Antibacterial Activity and Phytochemical Content of Silage Juice from Tropical Herbal Leaves

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Antibacterial Activity and Phytochemical Content of Silage Juice from Tropical Herbal Leaves

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Abstract. The objective of the present study was to evaluate silage juice from tropical herbal leaves from Indonesia, namely *Cordyline terminalis, Polyscias scutellaria, Cosmos caudatus, Hibiscus tileaceus, Syzygium aqueum* and *Swietenia mahagoni* against pathogenic bacteria based on phytochemical content and lactic acid bacteria. This study consisted of two phases, the first phase was to explore 6 tropical herbal leavesin combination with maizecrop in making silage juice, whereas the second phase was the evaluation of 2 types of selected silage juices with different harvesting times. Parameters measured were consisted of lactic acid bacteria population, phytochemical screening qualitatively, tannin, phenolic, flavonoid contents and its ability to inhibit pathogenic bacteria, i.e., *Eschericia coli* and *Salmonella thypimurium.* Data were analyzed by description test with table as the result. Results showed that from 6 silage juice from tropical herbal leaves, it was taken 2 best leaves based on phytochemical content and total lactic acid bacteria, those were *Cordyline terminalis* and *Polyscias scutellaria*. Result of second phase research showed *Cordyline terminalis* had better phytochemical content than *Polyscias scutellaria* so its ability to inhibit pathogenic bacteria was bigger. It is concluded that *Cordyline terminalis* and *Polyscias scutellaria* were the best combination of silage juice due to their ability to inhibit pathogenic bacteria.

1. Introduction

Prohibition of using synthetic antibiotic in livestock production since January 1st 2018 has encouraged researchers to find alternatives that do not cause any negative effect. Probiotic, prebiotic, organic acid, enzyme, phytochemical are materials that are usually used as antibacterial. However, their use either individually or in combination has not been able to replace synthetic antibiotic. Researchers have tried to combine antibacterial agent as one material. One of that combination materials is silage juice. Silage juice is a by-product of silage making that contain lactic acid bacteria, organic acids and enzymes. Total lactic acid bacteria which is contained in silage juice is 2.2 x 10^8 cfu/mL. Lactic acid bacteria in silage juice is able to inhibit *E. coli* even though the ability to inhibit is not as good as in inhibiting *Salmonella thypimurium*.

One way to optimize the ability of silage juice is by adding other antibacterial components such as phytochemical. Phytochemical is believed to be anticancer, anti-stress [2], etc. because in young plant, phytochemical is used as functional protection of bacteria who attack the plant. Combination of tropical herbal leaves and whole crop corn in the fermentation process is still a debate about the content of silage juice. One study told that fermentation can increase the phenolic content in legume [3] but another study told that there are some phytochemicals content that are lost during fermentation [4]. The reduction of

antibacterial ability can also be caused due to excessive ensiling period. Therefore, selection of tropical herbal leaves and silage harvest time are important keys in improving the ability against pathogenic bacteria in silage juice. This study therefore aimed to evaluate the silage juice against pathogenic bacteria based on harvest time and kind of tropical herbal leaves.

2. Materials and Methods

Silage juice preparation was divided into 2 steps. The first step was to determine best juice silage from 6 tropical herbal leaves (*Cordyline terminalis, Polyscias scutellaria, Cosmos caudatus, Hibiscus tileaceus, Syzygium aqueum* and *Swietenia mahagoni*) into 2 tropical herbal leaves. The second step was to determine the best silage juice from tropical herbal leaves which will be used for further research. Proportion of whole crop maize and tropical herbal leaves was 50 : 50 w/w (dry matter basis) [5]. Whole crop maize was harvested at about 60 days old at 08:00, and let its in the field for 3 hours, and then chopped using chopper machine to about 2-5 cm. First step, 10 kg of chopped whole crop was divided into 60 groups, 500 g each, and it was combined with 500 g of tropical herbal leaves without chopping. The crops were put in plastic container, compressed and properly sealed to ensure an aerobic condition. After 31 days, silage was press by hydraulic press until the water content came out. The treatments were 0 days silage juice (P1) and 21 days silage juice (P2). There were 20 plastic containers per treatment. All treatments were analyzed chemically and biologically. The result of this study was analyzed using descriptive test to illustrate the presence of lactic acid bacteria and phytochemical as antibacterial agents.

