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Physicochemical Characteristics Identification and Secondary Metabolite Analysis of Solid Herbal Waste as Source of Feed Rich Fiber and Supplement for Ruminants

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Abstract. A study was conducted to determine the potency, physicochemical characteristics, and to analyze the secondary metabolites content of solid herbal waste (SWH) as a substitute source of feed rich fibre and feed supplements in ruminants. The first study included an analysis of production potential, physical analysis, and chemical composition analysis of SWH. The second study was an analysis of secondary metabolites content of SWH. The results showed that SWH volume reached 6-8 m³/day or 4020-5360 kg/day, the chemical composition of herbal solid waste was similar to king grass with high lignin content (17.53%). SWH containing total phenols, flavonoids, tannins, saponins, alkaloids and essential oils which generally have antimicrobial activity. According to the potential availability, chemical composition and secondary metabolites content of SWH, it can be used as an alternative for substitute of feed rich fiber or feed supplements with attention to the content of secondary metabolites that can affect the process of fermentation and digestibility in the rumen. Further in vitro and in vivo research are needed to determine the effect of SWH on rumen fermentation parameters and its application in the ration in ruminant livestock.

Key words : Solid herbal waste, feed rich fiber, plant secondary metabolites, antimicrobial activities, rumen fermentation

Abstrak. Penelitian ini bertujuan untuk mengetahui potensi dan karakteristik fisikokimia limbah padat jamu (LPJ) dan melakukan analisis kandungan metabolit sekunder LPJ sebagai pengganti pakan sumber serat dan suplemen pakan pada ternak ruminansia. Penelitian pertama meliputi analisis potensi produksi, analisis fisik, dan analisis komposisi kimia limbah padat jamu. Penelitian kedua adalah analisis kadar metabolit sekunder LPJ. Hasil penelitian menunjukkan volume LPJ mencapai 6 – 8 m³/hari atau 4.020 – 5.360 kg/hari, komposisi kimia limbah padat jamu mendekati rumput raja dengan kandungan lignin yang tinggi (17,53%), LPJ mengandung total fenol, flavonoid, tanin, saponin, alkaloid dan minyak atsiri yang secara umum memiliki efek antimikrobia. Melihat potensi ketersediaan limbah, komposisi kimia dan kandungan metabolit sekundernya, LPJ dapat digunakan sebagai alternatif bahan pakan pengganti serat atau suplemen pakan dengan perhatian terhadap kandungan metabolit sekunder yang dapat mempengaruhi proses fermentasi dan pencernaan di dalam rumen, sehingga diperlukan penelitian lebih lanjut pengaruh LPJ terhadap parameter fermentasi rumen dan aplikasinya dalam ransum pada ternak ruminansia.

Kata kunci: Limbah padat jamu, pakan sumber serat, metabolit sekunder tanaman, efek antimikrobia, fermentasi rumen

Introduction

One potential feed source from industrial waste that can be developed in Java Island but still limitless researched is the solid waste produced from herbal medicinal industry. Industrial waste or residual dregs herbal medicine is extracted from various parts of herbs either the leaves, stems, bark, fruit, seeds, and roots or rhizomes. The parts of this

plant suspected to be the source of fibrous feed or animal feed supplements because they still contain nutrients such as carbohydrates, protein, crude fiber and various bio-active of plant secondary metabolites that can affect the digestion and performance of ruminants.

Solid herbal waste samples used in this study was a waste from medicinal products Antangin[®] containing residues of the roots or rhizomes, stems and leaves of the herb which composed

of mint plant (*Mentha piperita*), sembung (*Blumea balsamifera*) and ginger (*Zingiber officinale*).

In general, extracts of herbs not only contain one kind of secondary metabolites, but could be dozens or even hundreds of secondary metabolites of some kinds of structural group, so that the effect of the herb can be an interaction of a wide variety of secondary metabolites that can not be detected if only one kind of metabolites is evaluated (Wink, 2015). Therefore, it is necessary to study the chemical composition of solid waste herbal medicine, including the content of secondary metabolites, suspected to be contained therein. The objective of this study was to evaluate the solid waste herbal medicine as an alternative to animal feed in terms of its constituent components, nutritional value and plant secondary metabolites content.

