Tropical Life Sciences Research, 20(2), 109–118, 2009

SHORT COMMUNICATION

Preliminary Screening of Antibacterial Activity Using Crude Extracts of *Hibiscus rosa sinensis*

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Abstrak: *Hibiscus rosa sinensis* berasal daripada famili Malvaceae dan ditanam sebagai tumbuhan hiasan dengan luasnya di negara tropika, serta mempunyai pelbagai warna. Kegunaan tradisional bunga ini adalah untuk merawat sakit kepala, demam, kekejangan semasa haid, memudahkan dalam kelahiran anak, penawar bagi racun, ubat sakit mata serta merawat inflamasi. Kajian ini telah dilakukan untuk menilai aktiviti antibakteria melalui kaedah resapan agar. Ekstrak kasar petroleum eter, ekstrak kasar etil asetat dan ekstrak kasar metanol daripada daun, batang dan bunga telah diperolehi melalui kaedah rendaman sejuk. Kesemua ekstrak kasar pada kepekatan 4 mg/cakera hingga 0.017 mg/cakera telah diuji ke atas bakteria methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, *Eschericia coli*, *Pseudomonas aeruginosa* dan *Klebsiella pneumonia*. Ekstrak petroleum eter daripada batang, daun dan bunga serta ekstrak metanol daripada daun menunjukkan diameter zon perencatan > 12 mm terhadap MRSA. Secara keseluruhan, ekstrak petroleum eter daripada bunga pada kepekatan 4 mg/cakera dan 2 mg/cakera mempamerkan zon perencatan yang tertinggi iaitu 18.6 ± 2.85 mm dan 18.5 ± 0.29 mm masing-masing berbanding dengan vancomycin (30 µg/ml) yang tidak berbeza secara signifikan dari saiz zon perencatan vancomycin (30 µg/ml) ($p < 0.05$). Kesimpulannya, ekstrak *H. rosa sinensis* berpotensi sebagai agen antibakteria terhadap infeksi MRSA.

Kata kunci: *Hibiscus rosa sinensis*, Aktiviti Antibakteria, Zon Perencatan

Abstract: *Hibiscus rosa sinensis*, a member of the Malvaceae family, is widely cultivated in the tropics as an ornamental plant. It is often planted as a fence or hedge plant, and has several forms of flowers with varying colours. It is also used in traditional medicine to induce abortion, ease menstrual cramps, assist in childbirth and relieve headache, fever and inflammation. In this study, we evaluated the antibacterial activity of *H. rosa sinesis* extract using a disc diffusion method. Crude petroleum ether extract, ethyl acetate extract and methanol extract from the leaves, stems and flowers of the plant were prepared using a cold extraction technique. These extracts were tested at concentrations ranging from 4 mg/disc to 0.017 mg/disc against methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia.* The petroleum ether extract from the leaves, stems and flowers and methanol

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extract from the leaves showed inhibition zones with diameters > 12 mm against MRSA. Overall, the petroleum ether extract from flowers at concentrations of 4 mg/disc and 2 mg/disc displayed the strongest inhibition zones of 18.6 ± 2.85 mm and 18.5 ± 0.29 mm, respectively, as compared to vancomycin (30 µg/ml), which did not differ significantly from the 18.0 ± 0.10 mm size of the vancomycin (30 µg/ml) inhibition zone ($p < 0.05$). In conclusion, *H. rosa sinensis* extract is a potential antibacterial agent for treating MRSA infection.

Keywords: *Hibiscus rosa sinensis*, Antibacterial Activity, Inhibition Zone

Drugs from plants have played a dominant role in pharmaceutical care for the treatment of various diseases. In recent years, infections have been on the rise and antibiotic resistance has become a growing therapeutic problem (Austin *et al*. 1999). Microorganisms are the most important pathogens causing severe morbidity and fatal infections in humans. Natural products of higher plants may provide a new source of antimicrobial agents with potentially novel mechanisms of action (Nostro *et al*. 2000). Secondary metabolites from higher plants serve as defence agents against invading microorganisms (Fabry *et al*. 1998). The plants selected for evaluation as sources of antimicrobial agents are often those used in traditional medicine for the treatment of infectious and other diseases (Ahmad *et al*. 1998).

