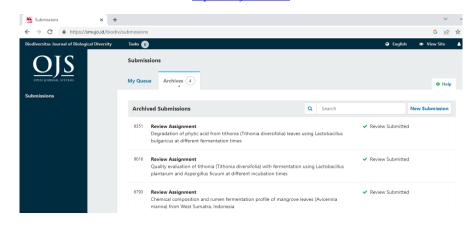
Biodiversitas Journal of Biological Diversity - Smujo (Q3)

Chemical composition and rumen fermentation profile of mangrove leaves (Avicennia marina) from West Sumatera, Indonesia

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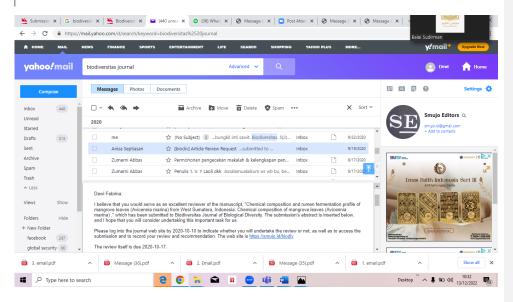


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Chemical composition and rumen fermentation profile of mangrove leaves (Avicennia marina) from West Sumatra, Indonesia

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Keywords Avicennia marina, mangroves, minerals, phytochemicals, proximate, rumen fluid

Chemical composition and rumen fermentation profile of mangrove leaves (Avicennia marina) from West Sumatera, Indonesia

Abstract. This study aimed to determine the potential of mangrove leaves (*Avicennia marina*) for ruminant animal feed. Laboratory tests were carried out on *Avicennia marina* with three replicates. Parameters measured were proximate and fiber contents, rumen fluid profile (pH, NH₃ and VFA), digestibility of nutrients (DM, Ash, CP, CF, NDF, ADF, cellulose, and hemicellulose), macro and micro mineral contents, and phytochemical compounds. The results showed the nutritional content of *Avicennia marina* were CP 13.37%; Ash 7.17%; lignin 7.34%; TDN 79%, rumen fluid profile is in reasonable condition, digestibility of food substances is more than 50%, rich in macro and micro minerals and contains phytochemical compounds such as phenols, steroids, triterpenoids, and tannins. This research concludes that *Avicennia marina* is very potential to be used as a ruminant animal feed.

Keywords: Avicennia marina, mangroves, minerals, phytochemicals, proximate, and rumen fluid.
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21 Running title: Chemical composition of mangrove leaves (Avicennia marina)

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INTRODUCTION

23 Indonesia is a country with the most extensive mangrove forests globally (Richards and Friess 2016;

Bunting et al. 2018). Indonesia's reliable mangrove forests are currently 3,361,216.61 ha (Rahardian
 et al. 2019). Mangrove forests help to reduce the impact of hurricanes, large waves, and winds from
 tropical cyclones. Mangrove trees reduce wave energy as they pass through mangrove forests and

become barriers between streams and land (United Nations Environment Program 2014). When the
sea is high tide, mangrove forests are flooded with water, and at low tide, thick mud covers the surface
of the soil, which stores wealthy organic material (FAO 2007).

Avicennia marina is a mangrove tree species almost always found in major mangrove ecosystems
 (Tomlinson 1986). Local people use this plant's stems and twigs for firewood, furniture, building
 materials, boat balancing joints, and fishing net dyes (Armitage 2002). These products are harvested

33 on a small and large scale, contributing to local livelihoods and national exports.

34 Avicennia marina leaves have a pointed shape at the tip and are green at the front and grayish at the 35 bottom with about 5-11 cm. The flowers are small round with a diameter of about 0.4 - 0.5 cm and 36 yellow to orange, while the fruit is round with a pointed tip and smooth-haired surface, green with a 37 length of 1.5 - 2.5 cm and a width of 1.5 - 2.0 cm (Kitamura et al. 1997). In the coastal areas of Indonesia, 38 people use their leaves to feed goats. These leaves fall off, and the amount is quite adequate as a forage 39 source for animal feed. Nevertheless, to date, there is little research that explores the potential of 40 Avicennia marina leaves as ruminant feed. This study aimed to evaluate the possibility of Avicennia 41 marina leaves as ruminant feed in terms of nutritional content, phytochemicals, digestibility and rumen 42 fluid profile in vitro.

43 44

MATERIALS AND METHODS

45 Sample Collection and Nutrient Analysis

46 The materials used in this experiment consist of Avicennia marina leaves and fruit, Tithonia diversifolia 47 leaves, Gliricidia sepium leaves, Leucaena leucocephala fruit, and leaves. Avicennia marina leaves were 48 taken from the South Coast mangrove forest, South Pesisir regency. Tithonia diversifolia, Gliricidia 49 sepium, and Leucaena leucocephala leaves were collected from the experimental gardens of the Faculty 50 of Agriculture, Andalas University. Leaves from these species have been traditionally used for feeding 51

ruminants and therefore used as references for evaluating *Avicennia marina* leaves' potency.

All the leaf samples were oven-dried at 60°C for 24h. Proximate content was analyzed by standard methods, according to AOAC (2000). Neutral detergent fiber (NDF), cellulose, and acid detergent fiber (ADF) were analyzed according to Van Soest et al. (1991). *In vitro* rumen incubation method followed the procedure of Tilley and Terry (1963), macro and micro minerals using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) while phytochemical compounds by the Harborne (1987). All the analyses were carried out at the Biochemistry Laboratory of the Faculty of Pharmacy and Water Laboratory of the Faculty of Engineering, Andalas University.

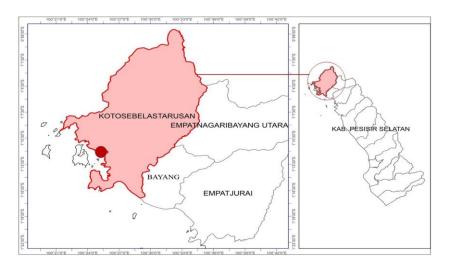


Figure 1. Research location (Avicennia marina leaf sampling) map using the Geographical Information System (GIS).