Materials and Method

Fresh herbs solid waste sample for physical analysis was taken from solid herbal waste disposal sites of PT. Deltomed Laboratories. Sample collected five times. Each sample in five

Research procedures

Sample of fresh herbs solid waste was taken from the PT. Deltomed Laboratories waste tank in five points at random each weighing of 1000 grams. Samples were kept in a box then taken to the laboratory of Animal Feed Science for analysis. Sampling was performed five times with an interval of two weeks. Examples of each solid waste sample was taken as much as 1000 grams with two replications, then the constituent parts were separated included leaves, stems, seeds, roots, rhizomes and crumbs / dirt. Furthermore, each part of these solid herbal waste constituent was weighed and the percentage was calculated. The characteristics of pH, density, odor, and color of solid waste herbal medicine were recorded. Data were tabulated and analyzed descriptively.

Dry matter (DM), Organic Matter (BO), Crude Protein (CP; N x 6.25) and Ether Extract (EE) of solid herbal waste were determined by proximate analysis (Wendee) according to AOAC (2005). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin were analyzed by the method of Goering and Van Soest (1970). Ca and P were analyzed by spectrophotometer according to AOAC methods (2005).

Total saponins and phenols were analyzed using procedures of Stahl (1985) and the method of Chanwitheesuk *et al.* (2005). Flavonoids were analyzed by spectrophotometry UV-Vis methods according to Farmakope Herbal Indonesia (Departemen Kesehatan RI, 2008). Total tannin content was analyzed by subtraction amount of polyvinylpyrrolidone that bind with tannin and acid equation expressed in tannin (tannic acid equivalents) (Makkar *et al.*, 1993). Alkaloid content was measured using a spectrophotometer at a wavelength of 420 nm. Essential oils measured using maceration method according to Materia Medica Indonesia (Departemen Kesehatan RI, 1995).

Observation variable

Physical analysis observation variable of SWH include the composition of the leaves, stems, seeds, roots, rhizomes and crumbs/dirt. The characteristics of pH, odor, color and density (BJ) also notes. Variable observations of chemical composition SWH was Dry Matter (DM), Organic Matter (OM), Crude Protein (CP; N x 6.25) and Ether Extract (EE), Neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, Ca, P, and secondary metabolites of SWH (total phenols, total tannins, flavonoids, saponins, alkaloids and essential oils).

Data analysis

Physical characteristics, chemical composition, and SWH secondary metabolites content were tabulated and analyzed descriptively (Steel and Torrie, 1995).

Results and Discussion

Physical characteristic evaluation of solid herbal waste

PT. Deltomed produce some instant herbal medicines. According to the catalog of products from PT. Deltomed Laboratories, Tbk. The main components of Antangin® instant herbal medicine composed of ginger extract (*Zingiber officinale*), mint (*Mentha piperita*), sembung (*Blumea balsamivera*), and panax ginseng, while the minor components were rhizome extract of *Curcuma domestica*, *Myristicae cement* and *Glycyrrhizae radix* (Deltomed, 2015). The composition of these plants in herbal medicine has been standardized. Samples for physical characteristics analysis was taken from the waste container vessel in five replication with an interval of two weeks. Solid waste constituent parts of these plant herbs were weighed and the percentage was calculated. The percentage of medicinal plant parts constituent of solid waste is presented in Table 1.

The components of solid waste plant herbs in this study are fine roots, rhizomes, stems and leaves, stems and roots, bark, seeds, and plant crumbs. Fine roots and rhizomes comprises 76.56%, other plant parts such as stems and leaves, stems and roots, bark, and fruit and seed is only only 5.8%, while the unidentified crumbs parts of the plant is 17.64%.

Medicinal herbal plants was extracted using water at high temperatures, around 70-80°C to obtain the extract. The boiling process at high temperatures allegedly can break or loosen lignocellulose ties in plants herbs. Effect of extraction of herbal plants with high temperature is thought to resemble the steaming process to increase the digestibility of fibrous feed ingredients such as rice or wheat straw. Research conducted by Viola et al. (2008) to improving the digestibility of wheat straw, barley, and oats showed using water heating at a temperature of 1980C for 2.5 minutes (steam

explosion) can improve the digestibility of straw up to 25%.

