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Antimicrobial, antioxidant and phytochemical activities of three orangutan plant foods in Wehea-Kelay Landscape, East Kalimantan

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Abstract. The Bornean orangutan (*Pongo pygmaeus*) is an umbrella species with a high population in the Wehea-Kelay Landscape. In this landscape, at least 227 forage plant species have been identified as potential medicine plants including *Eusideroxylon zwageri*, *Nephelium lappaceum*, and *Tristaniopsis whiteana*. The aim of this study was to determine the yield value of extracts produced from three different plant samples, qualitatively and quantitatively trace the phytochemical content, as well as test the antioxidant and antimicrobial activities. In this study, Qualitative phytochemical assays were conducted by observing the presence of colour changes. Quantitative analysis of phytochemicals was conducted using the colorimetric test method. The assessment of antioxidant activity involves the utilisation of the DPPH radical decolorization test method. An antimicrobial test was performed at a concentration of 62,5–500 µg/well with chloramphenicol and miconazole as positive controls. The qualitative phytochemical examination revealed the presence of alkaloid, flavonoid, tannin, triterpenoid, carbohydrate, carotenoid, and coumarin components in three samples. The quantitative phytochemical analysis revealed that the plant samples possessed a total phenolic content ranging from 2,397 to 4,163 µg GAE/mg extract. The plant samples were analysed and found to have a total flavonoid content ranging from 4,493 to 5,557 µg CE/mg extract. The plant samples demonstrated potent antioxidant activity, effectively inhibiting free radicals. The IC₅₀ values ranged from strong to extremely strong. Antimicrobial testing on medicinal plant extracts provides weak to strong inhibition.

1. Introduction

The Wehea-Kelay landscape spans over 532 thousand hectares and is under the management of the Wehea-Kelay Essential Ecosystem Area Forum. Around sixty-seven percent of the area is lowland and around 87% of the landscape is still forested. Wehea-Kelay has very high flora diversity. The Dipterocarpaceae and Euphorbiaceae families are the predominant tree species. This region exhibits a notable abundance of several fruit tree species, such as durian (*Durio* sp.), rambutan (*Nephelium* sp.), sukun (*Artocarpus* sp.), *Baccaurea* sp., and mangga (*Mangifera* sp.). This particular vegetation is of great significance to the indigenous community as well as the nearby fauna, which includes orangutans, langurs, and gibbons [1].

The Wehea-Kelay Landscape is home to a substantial population of Bornean orangutans (*Pongo pygmaeus*), which are considered an umbrella species. Based on the results of the *Population and Habitat Viability Assessment* (PHVA), an estimation has been made regarding the population of Bornean



orangutans in the landscape to range from 806-821 individuals. This suggests that approximately 26% of East Kalimantan's orang utans live in Wehea-Kelay and are dispersed throughout the management units that are members of the Wehea-Kelay KEE Management Forum [1].

Orang utans has a relatively close genetic kinship with humans of around 97% [2]. It is estimated that there is a wealth of knowledge that can be gleaned from the daily lives of orangutans in their habitat, particularly in relation to learning about the benefits of plants consumed as food and medicine. Several studies on orangutan food nutrition have been conducted in several places, including in Sebangau, Central Kalimantan. Orang utans in that location use the plant *Dracaena cantleyi* as a medicine for pain relief. The plant is frequently utilised by indigenous individuals for comparable intentions. Studies have demonstrated that the plant contains bioactive chemicals known as inflammatory cytokines [3].

The composition of the vegetation at Wehea-Kelay provides an indicator of the quality of the habitat for Bornean orangutans. Currently, around 227 species of orangutan food and nutrition plants have been identified [4] including *Eusideroxylon zwageri*, *Nephelium lappaceum*, and *Tristaniopsis whiteana*. The great potential of the diversity of medicinal plant species in Indonesia, especially in the Wehea-Kelay landscape, East Kalimantan Province to be utilized as medicinal plants, is the rationale for conducting research on the potential bioactivity and phytochemicals of several medicinal plants in Wehea-Kelay, East Kutai and Berau Regencies, East Kalimantan. It is expected that the results obtained can provide information about biological activities in the form of potential bioactivity and phytochemicals, as well as fundamental knowledge on the potential of conventionally used medicinal plants that serve as a source of therapeutic elements intended to alternative development and utilization of non-timber forest products.

