

INHIBITORY EFFECT OF ETHANOLIC AND WATER EXTRACTS OF TWO VARIETIES OF GINGER ON SELECTED BACTERIAL AND FUNGAL ISOLATES

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Summary

Ethanollic and water extracts obtained from Malaysian and Thailand gingers were evaluated for their inhibitory effect against eight bacterial isolates *viz.* *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Pseudomonas merobilis* and two fungal isolates *viz.* *Aspergillus niger* and *Candida albicans*. Ethanolic extracts of both Malaysian and Thailand gingers (MGE and TGE) showed good to excellent antibacterial activities whereas water extracts of both Malaysian and Thailand gingers (MGW and TGW) showed disparate results ranging from nil to average to good activity. A good to excellent antifungal activity was shown by all the extracts (MGE, TGE, MGW and TGW) against *C. albicans* and *A. niger*

Key words: Ginger, *Zingiber officinale*, water extract, ethanol extract, antibacterial, antifungal.

Introduction

Due to variation in chemical compositions and their relative proportions, herbal extracts often exhibit disparate or contradictory pharmacological results even among the same species if collected from different location. There are cases in which the extract and the pure compounds isolated from it showed such a disparity or contradiction. For example, the extract of soya did not show any inhibition on CYP enzymes but the genistein isolated from it inhibits several CYP enzymes [1]. Therefore, it is difficult to give quality guarantee on herbal products [2] and they always lack in quality control. Different experimental approaches and comparison of one herbal product with locally available herbal products of the same kind is often necessary to assess their medicinal efficacy.

Ginger (*Zingiber officinale*) is one of the most widely used herbal products since ancient time and its applications are widespread and are well documented [3,4]. Especially, the rhizome part of the ginger has been used in traditional medicine [5,6], has most potent medicinal properties [7] including antioxidant property [8-10] and also reported many other biological activities [4,11, 2]. The pungent smell of ginger is due to the presence of phenylpropanoids, shogaols and gingerols [13]; the latter one is being used to relieve migraine headache [14]. In fact, shogaols and zingerone are produced from gingerols when the ginger underwent processing such as drying and cooking [13]. Ginger has been used to aid digestion [15], to treat stomach upset, diarrhea, nausea, rheumatic complaints [16]; it is more effective in reducing symptoms associated with motion sickness [17] and as such it is better than any other medications; it lowers cholesterol and helps to prevent the blood from clotting. It is also reported that there is no interactions between ginger and blood thinning medications, such as aspirin and warfarin [17].

Although, a few varieties of gingers are available in Malaysia, the local Malaysian ginger (vernacular name is Halia Bentong) and Thailand ginger are the two main varieties widely used by all Malaysians in their day to day life. Several reports are available on the antibacterial studies of ginger [18]. However, our literature search revealed that Malaysian and Thailand gingers are not extensively studied for their biological studies, particularly for antimicrobial activities [18]. The anti-tumour promoter activity of Malaysian Zingiberaceae species including *Zingiber officinale* were reported [6]. The ethanolic extract of Malaysian ginger showed 81.7% inhibition of TPA-induced EBV-EV activation at a concentration of 80 µg/mL and a complete inhibition at a concentration of 160 µg/mL [6]. In the present study, we investigated and compared the inhibitory effect of ethanolic and water extracts of rhizomes of Malaysian and Thailand gingers on eight bacterial isolates *viz.* *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Pseudomonas merobilis* and two fungal isolates *viz.* *Aspergillus niger* and *Candida albicans* and the results are communicated herewith.

Materials and Methods

Plant Materials

10 kg each of rhizomes of Malaysian and Thailand gingers were purchased from a local market and a voucher specimen, PillaiMK/MY-Halia/06/2010 for Malaysian ginger and PillaiMK/TH-Halia/08/2010 for Thailand ginger, are separately deposited at the School of Pharmaceutical

Sciences, Univeristi Sains Malaysia, Penang, Malaysia and Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Penang, Malaysia.