Table 1. Parts of herb plants / 1000 grams of Solid Waste Herb

Parts of Plant	Sample	Percentage
Fine roots	598.2±107.70	60.79%
Rhizome	155.2±45.54	15.77%
Stems and leaves	32.6±35.12	3.31%
Leather trunk	14±19.00	1.44%
Stems and roots	6.8±9.55	0.69%
Fruit and seed	3.4±4.67	0.35%
Mix crumbs	173.6±54.76	17.64%
Total	984±38.29	100.00%

Allegedly, warming effect will also result in denaturation, coagulation and non-enzymatic browning process proteins in solid waste herbal medicine. Protein denaturation occurs when heated at a temperature of 60 - 90°C for approximately 1 hour. Denaturation reaction could not break the peptide bond so the primary structure of the protein molecule is not damaged (Winarno, 2006). It made protein denaturation easy to settle and digest by proteolytic enzymes (Winarno, 1997). Coagulation is defined as one that occurs as a result of proteins damage and clotting heating and hardening of the protein as it absorbs the water in the process (Makfoeld, 2008). Protein will undergo coagulation when heated at a temperature of 50°C or more. Coagulation occurs only when the protein is in iso electric point, at which point the protein is soluble at a pH in the outer of iso electric points (Purwaningsih, 2007). Browning process is the process of yellow pigment formation which will soon turn into dark brown. The formation of a brown color is triggered by oxidation reaction catalyzed by the phenol oxide or polyphenol oxide enzyme (Makfoeld, 2008).

Samples of solid herbal waste have an average density of 0.67±0.14 kg/l, with typical herbal bitter smell, greenish brown to dark brown color, and the average water content was 77.37%. The density under 1.0 shows that

Table 2. Gravity (BJ), odor and solid waste color

Sample	Volume (liters)	Wheight(kg)	BJ (kg/liter)	Odor	Color
1	1.00	0.53	0.53	Bitter herbs typical	Brown
2	1.00	0.56	0.56	Leaves smell, herbs	Greenish brown
3	1.00	0.84	0.84	Herbs typical odor	Brown
4	1.00	0.79	0.79	Bitter herbs typical	Dark brown
5	1.00	0.61	0.61	Herbs typical odor	Brown
Averages			0.67±0.14		

the mass of solid herbal waste is lighter than water mass so it will float. This solid waste is bulky that it will not solidify when placed in a container.

Bitter and pungent odor of solid waste medicinal herbs thought to have come from the content of plant secondary metabolites remaining in the effluent herbs such as alkaloids, glycosides (saponins), flavonoids, phenols, tannins, terpenes and essential oils (Dharmananda, 2010).

The brownish color of solid waste herbal medicine due to the original color of the herbal plant parts such as roots and branches, or allegedly because protein browning reaction. Browning process is divided into two parts, enzymatic browning and non-enzymatic browning process. Non Enzymatic Browning consists of three kinds reaction, Maillard reaction, caramelization and browning due to vitamin C (Makfoeld, 2008).

Enzymatic browning may occur because the total amount of phenols contained in solid waste herbal medicine. According to Makfoeld (2008) browning reactions are common in fruits and vegetables such as bananas, peaches, bark, nutmeg, strawberries, and apples that have phenolic compounds. The formation of a brown color is triggered by oksidation reaction catalyzed by phenol oxidase or polyphenol oxidase enzyme. Both of these enzymes can catalyze the oxidation of phenolic compounds into Quinon and then dipolimerize to be melaniadin brown pigment.

The chemical composition and content of secondary metabolites of solid herbal waste

Compared to some feed rich fiber that generally used as ruminant feed such as king grass, rice straw, corn stalks, and banana stems, solid herbal waste in this study had a higher crude protein content, while the fiber and fat were relatively equal except banana stems.