H. rosa sinensis is a glabrous shrub widely cultivated in the tropics as an ornamental plant. Previous studies have showed that *H. rosa sinensis* possesses many biological activities, such as anticomplementary, antidiarrhetic and antiphlogistic activity (Shimizu *et al*. 1993). It has also been reported that the plant's flower possesses antispermatogenic, androgenic (Sachdewa & Khemani 2003), antitumour and anticonvulsant properties; in addition, the leaves and flowers have been found to be hair growth promoters and aid in the healing of ulcers (Kurup *et al*. 1979).

The reported biological activities of *H. rosa sinensis* include antioestrogenic, anti-implantation, abortifacient, antipyretic, antispasmodic, hypotensive, embryotoxic, antispermatogenic, insect attractant, analgesic, antifungal and anti-inflammatory properties (Herbal Medicine Research Centre 2002). The objective of this study was to identify new potential plant antimicrobial agents from *Hibiscus* species that could be developed by the pharmaceutical industry, and also to promote the use of *Hibiscus* species in the treatment of various diseases.

Fresh *H. rosa sinensis* plants were collected in November 2003. A voucher specimen of the plant was identified and authenticated by the Herbal Unit at the Institute for Medical Research (IMR), Kuala Lumpur. The collected plants were dried in the oven (40°C), ground into a course powder and stored at –20°C. The dried, powdered leaves and stem were extracted using petroleum ether, ethyl acetate and methanol by cold extraction. The extract was filtered and concentrated to a small volume to remove all the solvent using a rotary evaporator at 40°C. The small volume was later dried and the gummy extract was kept in the freezer (–70°C).

A stock extract solution (200 mg/ml) was prepared by dissolving the extract in pure dimethylsulfoxide (DMSO) (Sigma, USA) and diluting it two times. This stock extract was sterilised and filtered using filter paper (0.2 µm) and stored in Eppendorf tubes at 4°C.

Test organisms were collected from the Bacteriology Unit of IMR. The organisms used in this research were MRSA, *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumonia*. Upon receipt, all isolates were subcultured onto selected culturing media to ensure purity and confirm their identification. The strains were maintained and tested on Mueller Hinton Agar (MHA) (Merck, Germany), which was stored at 4°C. The test organisms were cultured overnight at 37°C before being used in the antibacterial assay described below.

The crude extracts of *H. rosa sinensis* were tested for antimicrobial activity using the disc diffusion method (Kirby-Bauer method) (Bauer *et al.* 1966). Sterile commercial blank discs (Oxoid), 6.0 mm diameter, were impregnated with different dilutions of the extracts ranging from 4 mg/disc to 0.008 mg/disc. Discs were stored at –5°C prior to use. Overnight broth cultures were adjusted using a turbidometer to yield approximately 1.0 X 10^8 cfu per ml. Extract-impregnated discs (20 µl) were placed on agar plates and incubated at 37°C for 24 hours. Pure DMSO (20 µl) was used as a negative control, while vancomycin discs (30 µl) were used as a positive control. Antibacterial activities were then determined by measuring the clear zone of inhibition to the nearest millimetre (mm) ± S.E.M.

An *in vitro* test for antibacterial activity revealed that petroleum ether extract from the *rosa sinensis* flower inhibited most of the growth of MRSA, with an inhibition zone of 18.6 ± 2.85 mm at a concentration of 4 mg/disc (Table 1), and 18.5 ± 0.29 mm at 2 mg/disc (Fig. 1). The petroleum ether extracts from stems and leaves at a concentration of 4 mg/disc showed inhibition zones of 17.9 ± 0.06 mm and 12.3 ± 0.58 mm, respectively as shown in Figures 2 and 3. The ethyl acetate extract from stems showed weak inhibition zones of 7.2 ± 0.9 mm and 7.2 ± 0.56 mm at concentrations of 1 mg/disc and 0.5 mg/disc, respectively.

