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## Research Article

# Determination of Phytochemical Compounds and Antimicrobial Activities of Rendang Spices

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## Abstract

**Background and Objective:** Rendang is a delicious and long-lasting traditional food. This is partly due to the use of specific spices as well as the processing. The purpose of this study was to screen spice phytochemicals for rendang production as well as bioactivity, which included antibacterial properties. The several forms of rendang (curry, kalio, wet rendang and dry rendang) were investigated in relation to their manufacturing process. **Materials and Methods:** Rendang has been made traditionally and every stage of the process undergoes chemical composition analysis and antioxidant capacity test. The research was using a completely randomized design consisting of 4 treatments and 3 repetitions. Each raw material used is characterized chromatographically. The antimicrobial ability of each ingredient was also analyzed. The data were analyzed by F test and a further test of Duncan's New Multiple Range test at the 5% significance level. **Results:** The amount of the chemical components is obtained successively in rendang marinades such as onion (2695.5 ppm inulin), red pepper (494.5 ppm capsaicin), cloves (57.09% eugenol), lime leaves (13.32% trans-cinnamyl acetate), turmeric leaf (2,057 ppm curcumin), bay leaves (3.43% eugenol), ginger (2.84% zingiberene), cumin (2152.2 ppm safranal), cinnamon (4.52% cinnamaldehyde), nutmeg (49.91% linolenic acid), coriander (7.03% linalool), ginger (1.07% cineole), nutmeg (0.02 ppm myristin) and lemon grass (31.82% ascorbic acid). Antimicrobial power at the level of rendang against *Staphylococcus aureus* has a diameter of 7.33 to 10 mm, while the bacterium *Escherichia coli* has a diameter of from 0 to 10.33 mm. **Conclusion:** Rendang has a lot of seasoning, other possible bioactivities, like high antioxidant activity, can also be found. The utilization of spices improves the rendang goods that were generated.

**Key words:** Antimicrobial, food processing, phytochemical, secondary metabolites, self-life

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Rendang was voted the first ranked dish on the World's 50 Most Delicious Foods held by CNN International in 2011 and 2017<sup>1</sup>. Rendang is a traditional food from the West Sumatra (Minangkabau) Region. Rendang must meet certain criteria, namely, all ingredients used must be of the highest quality. The meat must be fresh; the coconut must be mature and squeezed perfectly. The chili should come from highland areas with a specific level of spiciness and fragrance. Onions and other spices must also be of high quality. The leaves, which are complementary ingredients, should also be fresh.

The process of making rendang takes a long time. It is grouped into 4 stages, which are gulai, kalio, wet rendang and dry rendang. Based on the cooking time, the final products of rendang are divided into two groups, which are wet rendang and dry rendang. Wet rendang is characterized by a shorter cooking time so the water content is higher, while dry rendang has a relatively long cooking time (drier). This cooking process uses a gradually increasing temperature according to the level of maturity of the rendang. The temperature used for cooking gulai at 75-85°C for 60 min, then in the process of making kalio the temperature becomes 85-95°C for 30 min. Afterwards, at the wet rendang stage, the cooking temperature is lowered to 80-90°C for 45 min. The formation of wet rendang is characterized by the brownish color of the meat, the coconut milk and spices thicken and become brownish. Heating is continued again at 75-80°C for  $\pm 45$  min until dry rendang is formed.

Rendang can be stored longer, because it contains lots of spices/seasonings, besides providing flavor to the rendang<sup>2</sup>. Spices that are commonly used in making rendang are shallots, garlic, red chilies, cloves, lime leaves, turmeric leaves, bay leaves, ginger, cumin, cinnamon, candlenuts, coriander, galangal, nutmeg and lemongrass<sup>3</sup>. Some of these spices are added directly in the form of leaves such as lime leaves, turmeric leaves and bay leaves and some are grounded together with red chilies mixed with coconut milk. The mixture of spices used will provide a delicious taste or the chemical components of spices can also act as antimicrobials so that rendang can be stored for a longer time<sup>4</sup>. This is important to pay attention to because it is semi-wet food with 40.50-42.50% water content, which makes it easy for microorganisms and mold to grow. Until now, there has been no comprehensive report or article about the chemical components of rendang spices that form the flavor, so this research aims to identify the main phytochemical components found in rendang spices, both qualitatively and quantitatively.

The main chemical components in spices or seasonings have quite strong antimicrobial activity, such as shallots, garlic, red chilies, ginger, turmeric and galangal<sup>5</sup>. In this research, the antimicrobial activity of each spice or seasoning used in making rendang are identified. Determination of antimicrobial activity is tested on groups of gram-positive and negative bacteria so that it can guarantee that food is safe for consumption. This research was conducted to obtain the phytochemical compounds contained in the spices used in making rendang, determine the amount of active ingredients (phytochemicals) that make up the rendang spices and determine the antimicrobial capabilities of each rendang spice.

