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by Muhammad Daud

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The Potential of *Ciplukan* (*Physalis angulata* l.) and patchouli waste extract (pogostemon patchouli pellet) as alternative sources of phytogenic feed additive

A I Daud[1]*, M A Yaman[1], Y Usman[1] and Y Aqmarina[1]
Animal Husbandry, Faculty of Agriculture, Syiah Kuala University, Banda Aceh, 23111 Indonesia

E-mail: daewood@unsyiah.ac.id

Abstract. The research was carried out using the method of extraction and identification of phytogenic components of *ciplukan* and patchouli waste. The observed variables include nutritional and antioxidant content, phytochemicals, phytogenic compounds, and antibacterial inhibition. Data were analyzed descriptively and tabulated according to the variables. The results showed that *ciplukan extracts* and patchouli waste produce a yield of 9.75% and 1.02%, respectively by weight of fresh ingredients. Furthermore, the nutritional content of *ciplukan* and patchouli waste contains crude protein of 27.79% and 14.19%, fibre of 7.08% and 17.09%, fat of 3.43% and 3.85%, BETN of 27.46 and 40.87%, and the antioxidant content of 75.70% and 73.53%, respectively. The phytochemical test result shows that patchouli waste extract contains bioactive substances such as alkaloids, flavonoids, phenols, tannins, saponins, steroids, terpenoids, and essential oils. However, *ciplukan* extract does not contain terpenoids and essential oils. Also, the antibacterial test result 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, respectively. Conclusively, patchouli waste extract has the potential to be used as an alternative source of phytogenic feed additive because it contains bioactive substances and acts as antibacterial.

1. Introduction

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

Feed additives are generally considered as products applied to livestock, which aims at improving their health status and production performance. In contrast, they are generally applied for prophylactic and therapeutic purposes in veterinary medicine for health problems that have been diagnosed for a limited or a waiting period basis. However, phytogenics and their application aspects are a relatively new group in feed additives.

Furthermore, complications arise because the phytogenic feed additives vary with respect to botanical origin, processing, and composition. Several research results indicated that these feeds have the potential to be used as an alternative growth enhancer for poultry because they have abilities that are not significantly different from antibiotics in maintaining the health tract of the digestive system

and increasing the immune response of livestock [3;4]. Furthermore, additives to phytogenic feeds have no negative effect, which is their advantage over antibiotics [5].

One of the herbal plants that are used as a source of phytogenic additive feeds is *ciplukan*. It has 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 a high nutritional value, which is rich in vitamins, minerals, and antioxidants [7]. Likewise, patchouli is an essential oil-producing plant that make an important contribution in the world of flavour and fragrance, especially for the perfume and aromatherapy industry. Meanwhile, this plant is used as a drug that functions as an anti-inflammatory, anti-depressant, divertic, antifungal, and antibacterial in the pharmaceutical industry [8].

However, there is not much information and reports on the use of *ciplukan* and patchouli oil refined waste, which have the potential to be used as a source of feed additives for livestock. With this in mind, it is necessary to investigate the potential of these plants, such that it is used as an alternative source of phytogenic feed additives, in an effort to providing a replacement for Antibiotic Growth Promoter (AGP) in livestock. Therefore, this research aims to investigate the potential of *ciplukan* leaves and patchouli oil refining waste as an alternative source of phytogenic feed additives to replace the Antibiotic Growth Promoter (AGP) in livestock.

2. Materials and methods

2.1. Material/sample handling

Leave samples of *Ciplukan* plants and patchouli waste was cleaned and dried by aerating for 7 days. Furthermore, these samples were mashed using a diskmill machine to produce mesh-shaped leaves and then stored for further testing.

2.2. Extraction

Ciplukan and patchouli waste were extracted by the maceration method, which is used to attract the required compounds by immersing the material into 96% ethanol solvent. The use of this solvent is intended to extract chemical components and to determine the yield and antioxidant content of each sample.