Petroleum ether extracts from flowers also inhibited the growth of *S. aureus* at concentrations ranging from 4 mg/disc to 0.25 mg/disc, with diameters of 15.4 mm, 14.2 mm, 13.2 mm, 11.4 mm and 11.2 mm (Table 2). The growth of *E. coli*, *P. aeruginosa* and *K. pneumonia* was not inhibited by any of the extracts from *H. rosa sinensis,* as there were no inhibition zones observed (data not shown). The DMSO negative control also showed no inhibitory effect, while the positive control (vancomycin) showed inhibition diameters ranging from 16.0 to 22.0 mm.

Table 1: Inhibition zones (mm ± S.E.M) of crude extracts of *H. rosa sinensis* against MRSA.

Solvent	Parts of extracts	Concentration of crude extracts of *H. rosa sinensis* against MRSA (mg/disc)									
		4	2	1	0.5	0.25	0.125	0.063	0.031	0.017	0.008
		Inhibition zone (mm)									
Petroleum ether	Stem	17.9 ± 0.06	17.8 ± 0.12	17.4 ± 0.42	16.7 ± 0.56	16.3 ± 0.76	15.0 ± 0.29	12.8 ± 0.79	9.44 ± 1.56	9.12 ± 0.65	8.68 ± 0.43
	Leaves	12.3 ± 0.58	11.8 ± 0.95	11.1 ± 0.46	10.2 ± 1.15	9.06 ± 0.72	0	0	0	0	0
	Flower	18.6 ± 2.85	18.5 ± 0.29	17.8 ± 0.2	17.6 ± 0.61	17.4 ± 0.4	13.1 ± 0.1	11.7 ± 0.5	9.90 ± 0.3	0	0

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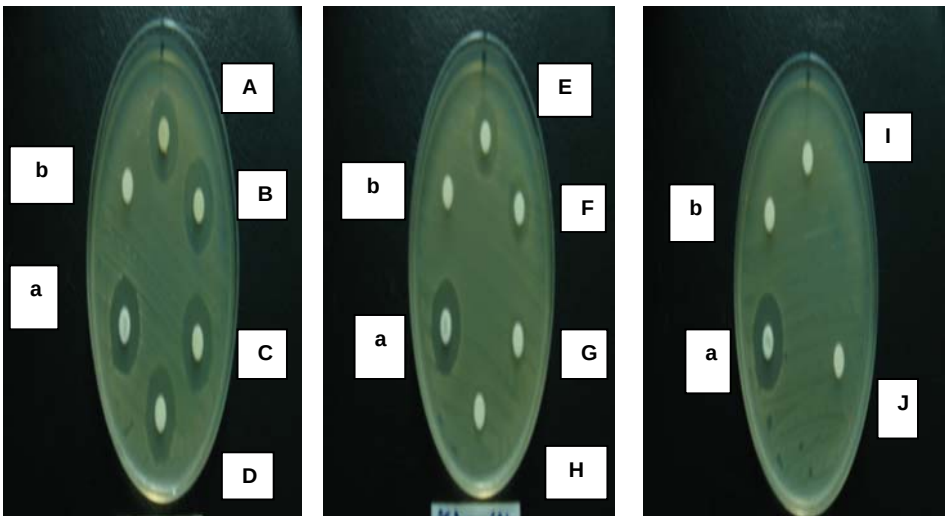
Table 1: *(continued)*

Solvent	Parts of extracts	Concentration of crude extracts of *H. rosa sinensis* against MRSA (mg/disc)									
		4	2	1	0.5	0.25	0.125	0.063	0.031	0.017	0.008
		Inhibition zone (mm)									
Ethyl acetate	Stem	11.8 ± 0.31	9.4 ± 0.31	7.2 ± 0.9	7.2 ± 0.56	0	0	0	0	0	0
	Leaves	0	0	0	0	0	0	0	0	0	0
	Flower	0	0	0	0	0	0	0	0	0	0
Methanol	Stem	0	0	0	0	0	0	0	0	0	0
	Leaves	12.8 ± 0.95	11.0 ± 0.58	0	0	0	0	0	0	0	0
	Flower	11.7 ± 0.2	0	0	0	0	0	0	0	0	0
Vancomycin (30 µg/ml)		16.0–22.0									