## MATERIALS AND METHODS

**Study area:** The study was carried out from December, 2019 to March, 2023. The study was conducted at the Laboratory Food and Agricultural Product Technology, Faculty of Agricultural Technology Andalas University.

**Raw materials, chemicals and instruments:** The raw material was collected from the local farmer in Padang City of West Sumatra Province Indonesia. The main spices were shallots, garlic, candlenut, ginger, galangal, cloves, cumin, cinnamon, coriander, nutmeg, red chilies, lemongrass, turmeric leaves, bay leaves, lime leaves. The spices were collected as approximately 100-200 g each (Fig. 1a). Four types of rendang based on the duration of cooking as shown in Fig. 1b.

The chemicals used in this research were n-hexane, ethyl acetate, methanol, hydrochloric acid, distilled water, magnesium powder, ethyl acetate, ammonia, acetic acid, sodium hydroxide, concentrated sulfuric acid, FeCl<sub>3</sub>, cytorbate reagent. Silica gel Brand 60 GF<sub>25</sub> 54 (230-400 mesh), H<sub>2</sub>SO<sub>4</sub> 2N, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O, Starch 1%, I<sub>2</sub>, KIO<sub>3</sub>, Meyer's reagent is used for identification of alkaloids, Lieberman Burchard's reagent for identification of terpenoids and steroids, Cyanidin test for identification of flavonoids and FeCl<sub>3</sub> for phenolic identification, distilled water, Whatman No. 1 filter paper, ethanol, test bacterial culture (*Staphylococcus aureus* was purchased from the Medical Microbiology Laboratory Andalas University) and Natrium Agar media. All chemical used as pro analyses grade and purchased from Merck Indonesia.

The instruments used for chemical analysis were a set of chromatography instruments (HPLC type UFLC Shimadzu and GC-MS type QP 2010 Ultra Shimadzu), analytical scales (excellent) and a set of tools for antimicrobial testing.

