

Potential of *ciplukan* extract (*Physalis angulata* L.) and patchouli waste (pogostemon patchouli pellet) as alternative sources of phytogetic feed additive

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Abstract. The research was carried out by using the method of extraction and identification of phytogetic components of *ciplukan* and patchouli waste as well for antibacterial and antioxidant tests. The variables observed nutritional content, phytochemicals, phytogetic compounds, antibacterial inhibition and antioxidant content. Data analysis was carried out descriptively and tabulated according to the research variables. The results showed that the extraction *ciplukan* produce yield, namely 9.75% and patchouli waste of 1.02% by weight of fresh ingredients. The nutritional content of *ciplukan* and patchouli waste respectively contains crude protein 27.79 and 14.19%, crude fibre 7.08 and 17.09%, fat 3.43 and 3.85%, BETN 27.46 and 40.87%, and the antioxidant content 75.70% and 73.53%. The results of the phytochemical test, patchouli waste extract contains bioactive (alkaloids, flavonoids, phenols, tannins, saponins, steroids and terpenoids as well as essential oils,) while the *ciplukan* extract does not contain terpenoids and essential oils. The results of the antibacterial test showed that patchouli waste extract had the largest inhibition zone against E.coli and Salmonella with an average diameter of 12.50 and 8.50 mm. Conclusion patchouli waste extract has the potential to as an alternative of phytogetic feed additive because it contains bioactive substances and can act as antibacterial.

1. Introduction

Phytogetic feed additives (phytobiotics or botanicals) are usually defined as additional compounds which are the result of plant secondary metabolites (either containing nutritional, non-nutritious, or anti-nutritional compounds) that are included in the ration to increase livestock productivity through improving feed properties, increasing digestive tract health by controlling pathogenic bacteria, increasing production performance, and improving the quality of livestock products [1], [2].

Feed additives are generally considered to be products applied to livestock for the purpose of improving health status and production performance. In contrast to veterinary medicines which are generally applied for prophylactic and therapeutic purposes for health problems that have been diagnosed for a limited period of time or on a waiting period basis. However, phytogenics are a relatively new group in feed additives and the aspects of phytogetic applications as feed additives.

Complications also arise because the phytogetic feed additives can vary with respect to botanical origin, processing, and composition. Several research results indicate that additive phytogetic feeds are potential to be used as an alternative growth enhancer for poultry because they have abilities that are not much different from antibiotics in maintaining the health of the digestive tract and increasing

the immune response of livestock [3;4] additives to phytogetic feed also have advantages, namely that they do not cause negative effects such as those caused by antibiotics [5].

One of the herbal plants that has the potential to be used as a source of additive phytogetic feeds is the *ciplukan* plant and patchouli waste. *Ciplukan* plants with a height of 0.1-1 m, grow wildly and evenly in lowland areas to an altitude of 1.550 above sea level [6] and have high nutritional value which is rich in vitamins, minerals and antioxidants [7]. Likewise, patchouli including essential oil-producing plants that make an important contribution in the world of flavor and fragrance, especially for the perfume and aromatherapy industry. In the pharmaceutical industry patchouli oil is used as a drug that functions as an anti-inflammatory, anti-depressant, divertic, antifungal and antibacterial [8].

However, there is not much information and reports on the use of *ciplukan* plants and patchouli oil refined waste which have the potential to be used as a source of feed additives for livestock. Observing this, it is necessary to conduct a study on the potential of *ciplukan* and patchouli waste which have not been utilized so that it can be used as an alternative source of phytogetic feed additives, in an effort to provide a strategy for providing feed additives to replace Antibiotic Growth Promoter (AGP) in livestock. The research aims to study the potential of *ciplukan* leaves and patchouli oil refining waste as an alternative source of phytogetic feed additives to replace the Antibiotic Growth Promoter (AGP) in livestock.

2. Materials and methods

2.1. Material / sample handling

Ciplukan plants, and patchouli waste used is the leaves, before use the leaves from each sample are cleaned and dried by aerating for 7 days. Furthermore, the *ciplukan* and patchouli waste are mashed using a diskmill machine to produce mesh-shaped leaves and then stored for further testing.

2.2. Extraction

Extraction of *ciplukan* and patchouli waste was carried out by maceration method. Maceration is a simple extraction method to attract the required compounds by immersing the material using 96% ethanol solvent. The use of this solvent is intended to extract chemical components and to determine the amount of yield and antioxidant content of each sample.

Maceration extraction was carried out with a 1: 4 ratio of sample and solvent for 48 hours, with a room temperature of 28 °C. Furthermore, the extraction results are filtered with filter paper using a vacuum filter. The filtered filtrate is then evaporated using a rotary evaporator so that it gets a concentrated extract and is then stored at a temperature of 0 °C for the next test process.