2. Material and Methods

2.1 Plants and chemical materials

The raw materials used in this study are the stem bark of several species of orangutan food plants found in the Wehea-Kelay Landscape, East Kalimantan. These plants include *Eusideroxylon zwageri* bark, *Nephelium lappaceum* bark and *Tristaniopsis whiteana* bark. Chemicals used in this study include: ethanol, methanol, acetone, distilled water, glucose, DMSO (*Dimethyl Sulfoxide*), *ascorbic acid*, DPPH (1,1-*diphenyl-2-picrylhydrazyl*) obtained from Tokyo Kasei Kogyo (Tokyo, Japan), *gallic acid*, *Folin-ciocâlteu*, catechin, Na₂CO₃, NaNO₂, AlCl₃, (CH₃COO)₂Pb 1%, dragendorff solution, HCl 1%, CH₃COOH, acetic acid anhydride C₄H₆O₃, concentrated H₂SO₄ solution, H₂SO₄ 85% solution, and NaOH.

2.2 Plant extraction

Plant specimens that have been collected, subsequently purified from soil through the process of washing. Next, cut into smaller sizes using scissors and knives/machete. Then, aerated to dry for ± 3 days and put in the oven at 40°C for ± 48 hours. Plant samples in the form of stem bark studied were made simplisia powder using a chipper. Samples that have been refined, then weighed using an analytical balance and dissolved with ethanol solution.

Plant samples of *Eusideroxylon zwageri* bark, *Nephelium lappaceum* bark, and *Tristaniopsis whiteana* bark were extracted using an ethanol solution at room temperature. The extraction process was facilitated by a shaker (7400 Tubingen, Edmun Buchler, Germany) for a duration of 48 hours. The plant samples were filtered using Whatman No. 2 filter paper (Maidstone, UK). The resulting filtrate was then evaporated using a *rotary vacuum evaporator* at a temperature of 38-40°C. The evaporated solution was subsequently dried in a *vacuum oven* to get the plant extract.

2.3 Qualitative phytochemical test

Qualitative phytochemical tests on plant extracts refer to [5], [6], [7], [8] and [9]. Each qualitative phytochemical test uses 1 mg of dry sample extract. Carbohydrate test using *molisch* reagent and concentrated H₂SO₄ if purple ring formation between two layers is indicative of the presence of carbohydrates. Alkaloid identification is done using Dragendorff solution. Flavonoid test using NaOH

and HCl, the presence of yellow to colorless color indicates positive flavonoids. In the triterpenoid test using chloroform and H₂SO₄, if a golden yellow color is formed, it indicates the presence of triterpenoids. Then, the tannin test uses 1% lead acetate (CH₃COO)₂Pb solution, tannin is positive if the reaction forms a yellow precipitate. The phytochemical analysis involving saponins is identified by the production of foam that lasts for 10 minutes, reaching a height of 1-10 cm, and remains unaffected even after the addition of 1 drop of HCl 2 N. Steroid test can be conducted by combining chloroform and concentrated H₂SO₄. If a blue or green colour is observed, it signifies the presence of steroids. In addition, a carotenoid test can be conducted by using chloroform and 85% H₂SO₄. If a colour ranging from blue to reddish brown appears on the surface, it signifies the presence of carotenoids. The presence of coumarin can be determined by doing a coumarin test using concentrated sodium hydroxide (NaOH) and ethanol. If a yellow colour is observed, it shows the presence of coumarin.

2.4 Total phenolic content

The quantification of phenolic compounds in plant extracts was conducted using the *Folin-Ciocalteu* reagent, following the methodology described by Biju [10], with minor adjustments. *Gallic acid* served as a reference substance for the creation of a calibration curve. The test stock was prepared by dissolving 1 mg of extract in 10 ml of distilled water. Subsequently, a solution containing 1 mg of *gallic acid* was prepared by dissolving it in 10 ml of distilled water. In addition, a solution containing 1 ml of *Folin-Ciocalteu* reagent was prepared by dissolving it in 9 ml of distilled water. Another solution was prepared by dissolving 7.5 g of Na₂CO₃ in distilled water to a final volume of 100 ml.

