Antimicrobial properties of kacangma (*Leonurus sibiricus*): The effect of extraction and heat treatment

(Potensi antimikrob herba kacangma (*Leonurus sibiricus*): Kesan pengekstrakan dan perlakuan haba)

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Abstract

Kacangma (*Leonurus sibiricus*) is a popular traditional herb that has been consumed for decades by the people of Sarawak as a herbal medicine and culinary ingredient. Several studies conducted worldwide have found that *Leonurus* species has high antimicrobial activities. Evaluation of antimicrobial effectiveness of kacangma extract was carried out using disc diffusion test and direct inhibition test. Results showed that both the water and ethanolic extracts reacted differently in inhibiting microorganisms growth. Ethanolic extracts at concentrations of 50 and 100 mg/ml inhibited *Staphylococcus aureus*. Water extracts at concentrations of 10, 25, 50 and 100 mg/ml inhibited *Aspergillus niger*, while concentrations of 25, 50 and 100 mg/ml inhibited *Saccharomyces cerevisiae* and a concentration of 100 mg/ml inhibited *S. aureus*. Effect of temperature treatments on antimicrobial activity of kacangma extract was studied using direct inhibition test by exposing the extracts to temperatures of 50, 100 and 121 °C. When the temperature of the heat treatments increased, the extract inhibitory effect on microorganisms growth also increased. Heat treatment at a high temperature of 121 °C did not reduce the inhibitory effect but instead increased it.

Keywords: *Leonurus sibiricus*, antimicrobial activity, direct inhibition test, disc diffusion test, heat treatment

Introduction

Antimicrobial agents are referred to as chemical compounds that can kill or inhibit the growth of microorganisms (bacteria, fungi and yeast) on a selective basis. These compounds may consist of either chemical or synthetic nature (Fasihuddin and Hasmah 1993). In the food industry, antimicrobial agents are often used to extend food storage.

According to the Malaysian Food Regulations (1985), permitted chemical preservatives in food are benzoic acid, sulfur dioxide, sorbic acid and propionic acid or salts derived from these compounds (Anon. 2015).

Long-term side effects associated with the intake of synthetic chemicals have been a guide to natural ingredient-based products.
Antimicrobial properties of kacangma (Rukayyah 2006). Interest in the search for alternative natural compounds for use in food and medicines is increasing. The search for alternative antimicrobial agents from natural sources such as herbs that have proven efficacy for safe food production has intensified in recent years (Alzoreky and Nakahara 2003; Girish and Satish 2008).

The family of Lamiaceae has been referred to as a family of many plants with medicinal importance. This is because many of its plants contain phytochemical compounds that show a wide range of biological activity, particularly antimicrobial activity (Huang et al. 2005; Topcu and Goren 2007). Several studies conducted in Korea, Japan, Mexico, Brazil and Europe have found herbs of *Leonurus* species with high potential in terms of antimicrobial activity (Mitscher et al. 1972; Woo et al. 1979; de Souza et al. 2004; Ahmed et al. 2006).

Kacangma (*Leonurus sibiricus*) is a herbaceous shrub from the Lamiaceae mint family. It is a popular traditional plant which has been consumed for decades by the people of Sarawak as medicinal and culinary herb (Chua and Aminah 2013). The role of kacangma as a local herb with potential economic value has been recognised (MOA 1995; Paulus and Lau 2004).

This study was conducted to evaluate the antimicrobial activity of both water and ethanolic extracts of dried kacangma. Microbial assays were carried out by disc diffusion test and direct inhibition test. Effect of temperature on microbial activity of kacangma extracts was studied by exposing the extracts at 50, 100 and 121 °C.

**Materials and methods**

**Raw materials**

Kacangma herb of pink-flowered variety from MARDI, Kuching, was harvested at 70-day maturity. The aerial parts of the plant which consists of leaves and young stems were chopped and oven-dried at 60 °C in a force-air oven for 5 – 6 h until the final moisture content reached below 6% (w/w). The dried herb was then ground into powder and stored in airtight containers (Chua et al. 2006).