Processing of materials

The rhizomes of both Malaysian and Thailand gingers were obtained in fresh condition and were cut into small pieces by a chopper. The materials were then dried in an oven at 40-50 °C for two to three weeks. The weight of the materials now reduced to about 5 kg and they were powdered using a miller.

Preparation of water extract

About 500 g of the powdered Malaysian ginger was taken in a glass jar and three liters of deionised and purified water was added and digested it on a water bath 70-80°C for one week. The water extract was obtained by simple filtration of this digested material over a filter paper and kept separately. To the residue, another three liters of water was added and repeated the digestion again for one week. The water extract obtained now was combined with previously obtained water extract. The combined water extract was concentrated by two steps. Buchi rotavapour was used first to remove as much as water possible followed by the use of Freeze dryer/Lyophiliser to remove the remaining water. The same procedure was repeated to obtain water extract from Thailand ginger. A dark brown dry residue of water extract was obtained on both cases and stored in a refrigerator.

Preparation of ethanol extract

The rest of the powdered Malaysian ginger was exhaustively extracted with ethanol using a Soxhlet's apparatus. The extract thus obtained was filtered over a filter paper to remove any contaminated solid particles and the solvent ethanol was completely removed using a Buchi rotavapour. The same procedure was repeated to obtain ethanol extract from Thailand ginger. A dark brown precipitate of ethanol crude extract was obtained on both cases and kept in a cupboard at room temperature.

Evaluation of antimicrobial activity

Disc diffusion assay

Antibacterial and antifungal activities were investigated by disc diffusion method as described in literature [19, 20]. Briefly, the MHA plates containing an inoculum size of 10⁶ colony-forming units (CFU)/mL of bacteria or 2 X 10⁵ CFU/mL yeast cells or molds spores on SDA and PDA plates, respectively, were spread on the solid plates with an L- shaped glass rod. Then discs impregnated with 25µL of each extracts, dissolved in water at a concentration of 100 mg/mL, were placed on the inoculated plates. Similarly, each plate carried a blank disc by adding solvent alone in the centre to serve as a control. Antibiotic discs (6.00 mm. dia.) of 30 µg/mL chloramphenical for bacteria and 30 µg/mL of miconazole nitrate for fungi were also used as positive controls. All the plates were incubated at 37°C for 18 to 24 hr. for bacteria and at 28 °C for 48 to 96 hr. for fungi. The sensitivity of microorganism species to the water and ethanolic extracts of rhizomes of Malaysian and Thailand gingers was determined by measuring the sizes of inhibition zones on the agar surface around the discs. All of the experiments were performed in triplicate and the results are reported here as the average of three experiments.

Results and Discussion

In general, both Malaysian Ginger-Ethanollic extract (MGE) and Thailand Ginger-Ethanollic extract (TGE) exhibited activities against all the bacterial strains tested except for *E. coli* for which case TGE did not show any activity but the MGE showed an inhibition zone of 12 mm (Table 1). MGE showed highest inhibition zone of 24 mm against both *M. luteus* and *S. typhi*. This is very much comparable with inhibition zone exhibited by TGE which are respectively, 23 and 20 mm. The antibacterial activity exhibited by MGE and TGE also very much comparable with each other against *P. aeruginosa* and the inhibition zones are, respectively, 18 and 16 mm. Both, MGE and TGE showed the same value of inhibitory effect of 14 mm against *S. aureus*. TGE showed highest inhibition zone of 27 mm against *B. subtilis* whereas MGE showed an inhibition zone of 23 mm against *B. subtilis*. Against *P. merobilis* MGE showed an inhibitory zone of 20 mm whereas TGE showed an inhibitory zone of 25 mm. A highest deviation was observed against *B. thuringiensis* for which case MGE showed an inhibitory value of 14 mm and the TGE showed an inhibitory value of 21 mm.

Table 1: Inhibitory effect of water and ethanollic extracts of Malaysian and Thailand gingers on selected bacterial and fungal isolates.