A 100 µl portion of the assay stock sample was combined with 0.4 ml of distilled water, 0.25 ml of Folin-Ciocalteu reagent, and 1.25 ml of 7.5% Na₂CO₃. Subsequently, the incubation process was conducted for a duration of 60 minutes. An experiment was conducted using a UV-VIS Spectrophotometer set at a wavelength of 760 nm. The presence of phenolic compounds is indicated by the production of a blue or purplish-blue colour.

2.5 Total flavonoid content

Quantifying the overall flavonoid concentration in plant extracts by colorimetric assays, following Biju's method [10] with some alterations. A quantity of 1 milligramme of extract was diluted in 10 millilitres of distilled water to create a testing stock solution. Catechin serves as a reference substance for generating a calibration curve. Subsequently, a quantity of 10 grammes of AlCl₃ was completely dissolved in ethanol, resulting in a final volume of 100 millilitres. In addition, a maximum of 5 grammes of NaNO₂ was dissolved in 100 millilitres of distilled water, while a maximum of 4 grammes of NaOH was dissolved in the same volume of distilled water.

Combine 100 µl of the test stock with 0,7 ml of distilled water. Next, add 0,1 ml of 5% NaNO₂, followed by 0,1 ml of 10% AlCl₃ and 0,5 ml of 1 M NaOH. Subsequently, a 10-minute incubation period was conducted. Experimentation was conducted with a UV-VIS Spectrophotometer set at a wavelength of 510 nm. Yellow colour formation indicates the presence of flavonoids.

2.6 Antioxidant assay

The ethanol extracts of *Eusideroxylon zwageri* bark, *Nephelium lappaceum* bark, and *Tristanopsis whiteana* bark were dissolved using DMSO. Three repetitions were performed to achieve the average test findings. The DPPH free radical test method employed is based on Shimizu's method [11], with minor adjustments. The UV absorption analysis was conducted using a Shimadzu UV-VIS 1240 Spectrophotometer, manufactured by Shimadzu Corp. in Kyoto, Japan.

A quantity of 3 milligrammes of sample extract was dissolved in 1000 microliters of DMSO. In the test, a total of 33 µL of sample, 467 µL of ethanol, and 500 µL of DPPH solution (dissolved in ethanol) were combined in the cuvette. The sample was adequately mixed when the sample volume reached 1000 µL. The samples were thereafter placed in an environment with little light and maintained at a room temperature ranging from 27°C to 30°C for approximately 20 minutes. The determination of antioxidant activity was conducted by measuring the decolorization of DPPH at a wavelength of 517 nm. Experiments were performed utilising test concentrations of 50 ppm, 25 ppm, 12.5 ppm, and 6.25 ppm, with *Ascorbic acid* used as a positive control.

2.7 Antimicrobial assay

The types of microbes used as test microorganisms are *Candida albicans* fungi, *Cutibacterium acnes* bacteria, *Salmonella typhi* bacteria, and *Staphylococcus aureus* bacteria. The antimicrobial test used the agar diffusion method [12] with modifications. The main material used for making microbial growth media is *nutrient agar* (NA). The plant extracts were prepared by measuring 25 mg of the crude extract and subsequently dissolving it in 1000 μ l of acetone. The negative control solution (-) is DMSO. Positive control solutions are *chloramphenicol* and *miconazole*.

The microbes were put into distilled water and then homogenized in laminar flow. The microbes used were adjusted to the *Mc. Farland* standard at 70-75% transmittance with a wavelength of 600 nm. Media as much as 5 ml was poured into each Petri dish that had been sterilized for 15 minutes at 121°C in an *autoclave*, allowed to stand until the media solidified. After that, 25 μ l of microbes were sprinkled on the surface of the media evenly and perforated the media in the center using a cork borer, then 20 μ l of test concentration was dripped. Then the media was tightly covered with plastic wrapping and put into an incubator for 18-24 hours for bacteria with a temperature of 37°C and a range of \pm 48 hours for fungi with a temperature of 28 ° C. Then the calculation of the inhibition area of each test concentration was carried out. Then the calculation of the inhibition area of each test concentration on x, y and z axes was carried out.

3. Results and Discussion

3.1 Plant extracts

The barks of *Eusideroxylon zwageri*, *Nephelium lappaceum*, and *Tristaniopsis whiteana* were soaked in ethanol at room temperature (Table 1). The process of extracting plant compounds using ethanol resulted in an extract yield ranging from 4.40% to 14.76% dependent on the weight of the dried plant sample.

