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[Research article]

Comparative studies of *Zingiber officinale* leaves and rhizomes on the antibacterial effect

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ABSTRACT

Phytochemicals are chemical compounds that occur naturally in plants. Different parts of a plant such as leaf, stem, root, flower or seed, contain different phytochemicals or various concentration of phytochemicals. These chemical compounds may have biological significance like antioxidants or antibacterial. This study was conducted to investigate the comparative effect of ginger (Zingiber officinale) rhizomes and ginger leaves on bacteria. The plants were extracted by percolation technique using soxhlet extractor. All extracts were tested against four bacterial strains which included gram positive (Staphylococcus aureus and Bascillus spp.) and gram negative (Escherichia coli and Salmonella spp.) bacteria using disc diffusion method. In this research gentamicin 10 µg were used as the antibacterial standard. The antimicrobial activity of the active extract was evaluated quantitatively using three different concentrations. The concentration of the leaves extract and rhizome extract had significant effect on the zone of inhibition for all the bacteria. The result from this study showed ginger rhizome has more antimicrobial activity than ginger leaves and it can be used alongside conventional antibiotic to fight infections.

Keywords: Zingiber officinale; Antibacterial activity; Pyhtochemical; Ginger rhizomes; Ginger leaves; Pathogenic bacteria

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INTRODUCTION

In every environment, competition for food and space is one of the major factors that determine which organisms succeed and become established as the regions micro flora. Organisms that grow fastest with the available nutrients and environmental conditions will predominate. These microbes often change the environment with their metabolic by-products, securing their prevalence in that habitat. The increasing reliance on drugs from natural source gas led to the extraction and development of several drugs and chemotherapeutic agents from traditional herbs and is present in abundance in the tropic (Falodunet al., 2006).

Many foods present function of antibiotic that are often unknown to the consumers which reduced or limited the growth of bacteria in their body (Hornick&Yarnell, 2007). In spite of thousands years of use, none of these bioactive plants compounds have been exploited for clinical uses as antibiotics, though some alkaloid compounds like quinine and emetine have been developed as chemotherapeutic agents. Among those antibacterial foods that are becoming more common are gingers (A. Sebiomoet al., 2011).

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Although ginger has been valued for its antibacterial properties, but specifically only the ginger rhizome is most commonly used. Thereby, the other parts of the ginger plants such as the stem and leaves are always wasted or thrown away. The aim of this research is to compare the antibacterial effects of the leaf and rhizome of the ginger. This will prove the beneficial effects of the leaves other than the rhizome. Hence, the ginger plants are fully utilized.

Figure 1:A) Ginger Rhizome

B) Ginger Leaf

MATERIALS AND METHODS

Plant collection and identification

The fresh rhizomes and leaves (See figure 1) of Zingiber officinale were collected from a nursery in Sungai Way New Village, Selangor. Identification and authentication of the plant was done at Forest Research Institute Malaysia (FRIM), Malaysia for taxonomic identity of the plant.

Extracts Preparation

Both of the ginger leaves and rhizomes were washed and dried using oven. The dried ginger leaves and rhizome were grinded into fine powder using electrical grinder separately. Finally, the ginger plant powder was stored into air tight plastic container separately. The powdered parts of plant were extracted by percolation method using soxhlet extractor. The extract was evaporated under reduced pressure using a rotary vacuum evaporator until the extract was dried out. The remaining thick semisolid extract was then scraped out from the evaporator flask using a spatula which was kept in a sterile container for further use in antimicrobial test.

Preparation of different concentration of the extracts.

For concentration of 100mg/ml, 100mg of the extracts was diluted with 100µl of DMSO solution and 100ml of distilled water. The same method was done to prepare concentration of 50mg/ml and 75mg/ml.

Microorganism's isolation

Escherichia coli, Salmonella spp., Staphylococcus aureus and Bacillus spp. were used in this study. All bacteria were cultured on the prepared Mueller Hinton agar where a loop full of each bacterial strain was inoculated in 5ml of nutrient broth. The completed plates agar were incubated in incubator for 24 hours at the temperature of 37^{o}C in order to the get the active strains. These will be used as inoculums for subsequent studies (Prashant T. et al., 2011).

Kirby-Bauer disk diffusion susceptibility test

Antimicrobial effects of the extracts of Zingiber officinale were determined by Kirby-Bauer disc diffusion method. Experiment was performed under aseptic conditions. Sterilized cotton swabs were dipped in the nutrient broth and touch on the surface of the bacterial culture before streaking. Then, the cotton swab was streaked on Mueller Hinton agar plates. The Whatmann filter paper discs (6mm) were dipped in the 3 different concentrations (100mg/ml, 75mg/ml, and 50mg/ml) of the plant extract and were placed onto 3 different spots surface of the agar plate. Standard disc of Gentamicin was used as a positive control

and distilled water as negative control. Finally, the plates were incubated at 37°C for 24 hours. The antimicrobial activity was assayed by measuring the diameter of clear inhibition zone formed around the discs. The diameter of zone of inhibition was measured in millimeters using a ruler. Test was repeated on three separate occasions for each microbial strain (Parekh et al., 2005).