59

60 Phytochemical Analysis

61 Before phytochemical analysis, Avicennia marina fruit and leaves, Tithonia diversifolia leaves, Gliricidia 62 sepium leaves, Leucaena leucocephala fruit and leaves were ground into flour, put into a bottle, added 63 with 90% methanol solvent in a ratio of 1: 3 (w / v), macerated with solvent methanol 3x24 hours and 64 every 24 hours the methanol solvent was replaced. The maceration results were then filtered using Whatman filter paper no. 42 so that the resulting filtrate. The filtrate was subjected to several 65 66 phytochemical screening tests, i.e., alkaloid, flavonoid, phenolic, saponin, steroid, and triterpenoid 67 tests. For the alkaloid test, the chloroform layer was added ten drops of H_2SO_4 and shaken slowly, allowed to form an acidic layer. A layer of acid (the part under the clear ring formed from the addition 68 69 of H₂SO₄) was taken, and one drop of Meyer reagent was added. A white mist characterized positive 70 reactions. The flavonoid Test layer of water as much as 2 ml from the preparation stage was taken and 71 put into a test tube. Then 1-2 grains of Magnesium were added, and three drops of HCl were added. 72 Positive samples contain flavonoids. If they form orange to Concerning the phenolic test, a layer of 73 water from the preparation stage was taken and put into a drip plate, then added ferric chloride to 74 each drip plate that has been sampled. The formation of blue and purple characterizes the presence of 75 phenolic compounds. A 2 ml layer of water from the preparation stage was taken and put into a test 76 tube then shaken for the saponin test. Positive samples contain saponins if they are formed 77 permanently, which do not disappear within 15 minutes. Steroid and triterpenoid test was performed 78 by taking the chloroform layer from the preparation stage and put into a Pasteur pipette, which 79 contains charcoal. The filtrate that comes out of Pasteur's pipette was inserted into three holes on the 80 drip plate, adding one drop of anhydrous acetic acid and one drop of H₂SO₄. Positive samples containing 81 steroid compounds were shown in blue to purple, while positive samples contain triterpenoid 82 compounds if produced in red.

83

84 Determination of Mineral Contents

85 Avicennia marina leaves and fruits, Tithonia diversifolia leaves, Gliricidia sepium leaves, Leucaena 86 leucocephala fruits and leaves were dried in an oven at 60 °C for 24 hours. Then the sample was ground 87 and filtered using a 20 mesh filter to obtain a powdered sample. One gram of powdered sample was added with 2 ml of distilled water, then dried in the furnace at 150 °C for 15 minutes. Then the sample 88 89 was cooled at room temperature. Dilute using aqua dest to a volume of 25 ml, and then the sample 90 was filtered using 45 mesh filter paper. The destruction results were analyzed in the mineral content of Fe, Zn, Mn, Cu, and Co using the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) 91 92 tool.

93 RESULT AND DISCUSSION

94 Chemical composition of Avicennia marina leaves

95 Avicennia marina leaves contain 13.37% crude protein (CP) with 79% Total Digestible Nutrient (TDN) 96 (Table 1). This value makes Avicennia marina leaves included in the category of high-quality forage. 97 (Jamarun and Zain 2013) classify forage quality in three categories based on CP and TDN content, 98 namely low quality forage (CP <4%, TDN> 40%), medium quality forage (CP 5-10%, TDN 40-50%) and 99 high-quality forage (CP> 10%, TDN> 50%). High forage CP and TDN are needed by livestock to optimize 100 their growth and production. Some CP in the rumen will be overhauled into NH₃ by proteolytic enzymes 101 produced by rumen microbes. NH₃ concentration is an important source of N for rumen microbes and 102 is used for microbial protein synthesis. NH₃ production is influenced by the amount of protein in feed 103 ingredients (Pazla et al. 2018). High TDN illustrates that these leaves have a high digestibility, so only a 104 few nutrients come out as feces.

Table 1. Chemical composition of Avicennia marina leaves						
	Chemical composition	%				
	Dry Matter	89.19±0.07				
	Ash	7.17±0.09				
	Organic Matter	92.83±0.11				
	Crude Protein	13.37±0.23				
	Crude Fiber	12.18±0.27				
	Crude Fat	3.18±0.39				
	NDF	45.99±0.41				
	ADF	35.95±0.43				
	Cellulose	23.10±0.42				
	Hemicellulose	10.03±0.67				
	Lignin	7.34±0.72				
	TDN	79.00±0.98				

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105

107 The high CP content in Avicennia marina leaves caused by soil organic matter (OM). (FAO 2007) states 108 that in mangrove areas, there is high organic matter in thick mud that lines the surface of the land at 109 low tide. Land influences nutrition, plant growth, and development. Plants will grow and develop 110 optimally if the soil conditions in which they live fit the nutritional and nutrient requirements. According to (Kennish 2000), mangrove roots can accumulate sediment and play a role in forming soil formations. 111 112 Mangroves are suppliers of organic material, so they can provide food for organisms that live in the 113 surrounding waters. Sedimentation that occurs in mangrove areas is different from other regions. 114 Sources of sedimentation come from the land, sea, and mangrove areas in the form of deposited leaf deposits, twigs, and dead organisms that are collected so that this region is rich in organic and mineral
materials such as N, P, K, Fe, and Mg (Nugroho et al. .2013). *Avicennia marina* leaves' crude protein
value in this study was higher than reported by (Handayani 2013), 11.04% and lower than (Ghosh et al.
2015), 15.14%. This variation in crude protein values can be caused by plant age, soil fertility, and the
source (Jama et al. 2000).

120 The high protein content of a feed ingredient will also increase the value of organic matter. This is due 121 to crude protein is part of organic material. Table 1 shows the organic matter content of the leaves is 122 also relatively high, at 92.83%. High organic matter will automatically reduce the value of ash content. 123 The higher the ash content, the worse the quality of feed ingredients (Suparjo 2010). (SNI 2017) 124 suggests that cattle's low ash content is 12% maximum, while poultry livestock is 8%. The low-crude fat 125 (CF) content in these leaves (3.18%) is advantageous in ruminant animals. The high-fat content in feed 126 ingredients has been reported to be a cause of digestive and metabolic disorders in cattle (Atteh, 2002). 127 Preston and Leng (1987) supported this, and Palmquist and Jenkins (1980) stated that ruminant animal 128 feed ingredients' standard fat content is below 5%.

129 Crude fiber is needed for ruminants to maintain the development of rumen microbes. Crude fiber that 130 is too low will interfere with the digestive system of ruminants. The *Avicennia marina* crude fiber 131 content (12.18%) is almost equal to the minimum requirement of crude fiber content in feed 132 ingredients, which is 13% for cattle, according to Sudarmono and Sugeng (2008).

133 The NDF content is closely related to feeding consumption because all its components meet the rumen space and are slow to digest. The lower the NDF content, the more food can be consumed. ADF's 134 135 content (cellulose, lignin, silica) is an indicator of forage digestibility because lignin's content is part of 136 an indigestible fraction (Pazla et al. 2020). NDF is always higher than ADF because ADF does not contain 137 hemicellulose. NRC (2001) suggests a minimum of NDF in feed 21% with ADF 19%. The percentage of 138 ADF and NDF content given to livestock should be 25-45% ADF and 30-60% NDF from forage dry matter (Anas et al. 2010). The average value of lignin that livestock can tolerate is 7% (Goering and Van Soest 139 140 1970). The NDF, ADF, and lignin values of these leaves are still within the tolerance range for ruminant 141 animal feed.

