

The extraction of antimicrobials component of andaliman (*Zanthoxylum acanthopodium* DC.) and its application on catfish (*Pangasius sutchi*) fillet

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Abstract. Andaliman (*Zanthoxylum acanthopodium* DC.) is a well known wild species in North Sumatera and used for seasoning in Batak's traditional cuisine. This study was aimed to examine the phytochemical constituents of andaliman fruit extracts after simple macerated in water, methanol, ethyl acetate and hexana using qualitative phytochemical analysis, and to determine its potential antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella sp* by using agar well diffusion method and minimum inhibitory concentration (MIC). Phytochemicals such as alkaloids, flavonoid, glycosides, saponins, tannins, triterpene/steroid and glycoside anthraquinones were detected in the methanol extracts, but steroids and glycoside anthraquinones were absent in the ethyl acetate extract. The ethyl acetate extracts showed maximum zone of inhibition and minimum inhibitory concentration against all the experimental microorganisms. The minimum zone of inhibition was determined in hexane extracts showing less antimicrobial activity against all the experimental microorganisms. The MIC of the ethyl acetate extracts was 0,5% w/v for all tested bacteria. Application of ethyl acetate extracts of andaliman fruits showed effective for catfish (*Pangasius Sutchi*) fillet stored in refrigerator (5 °C) for 3 days.

1. Introduction

Andaliman (*Zanthoxylum acanthopodium* DC.) fruits is known as the Batak society spicy and used to eliminate the smell of fish and raw meat. They are wildly grown in Tapanuli, North Sumatera at 1500 meters above sea level [1] at a temperature of 15-18 °C [2,3]. The fruits shape are like pepper, small round, green, but becomes black when they are dry. The fruits contain aromatic compounds with a bitter taste and produce the effect of thrilling sensation on taste buds and cause the tongue felt numb. Wijaya [4] found that citronellal and limonene were the most impacting compounds on the aroma of andaliman, but β -myrcene, (z)- β -ocimene, linalool, β -citronellol, neral, geraniol, geranial, geranyl acetate, unknown compound, and a sesquiterpene were also contributing to andaliman's fresh citrus and warm sweet-peppery aroma. It had been reported to have an anti-inflammatory activity [5] and antioxidant activity [6]. Based on Cahyana and Mardiana [7], andaliman has an antimicrobial activity. Siswadi [8] has evaluated the antibacterial activity of andaliman extract against *Bacillus stearothermophilus*, *Pseudomonas aeruginosa* and *Vibrio cholera*.

Fish product has more spoilage than other foods with high protein [9]. It should be noted that psychrophilic bacteria spoilage occurs in cold storage [10]. The most factors for determining the



quality of fish product are the smell, taste, freshness, absence of specific microorganism, size and composition [11]. The enzymatic and chemical deterioration occur in fish prior to microbial spoilage, because of the high content of saturated fatty acid, free amino acid and other reactive compound [9].

Nowadays consumers demand minimally preserved foods for maximum nutrient retention, without the addition of chemical preservatives. On the other hand, foods need to be safely preserved, with prolonged shelf-life [12]. An increasing interest in the use of natural antimicrobials as food preservatives has been recorded. Fortunately, antimicrobials from plants can be used as an alternative to chemical preservatives in order to satisfy consumers' demand for safe, convenient and wholesome food [13,14]. Therefore, the aim of this research is to study the antimicrobial component of andaliman extracts and to evaluate its potency on catfish fillet quality during storage in refrigerator.

2. Material and methods

2.1. Preparation of andaliman fruits

Andaliman fruits were collected from farmer at Brastagi Village North Sumatera. All fruits were washed with fresh water and dried in the oven at 40°C of temperature for 48 hours. The dried fruits were powdered by using electrical blender and stored in sterile container for further use.

2.2. Preparation of andaliman extracts

The dried fruit powder were divided into 4 groups, and kept in contact with the solvent (water, methanol, ethyl acetate and hexane) in a stoppered container for 72 hours with occasional shaking. The solvent from the extract was filtered, and concentrated by using vacuum evaporator at 50 °C to yield a crude extract and then weighed.

2.3. Qualitative analysis of phytochemical compound

The individual extract was subjected to the yield and qualitative phytochemical analysis for the presence of some chemical constituents as per standard methods described by Brain and Turner [15] and Evans [16] included alkaloids, flavonoid, glycosides, saponins, tannins, triterpene/steroid and glycoside anthraquinones.

2.4 Evaluation of Antimicrobial Activity

About 2 g of each extract was dissolved in 2 ml DMSO to obtain a stock solution (100% extract) and then the stock solution was diluted to obtain 75%, 50% and 25% of extract concentration. *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* were obtained from Biology Laboratory University of Sumatera Utara for the test and were performed by disc diffusion method according to the method described by Hussain *et al* [17]. Disc saturated with andaliman fruits extracts from different solvents with 25-100% concentration were placed on medium surfaces (Mueller-Hinton agar). Plates inoculated with tested organisms were incubated at 37 °C for 24 hours. Antimicrobial susceptibility testing was performed by the determination of the bacterial inhibition zone around the disc [17].

