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

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**Abstract.** This study aims to examine the potential of ciplukan and patchouli oil refining waste as alternative sources of phytogenic feed additive to replace the Antibiotic Growth Promotor (AGP) in livestock. The research was carried out by using the method of extraction and identification of phytogenic components of ciplukan and patchouli waste as well as in vitro for antibacterial and antioxidant tests of ciplukan leaf extract and patchouli waste. The variables observed were percentage yield, nutritional content, phytochemicals, phytogenic compounds, antibacterial inhibition and antioxidant content of ciplukan leaf extract and patchouli waste. Data analysis was carried out descriptively and tabulated according to the research variables. The results showed that the extraction using 96% ethanol solvent produced different yields, the highest yield was found in ciplukan, 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 fiber 7.08 and 17.09%, fat 3.43 and 3.85%, BETN 27.46 and 40.87%, and the antioxidant content tends to be higher in ciplukan, namely 75.70% and 73.53% patchouli waste. Based on the results of the phytochemical test, patchouli waste extract contains bioactive substances in the form of 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 *Escherichia coli* and *Salmonella* bacteria compared to the ciplukan extract with an average diameter of 12.50 and 8.50 mm. Conclusion patchouli waste extract has the potential to be used as an alternative source of phytogenic feed additive because it contains phytogenic bioactive substances and can act as antibacterial.

1. Introduction

Phytogenic 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 phytogenic applications as feed additives. Complications also arise because the phytogenic feed additives can vary with respect to botanical origin, processing, and composition. Several research results indicate that additive phytogenic 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 phytogenic 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 phytogenic 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 phytogenic 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 phytogenic feed additives to replace the Antibiotic Growth Promoter (AGP) in livestock.

2. Methodology
2.1. Material 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:

\[
Yield = \frac{\text{dry extract weight (g)}}{\text{initial sample weight (g)}} \times 100\%
\]

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 Eschericia coli and Salmonella bacteria was taken 1 ml 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 suspension 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 is immersed in each cup containing the sample for 10-15 minutes. The soaked disc paper is 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.

| Extraction method | Material / sample | Solvent type | Yield (%) |
|-------------------|------------------|--------------|-----------|
| Maceration        | Ciplukan         | 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.

| 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%. 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       | Ciplukan | patchouli waste |
|---------------------|---------------|----------|-----------------|
| Alkaloids           | Mayer         | +        | +               |
|                     | Wagner        | +        | +               |
|                     | Dragendorff   | +        | +               |
| Flavonoids          | Mg + HCl pekat| +        | +               |
| Phenol              | FeCl₃         | +        | +               |
| Tannins             | FeCl₃         | +        | +               |
| 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 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 phytogenic compounds and other bioactive substances. The results showed that ciplukan extracts and patchouli waste contain bioactive compounds such as glycosids, 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).

| 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).

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. Phytogenic compounds

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

| No. | Phytogenic compounds in the material / sample |
|-----|---------------------------------------------|
| 1.  | Alkaloids                                   |
| 2.  | Flavonoids                                  |
| 3.  | Phenol                                      |
| 4.  | Tannins                                     |
| 5.  | Saponins                                    |
| 6.  | Steroids                                    |
| 7.  | -                                           |
| 8.  | -                                           |

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 phytogenic 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. 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, 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 phytogenic 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](image1.png)

**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](image2.png)

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

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.

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

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