142 Rumen Fluid Profile and Nutrition Digestibility

143

The pH value of the rumen fluid from *Avicennia marina* leaves in this study was within the normal range for the growth and development of rumen microbes, mostly bacteria (Table 2). The ideal pH for fiber digestion is 6.4-6.8 (France and Siddon 1993). The pH below 6.2 will reduce plant fiber digestibility because cellulolytic bacteria's activity is inhibited (Erdman 1988). A pH value above 7.1 can reduce the microbial population drastically so that the energy generated from the rumen fermentation process is low (Van Soest 1982).

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- 154

155 Table 2. Rumen fluid profile and nutrition digestibility from Avicennia marina leaves

Parameters	Value
Rumen fluid profile	
рН	6.79±0.02
VFA (mM)	16.88±0.51
NH₃ (mM)	117.5±0.04
Nutrition Digestibility (%)	
DM	56.68±0.54
OM	63.74±0.67
CP	69.96±0.62
CF	61.37±1.58
NDF	57.44±0.96
ADF	51.44±0.92
Cellulose	60.24±0.73
Hemicellulose	62.03±1.04

156

157 Volatile fatty acid (VFA) is a source of energy for the growth and development of rumen microbes. The VFA value produced from Avicennia marina leaves sufficient for rumen microbes to grow and develop 158 optimally. Mc Donald et al. (2010) stated that the optimum VFA condition is 80-160 mM. The high value 159 160 of the resulting VFA indicates that Avicennia marina leaves are a feed material with a high level of 161 fermentability, which is suitable as a source of forage for ruminants. The low lignin content will make it 162 easier for enzymes from rumen microbes to penetrate cellulose and hemicellulose, which are the main 163 components of forming VFA. The high protein content of Avicennia marina leaves also contributed to 164 the high VFA value. There is a positive correlation between high crude protein values and VFA values (Jamarun et al. 2017b; Jamarun et al. 2018). 165

166 The concentration of NH₃ in Avicennia marina leaves in this study was included in the category of the 167 amount of NH₃ that supports rumen microbial growth, namely 6 mM - 21 mM (McDonald 2010). 168 Paengkoum et al. (2006) stated that the maximum NH₃ concentration required for rumen microbes to digest feed was 3.57-14.28 mM. Rumen microbes use NH_3 as a source of N for microbial protein 169 170 synthesis, and its value is also influenced by crude protein levels (Pazla et al. 2018). The pH, VFA, and 171 NH_3 values of Avicennia marina leaves in this study were almost the same as other forages such as 172 Tithonia diversifolia (6.78, 125.88mM, 22.48mM) and Elephant grass (6.79, 87.53 mM 20, 41mM) 173 (Jamarun et al. 2019).

174 Feed digestibility is a large amount of feed that livestock can utilize to meet basic needs and production. 175 Based on Table 2 above, it can be seen that rumen microbes can digest more than 50% of the nutrients 176 from these leaves; this is due to the low lignin content. Lignin in feed ingredients can reduce 177 digestibility, as reported by Jamarun et al. (2017a). Rumen microbes can digest food substances in feed 178 ingredients when the lignin content is low. Imsya et al. (2013) stated that lignin in plant cell walls limits 179 the feed material's digestibility. Crude protein content in feed ingredients will also affect the digestibility level of a feed ingredient. The high protein content of Avicennia marina leaves will provide 180 181 more nitrogen for the growth of rumen microbes. Profitable microbial growth will lead to better feed 182 digestibility (Febrina et al. 2016).

183 Macro and micro mineral contents

184

185 The amount of macro minerals (Ca, Na, Mg, K, S, P, and Cl) Avicennia marina leaves is higher than that 186 of Avicennia marina fruit, Tithonia diversifolia, Gliricidia sepium leaves, and Leucaena leucocephala 187 leaves (Table 3). The high mineral content is because the soil in the mangrove forest is rich in minerals and organic matter. Nugroho et al. (2013) explained that the sedimentation in the mangrove area is 188 189 different from other depositional environments. Sources of sediment in mangrove areas come from 190 land and sea (allochthonous) and from the mangrove area itself (autochtonous) in the form of heaps of 191 fallen leaves, twigs, and dead organisms deposited in the mangrove area and contain a lot of organic 192 and mineral matter (N, P, K, Fe, and Mg). The allochthonous sediment is deposited in mangroves 193 through sediment transport, where suspended particles are carried by tidal currents stored in the 194 mangrove area. Because mangroves have a unique root system, they can reduce tidal currents in the 195 mangrove area.

196 Macrominerals are needed by livestock to build body structures such as bones and teeth (Jamarun and 197 Zain 2013). P mineral is an important mineral to support the growth of rumen microbes digesting fiber 198 (Suyitman et al. 2020). Sulfur minerals are needed by rumen microbes to form amino acids that contain 199 sulfur (Bal and Ozturk 2006). Mineral P and S can stimulate rumen microbial performance to improve 200 feed digestibility (Pazla et al. 2018). Mineral P, S, and Mg were able to increase rumen VFA 201 concentrations. (Febrina et al. 2016). Minerals Ca, P, and Mg at normal levels in the rumen can increase 202 rumen microbial activity in digesting cellulose and VFA (Adriani and Mushawwir 2009). Na functions to 203 increase appetite and maintain osmotic pressure (Jamarun and Zain, 2013). Avicennia marina leaves' 204 mineral content is still in the normal range to help supply the mineral needs. According to McDowell et 205 al. (1983) the range of normal values for mineral content in animal feed for Ca is 0.17-1.53 %. Mg 206 0.05-0.25 %, P 0.17-0.59 %, K 0.50-0.70%, Na 0.01-0.06%, S 0.08-0.15%.

Fe's mineral content in Avicennia marina leaves relatively high compared to Avicennia marina fruit, *Tithonia diversifolia, gliricidia sepium*, and Leucaena leucocephala fruit, but Leucaena leucocephala
leaves have slightly higher Fe (Table 4). Nugroho (2008) states that Fe content in grass is usually
100 - 200 ppm while in legume 200 - 300 ppm. According to Darmono (2007), mineral Fe is used in the
enzymatic metabolism of hemoglobin in the livestock body.