Table 1. Plant extracts yield

Sample	Local Name	Family	Parts used	SW (g)	EW (g)	TY (%)
<i>Eusideroxylon zwageri</i>	Ulin	Lauraceae	Bark	5,0	0,338	6,76
<i>Nephelium lappaceum</i>	Rambutan	Sapindaceae	Bark	5,0	0,220	4,40
<i>Tristaniopsis whiteana</i>	Pelawan	Myrtaceae	Bark	5,0	0,738	14,76

Notes: SW = Sample Weight, EW = Extract Weight, and TY = Total Yield

Table 1 demonstrates that the bark of *T. whiteana* has a higher extract weight compared to other plant samples. The yield is expressed in percentage (%), with a higher yield value indicating a greater amount of extract produced. The extraction process employed can have an impact on the yield of an extract, as indicated by previous research [13]. The yield value can be affected by various factors such as the solvent type, solvent concentration, particle size of the sample, and duration of the extraction process [14].

3.2 Qualitative Phytochemical

The results of qualitative phytochemical screening of some medicinal plant samples on primary compounds and secondary compounds can be seen at table 2.

Table 2. Phytochemical screening results of medicinal plants

Sample	Alk	Fla	Tan	Ste	Tri	Karb	Sap	Caro	Cou
<i>Eusideroxylon zwageri</i>	+	+	+	-	+	+	-	+	-
<i>Nephelium lappaceum</i>	+	+	-	-	-	+	-	+	-
<i>Tristaniopsis whiteana</i>	+	+	+	-	+	+	-	+	+

*Notes: Alk (Alkaloid), Fla (Flavonoid), Tan (Tannin), Ste (Steroid), Tri (Triterpenoid), Carb (Carbohydrate), Sap (Saponin), Karo (Carotenoid), Kum (Coumarin)

**Test results: (+) = chemical compound content, (-) = no chemical compound content

Phytochemical testing was carried out qualitatively using primary compounds (carbohydrates) and secondary compounds (alkaloid, flavonoid, tannin, steroid, triterpenoid, saponin, carotenoid and coumarin). According to table 2, it is evident that all three medicinal plant extracts examined tested positive for alkaloid, flavonoid, carbohydrate, and carotenoid. *E.zwageri* bark comprises alkaloids, flavonoids, tannins, triterpenoids, carbohydrates, and carotenoids. Then, the bark of *N.lappaceum* contains alkaloid, flavonoid, carbohydrate and carotenoid. Meanwhile, the bark of *T.whiteana* contains alkaloid, flavonoid, tannin, triterpenoid, carbohydrate, carotenoid and coumarin.

In the medicinal plant samples studied, there were no secondary metabolites of steroids and saponins. Saponin and steroid compounds are not found in medicinal plant extract samples, because both compounds belong to the non-polar fat group [15]. Some studies reveal saponin and steroid compounds can dissolve in non-polar solvents such as chloroform or carbon tetrachloride [16]. In this study using the maceration extraction method with 99% ethanol solvent which is polar. Solvents have different abilities in taking bioactive compounds of a sample. Polar compounds have the ability to extract compounds from the range of polar to semi-polar compounds [16].

According to the test findings, variations exist in the composition of therapeutic plant components. The disparity arises from the diverse growing conditions that impact the development of identical plant species, including the chemical makeup of the molecules it generates, in terms of both quantity and structure. The phytochemical composition of secondary metabolites, such as flavonoids, in a plant varies across different regions due to the effect of several environmental conditions, including light intensity, temperature, pH, and altitude. These factors directly impact the phytochemical content of the plant. Additionally, the chemical composition of the extracted substances may be influenced by the processing of raw materials. Furthermore, the choice of solvent can also impact the compounds obtained from a plant [17].

3.3 Total fenolik content

Data from the measurement of total phenolic content of medicinal plant samples are attached in table 3.

