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Antioxidant and antibacterial activity of different parts of *Leucas aspera*

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ABSTRACT

Objective: To evaluate antioxidant, antimicrobial and cytotoxic activity of different parts (root, flower, leaf and stem) of *Leucas aspera* (*L. aspera*) (Labiatae). **Methods:** Different parts of *L. aspera* were extracted with 80% (v/v) methanol. The methanol extracts were subjected to antioxidant, antimicrobial and brine shrimp lethality assay. **Results:** All the extracts showed moderate to potent antioxidant activity, among which the root extract demonstrated the strongest antioxidant activity with the IC_{50} value of 6.552 μg/mL. Methanol extract of root possessed antioxidant activity near the range of vitamin E and thus could be a potential rich source of natural antioxidant. In case of antimicrobial screening, crude extracts of root, flower, leaf and stem showed notable antibacterial activity against tested microorganisms. The root extract showed the highest mean zone of inhibition ranging from 9.0-11.0 mm against tested microorganisms, at a concentration of 100 mg/mL. In the brine shrimp lethality bioassay, it was evident that the methanol root extract did not show significant toxicity. The LC_{50} value for 12 h and 24 h observation was 2.890 mg/mL and 1.417 mg/mL, respectively. **Conclusions:** The present finding suggests that the methanol root extract of *L. aspera* could be developed as pharmaceutical products.

1. Introduction

During the last few decades there has been an increasing interest in the study of traditional plants and their medicinal value in different parts of the world. The medicinal properties of plants have been investigated due to their potent pharmacological activities, low toxicity and economic viability[1]. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that green medicine is safe and more dependable than the costly synthetic drug, many of which may have adverse side effects[2]. This development could lead to new drug discovery or advance the use of indigenous herbal medicines.

The preservative effect of many plant spices and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissues. It has been reported that there is an inverse relationship between antioxidative status and incidence of human diseases such as cancer, aging, neurodegenerative disease, and atherosclerosis[3]. As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity. In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in treatment of infectious diseases. This situation forced scientists to search for new antimicrobial substances from various sources like medicinal plants which are good sources of novel antimicrobial agents[4].

Leucas aspera (*L. aspera*), belonging to the family of Labiatae, is a common aromatic herb found as weed in Africa, Asia-temperate and Asia-tropical countries. Traditionally, the whole plant is taken orally for analgesic, antipyretic, antirheumatic, anti-inflammatory and antibacterial treatment and its paste is applied topically to inflamed areas[5]. Its anti-inflammatory activity has been shown in rats[6,7] through prostaglandin inhibition[8,9]. The entire plant is also used as an insecticide and indicated in traditional medicine for coughs, colds, painful swelling and chronic skin eruption[10]. Apart from this, the plant possesses wound healing property and is used in cobra venom poisoning[11]. Leaves of *L. aspera* are useful in chronic rheumatism, psoriasis, scabies, chronic skin eruptions and their juice used as antibacterial agent. Its

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chloroform and ether extracts possess antifungal activity[12]. Compounds isolated from the plant include long chain aliphatic compound, triterpenes, sterols and novel phenolic compounds[11].

In this study, we would like to compare the antioxidant activity of methanol extract from different parts of *L. aspera* with commercial antioxidants α-tocopherol (vitamin E) and butylated hydroxytoluene (BHT). In addition, this study also evaluated the antibacterial activity of different parts of *L. aspera*. The toxicity effect of *L. aspera* root extract was also studied.

2. Materials and methods

2.1. Plant material

The plant *L. aspera* was collected from AIMST University campus and authenticated by the Botanist of the Department of Biotechnology, AIMST University, Kedah, Malaysia where a sample (voucher number LA01) was deposited. The plants were segregated into four parts consisting of the leaf, stem, root and flower. Then, the segregated parts were cut into small size and sun dried for 1 week.

2.2. Extract preparation

All the plant parts (root, flower, leaf and stem) were ground into powdered form with a grinder. All the plant parts (100 g) were then soaked in 500 mL methanol (80% v/v) separately in a beaker and let to soak for 4 days at room temperature [(26-28) ℃]. Removal of dry plant parts was done by filtration through cheesecloth and Whatman filter paper. The filtrate was then further concentrated using rotary evaporator. The extracts were all placed in glass Petri dishes. The dried plant extracts were then redissolved in 80% (v/v) methanol in order to obtain a solution containing 2.0 mg/mL of extract, respectively which were then used for assays.

