

Secondary Metabolites of Saponin and Tanin in Trembesi (*Samanea saman*) and Potential as Ruminant Feed

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ABSTRACT

The purpose of this study was to examine the content of secondary metabolites of saponins and tannins in Trembesi (*Samanea saman*) plants so that their potential as ruminant animal feed could be known. This research is a descriptive study. The data obtained will be presented in a table. The data obtained showed that the parts of the Trembesi plant, namely leaves, petioles, fruit, and fruit skins contained secondary metabolites and tannins. Saponins and tannins can be used to reduce methane production in ruminants. This research can be concluded that Trembesi (*Samanea saman*) contains secondary metabolites of saponins and tannins but their presence can be used as a methane gas remover so that Trembesi has the potential to become ruminant feed.

Keywords: trembesi, saponin, tanin

1. INTRODUCTION

In Indonesia, the population of trembesi trees (*Samanea saman*) is quite large and the distribution is evenly distributed. Trembesi trees are chosen for planting because they have a wide canopy so they are ideal for reforestation plants. Trembesi tree was once one of the commodities required to be planted by the Government of Indonesia, because the tree has a strong ability to absorb ground water, can absorb 28.5 tons/year of carbon dioxide and has roots that can symbiotic with rhizobium bacteria to fix nitrogen from the air. The existence of this rhizobium bacteria can be proven from the results of Dahlan's research which shows that every 100 grams of trembesi leaves contain 47.8 grams of water, 10.2 g of protein, 2.1 grams of fat, 22.2 grams of insoluble carbohydrates, 15 grams of fiber, and 2.0 grams of ash. The results of Sariri et al. [1] showed that the trembesi leaves contained 10.83% protein and 1.47% saponins.

On the other hand, trembesi also contains anti-nutrients. Trembesi leaves contain secondary metabolites, namely tannins. Besides tannins, trembesi leaves also contain flavonoids, saponins, steroids, terpenoids, and cardiac glycosides.

Indonesia is an area that only has two seasons. The dry season is a critical time for ruminants because

limited of forage. The current research results indicated that trembesi has potential as animal feed but has limitations due to the anti-nutritional content. Therefore, a study is needed that examines the content of secondary metabolites, especially saponins and tannins.

2. METHODS

This research was conducted at the Laboratory of Chemistry and Microbiology, University of Veteran Bangun Nusantara. The materials used in this study were young leaves, old leaves, young petioles, old petioles, fruit, seeds, and fruit skin. This research is a descriptive study. The data obtained will be presented in a table.

3. RESULT AND DISCUSSION

The secondary metabolites content of the trembesi parts were presented in Table 1. Plants produce substances as a defence against their predators, known as secondary compounds. Although considered dangerous, it can be beneficial to animals in some cases, especially in ruminants such as saponins and tannins. Table 1 showed that the body parts of the Trembesi plant contain saponins and tannins.

Table 1. Content of Saponins and Tannins in Trembes

Part of Trembesi	Kandungan Metabolit Sekunder	
	Saponin (%)	Tanin (%)
Young leaves	1.44	2.79
Mature leaves	0.86	4.98
Young petiole	0.87	1.61
Mature petiole	0.90	1.16
Fruits	0.95	0.58
Rind	1.44	0.96

3.1. Saponin

Saponins are secondary compounds found in many plants in the roots, bark, leaves, seeds, and fruits that function as a defence system. The presence of saponins can be characterized by a bitter taste, the formation of a stable foam in a liquid solution and the ability to form molecules with cholesterol. In general, immature plants had higher saponin content in the same plant than mature plants [2].

Saponins consist of sugars that usually contain glucose, galactose, glucuronic acid, xylose, rhamnose or methyl pentose, which is bound to hydrophobic aglycones (sapogenins), namely triterpenoids or steroids to form glycosides [2]. Saponins have broad structural diversification, and certain saponin compounds with surfactant properties can cause lysis of the cell wall of protozoa so that they can be used as defaunation of protozoa [3]. Saponins affect the production of methane gas. Many studies have been carried out using saponins derived from the extract or directly from the plant (without being extracted) and in vitro or in vivo. The study looked at the effect of adding saponins to feed on methane emissions, methanogenic populations, protozoa populations and their VFA characteristics (acetate, propionate, butyrate and valerate), gas production, body weight gain, dry matter and organic digestibility, and consumption of feed ingredients.