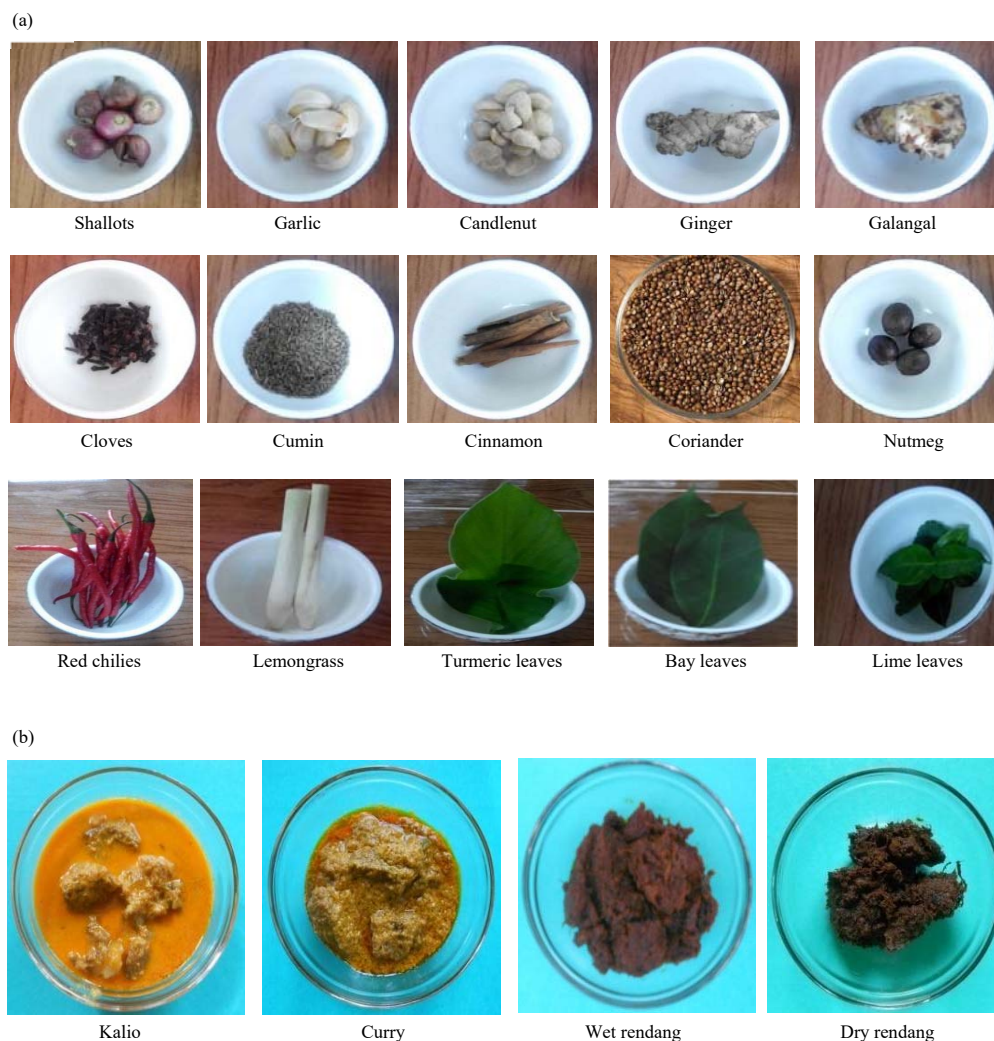


Fig. 1(a-b): (a) Rendang spices and (b) Types of Rendang

### Work procedures

**Testing of phytochemical compounds:** The powdered sample of 0.1 g was put into a test tube and then macerated with heated methanol (in a water bath) for 15 min. After that, filter it hot into another test tube and let the entire methanol evaporate until dry. Then 5 mL of chloroform and distilled water were added in a ratio of 1:1 each, shaken well, then transferred to a test tube and left for a moment until two layers of chloroform-water were formed. The chloroform layer at the bottom is used to examine triterpenoid and steroid compounds, while the water layer is used to test flavonoids, phenolics and saponins.

**Identification of active compounds in spices:** Tests for the identification of chemical compounds were carried out on spices that had been extracted using High Performance Liquid

Chromatography (UFLC 20AD, Shimadzu Japan) and GC-MS chromatography instruments (GC-MS QP2010, Shimadzu Japan). The use of HPLC chromatography was determined by using a DAD UV-Vis detector. The work continued with HPLC using an ODS/C18 column and mobile phases of acetone nitrile and distilled water to determine the levels of the main components that make up (general) rendang spices, then measured using a DAD UV-vis detector at a predetermined wavelength, while volatile spice ingredients were used. The GC-MS Shimadzu QP2010. A sample of 1  $\mu\text{L}$  was injected into the GC-MS operated using a glass column 30 m long, 0.25 mm in diameter and 0.25  $\mu\text{m}$  thick with a CP-Sil 5CB stationary phase with an oven temperature programmed between 60-220°C with a temperature increase rate of 10°C min<sup>-1</sup>, helium carrier gas pressure of 12 kPa, total rate of 30 mL min<sup>-1</sup> and split ratio of 1:50.

### Antimicrobial activity analysis

**Agar diffusion test Kirby-Bauer Method:** Dip a cotton swab in the bacterial culture then press the cotton swab against the side of the tube to let the water drain. Spread evenly over the entire surface of the agar plate (*Staphylococcus aureus* and *Escherichia coli* with NA media). Leave the cup for 5 min. Paper discs with a diameter of 6 mm were dipped in antimicrobial solution (pure extract). Remove, leave for a moment to drain and then place the disc paper on the surface of the agar. The disc paper is pressed using tweezers so that it sticks perfectly to the surface of the agar. Incubate at 37°C for 24 hrs. Measure the diameter of the inhibition zone (mm).

**Statistical analysis:** Statistical analysis was calculated using the SPSS package program version 11.5 (SPSS Inc., Chicago, Illinois, USA). After evaluating the data using One-way Analysis of Variance (ANOVA), the Duncan's Multiple Ranges *post hoc* test was run. The results were expressed using the triple samples' mean  $\pm$  SD. A significance level of  $p < 0.05$  was applied to differences.

## RESULTS AND DISCUSSION

**Phytochemical test:** Phytochemical testing was carried out on the extracts that make up rendang seasoning. This was done to determine the chemical compounds contained in the rendang spices. The test results were presented in Table 1.

Based on the phytochemical test, it is known that all forms of extracted ingredients that make up rendang seasoning do not all contain phenolics, alkaloids, flavonoids, terpenoids, steroids and saponins, except for turmeric leaves, greetings and galangal.

Phenolic compounds contained in ingredients (shallots, garlic, cloves, kaffir lime leaves, turmeric leaves, bay, cumin, cinnamon, galangal, nutmeg and lemongrass) tend to dissolve easily in water because they generally bind with sugar as glycosides and are usually found in vacuoles. However, some phenolic compounds are also lipophilic. Phenolic compounds can lignify bacterial cell walls so that the presence of phenolic compounds can inhibit bacterial growth<sup>6</sup>.