**Preparation of kacangma extracts**

The extraction was conducted based on the method of de Souza et al. (2004). In ethanolic extraction, a quantity of 100 g ground kacangma was soaked in 600 ml ethanol for 48 h at room temperature. The solvent was then filtered and evaporated at 36 – 40 °C using a rotary evaporator (Heidolph WB 2001). Prior to antioxidant test, stock solutions of the extracts (10, 25, 50 and 100 mg/ml) were prepared by dissolving the crude extracts in 99.5% ethanol.

In aqueous extraction, 100 g ground kacangma was refluxed with 1,000 ml distilled water for 3 h at 100 °C. The solvent was then filtered, concentrated to 1/3 of the initial volume and freeze dried. For antimicrobial testing, stock solutions of the extracts (10, 25, 50 and 100 mg/ml) were prepared by dissolving the freeze dried extracts in distilled water. Extracts were weighed and stored in sterile bottles wrapped in aluminum foil and stored in the refrigerator at 4 °C for further use.

**Media and microorganism strains**

Microbiological culture media used were Nutrient Agar (NA) (Oxoid CM3), Sabouraud Dextrose Agar (SDA) (Oxoid CM41), Mueller-Hinton Agar (MHA) (Oxoid CM0337), Nutrient Broth (NB) (Oxoid CM1), Sabouraud Dextrose Broth (SD) (Oxoid CM147), Ringer’s tablet (Oxoid BR52) and blank discs (Oxoid DD0014T).

Antimicrobial assay was performed against the following six microorganism strains: two Gram positive bacteria; *Bacillus cereus* (ATCC 11778) and *Staphylococcus aureus* (ATCC 25923), two Gram negative bacteria; *Escherichia coli* (ATCC 11775) and *Salmonella typhimurium* (ATCC 14028). The fungi strains used were *Saccharomyces cerevisiae* (ATCC 9763) and *Aspergillus*...
niger (ATCC 16404). Cultures were grown on agar slants and maintained in NB broth for bacteria and SD broth for fungi. Subcultures were freshly prepared before use.

Pure bacteria cultures were inoculated onto NA and incubated at 37 °C for 24 h. Whereas, pure yeast and fungi culture were inoculated onto SDA and incubated at 35 °C for 48 – 72 h until the formation of colonies (for yeast) or spores (for fungi). For the preparation of inoculum, four colonies were taken with a sterile wire loop and transferred into 10 ml of NB and incubated at 37 °C for 24 h (bacteria), and 10 ml of SD and incubated at 35 °C for 48 h (yeast and moulds). All culture broths were then diluted by serial dilution method to a concentration of 10^6 colony forming units per ml (CFU /ml) using a quarter-strength Ringer’s solution.

Antimicrobial screening

Antimicrobial activity for both water and ethanolic extracts of the kacangma herb was tested by direct inhibition test and disc diffusion test. Both of these tests were conducted inside a sterile laminar flow chamber (Nuair, USA). All culture media and small appliances such as forceps, micropipettes and paper discs were sterilised using an autoclave (Tomy SS-325, USA) under the pressure of 15 psi for 15 min at 121 °C. The laminar flow chamber surface was sterilised with ultraviolet radiation.

Disc diffusion test

Disc diffusion method was employed for the determination of antimicrobial activities of kacangma herb (NCCLS 1997a, Baydar et al. 2004). Bacterial inoculates were made in 5 ml of NA broth and grown for 24 h at 37 °C. The yeast and fungi were inoculated in SD broth and grown for 48 h at 25 °C.

Ten milliliters of molten sterile agar (MHA for bacteria or SDA for yeast and fungi) at 45 – 48 °C was inoculated with a broth culture (0.1 ml of 10^6 – 10^7 cfu/ml) of respective microorganism strains in a flask. The inoculated molten agar was then poured over base plates containing 10 ml solidified nutrient agar in sterile 9 cm petri dishes.