Table 3. Test results for total phenolic content

Sample	Absorbance	µg GAE/mg extract	mg GAE/g extract	STDEV
<i>Eusideroxylon zwageri</i>	0,0613	4,163	416,27	0,001
<i>Nephelium lappaceum</i>	0,0316	2,397	239,68	0,003
<i>Tristaniopsis whiteana</i>	0,0436	3,111	311,11	0,003

Based on the results obtained from testing the total phenolic content of medicinal plant samples including *Eusideroxylon zwageri* bark at 416.27 mg GAE/g, *Nephelium lappaceum* bark at 239.68 mg GAE/g and *Tristaniopsis whiteana* bark at 311.11 mg GAE/g. The total phenolic test findings indicated that the *N. lappaceum* bark extract had the lowest total phenolic content, measuring 239,68 mg GAE/g extract. Conversely, the *E. zwageri* bark extract had the greatest total phenolic content, measuring 416,27 mg GAE/g extract. The antioxidant activity of *E. zwageri* bark is strongly correlated with its high total phenolic content.

The level of antioxidant activity is closely correlated with the total phenol concentration. A substance with a higher phenol content would have a greater antioxidant activity [18]. Research [19] on mangosteen bark (*Garcinia mangostana*) further confirms that there is a positive correlation between the total phenol content and antioxidant activity. Moreover, the antibacterial activity is affected by the overall phenol concentration, wherein a higher phenol content in a plant extract corresponds to a stronger resistance of the plant extract against germs. Phenol chemicals exert their antibacterial effects by causing damage and penetration of cell walls, as well as precipitation of microbial cell proteins. Phenol components have the ability to disrupt the structure of proteins, including enzymes, leading to their denaturation. Protein denaturation is a condition where proteins undergo changes or destruction of their secondary, tertiary and quaternary structures [20].

3.4 Total flavonoid content

The following test results for total flavonoid content can be seen in table 4.

Table 4. Test results for total flavonoid content

Sample	Absorbance	µg CE/mg extract	mg CE/g extract	STDEV
<i>Eusideroxylon zwageri</i>	0,053	5,557	555,67	0,002
<i>Nephelium lappaceum</i>	0,043	4,493	449,29	0,002
<i>Tristaniopsis whiteana</i>	0,057	5,911	591,13	0,001

The high total flavonoid content in *T. whiteana* bark is related to the high antioxidant activity. Flavonoid compounds are known to have potential as free radical antidotes in biological systems and can provide antioxidant protection. Flavonoids are the most effective reducing agents for inhibiting oxidation processes, both enzymatically and non-enzymatically. Additionally, flavonoids have antioxidant properties that may contribute to the suppression of cancer cells [21]. The research conducted by [22] found a positive link between the quantity of total flavonoids and the level of antioxidant activity. Specifically, it was seen that greater levels of total flavonoids were associated with better antioxidant activity. Furthermore, the presence of total flavonoids has been shown to have antibacterial properties by forming complexes with extracellular proteins, soluble proteins, and microbial cell walls [23].

3.5 Antioxidant activity

Testing of medicinal plant sample extracts was carried out with four concentrations, namely 6,25 ppm; 12,5 ppm; 25 ppm and 50 ppm. The following percentage of free radical capture from medicinal plant sample extracts can be seen in Table 5.

Table 5. Percentage of Free Radical Capture in Medicinal Plant Samples

Sample	Percentage of Capture (%)				IC ₅₀ (ppm)
	6,25 ppm	12,5 ppm	25 ppm	50 ppm	
DPPH	0	0	0	0	-
<i>Ascorbic acid</i>	86	90	93	97	4,70
<i>Eusideroxylon zwageri</i>	12	20	27	34	49,88
<i>Nephelium lappaceum</i>	10	14	21	32	57,51
<i>Tristaniopsis whiteana</i>	9	14	25	41	47,81

The antioxidant efficacy of the bark extracts from *E. zwageri*, *N. lappaceum*, and *T. whiteana* was evaluated by subjecting them to DPPH free radicals. *Ascorbic acid*, often known as vitamin C, was used as the positive control, while DMSO was employed as the negative control. The ethanol extract samples underwent antioxidant testing, which demonstrated the plants ability to inhibit DPPH free radicals. The inhibition percentage varied between 32% and 41% when the concentration was 50 ppm. The *T. whiteana* bark sample exhibited the maximum inhibition percentage at 41%, while the *N. lappaceum* bark sample showed the lowest inhibition percentage at 32% (Figure 1).