Alkaloids are generally contained in various foods such as onions, garlic, kaffir lime leaves, turmeric leaves, bay leaves, ginger, coriander, galangal and lemongrass. Alkaloids are the largest group of secondary metabolite substances. In general, alkaloids include basic compounds that contain one or more nitrogen atoms. Alkaloids are often poisonous and used

extract have been proven to have antibacterial activity against *Staphylococcus aureus* and *E. coli*<sup>7</sup>. Flavonoids are generally found in plants that contain dyes and are bound to sugar as glycosides and flavonoid aglycones such as onions, turmeric leaves, bay leaves, ginger, cinnamon, galangal, nutmeg and lemongrass<sup>6</sup>. Terpenoids include a large number of plant compounds such as shallots, cloves, turmeric leaves, bay leaves, ginger, cumin, cinnamon, candlenuts, coriander, galangal, nutmeg and lemongrass. Terpenoids consist of several types of compounds, generally found in essential oils. Terpenoid groups that have antibacterial activity are borneol, cineol, pinene, camphene, camphor, nerolidol, linalool, indole and kadinen. This class of compounds is effective in inhibiting the growth of *B. subtilis*, *S. aureus*, *S. enterica* and *E. coli*. Steroids in red chilies, cloves, turmeric leaves, bay leaves, cumin, galangal and nutmeg are included in the triterpenoid group. Triterpenoids are compounds whose carbon skeletons come from six isoprene units and are biosynthetically derived from acyclic hydrocarbons, namely squalene<sup>6</sup>. Saponin is a surface-active compound that has soap-like properties and can be detected based on its ability to form foam. Long-lasting foam formation occurs while extracting the plant. This also happens when extracting turmeric leaves, bay leaves, galangal, shallots, garlic and cloves. Saponins, both triterpenes and steroids, have antimicrobial properties<sup>8</sup>.

**Bioactive component identification test:** Bioactive component testing was carried out on the extracts that make up rendang seasoning using an HPLC/GC-MS chromatogram instrument. This was done to determine the main bioactive components that act as antimicrobials. The test results are shown in Fig. 1-3 and Table 1-4.

**Shallots:** Shallots have excellent antimicrobial activity in processed foods such as rendang. Phytochemical analysis of shallot extract shows the presence of flavones, quercetin, ascalin, phenols and furostanol saponins. Phenolics are antibacterial from shallot bulbs which inhibit bacterial growth<sup>9</sup>. In general, HPLC instruments are used for materials that are not heat resistant and break down quickly, one of which is shallots. The results of HPLC analysis for the active fraction of shallots show that there was a component that has an antimicrobial effect.

The main component contained in shallots is inulin. The results obtained were that the content in shallot extract was 2695.5 ppm. The antimicrobial power of shallots is very influential in rendang preparations.

Table 1: Qualitative analyses of phytochemicals of rendang spices

| Material        | Phenolic | Alkaloids | Flavonoids | Terpenoids | Steroids | Saponins |
|-----------------|----------|-----------|------------|------------|----------|----------|
| Shallot         | +        | +         | +          | +          | -        | +        |
| Garlic          | +        | +         | -          | -          | -        | +        |
| Red chili       | -        | -         | -          | -          | +        | -        |
| Clove           | +        | -         | -          | +          | +        | +        |
| Lime leaves     | +        | +         | -          | -          | -        | -        |
| Turmeric leaves | +        | +         | +          | +          | +        | +        |
| Bay leaf        | +        | +         | +          | +          | +        | +        |
| Ginger          | -        | +         | +          | +          | -        | +        |
| Caraway         | +        | -         | -          | +          | +        | -        |
| Cinnamon        | +        | -         | +          | +          | -        | +        |
| Candlenut       | -        | -         | -          | +          | -        | +        |
| Coriander       | -        | +         | -          | +          | -        | +        |
| Galangal        | +        | +         | +          | +          | +        | +        |
| Nutmeg          | +        | -         | +          | +          | +        | +        |
| Lemongrass      | +        | +         | +          | +          | -        | -        |

+: Identified and -: Not identified

**Red chili:** The identification process of the chemical compounds contained in red chili was carried out using HPLC. Chilies which have a characteristic spicy taste can be used as an ingredient to inhibit microbes, one of which is bacteria. The result obtained from the standard comparison of capsaicin and red chili extract was 166.1 ppm, when absorbed by the human body; the capsaicin content found in red chilies has the effect of stimulating adrenaline secretion and causing sweating. Capsaicin levels found in Javanese red chili samples were 494.5 ppm<sup>10</sup>.

**Cloves:** Cloves are included in one of the terpenoid groups. Terpenoids are components that have antimicrobial activity<sup>6</sup>. Testing of the content of clove spice ingredients was carried out using an instrument in the form of GC-MS. The results of GC-MS analysis for the active fraction of cloves show that there was a component that had an antimicrobial effect. There were five quite large peaks which indicate that there are five types of components that influence cloves, each of which has a component level including 57.09% eugenol, which is the main peak that comes out with a retention time of 12.91 min, trans(beta)-caryophyllene 3.47%, pentadecanoic acid (CAS) 9.15%, cis-1-chloro-9-octadecene 13.60% and cyclopentylmethanol, 2-NI 1.45%, while Prianto's research characterization results showed that the retention time of eugenol in clove oil was 16.791 min, amounting to 81.2%<sup>11</sup>. Eugenol has a role as an antibacterial, antifungal, antioxidant, raw material for perfume and flavoring<sup>12</sup>.