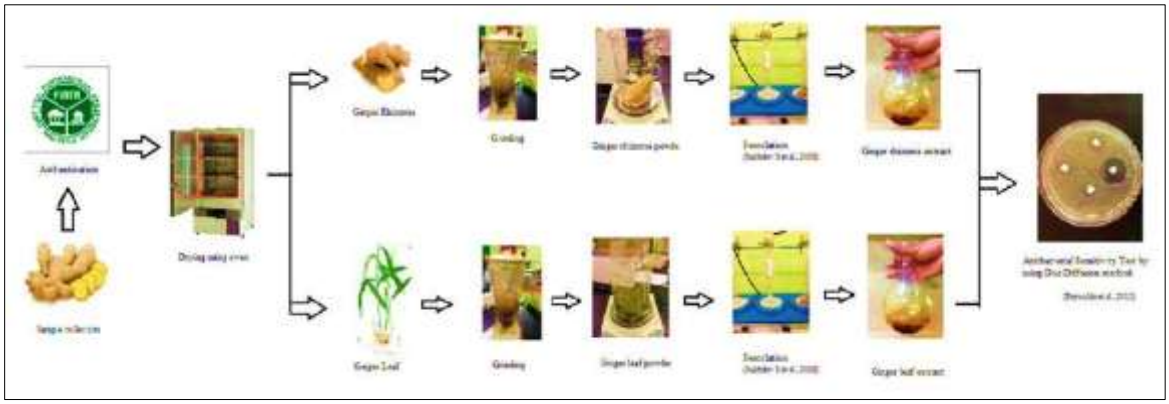

Figure 2: Flow chart of experimental work design

Data analysis

Microbial growth was determined by measuring the diameter of the zone of inhibition and the mean values are presented. The data collected was analyzed. The results of this experiment are presented as Mean+ SD triplicate experiments analyzed by using SPSS. Differences between mean is evaluated by one-way ANOVA at $p<0.05$.

RESULTS

The result of the mean of zone of inhibition observed in the plates of bacteria exposed to different concentrations of the leaves and rhizome extracts was shown in Table 1. It was observed that there were significant inhibition zone present in the pathogenic bacteria tested with leaves and rhizome extract at three different concentration tested (50mg/ml, 70mg/ml and 100mg/ml). Comparing between the leaf and rhizome extract, the rhizome extract has demonstrated higher mean of inhibition zone on tested microorganisms than the leaf extract.

Table 1. The mean of diameter of zone of inhibition of Zingiber officinale plant extracts against Escherichia coli, Staphylococcus aureus, Bacillus spp. and Salmonella spp.

Test Microorganism	Zone of inhibition (mm)							
	Ginger Rhizome Extract			Ginger Leaves Extract			Standard Drug	Negative Control
	50	70	100	50	70	100	Antibiotic	DW
Escherichia coli	5	8	10	6	7	7	25	0
Staphylococcus aureus	8	9	10	7	8	10	25	0
Bacillus spp.	9	13	15	10	11	12	23	0
Salmonella spp.	8	9	10	8	8	9	24	0

DW: Distilled water

Generally, the rhizome extract of Zingiber officinale has higher antimicrobial activity than the leaf extract against the four pathogenic bacteria**;** Escherichia coli, Staphylococcus aureus, Bacillus spp. and Salmonella spp. As the concentration of the plant extracts increased, the zone of inhibition is increased.

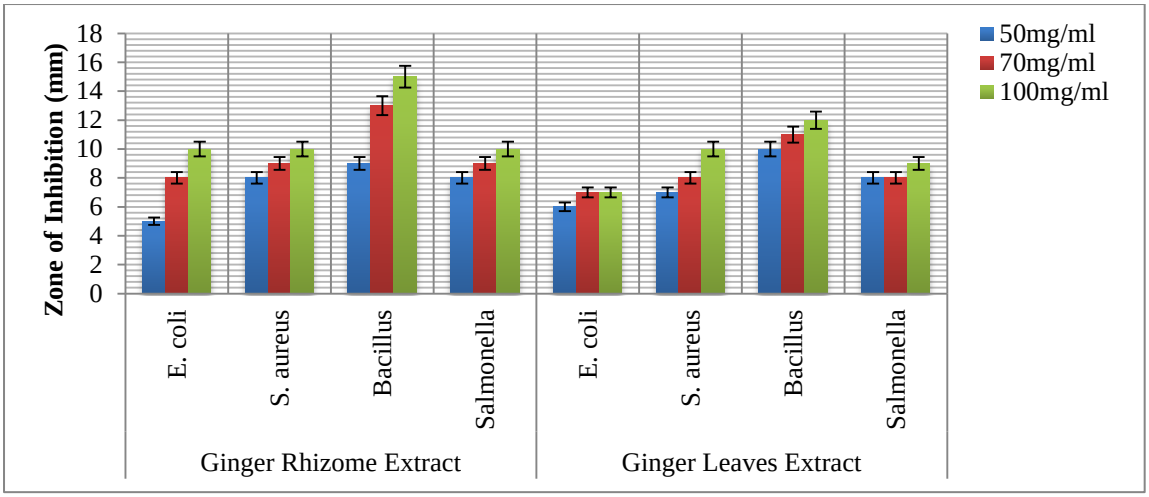

Figure 3: Mean of diameter of inhibition zone of Zingiber officinale plant extracts against the pathogenic bacteria

