026	$C_{12}H_{15}O_3$	93.28	Eugenol Acetate	Phenolic	
	6.97	241.1080	$C_{12}H_{17}O_5$	99.28	Tatarinoid B	Terpenoids	
	7.14	317.1389	$C_{18}H_{21}O_5$	83.07	Combretastatin A4	Phenolic	
	9.54	271.0612	$C_{15}H_{11}O_5$	72.72	Apigenin	Flavonoids	
	13.06	471.2384	$C_{27}H_{35}O_7$	96.99	Methyl Angolensate	Terpenoids	
	14.66	595.1606	$C_{34}H_{27}O_{10}$	96.87	7”-O-Methylsciadopitysin	Flavonoids	

Table 6. IC_{50} value of *Campnosperma auriculatum* and *Agathis borneensis*

Sample	IC_{50} (µg/mL)	Level
Quercetin	5.169±0.407 [a]	Very strong
Ascorbic acid	5.177±0.113 [a]	Very strong
A. borneensis	7.17±0.354 [b]	Very strong
C. auriculatum	4.63±0.111 [a]	Very strong

Note: Different letters in one column showed significant differences while similar letters showed no significant differences of IC_{50} between two plant species

Based on Table 6 of IC_{50} calculations, *C. auriculatum* (4.63±0.11µg/mL) and *A. borneensis* (7.17±0.354µg/mL) are classified as very strong antioxidants because their high concentrations of polyphenols further increase their antioxidant activity, as IC_{50} value <50 µg/mL is classified into the very strong group, 50-100 µg/mL into the strong category, 101-150 µg/mL into the medium category, and >150 µg/mL into the weak category (Lukman et al. 2024). Damaged polyphenols that are released due to heat treatment or that are liberated due to cell damage during the drying process may have low antioxidant activity, so even though they are present in greater quantities, their antioxidant activities do not increase (Bešlo et al. 2023).

In conclusion, based on proximate components, amino acids, bioactive components, and antioxidant activity, it is demonstrated that *C. auriculatum* possess the highest contents of proximate, amino acids, phytochemical component, total flavonoid content of the Tapanuli orangutan (P<0,05), and the bioactive compounds in *C. auriculatum* are (3alpha, 20R,24Z)-3-Hydroxy-21-oxoeupha-8,24-dien-26-oic acid in the terpenoid group and *A. borneensis* that is procyanidin B2 in the flavonoid group and the highest antioxidant activity is found in *C. auriculatum.* Therefore, future research should focus on developing strategies for cultivating these two plants and their use in pharmacognosy for the *P. tapanuliensis*.

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