Pen et al. [4] reported that different doses of saponins resulted in different responses to methane production and the protozoa population (in vitro). The addition of *Yucca schidigera* extract (80-100 g/kg saponins) with levels of 2, 4 and 6 ml/l rumen fluid was very significant ($P<0.001$), reduced methane production from 32-42%, decreased protozoa population to 56%, increased concentration propionate by 54%. The addition of *Quillaja Saponaria* extract (50– 70 g/kg saponins) did not decrease methane production. However, it significantly reduced the protozoa population ($P<0.001$) by 41% and increased the propionate concentration by 19%. This condition showed that the use of higher doses of saponins but still within favourable limits could reduce methane production, protozoa population and increase propionate concentration.

In vitro studies using saponins were also reported by Hu et al. [5]. The addition of saponin extract from tea seeds (60% saponins) with levels of 0.2 and 0.4 mg/ml rumen fluid was very significant ($P<0.01$) in reducing the protozoa population, methane production, but did not affect the increase in total and partial VFA. The protozoa population was $0.53-0.11 \times 10^5 \text{ ml}^{-1}$ with the addition of saponin extract, while the control population was $0.61 \times 10^5 \text{ ml}^{-1}$. Methane production decreased by 12.7 and 14.0% compared to the control. The research results of Suharti et al. [6] reported that the addition of Lerak (*Sapindus rarak*) extract containing 81.5% saponins at levels of 0.6 and 0.8 mg/ml rumen fluid significantly ($P<0.05$) reduced the protozoa population. At the level of 0.8 mg/ml significantly increased ($P<0.05$) the population of *P. ruminicola* (amylolytic bacteria) and the proportion of propionate and decreased the ratio of acetate and propionate. Different responses were reported by Hu et al. [5] that the addition of saponin extract from tea seeds with a level of 0.4 mg/ml did not significantly affect the total and partial VFA. This difference was due to the lower saponin content and level of saponin given compared to *Sapindus rarak*.

Many studies reported an increase in propionate when the feed contains much grain and is different when the feed is high in fiber or a mixture when added with saponins. Saponins can kill or lyse protozoa by forming complex bonds with sterols found on the surface of the protozoan membrane so that it interferes with the development of the protozoa, which causes the membrane to rupture, cell lysis, and the protozoa die. Protozoa are more susceptible to saponins than bacteria because the cell membrane walls of protozoa contain cholesterol, while bacteria are in the form of peptide bonds with glycerol (peptidoglycan). Bacteria do not have sterols that can bind to saponins. In addition, bacteria can metabolize these antiprotozoal factors by removing carbohydrate chains from saponins [3].

3.2. Tanin

Tannins are one of the secondary metabolites found in plants and are synthesized by plants [7]. Tannins are compounds with a molecular weight of 500-3000. They contain many phenolic hydroxy groups that allow them to form effective cross-links with proteins and other molecules such as polysaccharides, amino acids, fatty acids and nucleic acids [8]. Tannins are divided into two groups, namely easily hydrolyzed tannins and condensed tannins. Easily hydrolyzed tannins are polymers of gallic and ellagic acid which are ester-bonded to a sugar molecule, while condensed tannins are polymers of flavonoid compounds with carbon-carbon bonds in the form of catechin and gallocatechin [9].

Tannins derived from forage (leguminose) generally form condensed tannins and have a stronger complex bond with protein than hydrolyzed tannins [8]. Tannins can interact with proteins, and there are three forms of

bonds, namely: (1) hydrogen bonds, (2) ionic bonds, (3) covalent bonds. Hydrolyzed and condensed tannins bind to proteins by forming hydrogen bonds between the phenol groups of tannins and the carboxyl groups (aromatic and aliphatic) of proteins. Strong bonds between tannins and proteins will affect protein digestibility. Tannins also affect the production of methane gas. Many studies have been carried out using tannins derived from the extract or directly from the plant (without being extracted), from condensed or hydrolyzed tannins, *in vitro* or *in vivo*. The study investigated the effect of adding tannins to feed on methane emissions, methanogenic populations, protozoa populations and their VFA characteristics, gas production, body weight gain, dry matter and organic digestibility, and feed consumption.

In vitro study of the added pure condensed tannins from plant extracts of *Leucaena leucocephala* at levels of 0, 10, 15, 20, 25 and 30 mg in 500 mg samples showed that the production of methane gas, total VFA, population of protozoa and methanogenic bacteria would decrease. Methane gas production was significantly decreased ($P < 0.01$) from 10.0-5.5 mg/g BK compared to the control 14.9 mg/g BK. Total VFA production was significantly decreased ($P < 0.01$) from 47.6 to 46.7 mmol/L compared to the control of 57.3 mmol/L. Total protozoa decreased significantly ($P < 0.01$) from 4.73- 4.11 $\times 10^7$ compared to control 5.83 $\times 10^7$. Total methanogenic bacteria was significantly decreased ($P < 0.01$) from 3.24-1.65 $\times 10^7$ compared to 3.25 $\times 10^7$ control.

