The potency of active components containing on andaliman fruit [Zanthoxylum acanthopodium] for increasing the quality of salted eggs

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Abstract. A type of spice that is still limited to use with primary commodities is andaliman [Zanthoxylum acanthopodium DC]. Geographically, the spice is only found in the district of Toba Samosir which is closed to Lake Toba. The herb plant is a group of family Rutaceae and has identified with the name of Andaliman [Batak pepper]. The chemical compound that is thought to have the most role in various biological activities of andaliman fruit as an antioxidant and antimicrobial for food processing. Research conducted to determine the phytochemical compounds, the group of secondary metabolites within the extraction, and the influence of antimicrobial compounds from essential oils on salted eggs. Parameters were chemical compounds from different extracted and quality of the eggs. The chemical composition of each extract was examined by both the Harborne method and GC-MS. Results showed that phytochemical contents contained steroid and triterpenoid for three types of extraction with no detect for saponin. Antimicrobial compounds sourcing from andaliman extracts can inhibit the Staphylococcus aureus. Besides, the quality profile of salted eggs by ripening with andaliman extracts was significantly influenced comparing with control. It can be concluded that the active components of andaliman extract have potentially affected the quality of salted eggs.

1. Introduction
Andaliman [Zanthoxylum acanthopodium DC] is a plant that was commonly found in North Sumatera and has only been used as a spice for cooking, especially for traditional Batak dishes. This plant had potential as a medicinal plant because it contained a variety of aromatic compounds and essential oils including zanthalene and geranol acetate that were not found in other plants. Additionally, it has high economic value because it contained various types of aromatic compounds and essential oils. The species of Zanthoxylum generally had a pungent and bitter taste when the fruit is fully cooked [1]. Previous studies mentioned that Andaliman gained many chemical compounds such as alkaloid, amide, flavonoid, lignan, sterol, terpene and others, especially in the fruit section [2]. Regarding the compounds, it will be very important to know the phytochemical content on this plant.

The chemical content test has done through qualitative analysis. Results the end of the whole series are expected in finding the compounds that have pharmacological effects that can spur the discovery of new drugs, can be antibacterial, antiviral and so on. Phytochemical testing is a way to identify bioactive that has not been seen through a test or checks that can quickly separate between natural
ingredients that obtained the phytochemical contents with natural ingredients with no have to content certain phytochemicals.

Phytochemical screening is a post-stage in a phytochemical study that aimed to describe the group compounds contained in plants that are researched. Phytochemical screening methods are carried out by observing the color testing reaction by using the color reagents. Important things that figured out an important role in Phytochemical screening was the selection of solvents and methods extraction [3]. The chemical compounds will produce activated reactions in both primary and secondary on the biological metabolism. Reflecting on the tradition of Bataknese in terms of using the spices for food uses. Andaliman will be potential for many aspects with the massive types of processing. Biologically, the spice contains aromatic groups that can protect the plants from predators [4]. Furthermore, the extracts of the herb plant have potentially known to inactivate the activities for pathogenic species such as *Bacillus stearothermophilus, Pseudomonas aeruginosa, Vibrio colera*, and *Salmonella thyphimurium* [5]. The focus of the research is going farther for gaining the more potential extraction levels and the positive potency of active compounds from Andaliman especially in affecting the quality tested food [salted eggs].

### 2. Materials and Methods

Sample of Andaliman gained from Batu Nabolon Village that was geographically located in North Sumatera province, Indonesia. The variety of Andaliman was Simanuk that was the most massive with the population. A hundred grams of fresh andaliman distributed to the laboratory for chemical analysis and antimicrobial tests. Then, fresh andaliman passed the crude extraction before it tested for ripening the salted eggs. Organoleptic of salted eggs were measured by using organoleptic procedures and modified from [6]. Twenty-five blind panelists were set for quantitative assessment of the riped eggs.

For the chemical test, the residue was extracted twice with ethyl acetate [1: 4 w / v]. The ethyl acetate residue was then extracted twice with methanol [1: 4 w / v]. Each filtrate was evaporated using a rotary evaporator. Extract that obtained as a sample for phytochemical analysis. Phytochemical analysis of extracts and essential oils phytochemical analysis of n-hexane extract, ethyl acetate, and andaliman fruit methanol was carried out by the method, while the analysis of the chemical content of essential oils was carried out by the method GC-MS.