A quantity of 20 μl of the kacangma extracts (water and ethanolic) were pipetted on sterile filter paper discs (Whatman, 6 mm in diameter). The concentrations of the water and ethanolic extracts were set at 10, 25, 50 and 100 mg/ml. The paper discs were then allowed to dry in an open sterile petri dish in a biological safety cabinet with vertical laminar flow (Nuair Laminar Flow, USA). A sterile empty disc was used as control. A maximum number of 5 sterile discs were placed aseptically on the surface of the inoculated plate with a distance of at least 1.5 cm from each other and from the sides of the petri dish. After 20 min, petri dishes were inverted and incubated at 37 °C for 24 h for bacteria and 30 °C for 48 h for yeast and fungi. The diameters of the inhibition zones minus the disc diameter were measured in millimeters. Only inhibition zones with diameters exceeding 7 mm were considered as positive results. All tests were performed in duplicate.

Direct inhibition test

Direct inhibition tests were carried out in accordance to the NCCLS Standard (1997b). The effect of heat treatment on the antimicrobial activity of the water and ethanolic extracts of the kacangma herb was tested by exposing the extracts to three common processing temperatures of 50 °C (warming), 100 °C (boiling) and 121 °C (sterilisation), each at a duration of 15 min. The extracts at concentrations of 10, 25, 50 and 100 mg/ml, were added to separate growth medium (MHA for bacteria or SDA for yeast and fungi) in an aseptic manner in a sterilised laminar flow chamber.

To test the effect of heat treatment at 50 °C, the kacangma extracts were added to agar media that had been autoclaved, cooled and kept in a water bath maintained at 50 °C. Meanwhile, for 100 °C heat treatment, the kacangma extracts were added...
to agar media that had been autoclaved and re-heated up to 100 °C. For heat treatment at 121 °C, the extracts were added to agar media and autoclaved at 121 °C.

An amount of 0.1 ml of microorganism culture that had been diluted to $10^4$ cfu/ml was transferred onto the agar media containing kacangma extracts that were prepared earlier on. Agar media that did not contain any extract acted as control. The microorganism culture was spread evenly on the surface of the agar media by utilising a sterilised L-shaped glass rode. After that, the petri dishes that contained bacteria cultures were incubated at 37 °C for 24 h, whereas petri dishes with yeast and fungi cultures were incubated at 35 °C for 24 – 72 h. At the end of the incubation period, the number of colonies formed on every agar medium (both the media containing extracts and the control) were counted. The results obtained from the media containing extracts were compared with the results obtained from the controls. The inhibitory effect of kacangma extracts on the growth of microorganisms was computed as the difference between the amount of colonies formed on the agar media containing kacangma herb-extracts, and the amount of colonies found on their respective control media, and expressed in percentage as follows:

$$\text{Inhibitory effect} = \frac{\text{No. of colonies on control medium} - \text{No. of colonies on medium with extract}}{\text{No. of colonies on control medium}} \times 100$$

**Results and discussion**

**Disc diffusion test**

Antimicrobial screening was conducted to determine the kacangma extracts direct effect on bacteria (*B. cereus*, *S. aureus*, *E. coli*, *S. typhimurium*), yeast (*S. cerevisiae*) and fungi (*A. niger*). The results of the disc diffusion test are shown in Table 1. The minimum measurement of the inhibitory zone diameter detected was 7 mm, whereas the maximum reading reached 10 mm. Generally, the area of the inhibitory zones increased when the concentrations of the extract applied increased.

As compared to the ethanolic extracts, the water extracts of kacangma were found to have higher degree of inhibitory effect on microorganisms tested. The water extract inhibited the growth of *S. aureus* at a concentration of 100 mg/ml whereas *A. niger* could be inhibited by all four concentrations (10, 25, 50 and 100 mg/ml), and *S. cerevisiae* could only be inhibited by concentrations of 25, 50 and 100 mg/ml. This means that the water extracts were found to inhibit yeast and fungi growth more effectively as compared to bacteria. Meanwhile, the ethanolic extracts of kacangma could only inhibit *S. aureus* at concentration of 50 and 100 mg/ml. *E. coli* was inhibited by the ethanol extract at 100 mg/ml only.