The crude extract was weighed to determine the yield based on the extraction method, and the type of solvent using the formula:

$$\text{Yield} = \frac{\text{dry extract weight (g)}}{\text{initial sample weight (g)}} \times 100\% \quad (1)$$

2.3. Antibacterial test

The antibacterial activity test was carried out by the agar diffusion method using paper discs with a diameter of 6 mm with the *Escherichia coli* and *Salmonella* test bacteria. The bacterial activity test was carried out in three repetitions. Each of the *Escherichia coli* and *Salmonella* bacteria was taken 1 ose using a loop needle.

Then put it into a test tube that already contains as much as 9 ml of distilled water, then diluted 10^{-1} to 10^{-3} . Then inoculate 1 ml of suspense from 10^{-3} dilution into a petri dish, then pour Nutrient Agar (NA) and then homogenize it by turning the petri dish slowly and then let it stand until the media hardens. Disc paper measuring 5 mm was immersed in each cup containing the sample for 10-15 minutes. The soaked disc paper was placed on the agar surface using tweezers. Then the incubation was carried out for 24 hours at 37 °C. Observations were made on the formation of an inhibition zone

around the paper disc. The diameter of the drag zone formed is measured using a ruler in millimeters (mm).

3. Result and discussion

3.1. The ingredients of *ciplukan* extract and patchouli leaf waste

The yield obtained from the extraction of *ciplukan* and patchouli waste using 96% ethanol solvent produced different yields as shown in Table 1. The highest extract yields were found in *ciplukan* (9.75%) and patchouli waste of 1.02% of the weight of fresh ingredients. According to [9] factors that can affect the amount of yield produced from a sample of extracted material are: the extraction method used, the size of the sample particles, the conditions and storage time, the type of solvent used, the extraction time, and the ratio between the number of samples. with the amount of solvent used.

Table 1. Percentage of yield of *ciplukan* extract, and patchouli waste.

Extraction method	Material / sample	Solvent type	Yield (%)
Maceration	<i>Ciplukan</i>	Ethanol 96%	9.75
Maceration	Patchouli waste	Ethanol 96%	1.02

The results showed that the yield of patchouli waste extract obtained the lowest value, namely 1.02% ethanol 96% solvent in the extraction process. So that the yield of patchouli extract with ethanol 96% produces a low yield compared to *ciplukan*, where the *ciplukan* used are fresh which are then dried, milled into flour and then extracted.

The amount of yield produced also depends on the solubility properties of the bioactive components. The different extraction times are also thought to be the cause of the difference in yield. Stated that the maceration time that passes the optimum time will damage the solute contained in the material and has the potential to increase the loss of active compounds in the extracted solution because the interaction of the two causes mass deposition by diffusion between the concentration of the solution inside and outside the material inside evaporation process [10].

3.2. Nutrient, phytochemical and antioxidant content of *ciplukan* and patchouli waste

The results of proximate analysis of the nutritional content of *ciplukan*, and patchouli waste (patchouli leaves) are shown in Table 2, and the results of phytochemical and antioxidant analyzes are shown in Table 3 and 4.

Table 2. Nutritional composition of *ciplukan* and patchouli waste.

Nutrients	<i>Ciplukan</i> leaves	Patchouli waste
Dry matter (%)	79.25	90.55
Crude protein (%)	27.79	14.19
Crude Fiber (%)	7.08	17.09
Fat (%)	3.43	3.85
Ash (%)	13.49	14.55
Water (%)	20.75	9.45
BETN (%)	27.46	40.87

Source: Analysis results of the Lab. of Animal Feed Nutrition and Technology, Faculty of Agriculture, Syiah Kuala University (2020).

The results showed that *ciplukan* contains 27.79% crude protein, and it is higher than patchouli waste which is 14.19%. Crude fiber found in *ciplukan* is 7.08 and patchouli waste is 17.09%, fat is 3.43 and 3.85%, and BETN is 27.46 and 40.87% (Table 2).

Table 3. Phytochemical test results of *ciplukan* extract and patchouli waste.

