

Antioxidant Activity of Arsik Fish Seasoning from Indonesian

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Abstracts

Arsik fish seasoning is a traditional Batak Indonesian cooking spice that is a mixture of herbs, namely: andaliman, turmeric, ginger, galangal, lemongrass, garlic, shallots, Batak onions, red chili, pieces of tamarind, candlenuts, and salt to taste. The presence of andaliman, which has a distinctive aroma and flavor (spicy and warm) not found in other spices, gives this dish its unique taste. The combination of all the spices provides a taste profile: spicy, salty, sour, sweet, and savory in the cooked fish. This study aims to identify the compounds in the spices, their toxicity, and antioxidant properties. The extraction method used was a heat-based method (Soxhlet), and for toxicity testing, the BSLT (Brine Shrimp Lethality Test) method was used. For screening, it was carried out using Phytochemistry, and for antioxidants, it was conducted using the DPPH test. The results of the study showed the presence of bioactive compounds in arsik seasoning, namely alkaloids, flavonoids, terpenoids, tannins, and saponins. The LC50 (Lethal Concentration 50%) value on *Artemia salina* larvae tested against Batak's typical fish arsik seasoning was obtained. The result showed an LC50 value of 161.9892 µg/mL, then with the addition of 10% : 588.0848 µg/mL, and with 20% was 725.77 µg/mL, indicating a toxic category. Based on these LC50 values, it can be concluded that the arsik seasoning formulation used is toxic to *Artemia* larvae and shows good bioactivity. The antioxidant values for the original seasoning were: IC50: 142.885; with 10% spice concentration :50.395 and 20% : 42.85. It appears that the antioxidant strength is greater at the 20% concentration.

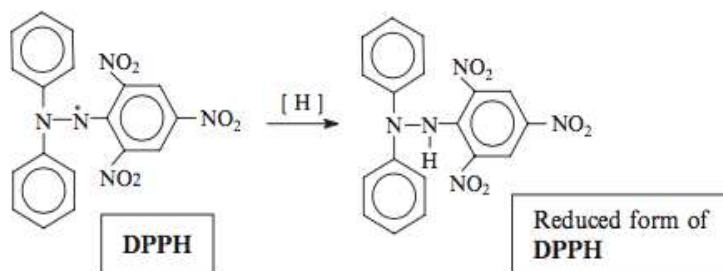
Keywords: Arsik seasoning, Batak toba traditional cuisine, toxicity, phytoscreening, and antioxidant activity

Introduction

Free radicals or oxidants are molecules that contain one or more unpaired electrons in their outermost orbitals, which makes them unstable and highly reactive in capturing paired electrons, consistent with the nature of electronic instability (Rosnagel,K,2018). In addition to genetic inheritance factors, several studies have shown that one of the most significant causes of human diseases is the uncontrolled production of free radicals highly reactive by products that possess one or more unpaired electrons (Mani, 2014). As a result, these free radicals can accumulate in the body and contribute to the development of various diseases when their levels become imbalanced (Suleman.M,2018). Flavonoids are found in plants, which have antioxidant properties and are capable of inhibiting free radicals.

Antioxidants work by donating one or more electrons to free radicals, thereby neutralizing them (Eric Chan, W. C., Lim, Y. Y., & Wong, S. K, 2011). Antioxidant activity can be measured using the DPPH method. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical that is marked by a deep purple color. When antioxidants are added, they donate electrons or hydrogen atoms to reduce DPPH to its non-radical form (DPPH-H), which appears colorless or pale yellow. The greater decrease in absorbance, the stronger the antioxidant's ability to reduce DPPH. The results are usually expressed as IC₅₀, which is the sample concentration required to reduce 50% of DPPH radicals. This method is widely the greater the decrease in absorbance, the stronger the antioxidant's ability to reduce DPPH. Results are usually expressed as IC₅₀, which is the sample concentration required to reduce 50% of DPPH radicals. This method is widely used because it is simple, fast, sensitive, and can be applied to various plant extracts, pure compounds, and food products used because it is simple, fast, sensitive, and applicable to various plant extracts, pure compounds, and food products.