**Lime leaves:** The results of component analysis of the active fraction in orange leaves were found as several components namely 1,6-octadien-3 ol, 3,7-dimethyl-12.06%, l-(+)-ascorbic acid 2,6-dihexadecanoate 13.21%, trans-cinnamyl acetate

research states that there are 29 components identified in kaffir lime leaves essential oil. Kaffir lime leaf essential oil consists of oxygenated monoterpenes in large quantities, which is 86.15% of the total essential oil. The main component is  $\beta$  citronellal, a monoterpene (66.85% of the total essential oil) followed by  $\beta$  citronellol, linalool and citronellol<sup>13</sup>.

**Turmeric leaves:** Identification process of the chemical compounds contained in turmeric leaf was also carried out. The most important components in turmeric rhizomes were curcuminoids and essential oils. Turmeric's bright yellow color comes primarily from fat-soluble, polyphenolic pigments known as curcuminoids<sup>14</sup>. Research results from the Research Institute for Spices and Medicinal Plants (Balittro) state that the curcumin content of turmeric rhizomes is an average of 10.92%. The curcuminoid content in turmeric is known to have many benefits and has antibacterial activity<sup>15</sup>. An important characteristic of curcumin is its sensitivity to light. When curcumin is exposed to light, structural decomposition occurs in the form of curcumin cyclization<sup>6</sup>. From the results obtained, the curcumin content of the material was 2,057 ppm. These results were obtained from a comparison of the standard curcumin and turmeric leaves tested. The high curcumin content was found in the older rhizomes and the older the color, the more durable and stronger they are<sup>16</sup>.

**Bay leaf:** Bay leaves are more widely used as a cooking spice mixture in several ready-to-eat food products, especially in making beef rendang with the aim of providing a distinctive aroma. The distinctive aroma of bay leaf is caused by the essential oils contained in it. The content of aromatic compounds in bay leaves was tested<sup>17</sup>. The compounds making up the bay leaf extract were analyzed using GC-MS.

The results of GC-MS chromatogram analysis show that bay leaves contain around 24 components. The components of the active fraction in bay leaves were found to give the two largest peaks. The names of the largest components in bay leaves are 1-(+)-ascorbic acid 2,6-hexadecenoic acid 36.19% and heptadecene-(8)-carbonic acid 27.99%. Apart from that, the eugenol content which is capable of acting as an antimicrobial is only 3.43% at a retention time of 12.76 min. Compounds that make up bay leaf extract, both in quantity and relative percentage, are influenced by the length of the curing process<sup>18</sup>. The extract resulting from the process contains 29 compounds with dominant contents of  $\alpha$ -ocimene, octanal, cis-4-decenal,  $\alpha$ -humulene and decanal with different relative percentages in each extract.

**Ginger:** The identification process of the chemical compounds contained in ginger was carried out using GC-MS. The results of GC-MS chromatogram analysis show that ginger contains around 27 components. Of the highest peaks contained in the components of the active fraction of ginger, the five highest peaks were obtained which exceeded 5%. The following are the names of the largest components in ginger, namely (IR)-2,6,6-trimethylbicyclo [3.1.1] hept, sabinene, 1,6-octadien-3-ol, 3,7-dimethyl-, pentadecanoic acid, heptadecene-(8)-carbonic acid. Another study showed that the highest ginger essential oil content was 3.71%, while the results obtained were only 2.84%<sup>19</sup>. The GC-MS data shows that ginger has a more dominant type of essential oil besides the amount of zingiberene.

**Cumin:** The largest component in cumin is safranal. Safranal is a water-insoluble component but soluble in methanol, ethanol, petroleum ether, glacial acetic acid and acetonitrile. Safranal exhibits various pharmacological effects such as antidepressant, anticonvulsant, antioxidant, antihypertensive, anti-ischemia, anti-inflammatory, antimicrobial and anticancer. Various techniques including HPLC have been used by researchers for qualitative and quantitative analysis of safranal in plant extracts<sup>20</sup>. In rendang preparations, cumin is one of the spices that play the greatest role in antimicrobial power. The test results obtained were 2152.2 ppm. White cumin which contains essential oils was reported to have antimicrobial properties when stored in dark bottles, namely the safranal component is 10.87%.

**Cinnamon:** The identification process of the chemical compounds in cinnamon was carried out using GC-MS.

The results of GC-MS chromatogram analysis show that cinnamon contains around 21 components. The highest peaks contained in the components of the active fraction of cinnamon were obtained by the four highest peaks. The following were the names of the largest components in sweet skin, namely cinnamaldehyde, 2H-1-benzopyran-2-one, fatty acids, pentadecanoic acid and oleic acid. The cinnamaldehyde content is 4.52%.

