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ORIGINAL RESEARCH ARTICLE

In vitro Antimicrobial Activity of Hexane: Petroleum Ether Extracts from Fruits of *Momordica charantia* L.

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ABSTRACT

In the present study, the antimicrobial activity from fruit extract of *Momordica charantia* L. using combination of hexane and petroleum ether at different concentrations (0%, 20%, 40%, 60%, 80% and 100%) was assessed and tested against four Gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis* and *Staphylococcus aureus*), four Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia* sp.) and a fungus (*Candida albicans*). Among the four Gram-positive bacteria tested, *Bacillus cereus* was found to be the most susceptible bacteria with the percentage inhibition of 109.5 ± 3.69% at 100% hexane (1mg/ml). Nevertheless, *Klebsiella pneumonia* was found to be the most susceptible Gram-negative bacteria with the percentage inhibition of 125.0 ± 5.27% using 40% hexane while the fungus *Candida albicans* demonstrated maximum inhibition with a percentage inhibition of 297.2 ± 6.80% using 100% hexane. Thus, the *in vitro* study of hexane : petroleum ether fruit extract of *Momordica charantia* L. has shown its potency as a promising antimicrobial agent towards a broad range of pathogenic microorganisms tested.

Key words: *Momordica charantia* L., Antimicrobial Activity, Hexane : Petroleum Ether Extract, Pathogenic Microorganisms

INTRODUCTION

Nature has been acknowledged as the rudimentary source of natural products with an abundance of profound and promising medicinal virtues since antiquity. The tremendous heterogeneity found in plants, animals, marine organisms and microorganisms makes it possible for nature to serve as an appealing source of novel therapeutic candidate compounds [19]. Natural products have long been the protagonist which plays a dominant role in the treatment of diseases in the history of mankind [14]. Approximately 62-80% of the world's population still depends on traditional herbal medicines as therapeutic medicines for their primary healthcare [24-25]. Higher plants are commonly used as sources of medicinal compounds with prominent roles in maintaining human health for centuries [7].

In recent year, microbial resistance to commonly used antibiotics has become a major solicitude to public health [16]. The alleged consumption should be done according to the dosage prescribed by doctor as over prescription of antimicrobial drugs is a crucial contributor to drug resistance [2,13]. Drug resistance towards pathogens was relatively low in three decades ago. However, the statistic is now in an appalling stage as numerous antibiotics and drug resistance to human pathogenic cases were reported worldwide, annually. A plausible explanation to drug resistance might be due to the genetic capability of microorganisms to mutate during their growths which eventually attain resistance to antimicrobial drugs. As infections have escalated to a great extent and antimicrobial drug resistance has become an ever increasing therapeutic problem [3], thus, this has the necessitated to search for new antimicrobial agents.

The aim of this study was to evaluate the antimicrobial activity from the fruits of *Momordica charantia* L. This plant is widely distributed throughout the tropics and used as folk medicament for various ailments which include healing of wounds, infections, measles, hepatitis

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and fevers [8,9,23]. To the best of my knowledge, there are only studies conducted on the leave extract of *Momordica charantia* L. to study its antimicrobial activity, but little information can be obtained from the fruit extract. Thus, it would be an attractive option to investigate the antimicrobial properties from the fruits of *Momordica charantia* L.

MATERIALS AND METHODS

Extraction of *Momordica charantia* : The unripe fruit of *M. charantia* L. was obtained from a farm located in Sitiawan, Perak, Malaysia from the duration of July to August 2008. The *M. charantia* fruits were washed and the seeds were removed. The fruits were then sliced into small pieces and dried in drying oven at 50^{o}C. The dried fruits were then ground prior to extraction. Two gram (2 g) of ground *M. charantia* was extracted with 20 ml solvents (1:10 ratio) by orbital shaking at 150 rpm at room temperature for 6 h. The solvents used were hexane and petroleum ether of different concentrations (0%, 20%, 40%, 60%, 80% and 100%). The extracts were then filtered through a Whatman No. 1 filter paper, concentrated with a rotary evaporator and diluted with 5% dimethyl sulfoxide (DMSO) at ratio 1 : 1 (v/v).

Test microorganisms : *In vitro* antimicrobial studies were carried out on four Gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis* and *Staphylococcus aureus*), four Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Serratia* sp.) and a fungus (*Candida albicans*). All microbial strains were obtained from the Laboratory of Microbiology, Faculty of Applied Sciences, UCSI University, Malaysia.