Regarding the antimicrobial test, compounds derived from andaliman fruit extract will be separated by the test method challenge for the pathogenic bacteria, the measurement conducted to a group of components in simplicia which was potential as an antimicrobial. The challenge was accompanied by a measurement of the total bacterial population at each phase of deep growth for every test. Measurement of total bacterial colonies was carried out by the pour plate method [method cup count] using MacKонkey agar specifically for pathogenic bacteria. After the incubation process, the total colony was measured using a bacterial count automatic [Automatic colony counter Protocol 2R]. Also, the bacteria tests were measured with two replications.

### 3. Results and Discussion

#### 3.1. Phytochemical Presence of Andaliman Fruit Extracts with Simanuk Variety

Compound secondary metabolites produced by plants are wrong only to defend themselves from conditions less favorable environment like temperature, climate, as well as pest and plant disease disorders [7, 8, 9]. Metabolite secondary of the compound groups is clustered into groups based on its chemical structure namely phenolic, saponin, flavonoid, tannin, triterpenoid, alkaloid, and steroid. The presence of phytochemical compounds was presented in Table 1.

Regarding Table 1, Andaliman fruit contained the various type of secondary metabolite compounds such as phenolic, saponin, flavonoid, tannin, triterpenoid, and alkaloid. The presence marks supported by color changes that occurred due to supply reagent to extract *Z. acanthopodium*. Higher plants have good flavonoids in the vegetative part, especially in flowers. Flavonoids as flower pigments represented an important role. Other functions of flavonoids were able to absorb ultraviolet rays for
directing insects, setting plants, setting photosynthesis, anti-microbial, and anti-viral work and so can work on insects. Flavonoids effected on many organisms very much and can explain why plants that contained flavonoids used in traditional medicine. Additionally, the flavonoid content could work as a strong respiratory inhibitor, inhibits the enzyme and non-enzyme oxidation reactions. The sugar content that was bound to flavonoids tended to cause the flavonoids to dissolve easily in water [10]. The antioxidant activity of flavonoids can explain active components of plants used traditionally to treat impaired liver function.

**Table 1.** Phytochemical presence of andaliman extracts with Simanuk variety

| Compounds  | Hexane Extract | Ethyl Acetate Extract | Methanol Extract |
|------------|----------------|-----------------------|------------------|
| Alkaloids  | -              | +                     | +                |
| Flavonoids | -              | +                     | +                |
| Phenolic   | -              | +                     | -                |
| Saponin    | -              | -                     | -                |
| Steroids   | +              | +                     | +                |
| Triterpenoid| +             | +                     | +                |
| Coumarin   | -              | -                     | -                |

+ : positively detected, - : no presence detected

The presence of tannin compounds in this test was marked the color changes to blackish inside test solution. This compound reacted with protein to form solid copolymers that were insoluble in water. Tannin has known to have several properties such as astringent, antidiarrheal, antibacterial and antioxidant. The mechanism of action in inhibiting bacteria was carried out by denaturing proteins and damaging membranes bacterial cells by dissolving the stacked up fat on the cell wall. This compound was able to migrate from the liquid phase to the lipid phase. Damage occurred to cell membranes resulting in inhibited activity and biosynthesis of specific enzymes needed in metabolic reactions and the condition that ultimately caused death in bacteria [11].

The chemical extract of Andaliman leaves with the highest obtained from triterpenoid substances and steroids, whereas flavonoids were found in low content. Steroids were the compounds derived from triterpenoids and their structure are multiples of 6 isoprene units commonly found in plants [12]. The most important included the use of steroids physiologically a very active compound involved in life processes that affected the hormonal system, like an adrenal hormone [cortisone], sex hormone [estrogen and testosterone] [13]. The biosynthetic trajectory before becoming steroid hormones are thoroughly derived from acetate and further changes into mevalonic acid [14]. Triterpenoid at fruit *Z. acanthopodium* was very attracted attention because of its similarities and possibilities of the relationship in biogenesis with steroids. Some activities interesting physiological showed by some triterpenoid. This compound had an active component in medicinal plants that have been used for diabetes, menstrual disorders, snakebite sores, skin disorders, liver damage, and malaria. Some possible compounds had ecological value for plants that contained because of the function as an antifungal, insecticide or antipredator, antibacterial and also the antivirus.