There are two types of antioxidant compounds, namely synthetic and natural. Secondary metabolites from plants, such as flavonoids, have antioxidant properties capable of inhibiting free radicals. Antioxidants work by donating one or more electrons to free radicals, thereby neutralizing them (Enrique Martínez Leo, E et al., 2024; Eric Chan, W. C., Lim, Y. Y., & Wong, S. K, 2011). Antioxidant activity can be measured using the DPPH method. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical identified by a deep purple color. When antioxidants are added, they donate electrons or hydrogen atoms to reduce DPPH to its non-radical form (DPPH-H), which appears colorless or pale yellow. The greater decrease in absorbance, the stronger the antioxidant's ability to reduce DPPH. The results are usually expressed as IC₅₀, representing the sample concentration required to reduce 50% of the DPPH radicals. This method is widely used because it is simple, rapid, sensitive, and applicable to various plant extracts, pure compounds, and food products.



Diphenylpicrylhydrazyl (free radical)

Diphenylpicrylhydrazine (nonradical)

Mechanism of DPPH Radical Reduction by Antioxidants (Mani, et.al., 2011)

Indonesia is rich in culture, and each ethnic group has its own distinctive cuisine, made with spices from various herbs and seasonings. These spices come from plants with antioxidant properties. Arsik is a traditional dish from the Batak Toba ethnic group. This carp-based dish is typical of Batak Toba cuisine and is usually served at traditional events such as weddings and thanksgiving ceremonies. The spices used in arsik include a variety of seasonings, with some herbs being characteristic of this dish. The composition of arsik spices and the potential health benefits of the traditional Batak arsik dish are known for its rich aroma and its sour, salty, and savory taste.

Arsik Spice Composition and Health Potential

The traditional Batak dish *arsik* is distinguished by its rich aroma, sourness, saltiness, and savory flavor. Its unique taste profile is derived from a blend of spices and herbs, including:

Andaliman (*Zanthoxylum acanthopodium*) • Turmeric (*Curcuma longa* Linn) • Ginger (*Zingiber officinale*) • Lemongrass (*Cymbopogon citratus*) • Galangal (*Alpinia galanga*) • Batak onion (*Allium chinense* L.) • Red onion (*Allium cepa* L.) • Garlic (*Allium sativum*) • Slices of asam gelugur (*Garcinia atroviridis* Griff.) • Red chili (*Capsicum annuum* L.) • Candlenut and table salt Each spice contributes synergistically to the flavor of the dish and its potential health benefits. Andaliman, in particular, provides a distinctive aroma and taste that no other spice has. Andaliman also shows antioxidant activity; chicken meat marinated in andaliman juice shows increased antioxidant levels and inhibited *Salmonella* growth (Silalahi, 2019). Turmeric, commonly used for its yellow color and distinctive aroma (Irham & Hardiyanti, 2021), has antioxidant, antimicrobial, anticancer, and antidiabetic properties (Santi, 2022). Ginger exhibits antioxidant properties and antimicrobial activity and has been shown to reduce cholesterol levels in male rats fed a high-fat diet (Niazi, A, 2024; V, 2016). Lemongrass has antioxidant properties and a distinctive aroma (Zhang et al., 2020), as well as antibacterial and antidiabetic effects. Antioxidant, anti-inflammatory, and antimicrobial properties of garlic and onions (Wilson EA, Demmig-Adams B (2007)). Characterization, antibacterial and antioxidant activity of mangosteen (*Garcinia mangostana* L.) Pericarp nanosized extract (Pericarp Nanosized Extract. (2017), while red chili adds spiciness and antioxidant activity (Can Dogan, 2018). Galangal also offers antimicrobial and antioxidant benefits (Reddy, 2022). In preparing *arsik*, all the spices are finely ground and cooked with the fish until the liquid is almost evaporated. The amount of each spice is adjusted according to taste, as preferences vary. Some prefer a stronger spicy, salty, or sour flavor, while others prefer a milder taste. Literature studies show that adding andaliman to *arsik* can inhibit bacterial growth (Silas, K. H., Ndubueze, C. W., Anorue, S. C., & Ahaneku, E. B. (2025)). The addition of a 20% spice mixture significantly affects the moisture, fat, and protein content ($p < 0.05$) and positively influences shelf life up to four weeks (Chatatikun et al., 2020). This synergistic spice blend, like traditional medicine (*jamu*), has promising potential as a functional culinary ingredient for health enhancement.