Culture Media : Nutrient agar (NA) (Himedia), nutrient broth (NB) (Merck, Germany) and potato dextrose agar (PDA) (Merck) were used during the study. NA and NB were used for the cultivation of bacteria while PDA were used to culture yeast. All bacterial cultures were incubated at 37 ºC for 24 h whereas yeast cultures were incubated at 30 ºC for 48 h.

Antimicrobial Susceptibility Test: The Kirby Bauer method was used. The cell concentration was standardized to 10^6 to 10^7 cells/ml (Chanwitheesuk *et al.* 2005)[4]. The bacterial cultures were inoculated in NB and incubated at 37 °C for 24 h while yeast was incubated at room temperature for 48 h; by orbital shaking at 150 rpm. Each microbial suspension of 100 µl was spread evenly on NA (for bacteria) and PDA (for yeast) plates with a sterilized swab.

Sterilized filter paper discs (diameter, 6 mm), was impregnated with 10 µl of different concentrations of DMSO-diluted fruit extracts and air-dried prior to placing on the surface of inoculated agar medium. Discs with 10 µg/disc of ampicillin served as positive control for bacteria while 30 µg/disc of tetracycline were used as positive control for yeast. The different concentrations of solvents (hexane and petroleum ether) and 5% DMSO were used as negative controls. The bacterial plates were incubated at 37 ºC for 24 h whereas plates for yeast were incubated at 30 ºC for 48 h. The test was carried out in triplicate. Zone of inhibition was then measured using a scale. The antimicrobial activity in terms of percentage relative to inhibition zone diameter was calculated and expressed as follow:

$$\text{Relative inhibition Zone (\%)} = \frac{\text{IZD sample} - \text{IZD negative control}}{\text{IZD standard antibiotic}}$$

Where, IZD is the diameter of inhibition zone (mm)

Statistical analysis: The antimicrobial activity of *Momordica charantia* L. was assessed in terms of inhibition zone diameters of the microorganisms, which was then converted into relative inhibition zone (%). Results were expressed as mean ± standard deviation (SD), with n = 3. Statistical analysis was determined using one-way ANOVA (analysis of variance), where a value of $P < 0.05$ is considered to be statistically significant. Then, Tukey's multiple comparison test with 95% confidence intervals was employed to indicate at which level of the factor that is actually responsible for the significant differences in the variables analyzed.

RESULTS AND DISCUSSION

A broad panel of disease-causing microorganisms commonly found in the environment had been included in this screening. As shown in (**Table 1 & 2**), the hexane: petroleum ether fruit extracts of *M. charantia* L. demonstrated antimicrobial activity towards two Gram-positive bacteria (*B. cereus* and *E. facealis*) and three Gram-negative bacteria (*E. coli*, *K. pneumonia* and *P. aeruginosa*). In Gram-positive bacteria, the largest percentage of inhibition was found in *B. cereus* with 109.5 ± 3.69% using 100% hexane while in *E. facealis*, the biggest inhibition percentage was seen at 40% hexane with 62.2 ± 1.95%. On the other hand, in Gram-negative bacteria, largest

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inhibition percentage was found in 40% hexane with 125.0 ± 5.27% in *K. pneumonia* while in *E. coli* and *P. aeruginosa*, the biggest inhibition percentage was 108.3 ± 0.00% at 0% hexane and 31.0 ± 1.35% at 60% hexane, respectively. As for *B. subtilis*, *S. aureus* and *Serratia* sp., these microorganisms were resistant to inhibition at all extract concentrations used. Interestingly, the *Momordica charantia* L. fruit extracts were found more potent towards fungus, *C. albicans* with inhibition percentage ranging from 116.7 ± 5.27% to 297.2 ± 6.80% when different combinations of hexane : petroleum ether were used as extraction solvent.