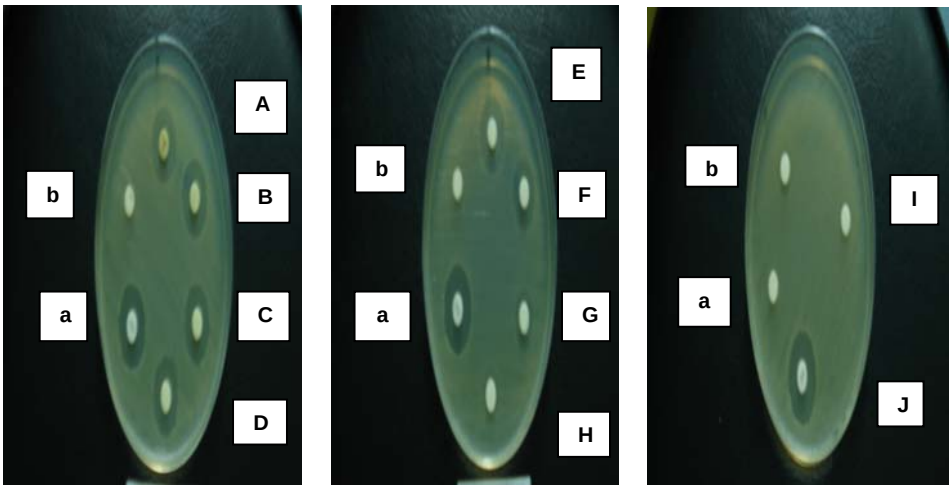
Table 2: Inhibition zones (mm) of crude extracts of *H. rosa sinensis* against *S. aureus.*

Solvent	Parts of extracts	Concentration of crude extracts of *H. rosa sinensis* against *S. aureus* (mg/disc)									
		4	2	1	0.5	0.25	0.125	0.063	0.031	0.017	0.008
		Inhibition zone (mm)									
Petroleum Ether	Stem	11.0	10.5	9.1	8.4	0	0	0	0	0	0
	Leaves	0	0	0	0	0	0	0	0	0	0
	Flower	15.4	14.2	13.2	11.4	11.2	0	0	0	0	0
Ethyl acetate	Stem	0	0	0	0	0	0	0	0	0	0
	Leaves	0	0	0	0	0	0	0	0	0	0
	Flower	0	0	0	0	0	0	0	0	0	0
Methanol	Stem	0	0	0	0	0	0	0	0	0	0
	Leaves	0	0	0	0	0	0	0	0	0	0
	Flower	0	0	0	0	0	0	0	0	0	0
Vancomycin (30 µg/ml)		16.0–22.0									



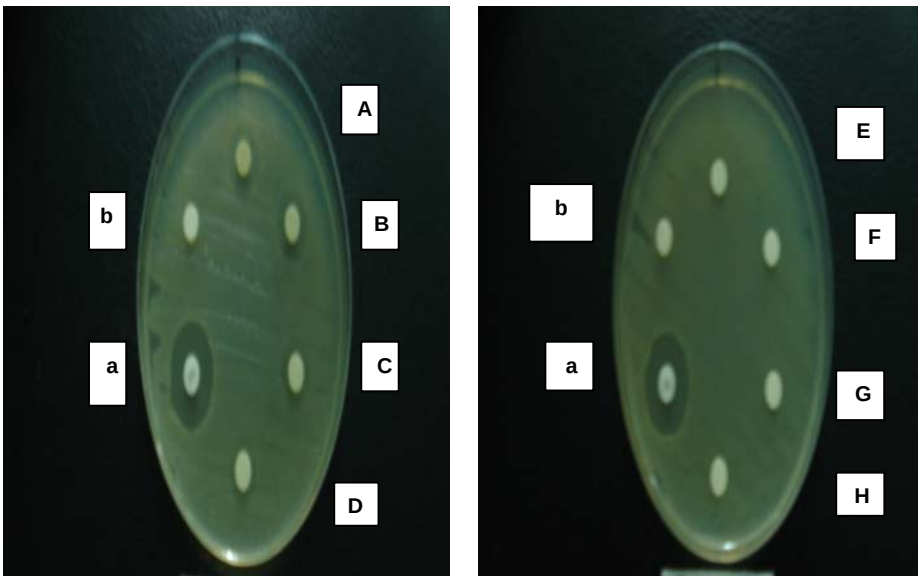
(a) (b) (c)