1. Andaliman
(*Zanthoxylum acanthopodium*)



2. Gelugur Acid (*Garcinia atroviridis* Griff ex T. Ander)



3. Red Chili (*Capsicum annuum* L.)



4. Cayenne pepper
(*Capsicum frutescens*)



5. Turmeric (*Curcuma domestica* Vahl)



6. Ginger (*Zingiber officinale*)



7. Lengkuas (*Alpinia galanga*)



8. Lemon Grass
(*Cymbopogon citratus*)



9. Shallot (*Allium cepa* L.)



10. Garlic (*Allium sativum* L.)



11. Batak onion (*Allium chinense* G. Don)



12. Pecan (*Aleurites moluccana* L.)

Method

Preparation of Arsik Spice Sample

The arsik spice sample, consisting of red chili, bird's eye chili, andaliman, shallots, garlic, Batak shallots, galangal, ginger, turmeric, candlenuts, lemongrass, and slices of asam gelugur, was washed clean under running water and sorted while wet. These ingredients were weighed according to the taste correction for 1 kg of arsik fish, which requires: 50 g andaliman, 20 g tamarind, 30 g red chili, 30 g bird's eye chili, 30 g galangal, 30 g lemongrass, 40 g garlic, 50 g shallots, 50 g turmeric, 80 g ginger, 50 g candlenut, 50 g Batak onion. After being weighed, these ingredients are chopped into small pieces and blended until smooth. For the 10% and 20% additions, each spice is increased by 10% and 20%, respectively.

Sample Extraction

The blended arsik spice samples were extracted using the Soxhlet method with 70% ethanol as the solvent. The extraction was carried out at 60 °C for 6 hours. The resulting filtrate was filtered through filter paper and evaporated using a rotary evaporator to remove the solvent. The obtained thick extract was stored in a closed container at 4 °C for further testing. Brine Shrimp Lethality Test (BSLT).

a. Preparation of Test Solution

A total of 0.2 g of the sample extract is dissolved in 100 mL of seawater (3.8% NaCl solution) to obtain a stock solution with a concentration of 2000 ppm. From this stock solution, 125, 250, 500, and 2500 µL are taken and diluted with 5 mL of seawater to produce solutions with concentrations of 50, 100, 200, and 1000 ppm. These solutions are homogenized and evaporated to dryness to ensure accurate concentration before testing.

b. Preparation of the Number of Artemia salina

Artemia salina eggs are hatched in a 3.8% seawater solution, which is made by dissolving 38 g of NaCl in 1 liter of distilled water. Hatching is carried out under constant lighting at room temperature for 48 hours until nauplii appear. Active and responsive nauplii are selected using a pipette for toxicity testing.

c. Toxicity Testing

Each vial containing a control solution (seawater) and test solutions (extract at concentrations of 10, 100, 200, 500, and 1000 ppm) was inoculated with 10 active nauplii using a micropipette, each in three replicates. The vials were then incubated at room temperature under constant light for 2 hours, after which the number of dead nauplii was recorded. Mortality was calculated using the formula:

$$\text{Mortality (\%)} = (\text{Number of dead nauplii} / \text{Total number of nauplii}) \times 100$$

The data were analyzed using SPSS version 20 with Probanalysis to determine the LC_{50} value, which is the concentration that causes 50% mortality.

Toxicity levels were categorized as follows:

- Highly toxic: $LC_{50} < 30 \mu\text{g/mL}$
- Toxic: $30 \mu\text{g/mL} \leq LC_{50} \leq 1000 \mu\text{g/mL}$
- Non-toxic: $LC_{50} > 1000 \mu\text{g/mL}$

Phytochemical Screening

Alkaloid Test

Each 0.5 g of *gompang batu* plant extract was dissolved in solvent and treated with 2–3 drops of Wagner's and Dragendorff's reagents. A positive result was indicated by a brown precipitate (Wagner) or brick-red precipitate (Dragendorff).

Flavonoid Test

Each 0.5 g of extract was dissolved in solvent, followed by the addition of 0.1 g magnesium powder and 1 mL concentrated HCl. After shaking and standing for 5 minutes, the appearance of orange, red, or yellow color indicated a positive result.