The addition of pure condensed tannins at the level of 15 mg/500 mg samples gave the best results in reducing methane production by 47% and did not have a negative effect on dry matter digestibility. A decrease in methane production with the addition of tannins was also reported by Jayanegara et al. [10] *in vitro*. The addition of pure tannins, either condensed tannins or hydrolyzed tannins at low concentrations of 0.5 mg/ml rumen fluid or equivalent to 25mg/500 mg of real samples ($P < 0.05$) decreased methane production, total and partial VFA (acetate, propionate, valerate, butyrate), organic matter digestibility and gas production compared with controls. Methane production with the addition of tannins was 13.3-13.5% while the control was 14.7%. The total VFA was 40.6-43.4 mM while the control was 46 mM. The digestibility of organic matter was 70.36-71.46% while the control was 74.83%. This shows that the addition of hydrolyzed tannins lowers methane emissions higher than that of condensed tannins. This is related to the protein precipitation capacity of tannins, namely that easily hydrolyzed tannins can precipitate more BSA protein than condensed tannins [10]. However, many studies have used condensed tannins because condensed tannins have a lower toxic effect than hydrolyzed tannins [11]. The addition of tannins in this low level, can still reduce the digestibility of organic matter and gas production during fermentation.

The addition of tannins was also investigated *in vivo* which showed different results and the same as *in vitro* results. Bauchemin et al. [11] reported that the addition of condensed tannin extract from the quebracho plant containing 91% condensed tannins with a level of 0.1, 2 % BK had no effect on methane emissions, dry matter digestibility, PBB in dairy cattle but decreased protein degradation. by 5 and 15% at levels 1 and 2%.

Different results were reported by Puchala et al. [12] who conducted a study that the addition of condensed tannins *in vivo* in goats was able to reduce methane production. The research of Puchala et al [12], This compares the feed added with condensed tannins at different doses. The addition of tannins derived from the plant *Sericea lepedeza* (17.7% condensed tannins) significantly ($P < 0.001$) reduced methane emissions (10.7 g/kg BK digestibility) and increased ($P < 0.01$) dry matter digestibility (0 ,71 kg/day) compared with the addition of a mixture of *Digitaria ischaemum* and *Festuca arundinacea* (0.5% condensed tannins), where methane production (21.5 g/kg BK digestibility) and dry matter digestibility were only (0.51 kg/day)).

The difference in the results of the study on the effect of adding tannins on methane production, total and partial VFA production, gas production, dry and organic matter digestibility, protozoa population, methanogenic bacteria population, body weight gain could be caused by different doses of tannins, types of tannins, basic substrate as feed and livestock used. The mechanism of decreasing methane production with the addition of tannins was mentioned by Tavendale et al. [13] there are two mechanisms, namely (1) directly inhibiting the growth and activity of methanogens, and (2) indirectly through inhibition of fiber digestion which reduces H₂ production. Condensed tannins have a toxic effect on methanogenic bacteria [13].

Hess et al. [14] reported that the study of measuring protozoa using PCR showed the addition of condensed tannins was able to reduce the population of protozoa. Tannins are reactive phophenols with bacterial cell walls and extracellular enzymes produced by bacteria. This interaction will inhibit the transport of nutrients into cells thereby inhibiting the growth of organisms. The addition of tannins tends to reduce the digestibility of feed ingredients and total VFA production. This decrease was due to the strong binding of tannins to proteins so that some proteins could not be degraded by rumen microbes. This mechanism will benefit livestock in optimal tannin conditions so that protein will be available to the host. Tannins are able to precipitate proteins with a number of functional groups that can form very strong complex bonds with protein molecules. The bond is the bond between the phenol group of tannins and keto proteins which is a hydrolysis bond between the aromatic ring of protein structure and tannins [8]. The presence of these bonds causes the protein cannot be degraded by rumen microbes. The tannin-protein complex bonds are stable at a pH of around 4-7 then will be broken in the abomasum

because the pH is 2.5-3.5 which then enters the small intestine so that the protein can be digested and absorbed [15].

This protein protection is very beneficial because it will increase the supply of quality feed protein to avoid excessive degradation by rumen microbes. It will increase the amount of feed amino acids absorbed by the host animal. However, suppose the concentration is not optimally regulated. In that case, the digestibility and absorption of nutrients can be disrupted because tannins interact not only with the protein but also with fiber and other components such as fiber, vitamins and minerals.

4. CONCLUSION

All parts of trembesi (*Samanea saman*) contain secondary metabolites of saponins and tannins. The presence of saponins and tannins in trembesi can be used as animal feed with the effect of suppressing the production of methane gas.

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