212 The minerals Zn, Mn, and Cu in Avicennia marina leaves show the highest value than other forages in 213 Table 3. Nugroho (2008) states that Mn functions as carbohydrate synthesis, mucopolysaccharide, and 214 enzyme systems, such as pyruvate carboxylase and arginine synthetase. In addition to enzymatic 215 reactions, Mn also functions for growth and reproduction of livestock, Onwuka et al. (2001), which states that Mn's mineral content in goats ranges from 2.98 - 13.9 mg/dl. Based on these data, it can be 216 217 concluded that the livestock reared with Avicennia marina leaf-based feed does not experience Mn 218 mineral deficiency because the Mn content in the forage is sufficient. Nugroho (2008) opinion states 219 that Mn mineral deficiency rarely occurs because Mn levels in the feed are enough for livestock needs. 220

Mineral	Avicenni	ia marina	Thitonia diversifolia	Gliricidia sepium	Leucaena le	ucocephala
content (%)	Leaf	Fruit	leaf	Leaf	Leaf	fruit
Са	0.38±0.007	0.35±0.014	0.21±0.007	0.25±0.014	0.28±0.014	0.24±0.007
Na	0.20±0.014	0.17±0.007	0.09±0	0.14±0.014	0.16±0.007	0.13±0
Mg	0.20±0.07	0.19±0.007	0.20±0.007	0.13±0.007	0.16±0.007	0.15±0.007
К	0.48±0.021	0.41±0.014	0.26±0.007	0.28±0.014	0.32±0.014	0.27±0.014
Р	0.51±0.014	0.47±0.014	0.32±0.014	0.23±0.007	0.42±0.014	0.23±0.014
S	0.01±0	0.0092±<0.001	0.0052±<0.001	0.0063±0	0.0071±<0.001	0.0054±<0.001
Cl	1.03±0.021	0.99±0	0.89±0.014	0.92±0.021	0.96±0.014	0.85±0.021

222 Table 3. Mineral Macro Content of Avicennia marina, Thitonia diversifolia, Gliricidia sepium and Leucaena leucocephala.

224 Table 4. Mineral Micro Content of Avicennia marina, Thitonia diversifolia, Gliricidia sepium and Leucaena leucocephala.

	Avicennia marina		Thitonia diversifolia	Gliricidia sepium	Leucaena le	eucocephala
Mineral content (ppm)	Leaf	Fruit	Leaf	Leaf	Leaf	fruit
Fe	388±<0.001	293±<0.001	293±<0.001	228±<0.001	390±<0.001	328±<0.001
Zn	164±<0.001	135±<0.001	76±<0.001	56±<0.001	88±<0.001	75±<0.001
Mn	211±<0.001	139±<0.001	75±<0.001	56±<0.001	88±<0.001	75±<0.001
Cu	128±<0.001	107±<0.001	40±<0.001	43±<0.001	83±<0.001	53±<0.001

			Test R	esult							
Parameters	Avicennia marina Fruit	Avicennia marina Leaves	Tithonia diversifolia Leaves	Gliricidia sepium Leaves	Leucaena leucocephala Leaves	Leucaena leucocephala fruit					
Alkaloid	+	-	-	-	-	-					
Flavonoid	-	-	-	-	-	-					
Fenolik	+	+	-	-	-	-					
Saponin	-	-	-	+	-	-					
Steroid	+	+	+	+	+	+					
Triterpenoid	+	+	+	+	+	+					
Tanin	+	+	+	+	+	+					

228 Table 5. Phytochemical composition test results of Avicennia marina, Tithonia diversifolia, Gliricidia sepium dan Leucaena leucocephala.

Zinc (Zn) is the micro-mineral often deficient for rumen microbial growth (Leng 1991). To maximize feed degradation in the rumen, the adequacy of Zn minerals is critical, given the strategic role of Zn in increasing rumen microbial growth and as an activator of many enzymes (Elihasridas et al. 2012). Mineral Zn can stimulate rumen microbial growth and improve the appearance of livestock. The Zn content in ruminant animal feed in Indonesia ranges from 20-38 mg/kg of dry ration material (Little 1986). This value is far below the need for rumen microbial metabolism and decrease enzyme activity. Therefore to achieve high feed degradation and microbial growth in the rumen, Zn must be available in sufficient and balanced amounts. The amount of Zn in *Avicennia marina* leaves still in the range to meet the needs of rumen microbes.

According to Darmono and Bahri (1989), the low Cu in animal feed sources will adversely affect Fe intake, even though the Fe content in the feed is adequate. It was reported that low Cu content in forage is one of the causes of anemia in livestock. According to Little (1985), several types of grass or forage are used as sources of feed for ruminants in Indonesia, especially on Sumatra, whose Cunya content is below average (low) limits. Reported by Prabowo et al. (1997) and Mathius (1988) from the results of field examinations that are commonly used as the main feed for goats generally have Cu content below the standard (critical) limit. The Cu content will be even lower during the dry season. This results in animals that consume them, thus experiencing mineral deficiencies. Mc Dowell (1992) states that Cu requirements are influenced by the levels of other mineral rations, which increases the need for ruminants in the presence of high molybdenum (Mo) levels. NRC (1988) recommends a Cu requirement figure of 10 mg/kg for ruminants. The mineral value of Cu in the leaves of the Avicennia marina is sufficient for livestock needs. The definition of Cu will cause bone disorders (paralysis), joint swelling, bone fragility. Pigment deficiency in Cu-deficient animals and humans. However, giving enough copper salt, especially to sheep, will cause accumulation in the liver. Sheep are sensitive to 20-30 mg Cu / kg of Cu ration (Tilman et al. 1998).

Phytochemical Contents

The phytochemical contents of the samples were varied (Table 5). Ruminant animals are more resistant to feed ingredients that contain phytochemicals than poultry. This is due to some phytochemicals that can be used to simplify the process of feed metabolism. Tannins are phytochemicals that function as by-pass protein agents. This means that the protein from feed ingredients eaten by livestock will be protected from rumen bacteria's degradation to enter the small intestine. This tannin can only release its bonds with feed ingredients by enzymes in the small intestine and low pH levels, while in the rumen, tannins are problematic in the rumen bacterial break and normal rumen pH (Jamarun and Zain, 2013). Tannin addition increased neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble CP (ADICP) (Jayanegara et al. 2019). However, the levels should not be excessive because if excessive phytochemicals can hurt livestock productivity. Phytochemicals in feed ingredients such as Tithonia diversifolia, gliricidia sepium, and Leucaena leucocephala have been tested in livestock, apparently still in normal conditions for consumption by ruminant animals and do not show a negative effect on livestock metabolic activities (Arief et al. 2020; Pazla 2018; Ningrat et al. 2019). Jamarun et al. (2019) tested tithonia diversifolia by using up to a 100% level, still having a positive effect on the digestibility of dry matter and organic matter and the fermentability of rumen fluids such as pH, NH₃, and VFA, even better when compared to elephant grass. Fasuyi et al. (2010) identified many phytochemicals found in tithonia diversifolia such as phytates, alkaloids, saponins, and far more than Avicennia marina leaves. This study confirms that Avicennia marina leaves are entirely safe for livestock consumption. In conclusion, the research showed that Avicennia marina leaves could be used as alternative feed ingredients for ruminant animals with CP content of 13.37%, lignin 7.34%, rich in macro and micro minerals, and containing phytochemical compounds such as tannins, steroids, and triterpenoids.