2.3. Antioxidant activity (DPPH assay)

The antioxidant activity (free radical scavenging activity) of all parts (root, flower, leaf and stem) was determined *via* 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Firstly, 50 μL of 2.0 mg/mL leaf extract (or 80% methanol as blank) was added into 5 mL of 0.004% (w/v) solution of DPPH in methanol. This was kept for 30 min of incubation in dark by using the dark box. The same procedure was performed on the other 3 plant parts which were root (2.0 mg/mL), stem (2.0 mg/mL) and flower (2.0 mg/mL). Known antioxidants such as vitamin E and butylated hydroxytoluene (BHT) were used as positive controls. The decrease in the solution absorbance was measured at 517 nm after 30 min of incubation. This test was done in triplicates. The DPPH scavenging activity (%) was calculated based on the following formula:

$$\text{DPPH scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the plant sample[13]. The percentage of scavenging of all the plant extracts (root, flower, leaf and stem) was compared with positive controls.

2.4. IC_{50} value of the root extract

Based on the screening results, it was indicated that the root had the highest scavenging activity which was 47.41%. Thus, the root was chosen for further determination of the IC_{50} value. The dried root extract was redissolved in 80% (v/v) methanol in order to obtain solution with 0.5 mg/mL, 1.0 mg/mL, 2.0 mg/mL, 3.0 mg/mL and 4.0 mg/mL concentrations. DPPH assay was performed to determine the free radical scavenging activity of all the concentrations at 1.0 mg/mL, 2.0 mg/mL, 3.0 mg/mL and 4.0 mg/mL of the root extract. Triplicate measurement was carried out. IC_{50} value was determined from the plotted graph of scavenging activity versus the concentration of root extract, which is defined as the amount of antioxidant necessary to reduce the initial DPPH radical concentration by 50%.

2.5. Antimicrobial activity of the plant extracts (root, flower, leaf and stem)

2.5.1. Microorganisms

Gram positive *Staphylococcus aureus* (*S. aureus*) and the Gram negative *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella typhimurium* (*S. typhimurium*), *Salmonella choleraesuis* (*S. choleraesuis*) and *Shigella flexneri* (*S. flexneri*) were used for antimicrobial study. All the stock cultures were from the collection of AIMST University Laboratory. All of the bacterial strains were grown and maintained on Muller Hinton agar at 37 ℃. The bacteria were subcultured overnight in Muller Hinton broth which was further adjusted to obtain turbidity comparable to McFarland (0.5) standard when required[14].

2.5.2. Antimicrobial screening by disk diffusion technique

The antimicrobial activity of the plant extracts (root, flower, leaf and stem) was determined by disk diffusion technique[15]. The test microbe was taken from the broth culture with an inoculating loop and transferred to a test tube containing 5.0 mL sterile distilled water. The inoculum was added until the turbidity was equal to 0.5 McFarland standard. Cotton swab was then used to inoculate the test tube suspension onto the surface of Muller Hinton agar plate and the plate was allowed to dry. By using a sterilized forcep, three sterilized Whatman paper disks (6 mm in diameter) were transferred onto the agar surface. Each sterile disk was impregnated with 20 μL of extract. This was done for all four types of plant extracts as well as methanol (negative control). The concentration of the extracts was 100 mg/mL. This was also done for chloramphenicol as positive control (30 μg/mL). The experiment was conducted in triplicates. The plates were incubated at 37 ℃ for 24 h. At the end of the period, the inhibition zone against each microorganism by each plant extract (root, flower, leaf and stem) was measured and analyzed by using descriptive statistic in SPSS 15.0.

2.6. Brine shrimp lethality bioassay of root extract

Brine shrimp lethality bioassay was carried out according to Meyer *et al*[16] to investigate the cytotoxicity of the root extract. The dried root extract preparation was redissolved in artificial sea water to obtain a solution of 10 mg/mL of root extract for toxicity test. 5 mL of artificial sea water was added into all the test tubes. Serial dilution was then carried out in order to obtain the concentration from 10 mg/mL to 0.25 mg/mL of root extract. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a beaker, filled with sterile artificial seawater (prepared by using sea salt 38 g/L and adjusted to pH 8.5 using 1N NaOH) under constant aeration for 24 h under the light. After the 24 h hatching period, active larvae were attracted to one side in a glass Petri dish by using a micropipette. The larvae were then separated from the eggs by aliquoting them in another glass Petri dish containing artificial sea water and used for the assay. Suspension containing 15 larvea was added into each test tube and was incubated at room temperature for 12 h under the light. The tubes were then examined after 12 h and the number of surviving larvae in each tube was counted. Experiments were conducted along with control, in a set of three tubes per dose. The percentage of mortality was plotted against the logarithm of concentration. Using the linear regression equation of the graph, the concentration that would kill 50% of the larvae (LC_{50}) was determined. Then observation was repeated again after 24 h incubation period. The LC_{50} was obtained again for this 24 h observation.