Both water and ethanolic extracts were found to inhibit the growth of Gram positive bacteria (*S. aureus*) more effectively than Gram negative bacteria (*S. typhimurium* and *E. coli*). This is because the Gram negative bacteria’s cell walls are formed by layers of liposaccharides that prevent lipophilic molecules from entering the bacteria through the cell walls. Apart from that, the cell walls of the Gram negative bacteria also have an additional external membrane that acts as an additional barrier and naturally selects hydrophilic molecules (Black 2002; Nikaido 2003). According to Chan et al. (2008), the antimicrobial compounds in kacangma extract most likely were unable to bypass the external membrane in order to react with the bacteria’s cell. On the other hand, the Gram positive bacteria (*S. aureus* and *B. cereus*) only have peptidoglycan layer in its cell walls. This layer helps to block the hydrophilic molecules but not the antimicrobial compounds that can penetrate it more easily (Thomas and John 2006).

The concentration of the extracts would also affect the inhibitory effectiveness. This was because all positive results that involved inhibitory zones
Table 1. Antimicrobial activities of kacangma extracts by disc diffusion method (n = 2)

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Concentration of kacangma extracts (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water extract</td>
</tr>
<tr>
<td></td>
<td>10  25  50  100</td>
</tr>
<tr>
<td>B. cereus</td>
<td>–  –  –  –</td>
</tr>
<tr>
<td>S. aureus</td>
<td>–  –  –  +</td>
</tr>
<tr>
<td>E. coli</td>
<td>–  –  –  –</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>–  –  –  –</td>
</tr>
<tr>
<td>S. cerevisae</td>
<td>–  +  +  +</td>
</tr>
<tr>
<td>A. niger</td>
<td>+  +  +  +</td>
</tr>
</tbody>
</table>

+ + : Diameter of inhibitory zone above 10 mm  
+ : Diameter of inhibitory zone above 7 mm  
– : No inhibitory zone

more than 10 mm were achieved by a concentration of 100 mg/ml. Extracts in low concentrations such as 10 mg/ml usually failed to form any inhibitory zone. These results highly matched the research work of Wan Norhanizan (2005), which also involved the use of kacangma herb as raw material. Nevertheless, a few other researchers had reported different findings. Heinrich et al. (1992) concluded that the ethanol extract of L. sibiricus could effectively inhibit E. coli, Micrococcus luteus and Bacillus subtilis. Meanwhile, Wadt et al. (1996) reported positive results on S. aureus, Pseudomonas aeruginosa and Candida albicans. According to de Souza et al. (2004), the ethanol extract of L. sibiricus could only inhibit the growth of B. subtilis. This could be due to the fact that different sources of herbs were used, and the agro-climate factor as well as the handling process may also affect the phytochemical contents of the herbs under study (Kyle and Duthie 2006; Erdman et al. 2007).

Direct inhibition test

In this test, the effect of heat treatments on antimicrobial activity of kacangma extracts was studied by exposing the extracts to different temperatures, i.e. 50, 100 and 121 °C. The inhibitory effect of the extracts on the growth of microorganisms was computed as percentage of the difference in the number of microorganism colonies found on the media with extract, to the quantity found in the control.

Extraction solvent factor

In this test, two types of solvents, i.e. water and ethanol, were used as the kacangma’s phytochemical extraction medium. The effectiveness of using the water extracts and ethanolic extracts of the kacangma herb to inhibit microorganisms growth are shown in Tables 2 and 3 respectively. The results of this study showed that the water extract of the kacangma herb could inhibit the growth of E. coli, S. aureus, A. niger and S. cerevisae. However, the water extract did not show such effectiveness on B. cereus and S. typhimurium, regardless of the levels of concentration used or the temperature applied in the heat treatments.

In the bacteria category, the highest inhibitory percentage for S. aureus (94.4%) was achieved when the water extract concentration was 100 mg/ml and the heat treatment temperature was at 121 °C. Meanwhile, the lowest inhibitory percentage of S. aureus (10.6%) occurred at 10 mg/ml at 50 °C. For E. coli, the highest inhibitory percentage (10.1%) was reached at 100 mg/ml at 121 °C, whereas the lowest inhibitory percentage (0%) was recorded
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at 10 and 25 mg/ml, regardless of the temperature used in the heat treatments.

In the yeast and fungi categories, the highest inhibitory percentage for *S. cerevisae* was achieved at 50 and 100 mg/ml, at all temperatures set for heat treatments. The lowest inhibitory percentage (16.3%) was recorded at 10 mg/ml at 50 °C. *A. niger* recorded its highest inhibitory percentage (67.6%) at 121 °C when the water extract concentration was 100 mg/ml, and achieved its lowest inhibitory percentage (0%) at 50 °C at 10 mg/ml.