As seen in (**Table 1**), both susceptible Gram-positive bacteria *B. cereus* and *E. faecalis* had an average of moderate inhibition percentage of 104.7 ± 4.52% and 53.9 ± 2.56%, respectively while in Gram-negative bacteria (**Table 2**), only *K. pneumonia* was more susceptible with an average of moderate inhibition percentage of 120.8 ± 4.84% whereas in *E. coli* and *P. aeruginosa*, both showed milder average of inhibition percentage with 71.3 ± 3.70% and 24.7 ± 1.37%, respectively. The finding that Gram-positive bacteria were more susceptible to inhibition as compared to Gram-negative bacteria in this investigation is in accord with numerous previous reports [12,17,21,22]. This activity could be ascribed to the presence of a broad spectrum of bioactive compounds from hexane: petroleum ether extracts of *M. charantia* L. such as fatty acids, essential oils, phenolic compounds and alkaloids [17]. As most of the bioactive compounds extracted using hexane : petroleum ethers were of low polarity, the relatively weaker antibacterial activity against Gram-negative bacteria might be attributed to the presence of hydrophilic polysaccharide chains in the outer membrane of Gram-negative bacteria, which serves as a barrier to hydrophobic essential oils [10]. Additionally, Gram-negative bacteria are found to possess enzymes in the periplasmic space which are capable of breaking down foreign molecules introduced from outside [5].

As for the anti-fungal activity (**Fig 1**), all concentrations of hexane : petroleum ether extracts used inhibited the growth of *Candida albicans* with the largest percentage inhibition was seen in 100% hexane with 297.2 ± 6.80%. Statistical analysis one-way ANOVA revealed the significance different ($P < 0.05$) when different concentrations of hexane: petroleum ether was used as an extraction solvent for antimicrobial activity. When further tested with Tukey's multiple comparison, 100% hexane was also found to be the best extraction solvent with the largest percentage of inhibition for most of the microorganisms tested. The finding pertaining to hexane being the best extraction solvent correlates with the results obtained by Rizvi *et al.* [18] and Al-Zubairi *et al.* [1].

Jagessar *et al.* [11] reported that there was no antimicrobial activity of the hexane and dichloromethane extracts from the leaves of *M. charantia* L. against *E. coli* and *C. albicans*. This study however contradicts with the results as reported by Jagessar *et al.* [11]. As shown in the results, hexane: petroleum extracts from fruit of *M. charantia* L. showed mild inhibition percentage against *E. coli* while strong inhibition percentage against *C. albicans*. According to Edeoga *et al.* [6], the seeds, leaves and pericarps of *M. charantia* showed a relatively higher accumulation of phenols and tannins but demonstrated lower constituents of other phytochemicals such as alkaloid, flavonoid and saponin. In another research conducted by Mwambete [15], the petroleum ether fruit extract of *M. charantia* L. showed mild antimicrobial activity against *C. albicans* (inhibition percentage of ~ 60%) but no activity was demonstrated in *E. coli*. Contrarily, in the present study, 80% and 100% hexane extracts used showed large inhibition percentage (186.1 ± 0.82% and 297.2 ± 0.52%, respectively) against *C. albicans* and strong inhibition percentage was also observed in *E. coli* with 0%, 20%, 40% and 60% hexane: petroleum ether extracts. This suggests that in using a low polarity solvent for extraction, a mixture of the solvents (hexane: petroleum ether) might be a better choice to extract antimicrobial compounds from fruit of *Momordica charantia* L. In addition, the 100% hexane extract from the fruit of *M. charantia* L. in this study also demonstrated a stronger antimicrobial activity against *E. coli* than the petroleum ether, methanol and ethanol, extracts of *Calendula officinalis* [20].

(**Table 3**) shows the antimicrobial activity for standard antibiotics, ampicillin (10 µg/disc) for bacteria and tetracycline (30 µg/disc) for fungus. From the result, it was found that *K. pneumoniae*, *Serratia* sp., *E. coli* and *C. albicans* were found to be resistant to the antibiotics tested. These resistances might be due to the mutation undergone by the microorganisms. On the other hand, the results obtained (**Table 2 & Fig 1**) showed that the extracts of hexane: petroleum

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ether from fruit of *Momordica chrantia* L. exhibited strong inhibition percentage for both *E. coli* and *C. albicans*. Thus, this study revealed the importance of hexane: petroleum ether extract from fruit of *Momordica charantia* L. as a potential natural antimicrobial agent. Further studies can be carried out to isolate and purify the active antimicrobial compounds present and subsequently, toxicity studies can be done to evaluate their safety.

Table 1: Percentage of inhibition (%) using different concentration of hexane: petroleum ether extracts against Gram-positive bacteria.