2.7. Statistical analysis

Statistical analysis was done using Microsoft Excel and SPSS 15.0 version. One way ANOVA and post hoc tests were conducted and P value less than 0.05 was considered as significantly different.

3. Results

3.1. Antioxidant activity of *L. aspera*

The results for the free radical scavenging of all the plant extracts and known antioxidants were presented in Figure 1. The results of one way ANOVA test and post hoc test indicated that there was a significant difference of mean percentage scavenging between all the tested extracts (BHT, vitamin E, root, flower, leaf and stem) at the concentration of 2 mg/mL. The results showed that root extract exhibited the greatest free radical scavenging activity among the plant extracts with a mean percentage of (32.36±1.19)%. On the other hand, the scavenging activity of flower, leaf and stem extracts was (26.39±0.07)%, (17.04±0.82)% and (13.42±0.56)%, respectively. The scavenging activity of these extracts was lower compared with both antioxidants, BHT (65.67±0.58)% and vitamin E (41.67±0.58)%.

Since the root exhibited the highest antioxidant activity, the root was further analyzed against the DPPH free radical. The results were illustrated in Figure 2. Figure 2 was obtained by plotting a graph of percentage scavenging against the log concentration of the root samples. The IC_{50} value of root sample was 6.552 mg/mL obtained from the linear regression equation, indicating that the concentration of root to scavenge 50% of the free radicals was at 6.552 mg/mL.

Table 1
Antibacterial activity (zone of inhibition) of different parts (root, flower, leaf and stem) of *L. aspera* methanol extract.

Microorganism	Zone of inhibition (mm)					
	Root (100 mg/mL)	Flower (100 mg/mL)	Stem (100 mg/mL)	Leaf (100 mg/mL)	Chloramphenicol (30 μg/mL)	Methanol (negative control)
E. coli	9.0 ± 0.5	7.0 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	24.0 ± 0.6	0.0 ± 0.0
S. aureus	10.0 ± 0.6	7.0 ± 0.6	7.0 ± 0.4	7.0 ± 0.5	23.0 ± 0.5	0.0 ± 0.0
S. choleraesuis	11.0 ± 0.5	8.0 ± 0.5	7.0 ± 0.6	8.0 ± 0.4	24.0 ± 0.6	0.0 ± 0.0
S. typhimurium	11.0 ± 0.6	9.0 ± 0.6	0.0 ± 0.0	7.0 ± 0.6	21.0 ± 0.4	0.0 ± 0.0
S. flexneri	11.0 ± 0.5	0.0 ± 0.0	7.0 ± 0.7	7.0 ± 0.5	24.0 ± 0.7	0.0 ± 0.0
P. aeruginosa	11.0 ± 0.6	7.0 ± 0.6	7.0 ± 0.6	0.0 ± 0.0	25.0 ± 0.5	0.0 ± 0.0

3.2. Antibacterial activity of *L. aspera*

Antibacterial activity results of methanol extracts of the different parts of *L. aspera* were given in Table 1. In general, the mean zone of inhibition produced by the commercial antibiotic, chloramphenicol, was between 21.0 to 25.0 mm and was larger than those produced by all methanol extracts which was between 7.0 to 11.0 mm. Based on the results, the root extract showed the highest zone of inhibition compared with all the extracts against all the tested microorganisms, followed by the flower extract which was more active against *Salmonella* species compared with other tested microorganisms. Leaf extract was active against 4 microbes. On the other hand, methanol (negative control) did not exhibit any effect on all the tested microorganisms.