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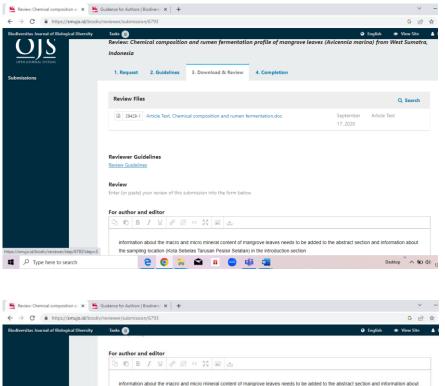
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2. GUIDELINES

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3. REVIEW



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Chemical composition and rumen fermentation profile of mangrove
 leaves (Avicennia marina) from West Sumatera, Indonesia

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Abstract. This study aimed to determine the potential of mangrove leaves (*Avicennia marina*) for ruminant animal feed.
Laboratory tests were carried out on *Avicennia marina* with three replicates. Parameters measured were proximate and
16 fiber contents, rumen fluid profile (pH, NH₃ and VFA), digestibility of nutrients (DM, Ash, CP, CF, NDF, ADF, cellulose, and
17 hemicellulose), macro and micro mineral contents, and phytochemical compounds. The results showed the nutritional
18 condition, digestibility of food substances is more than 50%, rich in macro and micro minerals and contains phytochemical
20 compounds such as phenols, steroids, triterpenoids, and tannins. This research concludes that *Avicennia marina* is very
21 potential to be used as a ruminant animal feed.

Keywords: Avicennia marina, mangroves, minerals, phytochemicals, proximate, and rumen fluid.

Commented [D1]: How many macro and micro mineral content is in mangrove leaves

25 Running title: Chemical composition of mangrove leaves (Avicennia marina)

26 INTRODUCTION

Indonesia is a country with the most extensive mangrove forests globally (Richards and Friess 2016; Bunting et al. 2018). Indonesia's reliable mangrove forests are currently 3,361,216.61 ha (Rahardian et al. 2019). Mangrove forests help to reduce the impact of hurricanes, large waves, and winds from tropical cyclones. Mangrove trees reduce wave energy as they pass through mangrove forests and become barriers between streams and land (United Nations Environment Program 2014). When the sea is high tide, mangrove forests are flooded with water, and at low tide, thick mud covers the surface of the soil, which stores wealthy organic material (FAO 2007).

34 Avicennia marina is a mangrove tree species almost always found in major mangrove ecosystems 35 (Tomlinson 1986). Local people use this plant's stems and twigs for firewood, furniture, building 36 materials, boat balancing joints, and fishing net dyes (Armitage 2002). These products are harvested on 37 a semal and large scale, contributing to lead lively backs and actional events.

a small and large scale, contributing to local livelihoods and national exports.

38 Avicennia marina leaves have a pointed shape at the tip and are green at the front and grayish at the 39 bottom with about 5-11 cm. The flowers are small round with a diameter of about 0.4-0.5 cm and yellow 40 to orange, while the fruit is round with a pointed tip and smooth-haired surface, green with a length of 41 1.5-2.5 cm and a width of 1.5-2.0 cm (Kitamura et al. 1997). In the coastal areas of Indonesia, people 42 use their leaves to feed goats. These leaves fall off, and the amount is quite adequate as a forage source 43 for animal feed. Nevertheless, to date, there is little research that explores the potential of Avicennia 44 marina leaves as ruminant feed. This study aimed to evaluate the possibility of Avicennia marina leaves 45 as ruminant feed in terms of nutritional content, phytochemicals, digestibility and rumen fluid profile 46 in vitro.

47

48 MATERIALS AND METHODS

49 Sample Collection and Nutrient Analysis

50 The materials used in this experiment consist of Avicennia marina leaves and fruit, Tithonia diversifolia 51 leaves, Gliricidia sepium leaves, Leucaena leucocephala fruit, and leaves. Avicennia marina leaves were

taken from the South Coast mangrove forest, South Pesisir regency. *Tithonia diversifolia, Gliricidia*

sepium, and Leucaena leucocephala leaves were collected from the experimental gardens of the Faculty

54 of Agriculture, Andalas University. Leaves from these species have been traditionally used for feeding

55 ruminants and therefore used as references for evaluating *Avicennia marina* leaves' potency.

All the leaf samples were oven-dried at 60°C for 24h. Proximate content was analyzed by standard
methods, according to AOAC (2000). Neutral detergent fiber (NDF), cellulose, and acid detergent fiber
(ADF) were analyzed according to Van Soest et al. (1991). *In vitro* rumen incubation method followed
the procedure of Tilley and Terry (1963), macro and micro minerals using Inductively Coupled Plasma
Optical Emission Spectroscopy (ICP-OES) while phytochemical compounds by the Harborne (1987). All
the analyses were carried out at the Biochemistry Laboratory of the Faculty of Pharmacy and Water
Laboratory of the Faculty of Engineering, Andalas University.

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Commented [D2]: information regarding the sampling locations (Kota Sebelas Tarusan, Pesisir Selatan) and mangrove characteristics should be added to the introduction

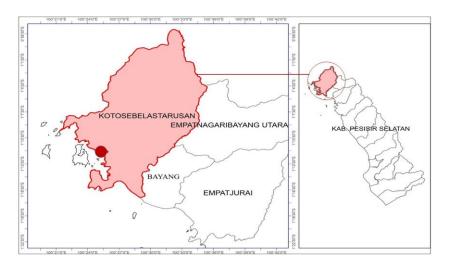


Figure 1. Research location (Avicennia marina leaf sampling) map using the Geographical Information System (GIS).

64

65 Phytochemical Analysis

66 Before phytochemical analysis, Avicennia marina fruit and leaves, Tithonia diversifolia leaves, Gliricidia 67 sepium leaves, Leucaena leucocephala fruit and leaves were ground into flour, put into a bottle, added 68 with 90% methanol solvent in a ratio of 1: 3 (w/v), macerated with solvent methanol 3x24 hours and 69 every 24 hours the methanol solvent was replaced. The maceration results were then filtered using 70 Whatman filter paper no. 42 so that the resulting filtrate. The filtrate was subjected to several 71 phytochemical screening tests, i.e., alkaloid, flavonoid, phenolic, saponin, steroid, and triterpenoid 72 tests. For the alkaloid test, the chloroform layer was added ten drops of H_2SO_4 and shaken slowly, 73 allowed to form an acidic layer. A layer of acid (the part under the clear ring formed from the addition 74 of H₂SO₄) was taken, and one drop of Meyer reagent was added. A white mist characterized positive 75 reactions. The flavonoid Test layer of water as much as 2 ml from the preparation stage was taken and 76 put into a test tube. Then 1-2 grains of Magnesium were added, and three drops of HCl were added. 77 Positive samples contain flavonoids. If they form orange to concerning the phenolic test, a layer of 78 water from the preparation stage was taken and put into a drip plate, then added ferric chloride to 79 each drip plate that has been sampled. The formation of blue and purple characterizes the presence of 80 phenolic compounds. A 2 ml layer of water from the preparation stage was taken and put into a test 81 tube then shaken for the saponin test. Positive samples contain saponins if they are formed permanently, which do not disappear within 15 minutes. Steroid and triterpenoid test was performed 82 83 by taking the chloroform layer from the preparation stage and put into a Pasteur pipette, which 84 contains charcoal. The filtrate that comes out of Pasteur's pipette was inserted into three holes on the 85 drip plate, adding one drop of anhydrous acetic acid and one drop of H₂SO₄. Positive samples containing 86 steroid compounds were shown in blue to purple, while positive samples contain triterpenoid 87 compounds if produced in red.