First phase of this research, the leaves and crop were collected on October 2018, P1 treatment was observed its physical analyzed (0 day not included), total lactic acid bacteria (0 day not included) and qualitative phytochemical screening. Physical analyzed measured were color, texture and flavor. Total lactic acid bacteria were measured by total plate count method. Qualitative phytochemical screening measurements consisted of alkaloid, flavonoid, phenol, steroid, triterpenoid, tannin and saponin (Harborne 1987)

Second phase of this research the leaves and crops were collected on December 2018, P2 treatment was quantitative phytochemical and minimum inhibitory (only 30 days of silage). Quantitative phytochemical consisted of phenol [6], flavonoid [6] and tannin [7] content and minimum inhibitory test measured by diffusion agar with bioassay method against pathogenic bacteria such as *Eschericia coli* and *Salmonella thypimurium*.

All data except inhibitory test were analyzed by descriptive with result in tables to describe the presence of lactic acid bacteria and phytochemical inside silage juice. Inhibitory test was analyzed by T-test to evaluate the ability of antibacterial against pathogenic bacteria such as *E. coli* and *S. thypimurium*

3. Result and Discussion

In origin plant, phytochemical play important role as a defense against prey, microorganism, stress as well as interspecies protections [2]. Hence, phytochemical screening as antibacterial activity served as the initial step in predicting the potential phytochemical content inside silage juice. Investigation on the preliminary qualitative phytochemical screening of silage juice from various tropical herbal leaves revealed the presence of diverse groups of metabolites such as alkaloid, flavonoid, phenolic, steroid, triterpene, tannin and saponin. Fermentation decreased the phytochemical content in most of silage juice.

The observations and inferences made in qualitative phytochemical test are presented in the following subsections:

3.1. Alkaloids

In this study a yellow precipitate was observed in the silage juice after fermentation and crop without fermentation. None contained alkaloids in crop without fermentation Most of silage juice contained alkaloids except *Hibiscus tileaceus*. It is unique when silage juice appears alkaloids but in origin plant not appear. It was like what [3] reported, fermentation makes phenolic content increase. It was caused there was enzyme that hydrolyze the β-glucosidic bonds so free polyphenols concentration increase.

3.2. Flavonoids

In this study a yellow colourations was observed in the silage juice after fermentation and crop without fermentation. Most of them has flavonoids content except in origin plant of *Cordyline terminalis* and *Cosmos caudatus*. Silage juice of *Syzygium aqueum* and *Swietenia mahagoni* is the biggest flavonoids content.

3.3. Phenol hydroquinone

The results showed a red colouration was observed in the silage juice after fermentation and crop without fermentation. Most of them indicated there was phenolic hydroquinone in all of material except in silage juice of *Hibiscus tileaceus* and *Syzygium aqueum*. Fermentation made the level of some phytochemicals decrease [4]. This was probably due to the degradation carried out by bacteria during the fermentation period [8].

3.4. Steroids and terpenoids

The results indicated that not all silage juice and origin crop contain steroids and terpenoids. Only origin plants like *Cordyline terminalis*, *Polyscias scutellaria* and *Syzygium aqueum* contained steroid. Different with steroids, terpenoids is contained in all origin plant and only in silage juice of *Polyscias scutellaria*

3.5. Tannins

A yellow precipitate was observed for the water extract in the silage juice after fermentation and crop without fermentation. Tannin was the second most phytochemicals contained in tropical herbs currently studied. Most of them contained tannin in their crop except in silage juice of *Cosmos caudatus, Hibiscus tileaceus, Syzygium aquaeum* and *Switenia mahagoni*. All tannin content decreased due to fermentation. This observation was contrary with observation of [9] as they observed increased phytate and tannin because of fermentation.

3.6. Saponin

The result indicated most of silage juice is not contained saponin and also origin plant like *Cordyline terminalis* and *Hibiscus tileaceus*. Only *Syzygium aquaeum, Switenia mahagoni, Cosmos caudatus and Polyscias scutellaria* contained saponin

Silage juice in this observation contained abundant lactic acid bacteria in most of silage juice. The result in this study are almost the same as the result which reported by [1]. Most lactic acid bacteria are *Cosmos caudatus*and *Cordyline terminalis* and the least lactic acid bacteria is *Polyscias scutellaria*. The result of total lactic acid bacteria is shown in Table 1.