**Candlenut:** The identification process of the chemical compounds contained in candlenut was carried out using GC-MS. The results of GC-MS chromatogram analysis show that candlenuts contain around 6 components. The main components contained in the active fraction of candlenuts are 9,12,15-octadecatrienoic acid, methyl ester, linolenic acid amounting to 49.81%. The results of mass spectrometer analysis show that this peak is a methyl-9,12-octadecadienoate compound of 87%.

**Coriander:** The identification process of the chemical compounds contained in coriander was carried out using GC-MS. The results of GC-MS chromatogram analysis show that coriander contains around 36 components. The highest peaks contained in the components of the active fraction of coriander were the four highest peaks. The following were the names of the largest components in coriander, namely 1,6-octadecenoic acid and 6-octadecenoic acid. Another study found that coriander oil had a linalool content of 57.13%<sup>21</sup>, while researchers only obtained a linalool content of 7.03%.

**Galangal:** Identification process of the chemical compounds contained in galangal was carried out using an instrument in the form of GC-MS. The results of GC-MS analysis for the active fraction of galangal show the presence of a volatile and non-volatile component which has a retention time of around 30 min showing three quite large peaks which indicate that there were three types of components that are well separated, including 1,6-octadien-3-ol, 3,7-dimethyl, pentadecanoic acid and octadec-9-enoic acid. The active components of galangal as antimicrobials include cineol and eugenol. However, from the results obtained, the levels of cineol and eugenol components were relatively low compared to the components seen in the three quite large peaks, namely 1.07 and 0.24%<sup>5</sup>. It can be seen that the components found are not much different from previous research.

Table 2: Main chemical compounds that makeup rendang seasoning

| Spice           | Chemical compounds     | Amount     |
|-----------------|------------------------|------------|
| Red onion       | Inulin                 | 2695.5 ppm |
| Garlic          | Aliin                  | -          |
| Red chili       | Capsaicin              | 166.1 ppm  |
| Clove           | Eugenol                | 57.09%     |
| Lime leaves     | Trans-cinnamyl acetate | 13.32%     |
| Turmeric Leaves | Curcumin               | 2.057 ppm  |
| Bay leaf        | Eugenol                | 3.43%      |
| Ginger          | Zingiberene            | 2.84%      |
| Caraway         | Safranal               | 2152.2 ppm |
| Cinnamon        | Cinnamaldehyde         | 4.52%      |
| Candlenut       | Linolenic acid         | 49.81%     |
| Coriander       | Linalool               | 7.03%      |
| Galangal        | Cineole                | 1.07%      |
| Nutmeg          | Myristin               | 0.02 ppm   |
| Lemongrass      | Ascorbic acid          | 31.82%     |

**Nutmeg:** Identification process of the chemical compounds contained in nutmeg was carried out using HPLC. Nutmeg oil has been widely used as an aroma component in large quantities in food products. The maximum permitted oil content was 0.08%. Nutmeg can also be used as a narcotic with hallucinatory effects but is very dangerous and can cause death because of the myristicin content<sup>6</sup>. The myristicin content contained in nutmeg extract was 0.02 ppm.

**Lemongrass:** The identification process of the chemical compounds contained in lemongrass was carried out using GC-MS. The results of GC-MS analysis for the active fraction of lemongrass showed the presence of a volatile and non-volatile component which had a retention time of around 30 min showing two quite large peaks which indicated that there were two types of components that were well separated. These components include 1-(+)-ascorbic acid 2,6-dihexadecanoate and OCTADEC-9-ENOIC acid, namely 31.82% and 31.60%.

**Overall metabolites in seasoning of rending:** Based on the main components of each chemical compound contained in the rendang spice mixture that has been used, the content of each compound is then analyzed quantitatively. Table 2 shows the levels of each of the main chemical components in rendang seasoning. It can be seen that the components that have been screened have been found and counted. It is known that the main component content of each spice ingredient is found in significant amounts in the ppm to percent range. This can be related to the taste of the rendang product produced.

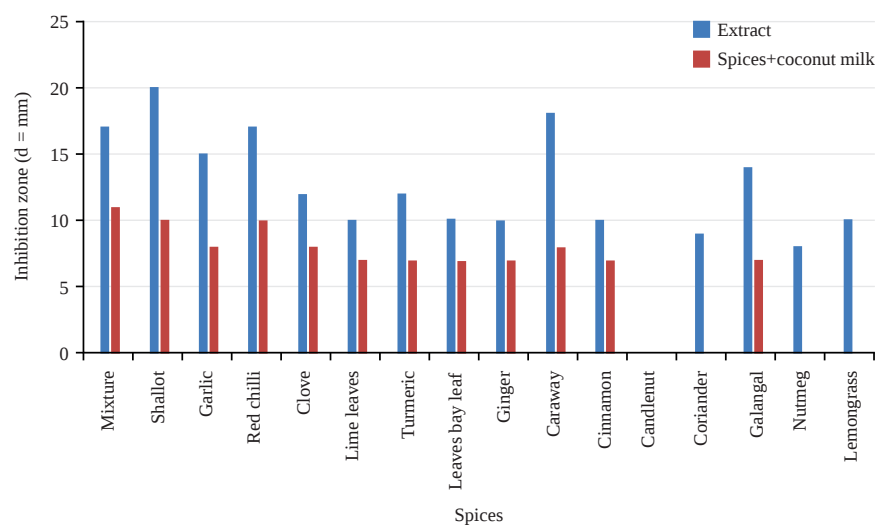
**Antimicrobial activity:** The antimicrobial activity test was carried out on the spices that make up rendang which