Figure 1: Inhibition zone against MRSA using petroleum ether extract from flowers (A = 4 mg/disc, B = 2 mg/disc, C = 1 mg/disc, D = 0.5 mg/disc, E = 0.25 mg/disc, F = 0.125 mg/disc, G = 0.063 mg/disc, H = 0.031 mg/disc, I = 0.017 mg/disc, J = 0.008 mg/disc, a = vancomycin 30 µg/ml and b = DMSO).



(a) (b) (c)

Figure 2: Inhibition zone against MRSA using petroleum ether extract from stems (A = 4 mg/disc, B = 2 mg/disc, C = 1 mg/disc, D = 0.5 mg/disc, E = 0.25 mg/disc, F = 0.125 mg/disc, G = 0.063 mg/disc, H = 0.031 mg/disc, I = 0.017 mg/disc, J = 0.008 mg/disc, a = vancomycin 30 µg/ml and b = DMSO).



(a) (b)

Figure 3: Inhibition zone against MRSA using petroleum ether extract from leaves (A = 4 mg/disc, B = 2 mg/disc, C = 1 mg/disc, D = 0.5 mg/disc, E = 0.25 mg/disc, F = 0.125 mg/disc, G = 0.063 mg/disc, H = 0.031 mg/disc, a = vancomycin 30 µg/ml and b = DMSO).

According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The medicinal value of plants lies in certain chemical substances that produce a definite physiological action on the human body. Disc diffusion methods are extensively used to investigate the antibacterial activities of natural antimicrobial substances and plant extracts. These assays are based on the use of discs as reservoirs containing the solution of substances to be examined (Bartner *et al.* 1994).

In this study, different microorganisms were used to screen the possible antimicrobial activities of *H. rosa sinensis* extracts. Petroleum ether extracts from flowers showed a promising activity against MRSA. There is great concern in the health care community about the rapid rise in resistance of *S. aureus* to antimicrobial agents. MRSA has emerged as an important endemic pathogen in hospitals. Due to the heterogeneous nature of methicillin resistance (Alborzi *et al*. 2000), the accurate detection of MRSA isolates also poses a major problem for most clinical microbiology laboratories. MRSA isolates are Gram-positive bacteria, which contain two layers of membranes: the outer layers, which are composed of peptidoglycan, and the inner cytoplasmic layer, which is easily penetrated (Nor Azfa 2002). New treatments are needed for this resistant strain of bacteria, and active compounds from natural products could be the answer.

Different solvents can be used for the extraction of plant compounds based on polarity. Even though petroleum ether is a nonpolar solvent, it showed a good inhibition zone against MRSA, likely because it extracted antimicrobial compounds from the plant. According to Veeramuthu *et al.* (2006), antimicrobial effects can also be seen using medicinal plants extracted with hexane and methanol. Indeed, extracts in their study prepared with hexane, a nonpolar solvent, contained some antimicrobial compounds that could inhibit MRSA. Similarly, this study also showed some antimicrobial activity, where we used a polar solvent, but the inhibition zone was less than that observed with the nonpolar solvent.

In conclusion, petroleum ether extracts from *H. rosa sinensis* flowers showed promising antimicrobial activity by inhibiting the growth of MRSA. Further studies should be carried out to extract the fractions of the plants that inhibit bacterial activity and, through bioassay guided fractionation, isolate the antimicrobial agent. The fractions that inhibit bacterial activity should then also be studied using Nuclear Magnetic Resonance (NMR).

ACKNOWLEDGEMENT

We would like to thank the Institute for Medical Research (IMR), Kuala Lumpur, Malaysia and Universiti Kebangsaan Malaysia (UKM), Kuala Lumpur for providing the grant to carry out this project. We also want to extend our gratitude to the Bacteriology Unit and Herbal Medicine Unit, IMR for providing test organisms for analysis and technical assistance.

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