On the inhibitory effectiveness of kacangma ethanolic extracts on microorganisms growth, the results from the study showed that the ethanolic extracts could inhibit the growth of *S. aureus* and *E. coli* only. The ethanolic extracts were unable to inhibit the growth of *B. cereus*, *S. typhimurium*, *S. cerevisae* and *A. niger*. The same outcome was recorded for all the extract concentrations and temperature treatments tested.

When the ethanolic extracts were used, highest inhibitory percentage (100%) for *S. aereus* was achieved at 100 mg/ml and treatment temperatures of 100 and 121 °C. Meanwhile, the lowest inhibitory percentage (35.3%) for *S. aereus* was recorded at 10 mg/ml and 50 °C. As for *E. coli*, the highest inhibitory percentage (100%) was achieved at 100 mg/ml, at 100 and 121 °C, whereas its lowest inhibitory percentage (19.2%) was recorded at 10 mg/ml at 50 °C. The higher the extract contraction, the higher the rate of the growth inhibitory percentage.

Both the water and ethanolic extracts reacted differently in inhibiting microorganism growth. The type of solvent used determines the content or form of the extract. The results of the ethanolic extract was in aqueous-oil state, whereas the water extract was in dried form.

In this study, the water extract was found to have higher inhibitory effectiveness compared to the ethanolic extract, especially in inhibiting the growth of yeast and fungi. According to Siedel (2005), water is more polar in nature and is able to extract phytochemical compounds such as flavonoids, glycosides, tannins, saponins and alkaloids. Some of these phytochemical compounds show good antibacterial and antifungal activities (Duke 2000).

**Extract concentration factor**
Different extract concentration would yield different results related to the inhibitory effect on microorganism growth. As expected, the kacangma extracts with higher concentrations had stronger inhibitory effect on microorganism growth even though heat treatments had been applied to the extracts. This is because extracts in high concentrations contain more antimicrobial compounds. Studies related to antimicrobial activity were also observed to yield similar results (Joshi and John 2002; Ali et al. 2005; Prasad et al. 2008).

**Treatment temperature factor**
Results of the study showed that the higher the treatment temperature on the kacangma extract, the stronger its inhibitory effect on microorganism growth. It is very interesting to find that treatments at high temperatures, i.e. 100 and 121 °C, not only did not reduce the extract’s inhibitory effectiveness, but also increased the extract’s antimicrobial effectiveness. This result does not coincide with the general perception that phytochemical compounds will be destroyed or damaged at high heat and thus, showed lower antimicrobial activity.

Similar research results were reported by Chan et al. (2008) where the herb samples were boiled for 20 min. After the heat treatment, it showed higher antimicrobial activity on *Staphylococcus aureus* and *Mycobacterium smegmatis* compared to the control that was not given any heat treatment.

According to Zhu (1998), heat treatments on herb extracts could lead to the elimination, separation or the release of certain active phytochemical elements.
Table 2. Inhibitory effect of water extracts of kacangma on microorganism growth (n = 2)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Concentration of water extract (mg/ml)</th>
<th>Inhibitory effect on microorganism growth at different temperatures (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50 °C</td>
</tr>
<tr>
<td>S. aureus</td>
<td>10</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>26.4</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>38.0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>59.3</td>
</tr>
<tr>
<td>E. coli</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>7.6</td>
</tr>
<tr>
<td>S. cerevisae</td>
<td>10</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>68.5</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>A. niger</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>48.8</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>56.7</td>
</tr>
<tr>
<td>B. cereus</td>
<td>0% for all concentrations and temperature treatments</td>
<td></td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>0% for all concentrations and temperature treatments</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Inhibitory effect of ethanolic extracts of kacangma on microorganism growth (n = 2)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Concentration of ethanolic extract (mg/ml)</th>
<th>Inhibitory effect on microorganism growth at different temperatures (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50 °C</td>
</tr>
<tr>
<td>S. aureus</td>
<td>10</td>
<td>35.3</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>43.6</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>50.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>63.3</td>
</tr>
<tr>
<td>E. coli</td>
<td>10</td>
<td>19.2</td>
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<td>31.4</td>
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<td></td>
<td>100</td>
<td>69.7</td>
</tr>
<tr>
<td>B. cereus</td>
<td>0% for all concentrations and temperature treatments</td>
<td></td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>0% for all concentrations and temperature treatments</td>
<td></td>
</tr>
<tr>
<td>S. cerevisae</td>
<td>0% for all concentrations and temperature treatments</td>
<td></td>
</tr>
<tr>
<td>A. niger</td>
<td>0% for all concentrations and temperature treatments</td>
<td></td>
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</tbody>
</table>

