Malaysian Journal of Science **20**: 65-69 (2001)

Screening for antimicrobial activity of extracts from *Zingiber officinale* Rosc. var. rubrum Theilade

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ABSTRACT Extracts from the rhizomes of *Zingiber officinale* Rosc. var. rubrum Theilade were tested for antimicrobial activity against 11 bacterial and 6 fungal species. Compared with standard antibiotics, extracts had low to moderate activity except for the petroleum ether extract. The activity spectrum is wide against gram-positive and gram-negative bacterial as well as fungal tested. The extracts that showed the highest antimicrobial activity have similar antifungal activity with 5-Fluorocytosine at 10µg/disc against *Trichophyton mentagrophytes*. Other extracts showed low to moderate antibacterial activity when compared with gentamicin at 10µg/disc against *Bacillus subtilis*, *Staphylococcus aureus* (ATCC 24213 and ATCC 29213), *Streptococcus faecalis*, *Micrococcus luteus* and *Proteus vulgaris*.

ABSTRAK Ekstrak dari *Zingiber officinale* Rosc. var. rubrum Theilade telah dikaji untuk mengesan aktiviti antimikrobia terhadap 11 spesis bakteria dan 6 spesis fungi. Dibandingkan dengan antibiotik piawai, didapati ekstrak mempunyai aktiviti yang rendah kepada pertengahan kecuali bagi ektrak petroleum eter. Spektrum aktiviti adalah luas terhadap bakteria Gram-positif dan Gram-negatif begitu juga dengan fungi. Ekstrak yang menunjukkan aktiviti antimikrobia tertinggi, menyerupai aktiviti antifungi dengan 5-Florositosin pada 10µg/disk terhadap *Trichophyton mentagrophytes*. Ekstrak yang lain pula menunjukkan aktiviti antibakteria yang rendah kepada pertengahan apabila dibandingkan dengan gentamisin pada 10µg/disk terhadap *Bacillus subtilis*, *Staphylococcus aureus* (ATCC 24213 and ATCC 29213), *Streptococcus faecalis*, *Micrococcus luteus* dan *Proteus vulgaris*.

(antibacterial, anti-fungal, natural product as part of the *Zingiber officinale* Rosc. var. rubrum Theilade)

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.), an important spice plant widely cultivated in South East Asia and Far East, is documented as a medicinal and food plant (Holttum, 1950; Theilade, 1996; Larsen et al., 1999). Thus, it is highly valued for its medicinal properties. Commercially, ginger is produced in various forms (Govindarajan, 1982).

Studies on some chemical constituent in ginger indicated that the pungent principles of ginger rhizomes, such as gingerols and shogaols, have pharmacological action. The phenolic pungent principles of ginger (PPPs), represent 4.5-7.5% of the dry weight (Connel et al., 1972; Shoji et al., 1982; McHale et al., 1989; Sakamura and Suga, 1989; Bhagyalakshmi et al., 1994).

Gingerols are 1-(4-hydroxy-3-methoxy-phenyl)-5-hydroxyalkan-3-ones with an S (+)-configuration, having side chains of varying length have been identified and designated as [3]-, [4]-, [5]-, [6]-, [8]- and [10]-gingerol. Gingerols can be synthesized from Zingerone (Shoji et al., 1982; Middleditch et al., 1989; Tang, 1992; Zarate et al., 1996). Shogoals are gingerol analogs with a 4, 5-double bond resulting from the elimination of the 5-hydroxy group. 1-(4-hydroxy-3-methoxyphenyl)-4-decen-3-one, was the first reported compound from the [6]-shogoal series and was synthesized by the condensation of Zingerone with hexanal (Shoji et al., 1982; Tang, 1992).

Ginger rhizome extracts have been extensively studied for a broad range of biological activities including antibacterial, anticonvulsant, analgesic, antiulcer, gastric, antisecretory, antitumor, antifungal, antispasmodic and antiallergenic. In this study, the different extracts from *Zingiber officinale* Rosc. var. rubrum Theilade were subjected to some tests for antimicrobial activity.

MATERIALS AND METHODS

1. Plant materials

Rhizomes of *Zingiber officinale* Rosc. var. rubrum Theilade were obtained from the local market and chopped into small pieces. The small pieces were subjected to 2 stages of drying process, i.e. air drying for 6 days and oven drying at 40°C for 4 days. The dried material was blended into powder for experimental use.

2. Extraction

2.1. Consecutive extraction

The powdered plant material (200g) was subjected to a 24 hr soxhlet extraction with 2l light petroleum ether (b.p. 40-60°C). This process was repeated with 2l chloroform in place of the petroleum ether for another 24 hr. Each extract was concentrated to dryness under reduced pressure using a rotary evaporator.