88

90 Determination of Mineral Contents

91 Avicennia marina leaves and fruits, Tithonia diversifolia leaves, Gliricidia sepium leaves, Leucaena 92 leucocephala fruits and leaves were dried in an oven at 60°C for 24 hours. Then the sample was ground 93 and filtered using a 20 mesh filter to obtain a powdered sample. One gram of powdered sample was 94 added with 2 ml of distilled water, then dried in the furnace at 150°C for 15 minutes. Then the sample 95 was cooled at room temperature. Dilute using aqua dest to a volume of 25 ml, and then the sample 96 was filtered using 45 mesh filter paper. The destruction results were analyzed in the mineral content of 97 Fe, Zn, Mn, Cu, and Co using the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) 98 tool.

99

100 RESULT AND DISCUSSION

101 Chemical composition of Avicennia marina leaves

102 Avicennia marina leaves contain 13.37% crude protein (CP) with 79% Total Digestible Nutrient (TDN) 103 (Table 1). This value makes Avicennia marina leaves included in the category of high-quality forage. 104 (Jamarun and Zain 2013) classify forage quality in three categories based on CP and TDN content, 105 namely low quality forage (CP<4%, TDN>40%), medium quality forage (CP 5-10%, TDN 40-50%) and 106 high-quality forage (CP>10%, TDN>50%). High forage CP and TDN are needed by livestock to optimize 107 their growth and production. Some CP in the rumen will be overhauled into NH₃ by proteolytic enzymes 108 produced by rumen microbes. NH_3 concentration is an important source of N for rumen microbes and 109 is used for microbial protein synthesis. NH₃ production is influenced by the amount of protein in feed 110 ingredients (Pazla et al. 2018). High TDN illustrates that these leaves have a high digestibility, so only a

111 few nutrients come out as feces.

112

Table 1. Chemical composition of Avicennia marina leaves

%
89.19±0.07
7.17±0.09
92.83±0.11
13.37±0.23
12.18±0.27
3.18±0.39
45.99±0.41
35.95±0.43
23.10±0.42
10.03±0.67
7.34±0.72
79.00±0.98

113 The high CP content in Avicennia marina leaves caused by soil organic matter (OM). (FAO 2007) states 114 that in mangrove areas, there is high organic matter in thick mud that lines the surface of the land at 115 low tide. Land influences nutrition, plant growth, and development. Plants will grow and develop 116 optimally if the soil conditions in which they live fit the nutritional and nutrient requirements. According 117 to (Kennish 2000), mangrove roots can accumulate sediment and play a role in forming soil formations. 118 Mangroves are suppliers of organic material, so they can provide food for organisms that live in the 119 surrounding waters. Sedimentation that occurs in mangrove areas is different from other regions. 120 Sources of sedimentation come from the land, sea, and mangrove areas in the form of deposited leaf 121 deposits, twigs, and dead organisms that are collected so that this region is rich in organic and mineral 122 materials such as N, P, K, Fe, and Mg (Nugroho et al. .2013). Avicennia marina leaves' crude protein 123 value in this study was higher than reported by (Handayani 2013), 11.04% and lower than (Ghosh et al. 124 2015), 15.14%. This variation in crude protein values can be caused by plant age, soil fertility, and the 125 source (Jama et al. 2000).

126 The high protein content of a feed ingredient will also increase the value of organic matter. This is due 127 to crude protein is part of organic material. Table 1 shows the organic matter content of the leaves is 128 also relatively high, at 92.83%. High organic matter will automatically reduce the value of ash content. 129 The higher the ash content, the worse the quality of feed ingredients (Suparjo 2010). (SNI 2017) 130 suggests that cattle's low ash content is 12% maximum, while poultry livestock is 8%. The low-crude fat 131 (CF) content in these leaves (3.18%) is advantageous in ruminant animals. The high-fat content in feed 132 ingredients has been reported to be a cause of digestive and metabolic disorders in cattle (Atteh, 2002). 133 Preston and Leng (1987) supported this, and Palmquist and Jenkins (1980) stated that ruminant animal 134 feed ingredients' standard fat content is below 5%.

Crude fiber is needed for ruminants to maintain the development of rumen microbes. Crude fiber that is too low will interfere with the digestive system of ruminants. The *Avicennia marina* crude fiber content (12.18%) is almost equal to the minimum requirement of crude fiber content in feed ingredients, which is 13% for cattle, according to Sudarmono and Sugeng (2008).

139 The NDF content is closely related to feeding consumption because all its components meet the rumen 140 space and are slow to digest. The lower the NDF content, the more food can be consumed. ADF's 141 content (cellulose, lignin, silica) is an indicator of forage digestibility because lignin's content is part of 142 an indigestible fraction (Pazla et al. 2020). NDF is always higher than ADF because ADF does not contain hemicellulose. NRC (2001) suggests a minimum of NDF in feed 21% with ADF 19%. The percentage of 143 144 ADF and NDF content given to livestock should be 25-45% ADF and 30-60% NDF from forage dry matter 145 (Anas et al. 2010). The average value of lignin that livestock can tolerate is 7% (Goering and Van Soest 1970). The NDF, ADF, and lignin values of these leaves are still within the tolerance range for ruminant 146 147 animal feed.