S. No	Microorganisms (bacteria/fungi)	Zone of inhibition (mm)				Positive controls
		MGE	TGE	MGW	TGW	
1	<i>E. coli</i>	12	-	17	-	22
2	<i>S. typhi</i>	24	20	-	-	27
3	<i>B. subtilis</i>	23	27	-	-	31
4	<i>B. thuringiensis</i>	14	21	-	-	27
5	<i>M. luteus</i>	24	23	-	13	29
6	<i>P. aeruginosa</i>	18	16	-	-	28
7	<i>S. aureus</i>	14	14	11	14	29
8	<i>P. merobilis</i>	20	25	22	12	30
9	<i>A. niger</i>	13	10	12	10	29
10	<i>C. albicans</i>	16	10	19	20	27

MGE: Malaysian Ginger- Ethanollic extract; TGE: Thailand Ginger-Ethanollic extract; MGW: Malaysian Ginger-Water extract; TGW: Thailand Ginger-Water extract; Dashes (-) indicate no inhibition and the extract is devoid of antibacterial activity; *E. coli*: *Escherichia coli*; *S. thypii*: *Salmonella thypii*; *B. subtilis*: *Bacillus subtilis*; *B. thuringiensis*: *Bacillus thuringiensis*; *M. luteus*: *Micrococcus luteus*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. aureus*: *Staphylococcus aureus*; *P. merobilis*: *Pseudomonas merobilis*; *A. niger*: *Aspergillus niger*; *C. albicans*: *Candida albicans*; S. No. 1-8 are bacterial isolates; S. No. 9 and 10 are fungal isolates. Discs with chloramphenical was used as positive control for bacteria and miconazole nitrate was used as positive control for fungi.

Both, Malaysian Ginger-Water extract (MGW) and Thailand Ginger-Water extract (TGW) did not show any activity against *S. typhi*, *B. subtilis*, *B. thuringiensis* and *P. aeruginosa* and for other bacterial isolates the results are disparate (Table 1). For example, MGW showed an inhibitory zone of 17 mm against *E. coli* whereas TGW showed no activity against the same bacterial isolate; similarly, MGW showed no activity against *M. luteus* but TGW showed an inhibitory zone of 13 mm against the same bacterial isolate. Against *S. aureus*, MGW showed an inhibitory zone of 11 mm but TGW showed slightly higher value of 14 mm. MGW and TGW showed highest level of deviation against *P. merobilis* with former showed an inhibition zone of 22 mm and the latter showed only 12 mm.

All the extracts (MGE, TGE, MGW and TGW) showed activities against the two fungal isolates tested viz. *A. niger* and *C. albicans*. MGE showed an inhibition zone of 13 and 16 mm, respectively, against *A. niger* and *C. albicans* whereas TGE showed an inhibition of 10 mm on both *A. niger* and *C. albicans*. Both, MGW and TGW showed antifungal activities against *A. niger* with former has an inhibition zone of 12 mm and latter has 10 mm which are comparable to each other. Similarly, MGW showed an inhibitory zone of 19 mm against *C. albicans* and TGW showed an inhibitory zone of 20 mm which are also very much comparable to each other.

Overall, ethanolic extracts of both Malaysian and Thailand gingers (MGE and TGE) showed good to excellent antibacterial activities against the above mentioned bacterial isolates, except for TGE on *E. coli*, whereas water extracts of both Malaysian and Thailand gingers (MGW and TGW) showed either nil or average activities except for a few cases for which they have a reasonable activity against the above mentioned bacterial isolates (Table 1). All the extracts (MGE, TGE, MGW and TGW) showed good antifungal activity against *A. niger*. TGE showed a good antifungal activity against *C. albicans* whereas MGE, MGW and TGW showed an excellent antifungal activity against *C. albicans*.