Maceration extraction was carried out with a sample and solvent ratio of 1:4 for 48 hours at a 28°C. Subsequently, the extraction results were filtered with a vacuum filter paper. Afterward, the filtrate was evaporated using a rotary evaporator such that it gets a concentrated extract and is then stored at 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:

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

2.3. Antibacterial test

The antibacterial activity test was carried out by the agar diffusion method using paper discs of diameter 6 mm with the *Escherichia coli* and *Salmonella* test bacteria. Also, the bacterial activity test was carried out in three repetitions. One dose of *Escherichia coli* and *Salmonella* bacteria was taken each using a loop needle and placed into a test tube that contains 9 ml of distilled water, then diluted 10^{-1} to 10^{-3}. Subsequently, 1 ml of suspense from 10^{-3} dilution was inoculated into a petri dish, filled with Nutrient Agar (NA), homogenize by turning the dish slowly and then allowed to stand until the media hardens. Furthermore, 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 and the incubation was carried out for 24 hours at 37°C. The formation of an inhibition zone around the paper disc was observed and the diameter of the drag zone formed is measured in millimeters (mm) with a ruler.

3. Result and discussion

3.1. The ingredients of ciplukan extract and patchouli leaf waste

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

Table 1. Yield percentage of *ciplukan* extract, and patchouli waste.

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

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The results showed that the yield of patchouli waste extract obtained the lowest value, namely 1.02% ethanol and 96% solvent in the extraction process. Therefore, ethanol (96%) produces a low yield compared to the fresh *ciplukan*, which was 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 believed to be the cause of the difference in yield. Furthermore, the maceration time that passes the optimum damage the solute contained in the material and has the potential to increase the loss of active compounds in the extract. This is because the interaction of the two causes mass deposition by diffusion between the concentration of the solution inside and outside the material during the evaporation process [10].

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

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

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

Nutrients	*Ciplukan* 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%. Also, the 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%, respectively (Table 2).

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

Bioactive compounds	Reactor	Material/sample	
		Ciplukan	patchouli waste

Alkaloids	*Mayer*	+	+
	Wagner	+	+
	Dragendorff	+	+
Flavonoids	Mg + HCl pekat	+	+
Phenol	$FeCl_3$	+	+
Tannins	$FeCl_3$	+	+
Saponins	Water	+	+
Steroids	*Lieberman-Burchard*	+	+
Terpenoids	*Lieberman-Burchard*	-	+

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

Phytochemical test is the initial stage of detecting the bioactive compound in a material using colour reagents. This test is carried to determine the potential of *ciplukan* extract and patchouli waste, which qualitatively contain phytogenic compounds and other bioactive substances. The results showed that they contain bioactive compounds such as glycosides, steroids, tannins, flavonoids, and anthraquinones. These compounds are sensitive to heat, therefore, they are easily damaged under high temperatures and for a long time [11]. The phytochemical test results showed that *ciplukan* contains no terpenoid compounds. However, patchouli waste contained bioactive compounds such as alkaloids, flavonoids, phenols, tannins, saponins, steroids, and terpenoids.

Furthermore, this test on ciplukan showed the presence of alkaloid, which are compounds with a bitter taste that are found in nature. The flavonoid phytochemical test was found in all extracts, which showed positive results. In plants, it functions in the process of photosynthesis, and as antibiotics on anti-microbial and anti-virus.

The phenol test showed positive results in all extracts, indicated by a change in colour from brownish green to blackish blue and also containing polyphenol compounds [12]. Furthermore, the usefulness of several groups of phenolic compounds is well known, for example, phenolic or polyphenolic are natural antioxidants in plants. These phenolic compounds are multifunctional and function as antioxidants because they have the ability to scavenge free radicals [13]. In addition, *ciplukan* extract contains steroid bioactive compounds but does not contain triterpenoid. However, 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 phenolic plant compounds that function as antioxidants [14] and dissolve in water and organic solvents [15]. These compounds in the extract are shown by changing the colour of the solution to greenish-brown. These changes occur because they 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 foam formation. 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. They are soluble in polar solvents such as water. Meanwhile, ethanol and methanol are soluble in semi-polar solvents, such that the saponins in the leaves are extracted.