148

149 Rumen Fluid Profile and Nutrition Digestibility

- The pH value of the rumen fluid from *Avicennia marina* leaves in this study was within the normal range for the growth and development of rumen microbes, mostly bacteria (Table 2). The ideal pH for fiber
- digestion is 6.4-6.8 (France and Siddon 1993). The pH below 6.2 will reduce plant fiber digestibility
- because cellulolytic bacteria's activity is inhibited (Erdman 1988). A pH value above 7.1 can reduce the

155 microbial population drastically so that the energy generated from the rumen fermentation process is

- 156 low (Van Soest 1982).
- 157

158 Table 2. Rumen fluid profile and nutrition digestibility from *Avicennia marina* leaves

159

Parameters	Value
Rumen fluid profile	
рН	6.79±0.02
VFA (mM)	16.88±0.51
NH ₃ (mM)	117.5±0.04
Nutrition Digestibility (%)	
DM	56.68±0.54
OM	63.74±0.67
CP	69.96±0.62
CF	61.37±1.58
NDF	57.44±0.96
ADF	51.44±0.92
Cellulose	60.24±0.73
Hemicellulose	62.03±1.04

160

161 Volatile fatty acid (VFA) is a source of energy for the growth and development of rumen microbes. The 162 VFA value produced from Avicennia marina leaves sufficient for rumen microbes to grow and develop 163 optimally. Mc Donald et al. (2010) stated that the optimum VFA condition is 80-160 mM. The high value 164 of the resulting VFA indicates that Avicennia marina leaves are a feed material with a high level of 165 fermentability, which is suitable as a source of forage for ruminants. The low lignin content will make it 166 easier for enzymes from rumen microbes to penetrate cellulose and hemicellulose, which are the main 167 components of forming VFA. The high protein content of Avicennia marina leaves also contributed to 168 the high VFA value. There is a positive correlation between high crude protein values and VFA values 169 (Jamarun et al. 2017b; Jamarun et al. 2018).

170 The concentration of NH₃ in Avicennia marina leaves in this study was included in the category of the 171 amount of NH_3 that supports rumen microbial growth, namely 6 mM-21 mM (McDonald 2010). 172 Paengkoum et al. (2006) stated that the maximum NH₃ concentration required for rumen microbes to 173 digest feed was 3.57-14.28 mM. Rumen microbes use NH_3 as a source of N for microbial protein 174 synthesis, and its value is also influenced by crude protein levels (Pazla et al. 2018). The pH, VFA, and 175 NH_3 values of Avicennia marina leaves in this study were almost the same as other forages such as 176 Tithonia diversifolia (6.78, 125.88 mM, 22.48 mM) and Elephant grass (6.79, 87.53 mM 20, 41 mM) 177 (Jamarun et al. 2019).

178 Feed digestibility is a large amount of feed that livestock can utilize to meet basic needs and production. 179 Based on Table 2 above, it can be seen that rumen microbes can digest more than 50% of the nutrients 180 from these leaves; this is due to the low lignin content. Lignin in feed ingredients can reduce 181 digestibility, as reported by Jamarun et al. (2017a). Rumen microbes can digest food substances in feed 182 ingredients when the lignin content is low. Imsya et al. (2013) stated that lignin in plant cell walls limits 183 the feed material's digestibility. Crude protein content in feed ingredients will also affect the 184 digestibility level of a feed ingredient. The high protein content of Avicennia marina leaves will provide 185 more nitrogen for the growth of rumen microbes. Profitable microbial growth will lead to better feed 186 digestibility (Febrina et al. 2016).

Commented [D3]: in vitro.....

187 Macro and micro mineral contents

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189 The amount of macro minerals (Ca, Na, Mg, K, S, P, and Cl) Avicennia marina leaves is higher than that 190 of Avicennia marina fruit, Tithonia diversifolia, Gliricidia sepium leaves, and Leucaena leucocephala 191 leaves (Table 3). The high mineral content is because the soil in the mangrove forest is rich in minerals 192 and organic matter. Nugroho et al. (2013) explained that the sedimentation in the mangrove area is 193 different from other depositional environments. Sources of sediment in mangrove areas come from 194 land and sea (allochthonous) and from the mangrove area itself (autochtonous) in the form of heaps of 195 fallen leaves, twigs, and dead organisms deposited in the mangrove area and contain a lot of organic 196 and mineral matter (N, P, K, Fe, and Mg). The allochthonous sediment is deposited in mangroves 197 through sediment transport, where suspended particles are carried by tidal currents stored in the 198 mangrove area. Because mangroves have a unique root system, they can reduce tidal currents in the 199 mangrove area.

200 Macrominerals are needed by livestock to build body structures such as bones and teeth (Jamarun and 201 Zain 2013). P mineral is an important mineral to support the growth of rumen microbes digesting fiber 202 (Suyitman et al. 2020). Sulfur minerals are needed by rumen microbes to form amino acids that contain 203 sulfur (Bal and Ozturk 2006). Mineral P and S can stimulate rumen microbial performance to improve 204 feed digestibility (Pazla et al. 2018). Mineral P, S, and Mg were able to increase rumen VFA 205 concentrations. (Febrina et al. 2016). Minerals Ca, P, and Mg at normal levels in the rumen can increase 206 rumen microbial activity in digesting cellulose and VFA (Adriani and Mushawwir 2009). Na functions to 207 increase appetite and maintain osmotic pressure (Jamarun and Zain, 2013). Avicennia marina leaves' 208 mineral content is still in the normal range to help supply the mineral needs. According to McDowell et 209 al. (1983) the range of normal values for mineral content in animal feed for Ca is 0.17-1.53 %, 210 Mg 0.05-0.25 %, P 0.17-0.59 %, K 0.50-0.70%, Na 0.01-0.06%, S 0.08-0.15%.

Fe's mineral content in *Avicennia marina* leaves relatively high compared to *Avicennia marina* fruit, *Tithonia diversifolia, gliricidia sepium,* and *Leucaena leucocephala* fruit, but *Leucaena leucocephala* leaves have slightly higher Fe (Table 4). Nugroho (2008) states that Fe content in grass is usually 100-200 ppm while in legume 200-300 ppm. According to Darmono (2007), mineral Fe is used in the enzymatic metabolism of hemoglobin in the livestock body.

216 The minerals Zn, Mn, and Cu in Avicennia marina leaves show the highest value than other forages in 217 Table 3. Nugroho (2008) states that Mn functions as carbohydrate synthesis, mucopolysaccharide, and 218 enzyme systems, such as pyruvate carboxylase and arginine synthetase. In addition to enzymatic 219 reactions, Mn also functions for growth and reproduction of livestock, Onwuka et al. (2001), which 220 states that Mn's mineral content in goats ranges from 2.98 - 13.9 mg/dl. Based on these data, it can be 221 concluded that the livestock reared with Avicennia marina leaf-based feed does not experience Mn mineral deficiency because the Mn content in the forage is sufficient. Nugroho (2008) opinion states 222 223 that Mn mineral deficiency rarely occurs because Mn levels in the feed are enough for livestock needs. 224

- 225
- 226
- 227
- 228

Mineral content (%)	Avicennia marina		Thitonia diversifolia	Gliricidia sepium	Leucaena leucocephala	
	Leaf	Fruit	leaf	Leaf	Leaf	fruit
Ca	0.38±0.007	0.35±0.014	0.21±0.007	0.25±0.014	0.28±0.014	0.24±0.007
Na	0.20±0.014	0.17±0.007	0.09±0	0.14±0.014	0.16±0.007	0.13±0
Mg	0.20±0.07	0.19±0.007	0.20±0.007	0.13±0.007	0.16±0.007	0.15±0.007
К	0.48±0.021	0.41±0.014	0.26±0.007	0.28±0.014	0.32±0.014	0.27±0.014
Р	0.51±0.014	0.47±0.014	0.32±0.014	0.23±0.007	0.42±0.014	0.23±0.014
S	0.01±0	0.0092±<0.001	0.0052±<0.001	0.0063±0	0.0071±<0.001	0.0054±<0.001
Cl	1.03±0.021	0.99±0	0.89±0.014	0.92±0.021	0.96±0.014	0.85±0.021

229 Table 3. Mineral Macro Content of Avicennia marina, Thitonia diversifolia, Gliricidia sepium and Leucaena leucocephala.