Solid herbal waste in this study has higher content of ash, ADF, lignin, Ca and P, and lower content of ETN, NDF, hemicellulose and silica compared to king grass. The high fiber and low protein content in solid herbal waste resembling to king grass, besides solid herbal waste also comes from various parts of the plant, so that solid herbal waste could be used as alternative feed sources for fibers substitute mainly grass forage. However the low content of ETN and high content of ADF on solid waste herbal medicine, indicates the low water soluble carbohydrates (non-structural carbs) and higher structurally carbohydrates than the king grass so the level of digestibility waste solid herbs will be relatively lower than king grass. Lignin content of solid waste herbal medicine is also relatively high, reaching 17.53%.

Lignin is a part of the plant that cannot be digested by anaerobic rumen microbes, most likely because of the low oxygen content and the structure of the condensed lignin that blocking hydrolysis, while the anaerobic microbes in the rumen also can't produce lignolitic enzymes.

Table 3. Comparison of the chemical composition of the Solid herbal waste, king grass (*Pennisetum hybrid*), corn, rice and banana stems.

Parameter	Solid herbal waste	King Grass	Corn ^{bc}	Rice ^{bd}	Banana Stems ^b
BK (%)	22.73	18	20	40	10
PK (%)	10.82	9.1	5.5	4.3	3.9
SK (%)	33.75	33.1	29.6	33.8	24.0
LK (%)	2.61	2.3	2.0	2.5	2.0
ETN (%)	33.33	40	55.3	35	56.1
Abu (%)	19.54	15.4	7.5	24.5	14.0
TDN (%)	52	55	60	49	60
NDF (%)	65.15	73.80	-	-	-
Hemiselulosa (%)	13.4	26.90	-	-	-
ADF (%)	51.75	42.36	-	-	-
Selulosa (%)	32.26	38.63	-	-	-
Lignin (%)	17.53	5.43	-	-	-
Si (%)	1.96	4.9	-	-	-
Ca (%)	0.78	0.51	0.6	-	0.35
P (%)	0.82	0.51	0.1	-	0.08
Energi Bruto (Kcal)	3988 kcal	-	-	-	-
DE (Kcal/kg)	2293 ^a	2420	2640	2150	2630

Description: ^acalculated according to Crampton et al. (1957) cit. Hartadi et al. (2005) ^bHartadi et al. (2005), ^c aerial part of maize, grown-up, without the rind, fresh, ^d aerial portions of rice, grown after harvest, fresh.

The large amount of cellulose that can be degraded in the rumen, especially depending on the degree of the plant material lignification (McDonald et al., 2002). The lignocellulose structure is very tight and strong making it difficult for microbial enzymes to break down cellulose and hemicellulose into simple sugars (Chahal and Chahal, 1998).

Cellulose crystalline structure is very dense and difficult to be broken down by enzymes produced by rumen microbes. Hydrogen bonding between cellulose molecules and straight and flat hydrophobic bonding on the top surface and bottom layers of cellulose, causing van der Waals interactions between cellulose layers to forming the unity between the strands of cellulose that is very stable and difficult to dissolve (Brandt et al., 2013).

The high content of lignin expected to affect the digestibility of solid herbal waste, but plant herbal medicine has been boiling at 80°C in the extraction process, so that the alleged structure of lignocellulose and crystalline cellulose bonding

portion has been disconnected or become more tenuous thus allowing crude fiber digesting enzymes to entrance and digest cellulose and hemicellulose.

When compared with other agricultural waste that are widely available in Indonesia, the solid waste herbal medicine has an advantage in terms of their chemical composition. The protein content of solid waste herbal approximately 10.8% while sorghum straw 3.8%, banana stems 3.9%, peel and pineapple fruit axis 3.4% (Hartadi et al., 2005), and cocoa pod husks 9.7% (Zain, 2009), in addition, the lignin content of solid waste herbal medicine is lower than the pod husks (20.15%) (Zain, 2009).

The existence of plant secondary metabolites in solid waste air herbs are still relatively high, although in general the typical bitter smell of herbs had been reduced. It was shown from the results of solid herbal waste secondary metabolites analysis in Table 4.