Further test results showed that the 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 (%)
Ciplukan extract	75.70±0.66

Patchouli waste extract	73.53±0.17

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

Antioxidant is a substance that neutralizes or reduces the negative impact of free radical, which is a molecule containing the collection of an unpaired electron in an outer circle. Various research have studied broadly the benefits of antioxidants to ward off free radicals. The results showed that antioxidants slow down processes caused by free radicals such as the presence of tocopherol, ascorbate, flavonoids, and lycopene [17].

3.3. Phytogenic compounds

The phytogenic compounds contained in the *ciplukan* extract and patchouli waste after the phytochemical test achieved very good results. Each test sample had a phytogenic content (Table 5).

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

No.	Phytogenic compounds in the material/sample	
	Ciplukan 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 containing nutritional, non-nutritious, or anti-nutritional substances used as feed additives in poultry ration formulations. This aims to increase livestock productivity through improving feed properties, the quality of livestock products, and channel health digestion by controlling pathogenic bacteria, as well as increasing production performance. The results showed that there were phytogenic compounds in the extract of *ciplukan*, and patchouli waste. Therefore, it is hoped that they are useful as feed additives to replace antibiotics in livestock rations. Phytogenic components of *ciplukan* extract and patchouli waste are shown in Table 5.

The phytogenic compounds contained in *ciplukan* and patchouli waste have pharmacological effects, therefore providing benefits to livestock. In addition, when a plant that contains active compounds are given to livestock, it improves their performance. Furthermore, *ciplukan* extract and patchouli waste in this study contain bioactive substances. Therefore, they are classified as phytogenic additives and are useful as a substitute for antibiotics.

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

3.4. Antibacterial test results (in vitro)

The test on antibacterial activity 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 test on *ciplukan* extract and patchouli waste against *Escherichia* coli and *Salmonella* bacteria using the disc paper method and that of measuring the inhibition zone diameter are shown in Figure 1 and Table 6.

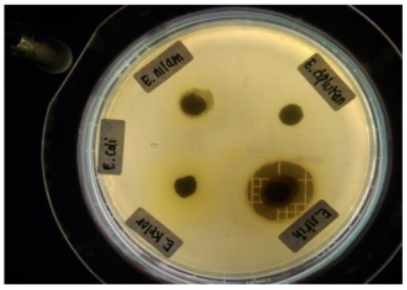

Figure 1. The test results for the antibacterial activity of *ciplukan* extract, and patchouli waste (patchouli leaves) on *Escherichia coli*.

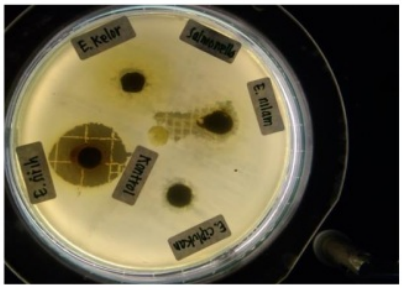

Figure 2. The test results for the antibacterial activity of *ciplukan* extract, and patchouli waste (patchouli leaves) on *Salmonella*.

The results 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* with an average diameter of 12.50 and 8.50 mm. The amount of inhibition zone in patchouli waste extract are shown in Figure 1 and 2. It contains a large enough potential to inhibit the growth of Escherichia coli and Salmonella bacteria. Furthermore, it contains essential oils, therefore, it has the potential to inhibit the growth of Escherichia coli and Salmonella. In line with [18], patchouli waste contains essential oils, flavonoids, saponins, tannins, glycosides, terpenoids, and steroids.

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

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

Ciplukan extract has the smallest zone of inhibition. However, it has quite a high flavonoid in addition to polyphenol compounds, which have antibacterial activity. However, the antibacterial reactivity depends on the protein content of polyamides in the bacteria. Furthermore, the result of inhibition 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 phytogenic feed additives because it contains bioactive substances. It also acts as an antibacterial because it has a high inhibition zone against Escherichia coli and Salmonella.

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