The inhibitory effect of ginger extract of several varieties have previously been reported against several bacterial isolates [3,13,18,21-23]. The ginger obtained from a retail market in Thailand and the subsequent obtainment of ethanolic extract from this ginger was screened against twenty *Salmonella* spp. and five other enterobacteria [21]. However, it did not show any activity against all the bacteria used for screening including *E. coli* [21] which is the only bacteria common to our study. The report is in good agreement with our study i.e. our study revealed that not only ethanolic extract of Thailand ginger (TGE) but also water extract of Thailand ginger (TGW) showed no antibacterial activity against *E. coli*. However, contrary to this result, the water and ethanolic extract of Malaysian ginger (MGW and MGE) showed an inhibition zone of, respectively, 17 and 12 mm against *E. coli*. The reason for this may be due availability of sufficient quantity of active chemical compositions or availability of some new chemical entities in Malaysian ginger to effect the inhibition of *E. coli*. The methanol, hexane, ethyl acetate and water extracts of Indonesian ginger were screened against four bacterial isolates viz. *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis* at a concentration of 50 and 500 mg/mL [18]. In particular, the water extract was inactive against all bacterial isolates tested at both concentrations. All these four bacterial isolates are also common to our study for water extract. Our studies showed that the water extract of both MGW and TGW did not inhibit *P. aeruginosa* and *B. subtilis* and thus the report was in good agreement. However, contrary to this report, our studies revealed that MGW and TGW showed an inhibition zone of, respectively, 11 and 14 mm against *S. aureus*. Against *E. coli*, TGW was inactive and therefore the report has good agreement but MGW showed an

inhibition zone of 17 mm. The *in vivo* antibacterial activity of 95% ethanolic extract of Cameroon ginger against four bacterial isolates viz. *S. aureus*, *S. pneumoniae*, *Haemophilus influenzae* and *S. pyogenes* were also reported previously [3]. *S. aureus* is the only bacterial isolate common to our study in this report and our *in vitro* study too has good agreement that the ethanolic extract was active against *S. aureus*. It was reported that the Indian variety of ginger showed insignificant antibacterial activity against *E. coli*, *S. aureus* and *B.cereus* [22]. The inhibitory effect of other bacterial isolates are that the hexane, ethylacetate and ethanolic extract of Nigerian ginger showed antibacterial activity against three fungal isolates viz. *Coliform bacillus*, *Staphylococcus epidermidis* and *Streptococcus viridans* [13]; however its water extract did not show any activity against the above mentioned three bacterial isolates [13]. It was reported that the hexane and ethanolic extracts of Korean ginger exhibited antibacterial activity against three bacterial isolates viz. *Porphyromonas gingivalis*, *Porphyromonas endodontalis* and *Prevotella intermedia* [23].

The antifungal studies of ethanolic extract of Iranian ginger on *C. albicans* were reported recently with an inhibition zone of ~12 mm. [24]. The essential oil obtained from rhizomes of Indian ginger exhibited antifungal activity against *C. albicans* (inhibition zone of ~12 mm.) [25]. In general, the inhibitory effect ginger extracts toward *C. albicans* is well over 10 mm. In the present study, the water extracts of MGW (inhibition zone of 19 mm) and TGW (inhibition zone of 20 mm) have higher activity than the ethanolic extracts of MGE (inhibition zone of 16 mm) and TGE (inhibition zone of 10 mm). Several antifungal gingerols were isolated from ethanolic extracts of African ginger (West Africa) and compared the gingerol contents with Indonesian ginger [12]. In general, all extracts, MGE, TGE, MGW and TGW, showed somewhat higher antifungal activity toward *A. niger* than values reported in the literature [25].

To conclude, the ethanolic and water extracts of Malaysian and Thailand gingers were screened for their antimicrobial activities. Ethanolic extracts both Malaysian and Thailand gingers showed a good to excellent activities whilst the water extracts showed relatively weak activities. On average, Malaysian ginger showed slightly higher antimicrobial activities than Thailand ginger. As ginger has been used widely throughout the world, further research is very useful for clinical applications.

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