2.2. Methanol extraction

Dried rhizome powder (200g) was soaked in methanol at room temperature for 3 days. The mixture was filtered and the filtrate evaporated *in vacuo*. The methanol extract was partitioned 3 times (25ml) with water and ethyl acetate (100ml). The ethyl acetate layer was evaporated *in vacuo* and subsequently dissolved in 30ml of methanol and extracted 3 times with 100ml n-hexane. The methanol layer was separated and evaporated using a rotary evaporator. The crude extract was dried and stored in a small vial.

3. Microorganism and culture methods

Bacterial cultures used were *Escherichia coli* (ATCC 25922 and ATCC 35213), *Staphylococcus aureus* (ATCC 24213 and ATCC 29213), *Enterococcus faecalis* (ATCC 29212, *Pseudomanas aeruginosa* (ATCC 27853) and several clinical isolate bacterial i.e. *Bacillus subtilis*, *Micrococcus luteus*, *Streptococcus faecalis*, *Proteus mirabilis*, *Proteus vulgaris*. All the bacterial cultures were obtained from the Department of Medical Microbiology and Department of Pharmacology, Faculty of Medicine, University of Malaya and maintained in the lab in Mueller Hinton broth media at 37°C in the dark.

The yeast, namely *Candida albicans*, *Candida glabrata* and *Candida parapsilosis* and the fillamentous fungus, namely *Aspergillus niger*, *Aspergillus flavus* and *Trichophyton mentagrophytes* were obtained from patients diagnosed as having fungal infections at the University Hospital, Petaling Jaya and maintained in Sabouraud Dextrose Agar at 25°C.

4. Antimicrobial Assay

4.1. Antibacterial activity test

Muller Hinton agar was prepared for culturing bacterial strains and broth media was prepared as suspension media for the test organism. The culture media were sterilized by autoclaving (121°C, 15psi, 20 minutes), poured (about 20ml each) into sterilized agar plates and left to solidify (Bauer et al., 1966; Perumal Samy et al., 1998).

The test organisms used were *Escherichia coli* (ATCC 25922 and ATCC 35213), *Staphylococcus aureus* (ATCC 24213 and ATCC 29213), *Bacillus subtilis*, *Enterococcus faecalis* (ATCC 29212), *Micrococcus luteus*, *Streptococcus faecalis*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomanas aeruginosa* (ATCC 27853). An inoculum of the bacterial strains was suspended in 10mL of broth media that were then incubated for 30-60 minutes. Optical density at 625nm was measured and adjusted to about 0.1 (OD_{625}=0.1) using NOVASPEC II Visible Spectrophotometer. The density was adjusted by adding sterile broth to the cultures. Swab inoculation was applied onto the surface of the agar plate (Brown and Blowers, 1978).

A sterile 6mm AA disc was saturated with the concentrated extract and dried, in the fume cupboard for 24hr. The impregnated disc was placed on the surface of a cultured agar plate. A drop of ultra pure water was applied on top of the saturated paper disc. Commercial antibiotic disc containing gentamicin (10μg) was used as a positive control while an AA disc applied with a drop of sterilized water was used as negative control. The plates were placed at 37°C in an incubator and the growth was observed after 24 hrs (Hernandez-Perez et al., 1994; Perumal Samy et al., 1998).

4.2. Antifungal test

Antifungal activity against yeasts was determined using the same procedure as in the antibacterial activity test. Sabouraud Dextrose Agar was used as the test medium. Antifungal activity against fillamentous fungi was determined using Agar Diffusion Assay (Drouhet et al., 1986; Arnone et al., 1994).

The 18 hours cultures of yeasts grown in Sabouraud Dextrose Broth was standardized to an optical density of 1 at 600nm (OD_{600}=1) using NOVASPEC II Visible Spectrophotometer. The density was adjusted by adding sterile broth to the cultures. The resultant suspension at approximately 10^7cells ml^{-1} (Rahalison et al., 1991) was diluted to 10^5cells ml^{-1} before use.

Conidial suspensions of the test fungi were prepared immediately before carrying out the assay by pouring 20ml Sabouraud Dextrose Broth containing 1 drop Tween 80 into 3-day-old cultures of *Aspergillus* species or 2-week-old culture of *Trichophyton mentagrophytes*. The cultures of *Aspergillus* species and *Trichophyton mentagrophytes* were grown on Sabouraud Dextrose Agar slants at 37°C and 27°C, respectively. After homogenizing with glass beads, the concentration of the resultant conidial suspensions of *Aspergillus* species and *Trichophyton mentagrophytes* were adjusted using the haemocytometer to 10^6conidia ml^{-1} and 10^8conidia ml^{-1}, respectively.