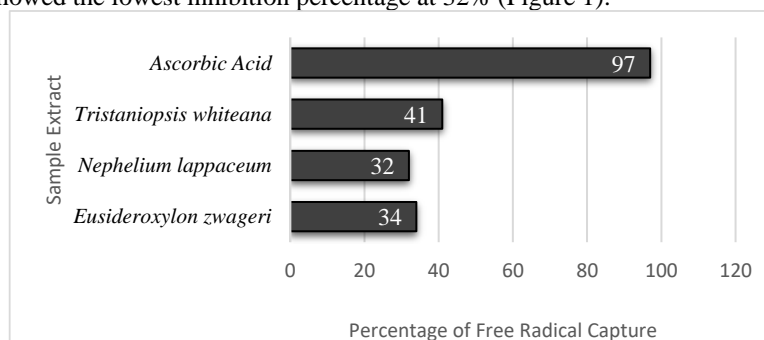


Figure 1. Antioxidant Activity of Plant Extracts at 50 ppm concentration

The antioxidant activity of plant extracts is related to the composition of the chemical compounds contained in them, especially the various types of compounds that have antioxidant activity. Antioxidant test using DPPH radical material on plant sample extracts causes a color change from purple to yellow or clear, which is measured using a spectrophotometer at a wavelength of 517 nm. Antioxidant activity is usually expressed as a percentage inhibition of DPPH, or as IC₅₀. The IC₅₀ value is the concentration that causes 50% loss of DPPH activity.

Table 6. Results of antioxidant activity test on three medicinal plant samples

Sample	IC ₅₀ (ppm)	Category
<i>Ascorbic acid</i>	4,70	Very Strong
<i>Eusideroxylon zwageri</i>	49,88	Very Strong
<i>Nephelium lappaceum</i>	57,51	Strong
<i>Tristaniopsis whiteana</i>	47,81	Very Strong

Based on table 6, two plant sample extracts, the bark of *Eusideroxylon zwageri* and the bark of *Tristaniopsis whiteana*, have IC₅₀ values of less than 50 ppm or classified as very strong. Meanwhile, the bark of *Nephelium lappaceum* has an IC₅₀ value with a strong category. The IC₅₀ value may be used to quantify the efficacy of antioxidant action in pure substances or extracts [24]. As the IC₅₀ value increases, the antioxidant activity decreases. The significant level of free radical scavenging in medicinal plant samples is attributed to the action of secondary metabolites present in the extract. According to the research [25], it was shown that total flavonoids and phenolics accounted for 58,1% and 57% of their antioxidant activity, respectively, while the remaining percentage was impacted by other substances.

3.6 Antimicrobial activity

Antimicrobial testing of *Cutibacterium acnes*, *Salmonella typhi*, *Staphylococcus aureus* and *Candida albicans* against 3 (three) medicinal plant species from the Wehea-Kelay landscape. The antimicrobial activity test aims to determine the ability of medicinal plant extracts to inhibit the growth of bacteria and fungi tested. The positive control used against *Cutibacterium acnes*, *Salmonella typhi* and *Staphylococcus aureus* is *chloramphenicol*. The positive control used against *Candida albicans* is *miconazole*. The results of the clear zone in antimicrobial testing of the three medicinal plant samples are then measured which forms a clear zone where it will be formed which has been tested against microbes. The following results of inhibition against microbes are attached in Table 7.

Table 7. Antimicrobial activity of medicinal plant samples at a concentration of 500 µg/well

Sample	<i>C. acnes</i>		<i>S. typhi</i>		<i>S. aureus</i>		<i>C. albicans</i>	
	IZ (%)	ID (mm)	IZ (%)	ID (mm)	IZ (%)	ID (mm)	IZ (%)	ID (mm)
<i>Chloramphenicol</i>	100	24,3	100	25,7	100	30,1	-	-
<i>Miconazole</i>	-	-	-	-	-	-	100	23,9
<i>Eusideroxylon zwageri</i>	70	16,3	54	13,8	46	13,9	66	13,9
<i>Nephelium lappaceum</i>	71	16,4	44	13,3	40	12,0	47	11,4
<i>Tristaniopsis whiteana</i>	52	12,7	31	8,7	49	14,7	42	10,0

*Notes: the percentage of inhibition zone (IZ) and diameter of inhibition (ID) were performed at a concentration of 500 µg/well.