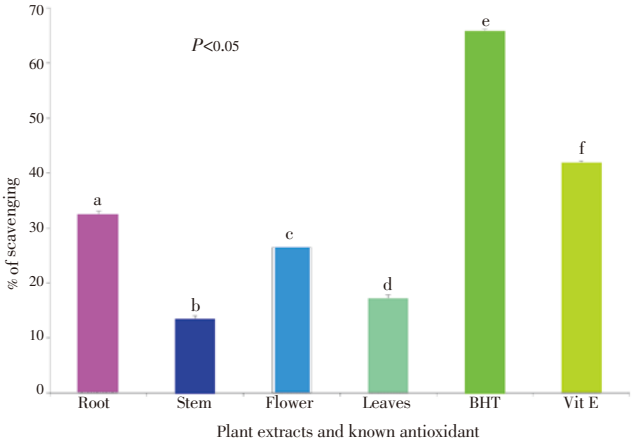

Figure 1. Scavenging effect (%) of plant extracts and known antioxidants at 2.0 mg/mL.
a, b, c, d, e and f indicate that the mean percentage scavenging between the tested populations is significantly different.

3.3. Cytotoxic activity of root extract of L. aspera

The results of *Artemia salina* testing were presented in Figure 3 for 12 h observation and Figure 4 for 24 h observation. The LC_{50} value indicated the concentration by which 50% of the shrimps were killed. The LC_{50} value for 12 h and 24 h observation were 2.890 mg/mL and 1.417 mg/mL, respectively.

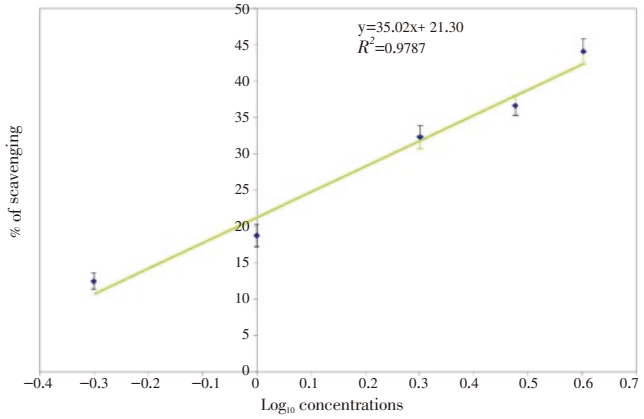

Figure 2. Dose inhibition curve of total antioxidant activity of root extract.

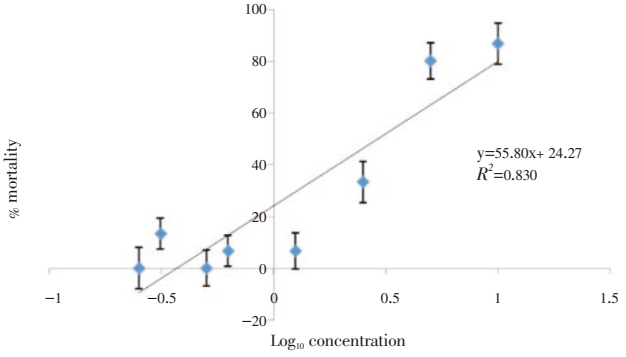

Figure 3. Toxicity of crude extract of *L. aspera* root against brine shrimp larvae (*Artemis salina*)-12 h observation.

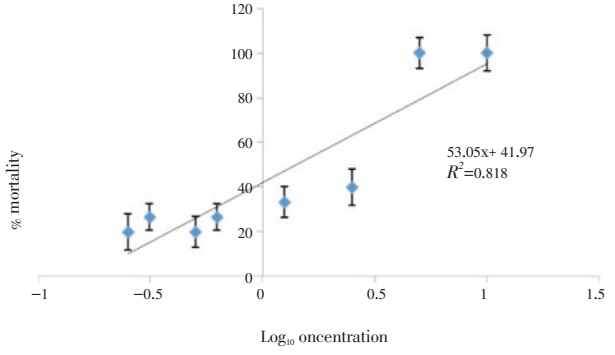

Figure 4. Toxicity of crude extract of *L. aspera* root against brine shrimp larvae (*Artemia salina*)-24 h observation.