The suspension was diluted ten times with molten Sabouraud Dextrose Agar at 40°C and agar was poured into each petri dish (20ml). Steriled AA discs (6mm) impregnated with various test extract solutions at 800mg ml^{-1} in methanol were transferred onto the surface of the solidified agar after evaporation of the solvent.

5-Fluorocytosine (10µg), saturated sterile 6mm AA disc of pet-ether, chloroform, MeOH and blank discs were also applied to the agar as controls. The zones of growth inhibition were measured after 24 hrs incubation at 37°C for yeasts and 3 days for *Aspergillus flavus* and *Aspergillus niger* while those for *Trichophyton mentagrophytes* were measured after one week at room temperature (30°C).

RESULTS AND DISCUSSIONS

The results for antimicrobial activity of *Zingiber officinale* Rosc. var. rubrum Theilade are shown in Table 1. Inhibitory effects of the extracts from *Zingiber officinale* Rosc. var Rubrum on 11 bacterial and 6 fungal species are shown in Table 1. Inhibition zones were measured in mm after 24 hrs. Results showed the petroleum ether extracts as having moderate activity against *Micrococcus luteus* and *Proteus vulgaris* while the methanol extracts showed activity against *Staphylococcus aureus* (ATCC 24213) and *Staphylococcus aureus* (ATCC 29213). The petroleum ether extracts also showed low activity against *Bacillus subtilis*, *Staphylococcus aureus* (ATCC 24213), *Staphylococcus aureus* (ATCC 29213) and *Streptococcus faecalis*. All the antibacterial activities were evaluated in comparison with the standard antibiotic (gentamicin) at 10µg/disc.

Antifungal screening revealed the pet-ether, chloroform and methanol extracts to have antifungal activity against *Candida glabrata*, *Candida parapsilosis*, *Aspergillus flavus* and *Trichophyton mentagrophytes*. The petroleum ether extract showed more pronounced activity than either the chloroform or methanol extracts. The petroleum ether extract showed a high activity against *Trichophyton mentagro-phytes* and low activity against *Candida glabrata* and *Candida parapsilosis* and *Aspergillus flavus* while methanol extracts exhibited stronger activity against *Aspergillus flavus* than *Trichophyton mentagrophytes*.

CONCLUSION

The petroleum ether extract showed the most antifungal activity with more pronounced activity against *Trichophyton mentagrophytes*. Other extracts i.e. chloroform and methanol extracts tested showed low to moderate activity against organisms tested. Antibacterial activity was more active using the methanol extract and showed positive activity against *Escherichia coli* (ATCC 25922 and ATCC 35213). There was no antibacterial activity using chloroform extract but it showed weak activity towards antifungal.

Acknowledgement We thank the University of Malaya, Malaysia for the financial support to carry out this work, Professor Soo Hoo Tuck Soon and Professor Hamimah Bt Hassan (Department of Medical Microbiology, University of Malaya) for supplying fungal and bacterial cultures. We also thank Professor Halijah Ibrahim (Institute of Biological Sciences, University of Malaya) for the identification of the plant material and Professor A. Hamid A. Hadi (Department of Chemistry, University of Malaya) for his help in this work.

Table 1. Antimicrobial activity of *Zingiber officinale* Rosc.var. rubrum Theilade

Extract or Product[a]	1[b]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Petroleum Ether	+	-	+	+	++	+	-	-	-	++	-	-	+	+	+	-	++++
Chloroform	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	++
Methanol	-	-	++	++	-	-	-	-	-	-	-	-	-	-	++	-	+
Control	+++	++	+++	+++	++++	+++	++	++	+++	+++	++	nd	nd	nd	nd	nd	nd
Gentamicin[c]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+++	++	++	++++	+++	++++
5-Fluorocytosine[d]																	

Key:
[a]each disc received 20µl extract or fraction, solvent
[b]1=*Bacillus subtilis*; 2= *Enterococcus faecalis* (ATCC 29212), 3= *Staphylococcus aureus* (ATCC 24213);
4= *Staphylococcus aureus* (ATCC 29213); 5=*Micrococcus luteus*; 6=*Streptococcus faecalis*; 7=*Escherichia coli* (ATCC 25922);
8= *Escherichia coli* (ATCC 35213); 9=*Proteus mirabilis*; 10=*Proteus vulgaris*, 11=*Pseudomanas aeruginosa* (ATCC 27853);
12=*Candida albicans*; 13=*Candida glabrata*; 14=*Candida parapsilosis*; 15=*Aspergillus flavus*; 16=*Aspergillus niger*; 17=*Trichophyton mentagrophytes*
[c] and [d]=positive control
nd= not determined; - = no inhibition zone; += inhibition zone (>6-9mm); ++= inhibition zone (10-20);
+++ = inhibition zone (21-30mm); ++++ =inhibition zone (31-50mm).

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