The minimum inhibitory concentrations (MIC) was performed by a serial dilution technique (0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0 and 5.0%) of concentrate according to the method used by Kubo *et al.*, [18, 19] with some modifications. The extracts were mixed with 10 µL of bacteria test culture in a shaker incubator at speed 150 rpm for 24 hours. MIC value is the minimum extract concentration that inhibits 90% of the growth of bacteria during a 24 hours incubation.

2.5 Preparation of catfish fillet

Fresh catfish (*Pangasius sutchi*) were bought from catfish pond in Medan. The live catfish of similar weight and size were selected for each experiment. The fishes were cleaned and filleted. They were transported in isothermal ice boxes to the laboratory. The catfish fillet then dipped in andaliman extract at its MIC (according to the result of MIC determination) for 15 min, removed, placed in plastic bags,

stored in refrigerator (5 °C) and taken for analysis on 1, 2 and 3 days. The quality of catfish fillet observed were total plate count and total volatile base nitrogen (TVBN) according to method of AOAC [19]. Sensory analysis was conducted by a panel of 15 non-trained taste panelists using 5-point hedonic scale [20]. The panelists scored the sample based on the characterization and differentiation of the various sensory characters such as odor, color and texture [21]. The experiment were performed with three replications.

The statistical analysis was used to carry out mean values, standard deviation in addition to an overall analysis of variance (ANOVA) and Least Significant Differences (LSD) at 0.05 level.

3. Results and discussions

3.1. Phytochemical compounds of andaliman extracts

The single stage extractions was performed in this research and the yield of extraction of andaliman fruit is shown in Table 1. The highest yield of andaliman fruit extract was found in methanol extract and followed by ethyl acetate and water. Results of the analysis of the phytochemical compounds of andaliman fruit extract (Table 2) extracted with methanol has more phytochemical compounds and hexana extracts only have alkaloids and triterpens/steroids. The results revealed the presence of phytochemicals compound such as alkaloids, flavonoids, glycoside, saponins, tanins, triterpen/steroids, and glycoside anthraquinones [22]. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure [23]. Phenolic compounds such as saponins and tannins are generally soluble in organic solvents that are polar such as methanol. Phenolic compounds are substances which have aromatic rings with one or more hydroxyl groups so it is easy soluble in polar solvents [24].

Table 1. The yield of andaliman fruits extract

Solvent	Yield (%)
Water	3.23
Methanol	4.15
Ethyl Acetate	3.97
Hexana	3.02

Table 2. Qualitative phytochemicals analysis of andaliman fruits extract

Phytochemical	Solvent Extract [(+) means present, (-) means absent]			
	Water	Methanol	Ethyl Acetate	Hexana
Alkaloids	-	+	+	+
Flavonoids	+	+	+	-
Glycoside	+	+	+	-
Saponins	-	+	+	-
Tanins	+	+	+	-
Triterpens/steroids	-	+	-	+
Glycoside anthraquinones	-	+	-	-

The presence of alkaloids, flavonoids, and tanins have been confirmed to have antimicrobial activity [25,26]. In general, plant phenolic compounds have been shown to have antibacterial activity. For example, the antimicrobial activity in germinated fenugreek seeds which may be due partly to the presence of flavonoids and polyphenols has been reported [27]. Scazzocchio *et al.* [28] reported that alkaloids (berberine, beta-hydrastine, canadine and canadoline) of *Hydrastis canadensis* L. (Ranunculaceae) have antibacterial activity against 6 strains of microorganism: *Staphylococcus aureus*, *Streptococcus sanguis*, *Escherichia coli* and *Pseudomonas aeruginosa*. Steroids of *Alstonia macrophylla* plants have been reported to possessed antibacterial activity against *E. coli*, *Salmonella*

Typhi, *Staphylococcus aureus* and *B. subtilis* [29]. Triterpenoids compounds that have antimicrobial activity include merediol, linalool, indole and kadinen that effective to inhibit the growth of *B. subtilis*, *S. aureus* and *E. coli* [19].

3.2. Antimicrobial activity of andaliman extracts

The results of the antibacterial activity of an andaliman fruits extracts are tabulated in (Table 3). Minimum inhibitory concentrations of extracts against the tested organisms of andaliman fruit extracts exhibited MIC (Table 4) against the tested organisms. Table 2 shows that ethyl acetate extract has the higher antibacterial activity against *S.aureus* and *S.typhimurium* at the concentration 100% while methanol extract has the higher antibacterial activity against *E.coli* at the 100% concentration. In the methanol extract, phenolic is more polar than flavonoid. Phenolic has higher antimicrobial activities than flavonid [30].