3.7. Selection of the best tropical herbal leaves

The best selection of tropical herbal leaves as a component of silage juice was based on the phytochemical content and total lactic acid bacteria. Based on Table 1, it was known that *Cordyline terminalis* was the best tropical herbal leaves based on these two aspects. *Polyscias scutellaria* leaf was chosen as the second-best leaf based on phytochemical content which was left in silage juice due to the fermentation. Although the content of lactic acid bacteria from leaf *Polyscias scutellaria* is the lowest content, it is not capable enough to inhibit pathogenic bacteria such as *E. coli* [1]. So, in this study, research focus more on phytochemical content with total lactic acid bacteria which is not far from other LAB content of another leaves.

Table 1. The phytochemical screening and total lactic acid bacteria of various silage juice from tropical herbal leaves.

Silage juice (+ whole crop maize	Fermentation	Phytochemical							LAB (cfu/mL)
		A	F	P	S	T	Tn	Sp	
Cordyline terminalis	0 day	-	-	+++	++	+	++	-	1.74×10^8
	31 days	++	+	++	-	-	++	-	
Polyscias scutellaria	0 day	-	-	+++	++	+	+	+++	8.13×10^7
	31 days	++	+	+	-	+	+	-	
Cosmos caudatus	0 day	-	+	++	-	+	++	++	1.99×10^8
	31 days	++	+	+	-	-	-	-	
Hibiscus tileaceus	0 day	-	+	+++	-	+++	+	-	1.42×10^8
	31 days	-	+	-	-	-	-	-	
Syzygiumaqueum	0 day	-	+	++	+	+	+++	++	9.26×10^7
	31 days	+	+++	-	+	-	-	-	
Swieteniamahagoni	0 day	-	+	+++	-	+++	+++	+	1.00×10^8
	31 days	++	+++	++	-	-	-	-	

Note: A, alkaloid; F, flavonoid; P, phenol hydroquione; S, steroid; T, triterpenoid; Tn, tannin; Sp, saponin; -, absence; +, poor; ++, moderate; +++, abundant.

3.8. Total phenolic, flavonoid and tannin against pathogenic bacteria

Poluscias scutellaria and *Cordyline terminalis* were analyzed for their total phenolic, flavonoid and tannin content using different extraction content. The phytochemicals content can be seen on Table 2. Phenolic, flavonoid and tannin content on *Cordyline terminalis* are 0.3 mL/L; 967.53 mg/L and 0.09 mL/L respectively and for *Polyscias scutellaria* are 0.19 mL/L; 683.66 mg/L and 0.03 mL/L respectively. From these contents, we could link this content with inhibitory test against pathogenic bacteria. The minimum inhibitory test was continued on *Cordyline terminalis, Polyscias scutellaria* at Table 2 against *E. coli* and *S. thypimurium*. The silage juice was given 60 µL. The result of 24-hour incubation showed silage juice from *Polyscias scutellaria* and *Cordyline terminalis* can inhibit both pathogenic bacteria. *Cordyline terminalis* and *Polyscias scutellaria* could inhibit 6.72±0.95 and 4.85±1.21 mm for *E. coli* respectively and 6.52±1.21 and 6.49±1.89 mm respectively for *S. tyhpimurium*. Based on T-test of minimum inhibitory test, *Cordyline terminalis* and *Polyscias scutellaria* had no significant different (p>0.05) against both pathogenic bacteria. [10] reported that antibacterial agent can be categorized as follow; inhibitory area 10-20 mm (strong), 5-10 mm (medium) and <5 (weak). So, both of silage juice has a medium antibacterial agent except *Polyscias scutellaria* has a weak antibacterial agent. As we can see on phenolic, flavonoid and tannin content on *Cordyline terminalis* is higher than *Polyscias scutellaria* has. [11] reported phenolic content such as flavonoid and tannin can diffuse through the microbial membrane and it can reduce membrane fluidity of bacterial cells and causing cell fluid imbalance.

Table 2. Total phenolic, flavonoid, tannin and inhibitory against pathogenic bacteria from 2 types of silage juices.

Silage Juice	Phytochemicals			Inhibitory Test (cfu/mL)	
	Flavonoid	Phenolic	Tannin	*E. coli*	*S. thypimurium*
Cordyline terminalis	0.3 mL/L	967.53 mg/L	0.09 mL/L	6.72±0.95	6.52±1.21
Polyscias scutellaria	0.19 mL/L	683.66 mg/L	0.03 mL/L	4.85±1.21	6.94±1.89

4. Conclusion

Cordyline terminalis and *Polyscias scutellaria* are the two best combinations of silage juice based on total lactic acid bacteria and phytochemical content. Inhibitory test show that both leaves has antibacterial ability due to phytochemical content such as flavonoid, phenolic and tannin.

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