Terpenoid Test

Each 0.5 g of extract was dissolved in solvent, then 10 drops of acetic anhydride and 2 drops of concentrated sulfuric acid were added. After gently shaking and letting it sit, a green or blue color indicates the presence of steroids, while a red or orange color indicates the presence of terpenoids. The extract was dissolved in solvent, placed in a test tube, and treated with a few drops of 1% FeCl₃. A greenish-black color indicated the presence of tannins.

Saponin Test

A total of 0.5 g of ethanol extract was placed in a test tube, mixed with 3 mL of distilled water, and shaken for 1 minute. If foam formed, add 4 drops of 1M HCl. If no foam appeared, the mixture was heated for approximately 3 minutes, cooled, and shaken vigorously. Stable foam formation for about 10 minutes indicated the presence of saponins.

Antioxidant

DPPH Antioxidant Activity Test

- a. Preparation of DPPH Solution (0.4 mM)** A total of 15.7 g of DPPH (Molecular Weight 394.32) was weighed and dissolved in analytical grade methanol (p.a) up to 100 mL in a volumetric flask. The solution was then homogenized and stored in a dark bottle.
- b. Preparation of Vitamin C (Ascorbic Acid) Standard Solution** A total of 2.5 g of Vitamin C (ascorbic acid) was dissolved in analytical grade methanol (p.a) and transferred into a 50 mL volumetric flask up to the calibration mark (500 ppm). The solution was stored in a reagent bottle as the stock standard solution.
- c. Preparation of Arsik Spice Extract Stock Solution** A total of 5 mg of arsik spice extract was weighed and dissolved in 10 mL of analytical grade methanol. The solution was stored as a 500 ppm sample stock solution.
- d. Antioxidant Activity Testing of Samples** Volumes of 50, 100, 250, 500, and 1000 µL of the sample stock solution were pipetted into 5 mL-calibrated test tubes to obtain concentrations of 5, 10, 25, 50, and 100 ppm. To each tube, 1.0 mL of DPPH solution was added, followed by analytical grade methanol (p.a) up to the 5 mL mark. The mixture was homogenized and covered with aluminum foil. The solutions were incubated in a water bath at 37°C for 30 minutes. Absorbance was then measured at the maximum absorption wavelength of 517 nm using a UV-Vis spectrophotometer, with three repetitions (triplicate). Vitamin C solutions at concentrations of 5, 10, 25, 50, and 100 ppm were used as positive controls, and methanol with 1.0 mL DPPH was used as the negative control.
- e. Measurement of DPPH Free Radical Scavenging Absorbance** The percentage of inhibition was calculated using the following formula:

DPPH Radical Inhibition (%) = $\frac{\text{Absorbance of Blank} - \text{Absorbance of Sample}}{\text{Absorbance of Blank}} \times 100\%$ (Gulcin, 2020).

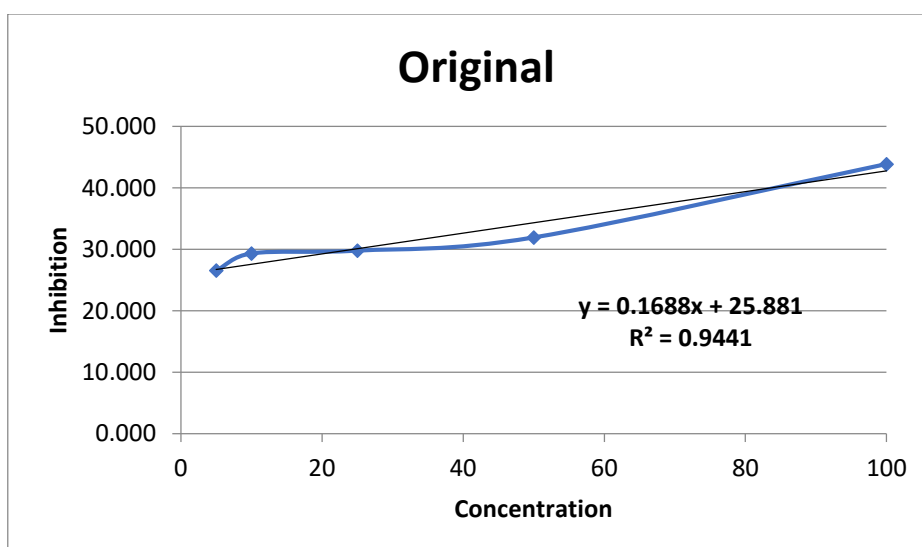
The IC₅₀ value (Inhibition Concentration 50) is the antioxidant concentration (µg/mL) required to inhibit 50% of free radicals. The IC₅₀ is obtained from the intersection point of the 50% inhibition line with the concentration axis, and then calculated using the equation:

$$Y = a + bX$$

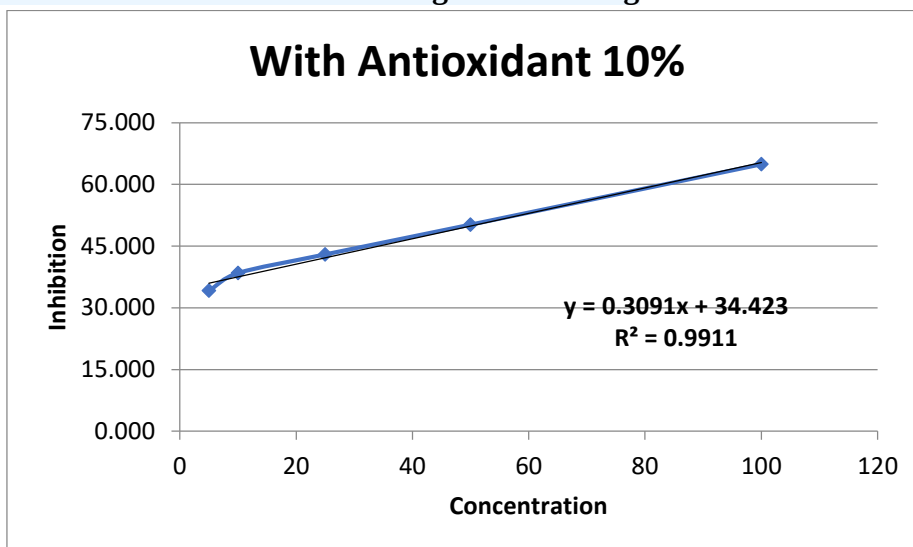
where Y is the percentage of inhibition (i.e., 50), and X represents the IC₅₀ value.

Antioxidant Table Data of Batak Arsik Spices

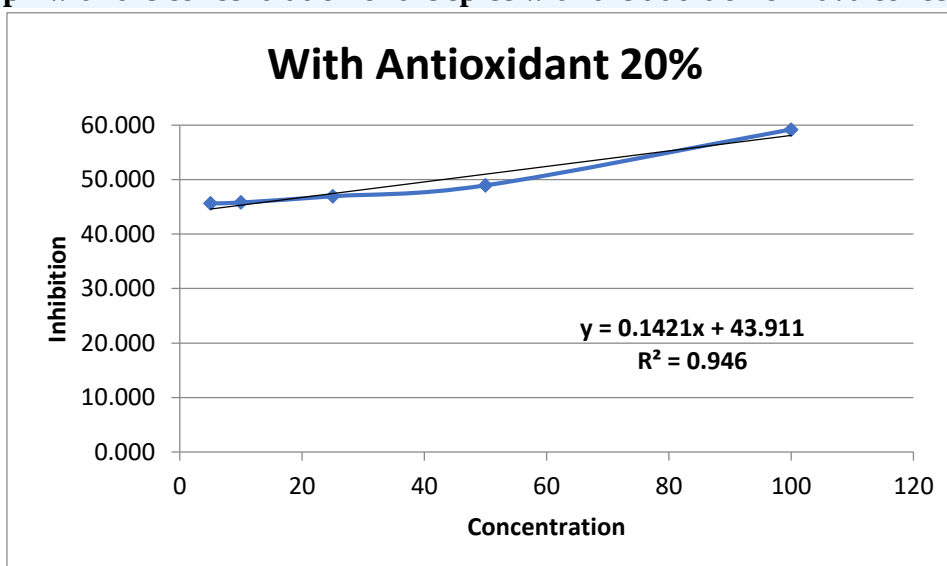
Type	Concentration (ppm)	Absorbance	% Inhibition	IC50
Original	5	0.491	26.547	142.885
	10	0.472	29.341	
	25	0.469	29.790	
	50	0.455	31.936	
	100	0.375	43.862	
With 10% Antioxidant	5	0.440	34.182	50.395
	10	0.411	38.473	
	25	0.381	43.014	
	50	0.332	50.250	
	100	0.234	64.920	
With 20% Antioxidant	5	0.363	45.659	42.85
	10	0.362	45.808	
	25	0.354	46.956	
	50	0.341	48.952	
	100	0.273	59.182	