Analysis of the six types of secondary metabolites expected in solid waste herbal

medicine showed positive results. Plant secondary metabolites levels from the highest to the low were tannins, phenols, saponins, alkaloids, flavonoids and essential oils. These solid herbal waste are known mainly composed of mint plant (*Mentha piperita*), sembung (*Blumea balsamifera*), ginger (*Zingiber officinale*) and *Panax ginseng* (Deltomed, 2015). The literature study shows ginger, mint, and sembung containing more than one secondary metabolites (Table 5). The accumulation of a variety of plant secondary metabolites is thought to cause the levels of several types of secondary metabolites such as total phenols, flavonoids, tannins, saponins and alkaloids is quite high, although it has undergone a solvent extraction process using water at about 80°C.

Plant secondary metabolites have different mode of action and an effect depending on their chemical structure in which each has a reactive functional group (Wink, 2015).

Plants secondary metabolites are often characterized as both poisonous and medicinal, and a beneficial or an adverse result may depend on the amount eaten and the context of intake (Bernhoft, 2010). Polyphenol

compounds commonly found in plants, both nutrients or non-nutrients. Polyphenols are known to have antioxidant potential in vitro (Kahkonen et al., 2003). Polyphenols are compounds that are biologically active as it can donate hydrogen atoms to free radicals and can prevent lipid oxidation chain reaction at the beginning of the initiation phase (Gulcin et al., 2004). Simple phenols, phenolic acids, coumarin, tannins and flavonoids are part of polyphenol compounds (Kahkonen et al., 2003). Flavonoids are the largest group of plant secondary metabolites in phenol class (Patra, 2012).

Flavonoids are known to have antibacterial, anti-fungal and anti-virus activity (Edziri et al., 2012; Andrew et al., 2009), antioxidants (Patra, 2012; Harborne, 1973), anti-inflammatory and analgesic effects (Harborne, 1973). In general flavonoids react against microbes by inhibiting microbial cytoplasmic membrane function, inhibits bacterial cell wall synthesis, or inhibit the synthesis of nucleic acids (Chusnie and Lamb, 2011).

Table 4 . Plant secondary metabolites content of solid herbal waste.

Forms	Plant secondary metabolites (%)					
	Total	Flavonoids	Tanins	Saponins	Alkaloids	EO
FDSHW	4,71	1,86	11,50	3,28	3,44	0,21

FDSHW = Freeze dried solid herbal waste

EO = Essential oils

Table 5. The presence of plant secondary metabolites in herbal medicinal plants constituent of solid herbal waste.

Herbal plants	Plant secondary metabolites ingredients					
	Phenol	Flavonoids	Tanins	Saponins	Alkaloids	EO*
<i>Blumea balsamifera</i>	+ ^a	++ ^a	+ ^{bc}	-	+ ^c	+ ^c
<i>Mentha piperita</i>	+ ^{ef}	+ ^e	+ ^d	+ ^{de}	+ ^{de}	+ ^{de}
<i>Zingiber officinale</i>	+ ^g	+ ^k	+ ^h	+ ⁱ	+ ^l	+ ^j

* = essential oils, + = exist, - = nonexist, a = Xu et al. (2013); N.Saewan et al. (2011); Nessa et al. (2004), b = Susiarti (2000), c = Dalimartha (1999); Hariana (2013); Bhuiyan et al. (2009), d = Schuhmacher (2013), e = Adham (2015), f = Riahi et al. (2013), g = Wolmuth et al. (2005); Mishra (2009); suryani (2012), h = Ajayi et al. (2013), i = Nwinuka et al. (2005), j = Govindarajan (1982); Eze dan Agbo (2011), k = Ghazsemzadeh et al. (2010), l = Raaof et al. (2013).