4. Discussion

4.1. Antioxidant activity of L. aspera

To evaluate the scavenging effect of the extracts in this study, DPPH reduction was investigated against positive controls (BHT and vitamin E). The more antioxidants occurred in the extract, the more DPPH reduction will occur. High reduction of DPPH is related to the high scavenging activity performed by particular sample. At a higher concentration, these extracts may exhibit more significant free radical scavenging activity. IC_{50} was calculated as amount of antioxidant present in the extracts. The IC_{50} value (6.552 mg/mL) recorded in this study was varying from other report with the same extract. According to Rahman *et al*[12], the ethanolic extract of *L. aspera* root showed a significant free radical scavenging activity with an IC_{50} value of 7.5 μg/mL. This difference in the results is probably due to the different solvent system that was used to extract the samples. These results imply that the ethanolic root extract shows slightly higher radical scavenging activity which may be attributed to its stronger proton-donating abilities.

4.2. Antibacterial activity of L. aspera

Plant has long been a very important source of drug and many plants have been screened if they contain compounds with therapeutic activity[17]. Therefore, it is vital to evaluate the antimicrobial activity of *L. aspera*. In this study, the antibacterial activity of the different parts of *L. aspera* was evaluated by using disk diffusion method. The microorganisms chosen to be studied were Gram positive, *S. aureus* and Gram negative *E. coli*, *P. aeruginosa*, *S. typhimurium*, *S. choleraesuis* and *S. flexneri*. These bacteria were chosen to be studied as they are important pathogens and also due to rapidly developed antibiotic resistance as antibiotic use increases.

In this study, the mean zone of inhibition produced by the commercial antibiotic, chloramphenicol, was larger than those produced by all methanol extracts. It may be attributed to the fact that the plant extracts being in crude form contain a smaller concentrations of bioactive compounds[18]. In classifying the antimicrobial activity it would be generally expected that much greater number would be active against Gram positive than Gram negative bacteria[19]. However, in this study, the plant extracts (root, stem and flower) were effective against both Gram positive and Gram negative bacteria. The activity against both types of bacteria may be indicative of the presence of broad spectrum of antibiotic compounds or simply general metabolic toxin[20].

In a research conducted by using the entire plant of *L. aspera*, Srinivasan *et al*[20] indicated that the aqueous extract of the whole plant exhibited a higher range of zone of inhibition against *S. aureus*, *E. coli* and *P. aeruginosa*. However, the aqueous extract did not exhibit any activity against *S. typhimurium*. The difference in the zone of inhibition is probably due to the different solvents used for extraction and also the fact that the extracts in the previous study were from the entire plant whereas in this study the plant parts were segregated and tested individually. Moreover, growth area also affects the chemical components of the plants and leads to the activity difference. Apart from that, research done by Mangathayaru *et al*[21] indicated that the methanol extract of *L. aspera* (flower) was effective against *S. aureus*, *E. coli* and *P. aeruginosa* which were similar to the results from this study.

4.3. Cytotoxic activity of root extract of L. aspera

The isolation of bioactive compounds from natural sources requires toxicity information on the constituent of interest. It should be emphasized that toxic effect of the antioxidant and antimicrobial agent on the host cells must be considered, as a substance may exhibit antioxidant and antimicrobial activity by virtue of its toxic effect on the cells[22]. Therefore, this was the reason of the brine shrimp lethality test being conducted on the root extract.

The results observed at 12 h and 24 h indicated that the brine shrimp sensitivity to root extract was dose dependent. Since the LC_{50} values for both the 12 h and 24 h observation were higher than 1.0 mg/mL, which is the cutoff point in detecting cytotoxicity, the root extract is concluded to be non toxic. In a research done with ethanol extract of *L. aspera* (root) by Rahman *et al*[12], the LC_{50} value for brine shrimp lethality bioassay was 52.8 μg/mL. Since the LC_{50} value is less than 1 000 μg/mL, which is the cutoff point in detecting cytotoxicity, the ethanolic root extract is concluded to be toxic. The deviating result in comparison with this study might be due to the difference in the solvent system used. Further toxicity and pharmacological studies should be done to confirm the toxic effect of this extract.

In conclusion, the study demonstrates that methanol extracts from various parts of *L. aspera* are different in their antioxidant and antimicrobial effects. The results indicate that the root extract possess the highest antioxidant as well as antimicrobial activity. The root extract was shown to be non toxic based on the toxicity assay. However, further pharmacological and toxicity studies are necessary to confirm this suggestion. Phytochemicals analysis should be carried out to characterize the compounds in *L. aspera* that act as antioxidant and antibacterial agents. This work demonstrates that *L. aspera* should be considered as a useful source of material for human health, as an antioxidant and antimicrobial agent.

Conflict of interest statement

We declare that we have no conflict of interest.

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