Inhibition graph with concentrations of the original seasoning

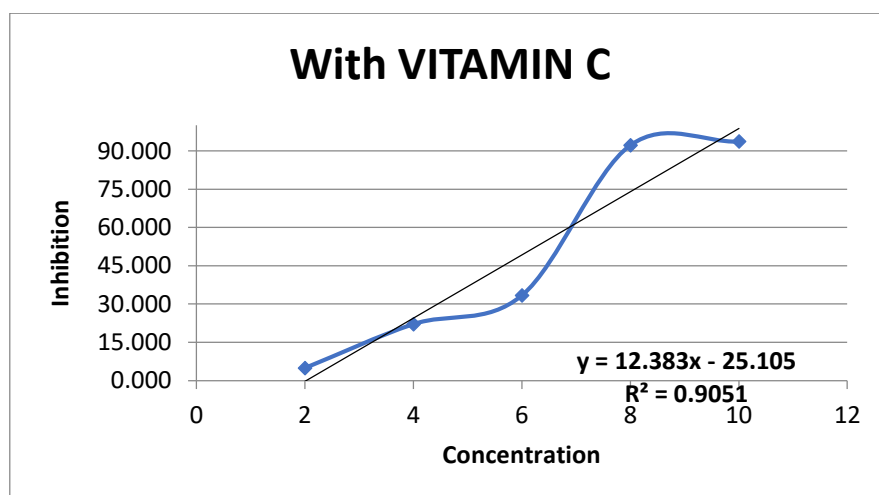


Inhibition graph with the concentration of the spice with the addition of 10% concentration



Inhibition graph with the concentration of the spice with the addition of 20% concentration Vitamin C Antioxidant Table

Type	Concentration (ppm)	Absorbance	% Inhibition	IC50
Vitamin C	2	0.636	4.840	6.065
	4	0.521	22.056	
	6	0.446	33.283	
	8	0.052	92.166	
	10	0.043	93.613	



Inhibition and Vitamin C concentration graph

Result:

The antioxidant values for the original seasoning were: IC₅₀: 142.885; with 10% spice concentration :50.395 and 20% : 42.85. It appears that the antioxidant strength is greater at the 20% concentration. Addition of 20% spice concentration significantly increases antioxidant activity, although still lower compared to Vitamin C (recognized as the gold standard of antioxidants). The synergy of fresh spices has already shown strong antioxidant activity, showing great potential to be developed as a natural source of antioxidants.

Conclusion

From the data and graph, it is shown that the addition of 20% spice concentration can enhance antioxidant strength and is classified as a strong antioxidant, although still lower than Vitamin C. Further observation is needed to evaluate the effect when using dried spices.

Antioxidant activity can be categorized as follows:

- **Very active** (IC₅₀ < 50 ppm)
- **Active** (IC₅₀ between 50–100 ppm)
- **Moderate** (IC₅₀ between 101–250 ppm)
- **Weak** (IC₅₀ between 250–500 ppm)
- **Inactive** (IC₅₀ > 500 ppm)

This classification helps in determining the efficiency and potential of various compounds in combating free radicals, which can be linked to their ability to prevent oxidative damage in the body (Flieger *et al.*, 2021).

The antioxidant activity of Batak traditional Arsik seasoning is strongly influenced by spice concentration. The higher the concentration of spices such as andaliman, cikala fruit, and turmeric, the stronger the seasoning's ability to neutralize free radicals. With proper formulation, Arsik can be developed not only as a traditional culinary heritage but also as a functional food that promotes health.

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