Hexane : Petroleum ether	Relative Percentage of Inhibition (%)			
	B. cereus	***B. subtilis***	***E. faecalis***	***S. aureus***
0%	105.7 ± 5.37	0.0 ± 0.00	45.2 ± 3.24	0.0 ± 0.00
20%	98.8 ± 2.92	0.0 ± 0.00	57.6 ± 1.69	0.0 ± 0.00
40%	105.9 ± 5.37	0.0 ± 0.00	62.2 ± 1.95	0.0 ± 0.00
60%	104.8 ± 5.83	0.0 ± 0.00	53.4 ± 2.32	0.0 ± 0.00
80%	103.6 ± 3.91	0.0 ± 0.00	56.0 ± 1.95	0.0 ± 0.00
100%	109.5 ± 3.69	0.0 ± 0.00	48.8 ± 4.22	0.0 ± 0.00
Average	104.7 ± 4.52	0.0 ± 0.00	53.9 ± 2.56	0.0 ± 0.00

Values are mean ± SD, where n = 3.

Table 2: Percentage of inhibition (%) using different concentration of hexane: petroleum ether extracts against Gram-negative bacteria.

Hexane : Petroleum ether	Relative Percentage of Inhibition (%)			
	E. coli	***K. pneumonia***	***P. aeruginosa***	***Serratia* sp.**
0%	108.3 ± 0.00	120.6 ± 5.34	0.0 ± 0.00	0.0 ± 0.00
20%	106.9 ± 3.40	116.7 ± 5.27	28.0 ± 1.42	0.0 ± 0.00
40%	105.6 ± 4.30	125.0 ± 5.27	31.0 ± 2.69	0.0 ± 0.00
60%	106.9 ± 3.40	119.4 ± 4.30	31.0 ± 1.35	0.0 ± 0.00
80%	0.0 ± 0.00	120.8 ± 4.56	30.2 ± 1.35	0.0 ± 0.00
100%	0.0 ± 0.00	122.2 ± 4.30	28.0 ± 1.42	0.0 ± 0.00
Average	71.3 ± 3.70	120.8 ± 4.84	24.7 ± 1.37	0.0 ± 0.00

Values are mean ± SD, where n = 3.

Table 3: Antimicrobial activity of standard antibiotics, ampicillin and tetracycline.

Microorganisms	Zone of inhibition (mm)	
	Ampicillin (10 µg/disc)	**Tetracycline (30 µg/disc)**
Gram-positive bacteria :		
B. cereus	7.0 ± 0.00	
B. subtilis	7.0 ± 0.00	nd
E. faecalis	16.0 ± 0.00	nd
S. aureus	22.0 ± 1.41	nd
		nd
Gram-negative bacteria :		
E. coli	-	nd
K. pneumonia	-	nd
P. aeruginosa	22.5 ± 0.71	nd
Serratia sp.	-	nd
Fungus :		
Candida albicans	nd	0.0 ± 0.00

Values are mean ± SD, where n = 3.
(-) indicates No zone of inhibition; 'nd'= Not tested

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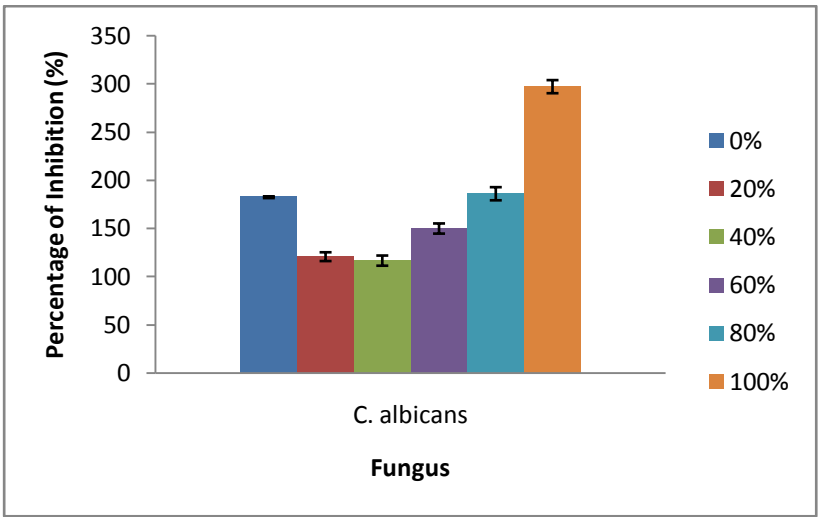
Fig 1.Antifungal activity (relative percentage inhibition) of the hexane : petroleum ether fruit extracts of *Momordica charantia* L. against *C. albicans*.



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