231 Table 4. Mineral Micro Content of Avicennia marina, Thitonia diversifolia, Gliricidia sepium and Leucaena leucocephala.

	Avicennia marina		Thitonia diversifolia	Gliricidia sepium	Leucaena leucocephala	
Mineral content (ppm)	Leaf	Fruit	Leaf	Leaf	Leaf	fruit
Fe	388±<0.001	293±<0.001	293±<0.001	228±<0.001	390±<0.001	328±<0.001
Zn	164±<0.001	135±<0.001	76±<0.001	56±<0.001	88±<0.001	75±<0.001
Mn	211±<0.001	139±<0.001	75±<0.001	56±<0.001	88±<0.001	75±<0.001
Cu	128±<0.001	107±<0.001	40±<0.001	43±<0.001	83±<0.001	53±<0.001

Parameters	Test Result						
	Avicennia marina Fruit	Avicennia marina Leaves	Tithonia diversifolia Leaves	Gliricidia sepium Leaves	Leucaena leucocephala Leaves	Leucaena leucocephala fruit	
Alkaloid	+	-	-	-	-	-	
Flavonoid	-	-	-	-	-	-	
Fenolik	+	+	-	-	-	-	
Saponin	-	-	-	+	-	-	
Steroid	+	+	+	+	+	+	
Triterpenoid	+	+	+	+	+	+	
Tanin	+	+	+	+	+	+	

235 Table 5. Phytochemical composition test results of Avicennia marina, Tithonia diversifolia, Gliricidia sepium dan Leucaena leucocephala.

Zinc (Zn) is the micro-mineral often deficient for rumen microbial growth (Leng 1991). To maximize feed degradation in the rumen, the adequacy of Zn minerals is critical, given the strategic role of Zn in increasing rumen microbial growth and as an activator of many enzymes (Elihasridas et al. 2012). Mineral Zn can stimulate rumen microbial growth and improve the appearance of livestock. The Zn content in ruminant animal feed in Indonesia ranges from 20-38 mg/kg of dry ration material (Little 1986). This value is far below the need for rumen microbial metabolism and decrease enzyme activity. Therefore to achieve high feed degradation and microbial growth in the rumen, Zn must be available in sufficient and balanced amounts. The amount of Zn in *Avicennia marina* leaves still in the range to meet the needs of rumen microbes.

According to Darmono and Bahri (1989), the low Cu in animal feed sources will adversely affect Fe intake, even though the Fe content in the feed is adequate. It was reported that low Cu content in forage is one of the causes of anemia in livestock. According to Little (1985), several types of grass or forage are used as sources of feed for ruminants in Indonesia, especially on Sumatra, whose Cunya content is below average (low) limits. Reported by Prabowo et al. (1997) and Mathius (1988) from the results of field examinations that are commonly used as the main feed for goats generally have Cu content below the standard (critical) limit. The Cu content will be even lower during the dry season. This results in animals that consume them, thus experiencing mineral deficiencies. Mc Dowell (1992) states that Cu requirements are influenced by the levels of other mineral rations, which increases the need for ruminants in the presence of high molybdenum (Mo) levels. NRC (1988) recommends a Cu requirement figure of 10 mg/kg for ruminants. The mineral value of Cu in the leaves of the Avicennia marina is sufficient for livestock needs. The definition of Cu will cause bone disorders (paralysis), joint swelling, bone fragility. Pigment deficiency in Cu-deficient animals and humans. However, giving enough copper salt, especially to sheep, will cause accumulation in the liver. Sheep are sensitive to 20-30 mg Cu/kg of Cu ration (Tilman et al. 1998).

Phytochemical Contents

The phytochemical contents of the samples were varied (Table 5). Ruminant animals are more resistant to feed ingredients that contain phytochemicals than poultry. This is due to some phytochemicals that can be used to simplify the process of feed metabolism. Tannins are phytochemicals that function as by-pass protein agents. This means that the protein from feed ingredients eaten by livestock will be protected from rumen bacteria's degradation to enter the small intestine. This tannin can only release its bonds with feed ingredients by enzymes in the small intestine and low pH levels, while in the rumen, tannins are problematic in the rumen bacterial break and normal rumen pH (Jamarun and Zain, 2013). Tannin addition increased neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble CP (ADICP) (Jayanegara et al. 2019). However, the levels should not be excessive because if excessive phytochemicals can hurt livestock productivity. Phytochemicals in feed ingredients such as Tithonia diversifolia, gliricidia sepium, and Leucaena leucocephala have been tested in livestock, apparently still in normal conditions for consumption by ruminant animals and do not show a negative effect on livestock metabolic activities (Arief et al. 2020; Pazla 2018; Ningrat et al. 2019). Jamarun et al. (2019) tested tithonia diversifolia by using up to a 100% level, still having a positive effect on the digestibility of dry matter and organic matter and the fermentability of rumen fluids such as pH, NH₃, and VFA, even better when compared to elephant grass. Fasuyi et al. (2010) identified many phytochemicals found in tithonia diversifolia such as phytates, alkaloids, saponins, and far more than Avicennia marina leaves. This study confirms that Avicennia marina leaves are entirely safe for livestock consumption. In conclusion, the research showed that Avicennia marina leaves could be used as alternative feed ingredients for ruminant animals with CP content of 13.37%, lignin 7.34%, rich in macro and micro minerals, and containing phytochemical compounds such as tannins, steroids, and triterpenoids.

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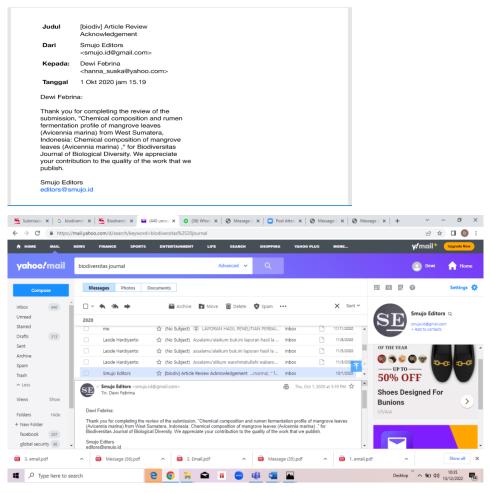
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