According to table 7 shows the antimicrobial activity of the three medicinal plant samples against *Cutibacterium acnes* bacteria, *Salmonella typhi* bacteria, *Staphylococcus aureus* bacteria and *Candida albicans* fungi. All plant extracts inhibited microbes with a percentage inhibition of 31% to close to the percentage inhibition of positive controls, both *chloramphenicol* and *miconazole*. Based on the classification of microbial growth inhibition response, all inhibition zone diameter of medicinal plant

samples against *C.acnes*, *S.typhi*, *S.aureus* and *C.albicans* at a concentration of 500 µg/well were classified as strong (11.4-16.4 mm), medium (10.0 mm) and weak (8.7 mm). The percentage inhibition of plant extracts against microbes including *C.acnes* bacteria shown in table 7 in the range of 52-72%, *S.typhi* bacteria (31-54%), *S.aureus* bacteria (40-49%) and *C.albicans* fungi (42-66%).

The research used the agar diffusion technique using wells. This technique involves the introduction of antimicrobial chemicals into wells created in a petri dish containing agar medium that has been inoculated with bacterial cultures for testing purposes. The antibacterial activity of ethanol extracts from the barks of *Eusideroxylon zwageri*, *Nephelium lappaceum*, and *Tristaniopsis whiteana* was tested using four different concentrations: 500 µg/well, 250 µg/well, 125 µg/well, and 62,5 µg/well. Each well was then filled with 20 µl of the corresponding concentration of the medicinal plant sample, as indicated on a petri dish.

The extracts of the medicinal plant samples studied contain compound groups such as alkaloids, flavonoids, triterpenoids, phenolics, and tannins. These compounds inhibit microbial growth. Alkaloids, flavonoids, phenolics, triterpenoids, and tannins all have different mechanisms of action [26]. Specifically, alkaloids work by preventing the production of cell walls, particularly in the peptidoglycan component, which affects cell death. Then, flavanoids can bind to extracellular proteins to form complex compounds that have an impact on disrupting the integrity and damage to cell membranes, especially in the phospholipid section so that their permeability decreases. The activity of proteins (enzymes) contained in the cell membrane will die due to the presence of phenol compounds.

Meanwhile, triterpenoids can bind to proteins, carbohydrates and lipids in the cell membrane which results in decreased or even loss of membrane permeability which ultimately causes cell lysis. Tannins have the ability to interfere with the work of enzymes, namely adhesin and protein transport which results in decreased cell wall permeability and eventually the cell will die [27]. Based on the results of qualitative phytochemical tests, it shows that medicinal plant extracts have secondary metabolite compounds that act as antimicrobials. This is what causes all the medicinal plant extracts tested to produce inhibition zones against microbial growth, both bacteria and fungi.

The antibacterial activity of plant samples is regulated by several parameters, including the quantity of secondary metabolites, the diffusion strength of the extract, and the specific kind of bacteria being inhibited [28]. Concurrently, the level of concentration of the specific chemical plays a crucial role in determining its efficacy. Greater concentrations of plant extracts, together with larger levels of active antimicrobial components, result in increased capacity to inhibit the development of bacteria and fungus [29].

4. Conclusion

This research presents an analysis of the qualitative and quantitative phytochemical composition and bioactivity of three plant species used by Bornean orangutans in the Wehea-Kelay region for their dietary and nutritional needs. The qualitative phytochemical study findings indicate that the samples of medicinal plants consumed by orangutans include a range of primary and secondary metabolite components, such as alkaloids, flavonoids, tannins, triterpenoids, carbohydrates, carotenoids, and coumarins. Furthermore, the three ethanol extracts obtained from the plant samples exhibit elevated levels of total phenolic content and total flavonoid content, making them effective as antioxidant and antibacterial agents. The ethanol extracts of the plant samples demonstrated potent antioxidant activity, as shown by their capacity to effectively suppress free radicals. The IC₅₀ values, which indicate the concentration required to block 50% of the free radicals, fell within the strong range (50-100 ppm) and even the very strong range (<50 ppm). All ethanol extracts of plants samples inhibited microbes with a percentage inhibition of 31% to approach the percentage inhibition of positive controls, both *chloramphenicol* and *miconazole*. The plant sample extracts used have natural antioxidant content and have the potential to be further developed into a cosmetic product or others, although further testing is needed.

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