Bacillus spp. was the most susceptible microorganism towards the plant extracts and the study showed that Bacillus spp. is more sensitive to rhizome extract than leaf extract. The highest inhibition zone was observed on the concentration of 100mg/ml of the rhizome extract with the diameter of 15mm, which is the highest of all the extracts used. This indicated that antimicrobial testing at highest concentration of 100mg/ml for rhizome extract demonstrated a potential activity against Bacillus spp.

The ginger extracts demonstrated similar antimicrobial activities against Salmonella spp. and Staphylococcus aureus with almost same zone of inhibition. The highest inhibition zone was observed on the concentration of 100mg/ml of both leaf and rhizome extract with the diameter of 9mm to 10mm.

The inhibition against Escherichia coli was the lowest in both the leaf and rhizome extract compare to the other three bacteria. The lowest zone of inhibition was observed on the concentration of 50mg/ml of the ginger rhizome extract with the diameter with only 5mm. Overall, Escherichia coli showed the lowest diameter of inhibition zone I all extract tested.

The standard antibiotic used in the study was gentamicin. It inhibited the growth of all four test organisms indicating that the organisms are not resistant to gentamicin. It demonstrated the highest mean of inhibition zone in Escherichia coli and Staphylococcus aureus with the diameter of 25 mm followed by Salmonella spp. with diameter 24 mm. The lowest mean of inhibition zone was Bacillus spp. with diameter of 23 mm. There was no zone of inhibition observed on the disk introduced with distilled water.

The statistical analysis using SPSS indicates that there was no significant difference between the zone of inhibition when different parts of plant wasused with the value of P>0.05for the respective concentrations. As for the increasing concentration of the plant extracts, there were also no significant difference between the zone of inhibition with the value of P>0.05.

DISCUSSION

Background

Zingiber Officinale is a common condiment for various foods and beverages and a long history of important Traditional Medicine herb for the treatment of stomach disorders. The constituents present in ginger have potent antioxidant and anti-inflammatory activities. The study deals with antimicrobial activity of Zingiber Officinale (ZO) extract and their phytochemical composition. Phytochemical screening revealed the presence of alkaloids, saponins, tannins, flavonoids, and terpenoid and phlobo tannins in both the extracts. Based on the study conducted by Shipra B. et al., 2012, the ZO extracts were obtained by soxhlet apparatus and their chemical profile was determined through GC and GC-MS analysis resulted in the identification of 40 compounds in methanolic and 32 compounds in ethanolic extract. Their antimicrobial activity was tested against nine microorganisms that cause various diseases in human. Zingiber extracts showed selective antimicrobial activities.

According to the results obtained from this study, it showed that in leaf and rhizome extracts the mean

of inhibition zone increased with increasing concentration of the plant extract. This is likely due to the increasing amount of active compound present in higher extract concentration tested. Thus result in larger diameter of inhibition zone.

Phytochemical constituents

The better inhibition by rhizome extract is probably due to the presence of active components in the extract. Based on the qualitative phytochemical analysis of the Zingiber officinale done by previous research (Shipra B. et al., 2012), it was reported that the rhizome contains alkaloid, phlobo tannins, flavonoids, glycosides, saponins, tannin and terpenoid (Amla B. et al., 2012). Whereas the composition of leaf of Zingiber officinale only shows the presence of flavanoids, tannins and saponins (Sasidharan, 2010). This shows that the rhizome extract has more amount of antimicrobial agents present than in leaf extract.

Antimicrobial activity against pathogenic bacteria

The results also showed that antimicrobial activity between all four pathogenic bacteria test exhibit different measurement of diameter in the inhibition zone. The various zone of inhibition suggest the varying degree of efficacy and different phytoconstituents of herb on the target organisms (C. Baskaran, 2012). Bacillus spp. was more susceptible to the plant extracts followed by Salmonella spp. and Staphylococcus aureus respectively. However, the plant extracts showed the lowest antimicrobial activity against Escherichia coli. Types of bacteria may contribute to the variation of antimicrobial activity. This is due to the properties of each bacterium that are different from each other.

CONCLUSION

This study proved that the extracts of rhizome and leaf of the Zingiber officinale have potential natural antimicrobial activity against the pathogenic bacteria which include Escherichia coli, Staphylococcus aureus, Bacillus spp. And Salmonella spp. Demonstration of antimicrobial activity against the test isolates is an indication that there is possibility of sourcing alternative antibiotics substances in these plants for the development of newer antibacterial agents to combat various diseases. Although ginger rhizome has been proved having better antibacterial activity, ginger leaves should be fully utilised despite of its slightly weaker antibacterial activity. Therefore, ginger plants are useful natural sources to fight against certain bacterial infection

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