included an agar diffusion test using paper discs using the Kirby-Bauer method against gram-positive and negative bacteria (*Staphylococcus aureus* and *Escherichia coli*). This was done to determine the antimicrobial activity of a compound.

**Agar diffusion testing:** Observations of the agar diffusion test were carried out after 24 hrs. This test was carried out on each spice/spice extract that makes up rendang and spices heated with coconut milk which formed an inhibition zone which can be seen in Table 3.

The results of the tests carried out show that the processed rendang spice ingredients are capable of being antimicrobial. Tests on spices that had been heated with coconut milk experienced a decrease in quality compared to direct extracts because the ingredients would be damaged due to repeated heating. Antimicrobial sensitivity can be seen in comparison to other antibiotics such as gram-negative antibiotics enterics if the diameter of the inhibition zone = 13 it was said resistant, 14-16 = intermediate and = 17 susceptible, whereas in staphylococci if the diameter of the inhibition zone = 28 resistant and = 29 susceptible. In addition, ingredients that tend to be oilier can indirectly reduce antimicrobial effectiveness. To find out more clearly the changes in each of the basic spice extracts that make up rendang and spices heated using coconut milk can be seen in Fig. 2 and 3.

The basic difference between gram-positive and negative bacteria was the components of their cell walls. Gram-positive bacteria have a single membrane coated with thick peptidoglycan while negative bacteria have a thin peptidoglycan layer. Apart from that, in gram positive, the inhibition by the clear zone is more inhibited<sup>6</sup>.

Fig. 2: Histogram of agar diffusion test results using *Staphylococcus aureus*Table 3: Inhibitory power of extracts of spices and condiments heated with coconut milk against *Staphylococcus aureus* and *Escherichia coli*

| Spices        | Inhibition zone (mm)         |                                      | Inhibition zone (mm)    |                                      |
|---------------|------------------------------|--------------------------------------|-------------------------|--------------------------------------|
|               | <i>Staphylococcus aureus</i> |                                      | <i>Escherichia coli</i> |                                      |
|               | Extract                      | Spices+coconut milk (70°C for 5 min) | Extract                 | Spices+coconut milk (70°C for 5 min) |
| Mix           | 17                           | 11                                   | 10                      | 8                                    |
| Onion         | 20                           | 10                                   | 17                      | 13                                   |
| Garlic        | 15                           | 8                                    | 13                      | 11                                   |
| Red chili     | 17                           | 10                                   | 15                      | 12                                   |
| Clove         | 12                           | 8                                    | 11                      | 7                                    |
| Lime leaves   | 10                           | 7                                    | 10                      | 7                                    |
| Turmeric leaf | 12                           | 7                                    | 9                       | 7                                    |
| Bay leaf      | 10                           | 7                                    | 9                       | 8                                    |
| Ginger        | 10                           | 7                                    | 9                       | 7                                    |
| Cumin         | 18                           | 8                                    | 16                      | 10                                   |
| Cinnamon      | 10                           | 7                                    | 8                       | 7                                    |
| Candlenut     | -                            | -                                    | 8                       | -                                    |
| Coriander     | 9                            | -                                    | 7                       | -                                    |
| Galangal      | 14                           | 7                                    | 12                      | 9                                    |
| Nutmeg        | 8                            | -                                    | 8                       | -                                    |
| Lemongrass    | 10                           | -                                    | 8                       | -                                    |

Inhibition of microbial activity by active plant compounds can be caused by several factors: (1) Interference with the compounds that make up cell walls, (2) Increased cell membrane permeability which causes loss of cell constituent compounds, (3) Inactivating metabolic enzymes and (4) Destruction or damage to the function of genetic material. This process occurs due to the attachment of antimicrobial compounds to the surface of microbial cells or the compounds diffuse into the cells<sup>6</sup>.

**Rendang product grade antimicrobial power:** Observation of the agar diffusion test at the low level after 24 hrs. This test

was carried out on each level of rendang making where an inhibition zone was formed and can be seen in Table 4.