Bioactive compounds	Reactor	Material / sample	
		<i>Ciplukan</i>	patchouli waste
Alkaloids	<i>Mayer</i>	+	+
	<i>Wagner</i>	+	+
	<i>Dragendorff</i>	+	+
Flavonoids	Mg + HCl pekat	+	+
Phenol	FeCl ₃	+	+
Tannins	FeCl ₃	+	+
Saponins	Water	+	+
	<i>Lieberman-</i>		
	<i>Burchard</i>	+	+
Steroids	<i>Lieberman-</i>		
	<i>Burchard</i>	-	+

Source: Lab. of Pharmacology, Faculty of Veterinary Medicine, Syiah Kuala University (2020). Information: + (contains bioactive compounds) - (does not contain bioactive compounds)

Phytochemical test is an initial testing stage to detect whether there is a bioactive compound in a test material using color reagents. Phytochemical testing was carried out to determine the potential of *ciplukan* extract, and patchouli waste qualitatively containing phytochemical compounds and other bioactive substances. The results showed that *ciplukan* extracts and patchouli waste contain bioactive compounds such as glycosides, steroids, tannins, flavonoids and anthraquinones. These compounds are sensitive to heat so they will be easily damaged if heated at high temperatures and for a long time [11]. The results of the phytochemical test showed that the *ciplukan* did not contain any terpenoid compounds, and patchouli waste contained the bioactive compounds tested (alkaloids, flavonoids, phenols, tannins, saponins, steroids and terpenoids).

The results of phytochemical tests on *ciplukan* and patchouli waste showed the presence of alkaloid compounds. Alkaloid compounds are organic compounds that are found in nature. This compound is usually found in that have a bitter taste. The flavonoid phytochemical test was found in all extracts, which showed positive results. In plants, flavonoids function in the process of photosynthesis, anti-microbial and anti-virus and function as antibiotics.

The phenol test showed positive results in all extracts of the test sample, indicated by a color change from brownish green to blackish blue and containing polyphenol compounds [12]. The usefulness of several groups of phenolic compounds is well known, for example phenolic or polyphenolic compounds are natural antioxidant compounds in plants. These phenolic compounds are multifunctional and function as antioxidants because they have the ability to scavenge free radicals [13]. *Ciplukan* extract contains steroid bioactive compounds but does not contain triterpenoid compounds while patchouli waste contains triterpenoid compounds. Steroid compounds have several benefits for plants, including growth regulators.

The phytochemical test of tannins on *ciplukan* extract and patchouli waste showed positive results. Tannins are a group of active plant compounds that are phenolic. Tannin compounds function as antioxidants [14] and can dissolve in water and organic solvents [15]. The tannin compounds in the extract are shown by changing the color of the solution to greenish brown. These changes occur because tannins are soluble in water, alcohol and acetone [16]. Furthermore, the phytochemical test for saponin content in *ciplukan* extract and patchouli waste showed positive results and showed the formation of foam. Saponins found in certain parts of the plant have a high concentration and are influenced by the type of plant and the stage of growth. Saponins are soluble in polar solvents such as water, while ethanol and methanol are semi-polar solvents so that the saponins in the leaves can be extracted.

The results of the antioxidant test results of *ciplukan* extract and patchouli waste showed that antioxidant levels tended to be higher in the *ciplukan* extract with a percentage of 75.70% and patchouli waste (patchouli leaves) of 73.53% (Table 4).

Table 4. Antioxidant test results.

Material/sample type	Antioksidan (%)
<i>Ciplukan</i> extract	75.70±0.66
Patchouli waste extract	73.53±0.17

Source: Lab. of Food and Agricultural Product Analysis, Syiah Kuala University (2020).

Antioxidants are substances that can neutralize or reduce the negative impact of free radicals. Free radical itself is a molecule that has an unpaired electron collection in an outer circle. The benefits of antioxidants to ward off free radicals have been very widely studied by researchers. Various research results show that antioxidants have been reported to slow down processes that can be caused by free radicals such as the presence of tocopherol, ascorbate, flavonoids, and the presence of lycopene [17].

3.3. Phytogetic compounds

The phytogetic compounds contained in the *ciplukan* extract and patchouli waste after the phytochemical test were carried out very good results, each test sample had a phytogetic content (Table 5).

Table 5. Phytogetic compounds of *ciplukan* extract, and patchouli waste.

No.	Phytogetic compounds in the material / sample	
	<i>Ciplukan</i> leaves	Patchouli waste (patchouli leaves)
1.	Alkaloids	Alkaloids
2.	Flavonoids	Flavonoids
3.	Phenol	Phenol
4.	Tannins	Tannins
5.	Saponins	Saponins
6.	Steroids	Steroids
7.	-	Terpenoids
8.	-	Essential oil

Source: Lab. of Pharmacology, Faculty of Veterinary Medicine, Syiah Kuala University (2020).

Phytogenics are bioactive compounds which are the result of plant secondary metabolites (both containing nutritional, non-nutritious, or anti-nutritional compounds) which can be used as feed additives in poultry ration formulations with the aim of increasing livestock productivity through improving feed properties, improving channel health digestion by controlling pathogenic bacteria, increasing production performance, and improving the quality of livestock products. The results showed that there were phytogetic compounds in the extract of *ciplukan*, and patchouli waste and it is hoped that they can be used as feed additives to replace antibiotics in livestock rations. Phytogetic components of *ciplukan* extract and patchouli waste are shown in Table 5.