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and this may lead to a change in the extract’s biological nature or the creation of a new biological characteristic. For example, the frying process on the *Rheum palmatum* roots (Radix et Rhizoma Rhei) had created the antimicrobial characteristic of the herb. This was because, during the frying process, the heat treatment had broken down the herbs’ phytochemical complex known as anthraquinone glycoside, and the anthraquinone that was released had antimicrobial activity (Wang 1985; Su and Qiao 1989).

A similar study was also conducted by Traub and Leonhard (1995) where 62 types of antimicrobial materials were heated at 56 °C for 30 min, and at 121 °C at 15 min. Results of the study showed that 25 types of the materials tested were found stable against heat and therefore not affected by heat treatments. β-lactan, azlocillin, aztreonam, mezlocillin and oxacillin were among those materials. The ability to remain stable under high heat is a very important characteristic of an antimicrobial agent for food as food is very likely to undergone thermal processing.

**Conclusion**

As compared to ethanolic extracts, water extracts of the kacangma herb was found to have a higher degree of inhibitory effect on microorganism growth. The water kacangma extract was able to inhibit *S. aureus* at a concentration of 100 mg/ml, *A. niger* at all four concentrations tested (10, 25, 50 and 100 mg/ml) and *S. cerevisiae* at concentrations of 25, 50 and 100 mg/ml. On the other hand, the ethanolic kacangma extract could only inhibit *S. aureus* at concentrations of 50 and 100 mg/ml, and inhibit *E. coli* at a concentration of 100 mg/ml. In the direct inhibition tests, the water kacangma extracts were able to inhibit the growth of *E. coli*, *S. aureus*, *A. niger* and *S. cerevisiae*, whereas the ethanolic extracts could only inhibit the growth of *E. coli* and *S. aureus*. When the kacangma extract concentrations, or the temperature of the heat treatments increased, the extract inhibitory effect on microorganisms growth also increased. Heat treatments at a high temperature of 121 °C did not reduce but increased its inhibitory effect.

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**References**


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Abstrak

Kacangma (Leonurus sibiricus) ialah herba tradisional yang popular dan digunakan sejak dahulu oleh masyarakat Sarawak sebagai herba ubatan dan ramuan masakan. Beberapa kajian yang telah dijalankan di merata dunia mendapati herba daripada spesies Leonurus mempunyai potensi tinggi dari segi aktiviti antimikrob. Kajian keberkesanan sifat antimikrob ekstrak kacangma dijalankan menggunakan ujian resapan cakera dan ujian perencatan langsung. Hasil menunjukkan kedua-dua ekstrak air dan etanol memperlihatkan kesan berlainan dalam perencatan pertumbuhan mikroorganisma. Ekstrak etanol didapati merencat pertumbuhan Staphylococcus aureus pada kepekatan 50 dan 100 mg/ml manakala ekstrak air pula didapati merencat pertumbuhan Aspergillus niger pada kepekatan 25, 50 dan 100 mg/ml. Ekstrak air juga didapati merencat Saccharomyces cerevisae pada kepekatan 25, 50 dan 100 mg/ml, dan S. aureus pada kepekatan 100 mg/ml. Kesan perlakuan haba ke atas aktiviti antimikrob pada ekstrak kacangma dikaji menggunakan ujian perencatan langsung melalui pendedahan pada suhu 50, 100 and 121 °C. Perlakuan pada suhu tinggi 121 °C tidak menunjukkan sebarang perbezaan ke atas perencatan mikroorganisma yang dikaji.