Based on statistical tests of antimicrobial power at rendang levels (with meat), it can be seen that the effect of heating at each level of curry to dry rendang has a significant effect on bacterial inhibition. Low level of antimicrobial power against *Staphylococcus aureus*. The diameter ranges between 7.33-10 mm, while *Escherichia coli* ranges from 0-10.33 mm. This shows that with different heating up to dry rendang, there was a decrease in the active components that make up rendang, marked by different clear zones for each diameter. Food is mostly contaminated through human handling, but in

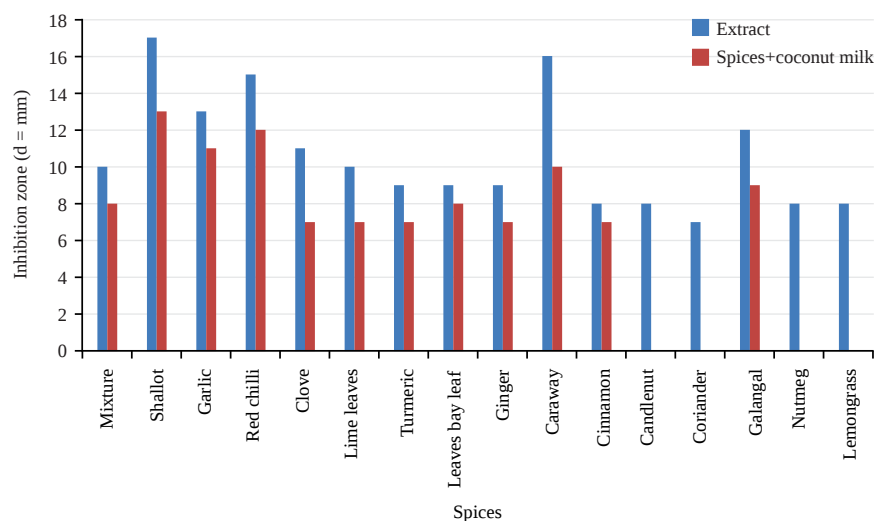
Fig. 3: Histogram of agar diffusion test results using *Escherichia coli*

Table 4: Average antimicrobial power level of rendang products

| Treatment       | Inhibition zone (d = mm)     |                           |
|-----------------|------------------------------|---------------------------|
|                 | <i>Staphylococcus aureus</i> | <i>Escherichia coli</i>   |
| D (Dry rendang) | 7.33 ± 0.58 <sup>a</sup>     | 0.00 ± 0.00 <sup>a</sup>  |
| C (Wet rendang) | 7.67 ± 0.58 <sup>a</sup>     | 7.00 ± 0.00 <sup>b</sup>  |
| B (Potassium)   | 8.67 ± 0.58 <sup>a</sup>     | 9.33 ± 0.58 <sup>c</sup>  |
| A (Goulash)     | 10.00 ± 1.00 <sup>b</sup>    | 10.33 ± 0.58 <sup>d</sup> |
| CV              | 0.08%                        | 0.06%                     |

A (Goulash at a temperature of 75-85°C for 60 min), B (Kalio with a temperature of 85-95°C for 30 min), C (Wet drum with a temperature of 80-90°C for 45 min), D (Dry rendang at a temperature of 75-85°C for 45 min) and numbers in the same column followed by different lowercase letters are significantly different according to DNMRT at the 5% level of significance

foods that have been cooked or salted, where the organisms present have been damaged by heating or whose growth has been inhibited by the concentration of salt, cells of *Staphylococcus aureus* can continue to grow to dangerous levels<sup>22</sup>.

Antimicrobial components that are hydrophobic, such as fatty acids, tend to combine with fat-containing components in foodstuffs and stay away from watery conditions in these foodstuffs, even though microbes usually grow in watery conditions. Apart from that, the addition of this oil can indirectly reduce the effectiveness of antimicrobials. Heated coconut milk will always cause oil to form in the ingredients. In addition, it was suspected that the antimicrobial components of spices, such as phenol, after heating decompose into components that more easily penetrate into cells, thereby causing microbial death<sup>22-26</sup>.

## CONCLUSION

Based on this research, the components of the spices used to prepare rendang have been presented. Research on the antibacterial characteristics of spices has also been

carried out, demonstrating the significance of rendang spices for the durability of a rendang product. Furthermore, rendang may have other bioactivities, such as high antioxidant activity, due to its substantial seasoning. The rendang products that were produced have been improved by the use of spices. This research data was also useful for strengthening the research that has been carried out to develop rendang products. A deeper study can be carried out by looking at the influence of the region of origin of the spice raw materials on the phytochemical characteristics and their influence on the quality of the rendang produced.

## SIGNIFICANCE STATEMENT

The phytochemical composition and bioactivity of rendang spices have been demonstrated by this study. This article's content can give a general summary of the advantages and explain why rendang has been described as a tasty cuisine with a long shelf life. In addition, the information gathered from this study may serve as a guide for future researchers creating new rendang products.

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