The phytogetic compounds contained in *ciplukan* and patchouli waste have pharmacological effects, thus providing benefits to livestock. In addition, plants containing active compounds when given to livestock can improve livestock performance. *Ciplukan* extract and patchouli waste obtained

in this study contain bioactive substances so that they can be classified as phyto-genic additives and can be used as a substitute for antibiotics.

Plants that are classified as herbal plants can be used as natural growth promoters and are safe for human consumption because they do not leave residues on livestock products. The benefits of plants containing bioactive substances for poultry include improving the condition of the digestive tract and feed conversion, increasing digestibility of nutrients, increasing body weight, increasing immunity and reproductive performance, reducing mortality.

3.4. Antibacterial test results (in vitro)

Antibacterial activity testing was carried out to determine the differences in the inhibitory ability of *ciplukan* extract and patchouli waste. Each sample has a potential inhibition as evidenced by the formation of a clear zone (zone of inhibition) around the disc paper. The results of the antibacterial activity test of *ciplukan* extract and patchouli waste against *Escherichia coli* and *Salmonella* bacteria using the disc paper method and the results of measuring the inhibition zone diameter are shown in Figure 1 and Table 6.

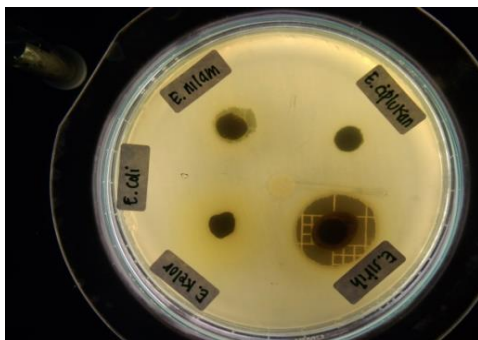


Figure 1. The results of the test results for the antibacterial activity of *ciplukan* extract, and patchouli waste (patchouli leaves) on *Escherichia coli*.

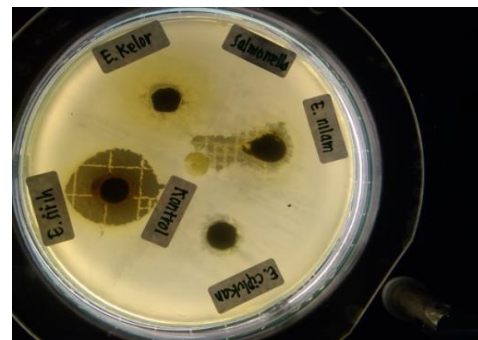


Figure 2. The results of the test results for the antibacterial activity of *ciplukan* extract, and patchouli waste (patchouli leaves) on *Salmonella*.

The results obtained from the antibacterial test showed that the patchouli waste extract had the largest clear zone (inhibition zone) against *Escherichia coli* and *Salmonella* bacteria compared to the *ciplukan* extract with an average diameter of 12.50 and 8.50 mm. The amount of inhibition zone in patchouli waste extract can be seen in Figure 1 and 2. Patchouli waste extract has a large enough potential to inhibit the growth of *Escherichia coli* and *Salmonella* bacteria. Patchouli waste still contains essential oils, so it has the potential to inhibit the growth of *Escherichia coli* and *Salmonella*. In accordance with the opinion of [18], patchouli waste contain essential oils, flavonoids, saponins, tannins, glycosides, terpenoids and steroids.

Table 6. Diameter of inhibition zone of *ciplukan* extract, and patchouli waste against *Escherichia coli* and *Salmonella*.

Treatment	Average bacterial inhibition zone diameter (mm)	
	<i>Escherichia coli</i>	<i>Salmonella</i>
<i>Ciplukan</i> leaf extract	2.50	2.75
Patchouli waste extract	12.50	8.50

Ciplukan extract has the smallest zone of inhibition. *Ciplukan* extract has quite high flavonoid compounds, besides that it also has polyphenol compounds. Polyphenols have antibacterial activity, but the antibacterial reactivity depends on the protein content of polyamides in bacteria. The result of

inhibition of bacteria by phenol components is due to the formation of phenol hydrogen bonds with core proteins [19].

4. Conclusion

Patchouli waste extract has the potential to be used as an alternative source of phytogetic feed additives because it contains phytogetic bioactive substances and can act as an antibacterial (it has a high inhibition zone against *Escherichia coli* and *Salmonella*).

Acknowledgement

The author would like to thank the research team, LPPM Syiah Kuala University, and all those who have helped carry out the research.

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