Table 3. Antimicrobial activity (Inhibition zone in mm) from andaliman fruit extracts

Inhibition zone (mm) of andaliman fruit extracts				
Test Bacteria	Concentration of andaliman fruit extracts (%)			
	25	50	75	100
Solvent : Water				
<i>Escherichia coli</i> ,	8.25	8.75	9.00	8.88
<i>Staphylococcus aureus</i>	9.77	11.26	13.76	15.40
<i>Salmonella typhimurium</i>	8.49	8.72	9.14	15.46
Solvent : Methanol				
<i>Escherichia coli</i> ,	10.75	10.38	10.75	11.75
<i>Staphylococcus aureus</i>	11.34	13.88	15.81	17.78
<i>Salmonella typhimurium</i>	10.86	12.86	15.31	17.76
Solvent : Ethyl Acetate				
<i>Escherichia coli</i> ,	10.30	10.48	11.28	10.73
<i>Staphylococcus aureus</i>	12.23	14.97	17.22	20.06
<i>Salmonella typhimurium</i>	14.41	16.15	18.52	20.19
Solvent : Hexana				
<i>Escherichia coli</i> ,	10.38	10.13	10.00	9.63
<i>Staphylococcus aureus</i>	7.70	8.69	10.28	13.13
<i>Salmonella typhimurium</i>	9.42	10.30	10.86	13.67

Table 4. MIC determination of ethyl acetate extract of andaliman fruit against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium*

Extract Concentration (%)	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella typhimurium</i>
5	+	+	+
4	+	+	+
3	+	+	+
2	+	+	+
1	+	+	+
0.5	+	+	+
0.25	+	-	-
0.1	-	-	-

(+) means that andaliman fruit extract still be able to inhibit the bacteria growth

(-) means that andaliman fruit extract is unable to inhibit the bacteria growth

Further testing of the extract of ethyl acetate was conducted to determine the value of MIC against *E. coli*, *S. aureus* and *S. typhimurium* with the direct method on nutrient broth media. MIC values of extracts of andaliman fruit ranged from 0.1 – 0.25%, with *E. coli* and *S. typhimurium* showing MIC of 0.25% and *S. aureus* showing MIC of 0.1%. The differences observed in the susceptibilities of the bacteria tested against the ethyl acetate extracts could be due to their cell wall component, such as the porin protein [31]. For the application of andaliman fruit extract in catfish fillet, the extract concentration used was 0.5%.

3.3. Application of andaliman fruit extract on catfish fillet

The addition of 0.5% (volume per mass) andaliman fruit extract to catfish fillet can reduce the population of spoilage microflora as shown in Table 4. Total plate count (TPC) reached the level 4.50×10^6 in treated catfish sample within 3 days of storage at 5°C. Unacceptable TPC (almost 10^6) were found in control catfish fillet (day 3).

The TVBN value at the beginning of experiment was 16.66 mg N/100 g and reached the level of 26.18 mg N/100 g and 30.62 mg N/100 g for catfish with and without addition of andaliman fruit extract, respectively. The limit of acceptability of TVBN in fish and fishery product is 30-35 mg N/100 g [32], therefore, the value of TVBN for untreated catfish fillet stored in refrigerator was unacceptable after 3 days of storage. TVBN is an indicator of freshness of fish [33].

Table 5. The TPC and TVBN value of catfish fillet with and without the addition of andaliman fruit extract for 3 days of storage in 5°C.

Days	Samples *	Total Plate Count (CFU/ml)	TVBN (mg N/100g)	Sensory Value		
				Odor	Color	Texture
1	Treated catfish fillet	4.18×10^5	18.10	7.5	7.3	7.3
	Control	4.25×10^5	22.14	5.0	5.0	5.0
2	Treated catfish fillet	4.35×10^5	21.53	6.5	6.5	6.5
	Control	3.26×10^6	25.01	5.0	5.0	5.0
3	Treated catfish fillet	4.50×10^6	26.18	6.5	6.5	6.3
	Control	5.60×10^6	30.62	5.0	3.0	1.0

* Treated catfish was catfish fillet with the addition of ethyl acetate andaliman extract with the 0.5% concentration, and control was catfish fillet without the addition of andaliman fruit extract

Table 4 showed the changes in sensory evaluation (odor, color and texture) for 3 days of catfish storage. The sensory scores of fillets in both groups decreased with the storage time, and the sensory scores of the control catfish fillet was lower than the treated one. It can indicate that the ethyl acetate extract of andaliman fruit extract with 0.5% concentration could be used as natural source of antimicrobial since it has better consumer acceptance. Fan *et al* [32] reported that sensory scores in common carp treated with tea polyphenols had a higher score than the control.

4. Conclusions

Andaliman fruit extracts contain various bioactive compounds such as alkaloids, flavonoids, glycoside, saponins, tanins, triterpene/steroids and glycoside anthraquinone. These bioactive compounds have antimicrobial activity against the *E. coli*, *S. aureus* and *S. typhimurium*. The higher antimicrobial activity was found in the ethyl acetate extract with the MIC value of 0.5%. The andaliman fruit extract can be used to inhibit the growth of spoilage microbial in the catfish fillet during 3 days of storage at 5°C. Consequently, the application of grape extract improved the quality of fillets which is quite promising for the food industry.

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