3.2. **Antibacterial Counts from Salted Eggs with Ripening by Using The Andaliman Extracts**

The antibacterial component was performed with an appealing activity zone that is produced from bacteria. Regarding the standard growth of bacteria, the clear zone has to be enlarged as the indicators for bacteria production. After rearing the media containing the Andaliman extracts and tested with *Staphylococcus aureus*, it showed the minimum clear zone and indicated the lower growth of pathogenic bacteria. The potential compounds' source from Andaliman fruit significantly affected the frequency of bacteria to use the media [15].

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After the Andaliman extracts were blended with traditional media or paddy mud to test the potential for ripening the salted eggs. Regarding the microbial counts, the tested products or salted eggs performed the fluctuating amounts of the alive bacteria as represented in Table 2.

| Treatments*                  | Bacteria Species | E. Coli  | Michobacteria sp | Pseudomonas sp |
|-----------------------------|------------------|----------|------------------|----------------|
| Control                     |                  | 0.04 x 10^7^a | 0.13 x 10^7^b   | 0.07 x 10^7^b  |
| Media + PM                  |                  | 0.03 x 10^7^a | 0.22 x 10^7^a   | 0.11 x 10^7^a  |
| Media + 25% CEA             |                  | 0.02 x 10^7^a | 0.19 x 10^7^a   | 0.09 x 10^7^a  |
| Media + 50% CEA             |                  | 0.03 x 10^7^a | 0.14 x 10^7^b   | 0.07 x 10^7^b  |
| Media + 75% CEA             |                  | 0.01 x 10^7^b | 0.11 x 10^7^b   | 0.06 x 10^7^b  |

* Control: fresh eggs were ripened with standard media, PM: fresh eggs were ripened with paddy mud as the main media, CEA: each media used the crude extracts of andaliman with different levels in the combination.

Regarding Table 2, supplementation of crude extract andaliman within the media for ripening the fresh eggs was significantly influenced the total counts of pathogenic bacteria on the salted eggs. It has confirmed that the active compounds of the herb plant in line with the trend of microbial counts, especially for the negative gram bacteria. There was a decreased count of the bacteria along with the level addition of the CEA. Then, the most influenced by the active bio components from the extract were steroids and triterpenoids. Those chemical compounds performed better in affecting the bacteria growth. Potentially, the extraction products were more optimum in spending the active particles to inhibit the bacteria initiation in the salted eggs [16]. Another hand, the total number of bacteria by using the paddy mud performed better compared to previously conducted research [1]. The total counts of bacteria were slightly lowered after testing with the extract of the spice fruits. However, using the media by adding the CEA from 25% to 50% showed a fluctuating number of total bacteria.

3.3. The Organoleptic Profile of Salted Eggs after Wrapped by Using Andaliman Extracts

The potential active compounds can be useful for the security of food products. Knowing the chemical components from Batak pepper will inform the basic dietary formulation to implement the potencies of the spice plant [Figure 1].

Applying the sensor measures by combining the crude extracts of Andaliman in the curing of salted eggs. The general appearance [overall panelist] of salted eggs is referred to as the main hedonic conclusion in influencing consumer acceptance. General appearance will describe the whole of salted eggs after being split into two parts. The salty taste of egg whites is influenced by the amount of NaCl salt that enters the egg white after the salt ionizes Na^+ and Cl^- . Statistically, the addition of andaliman fruit extracts in egg marinating pasta was not significantly different from the level of preference by panelists but the high concentration showed a rather favorable assessment of salted egg products.
Figure 1. Organoleptic from the overall profile test. P0= salted eggs with no receive the chemical compounds of Andaliman extracts, P1, P2, P3, P4: salted eggs with receiving the chemical compounds from Andaliman extracts with level 25%, 50%, and 75% respectively.

4. Conclusion

Andaliman extract has a positive potency for food acceptance in terms of the overall score with extract level until 75%. Additionally, the steroid and triterpenoid resulted in three different sources of extraction especially with the presence of the active compounds.

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