Tannins are polyphenols compounds that dissolve in water with a molecular weight of 500 - 5000 and has the ability to bind alkaloids, gelatin and other proteins (Ramakrishnan and Krishnan, 1994), because it has a large number of hydroxy phenolic (Patra et al., 2012). Tannins are classified into two groups, namely hydrolyzed tannins and condensed tannins (Patra et al., 2012). Hydrolyzed tannins are easy for chemical hydrolyzation by rumen microbes enzyme to the form of phenolic compounds that can be absorbed by the bloodstream. While condensed tannins cannot be degraded by rumen microbial and will bind with fiber and protein fractions in the gastro intestinal tract. Condensed tannins are not absorbed in the bloodstream, resulting in normal physiological conditions that will not damage organs such as the liver and spleen (Makkar, 2003). Tannins bind proteins with hydrogen bonds which are sensitive to pH changes. Condensed tannins will bind stable at pH 4-7 in the rumen, whereas at extremes pH, tannins-proteins bond will be separated, ie at a pH of less than 3, in abomasum (El-Wazyri et al., 2005) and at pH of more than 7 in the intestine (Perez-Maldonado et al., 1995; Diaz-Hernandez et al., 1997 cit. Andrabi, 2005). Tannin also can bind and reduced cellulose degradations by rumen microbes (Klita et al., 1996). According to Min et al. (2005) the ideal tannin levels in the ration is 2-4% of DM, tannin levels between 4-9% of DM is already too high. The high levels of tannin in forage can be reduced by wilting and drying under the sunlight (Kustantinah et al., 2005). Saponins have the ability to lyse protozoa in the rumen (Wallace et al., 1994). Saponins react against protozoa membrane sterols, particularly cholesterol (Glauert et al., 1962). The addition of saponin extract decrease protozoa population and increase the flow of lactic acid and microbial protein from the rumen to the small intestine (Wallace et al., 1994). A decreased ruminal ciliate protozoa population may enhance the flow of microbial N from the

rumen, increase efficiency of N utilisation (Newbold et al., 1995). When amount of protozoa in the rumen pressed, there will be an increase in rumen bacteria that are a source of protein for ruminants. This resulted in the intake of protein to ruminant increasing performance. Alkaloids are nitrogen-containing compounds with a carbon skeleton showing the isoprenoid derived compounds. The most important members of this group are akonitum alkaloid and steroid alkaloids, and among these alkaloid compounds there serves as antifungus and insect repellent (Robinson, 1995). Furthermore in general it is believed that the alkaloid compounds cause a bitter taste. Feeding contains the alkaloid compounds in ruminant livestock can negatively impact the quality of the meat. For example the provision of papaya leaves contain alkaloids carpain in goats bligon feed cause bitter taste in meat, liver and lungs (Robinson, 1995; Harris, 2005). Alkaloids are known to have antibacterial activity, anti-virus, and together improve the antibiotics effect (Cushnie et al., 2014). The mechanism of action as an anti-bacterial alkaloids vary widely, depending on the chemical structure or class. For example, alkalod indolizidine class like pergularinine and tylophorinidine inhibits bacterial nucleic acid synthesis, by blocking the action of the enzyme dihydrofolate reductase (Rao and Venkatachalam, 2000). benzophenanthridine and protoberberine isoquinolines, the class of isoquinoline alkaloid, react by disturbing ring-Z and inhibit cell division of bacteria (Cushnie et al., 2014), while phenanthridine isoquinoline ungeremine alkaloids react by inhibiting the synthesis of nucleic acids by interrupting the enzyme type I topoisomerase (Casu et al. , 2011). Essential oils (EO) is a mixture of secondary metabolites from herbal, spices, and aromatic plants that give its smell characteristic (Ultee et al., 1999). Essential oils have antimicrobial properties, by affecting the bacterial cell membrane (Ultee et al., 1999),

deactivated bacteria enzymes (Benchaar and Henry, 2011), or inhibit microbial growth indirectly by causing bacterial cells lose much energy (Griffin et al., 1999; Ultee et al., 1999). Essential oils can inhibit the growth of methanogenic bacteria. Variations of essential oil components include phenolic compounds and aldehydes, intervene the ion transport in the cytoplasmic membrane and deactivate microbial enzymes (Benchaar and Henry, 2011).

Conclusions

Solid herbal waste contains a variety of plant secondary metabolites that have antimicrobial effects and binding protein and carbohydrates. According to the potential availability of solid waste and chemical composition, solid waste herbal medicine can be used as an alternative of feed ingredients to substitute feed rich fibers with attention to the content of secondary metabolites that could be expected to affect the process of fermentation and digestibility in the rumen.

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