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Evaluation of antimicrobial activities of the crude extracts from *Garcinia atroviridis* and *Solanum torvum*

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ABSTRACT Crude ethyl acetate (EtOAc) and ethanol (EtOH) extracts from the fruits of *Garcinia atroviridis* and *Solanum torvum* were screened for their antimicrobial activities against 7 bacterial strains; 3 Gram-positive (*Staphylococcus aureus* ATCC 25923, *S. epidermidis* and *Bacillus subtilis*) and 4 Gram-negative bacteria (*Escherichia coli* O157:H7, *Salmonella typhimurium* NCTC 74, *S. enteritidis* NCTC 5188 and *Pseudomonas aeroginosa* ATCC 27853) and 2 yeast strains (*Candida glabrata* and *C. parapsilosis*). Blank discs of 6 mm diameter were loaded with 10 mg/ml of the extracts applied to the inoculated plates. The results showed that the EtOAc and EtOH extracts from *G. atrovoridis* are active against all the microorganisms studied. Strongest inhibition zones were observed from EtOAc extract of *Garcinia atroviridis* against *Staphylococcus epidermidis* (25.27 ± 1.30 mm) and *S. aureus* (23.87 ± 1.10 mm) compared to positive control (Gentamycin at 10 μg/disc) with inhibition zones of 20.7 mm and 17.8 mm, respectively. Both the crude extracts from *S. torvum* displayed weak inhibitory effect against all Gram-positive bacteria tested. Among all the microorganisms in the study, *S. enteritidis* NCTC 5188, *C. glabrata* and *C. parapsilosis* were resistant towards the extracts from *S. torvum.*

ABSTRAK Ekstrak kasar etil asetat (EtOAc) dan etanol (EtOH) daripada buah *Garcinia atroviridis* dan *Solanum torvum* disaring untuk aktiviti antimikrob ke atas 7 strain bakteria : 3 bakteria Gram-positif (*Staphylococcus aureus* ATCC 25923, *S. epidermidis* dan *Bacillus subtilis*) dan 4 bakteria Gram-negatif (*Escherichia coli* O157:H7, *Salmonella typhimurium* NCTC 74, *S. enteritidis* NCTC 5188 dan *Pseudomonas aeroginosa* ATCC 27853) dan 2 strain yis (*Candida glabrata* dan *C. parapsilosis*). Disk kosong berukuran 6 mm diameter yang ditepukan dengan 10 mg/ml ekstrak diletakkan pada piring yang telah diinokulasi. Hasil menunjukkan bahawa ekstrak etanol dan etil asetat daripada *G. Atroviridis* didapati berupaya merencat pertumbuhan semua mikroorganisma dalam kajian. Antara mikroorganisma yang didapati rentan oleh ekstrak etil asetat daripada *G. atroviridis* adalah *Staphylococcus epidermidis* (25.27 ± 1.30 mm) dan *S. aureus* (23.87 ± 1.10 mm) berbanding kawalan positif (Gentamycin pada 10 μg/disk) dengan nilai zon perencatan iaitu masing-masing, 20.7 mm dan 17.8 mm. Ekstrak kasar etil asetat dan etanol daripada *S. torvum* sekadar mempamerkan kesan perencatan yang lemah ke atas bakteria Gram-positif yang diuji. Antara semua miroorganisma dalam kajian, *S. enteritidis* NCTC 5188, *C. glabrata* dan *C. parapsilosis* didapati resistan terhadap ekstrak *S. torvum.*

Keywords : *Garcinia atroviridis*; *Solanum torvum*; Antimicrobial; Disc diffusion assay

INTRODUCTION

Garcinia atroviridis is classified under the genus *Garcinia* and family *Guttiferae* [1,2]. It is a medium sized tree, wildly distributed in Peninsular Malaysia. Sun-dried slices of the fruits, known as 'asam keping' are commercially available and are used popularly as a seasoning in curries, sour relish and also for dressing fish [3,4]. The young leaves are also being used for culinary purposes and as a traditional vegetable [5]. In folklore medicine, *G. atroviridis* is used as post-partum medications and for treating diseases such as earache, throat irritation, cough, dandruff and stomach pains associated with pregnancy [6,7]. Throughout evaluations of antimicrobial, antinematodal, antitumor promoter and antiviral properties of *G. atroviridis*, it is proven that only antibacterial activity is found to be significant [8,9,10,11]. The occurrence of xanthones, biflavonoids and benzophenones are common in *Garcinia* genus, while isolation of atroviridin has been reported [12].

Solanum torvum, which can be found along roadsides, grows wildly especially in Peninsular Malaysia. Classified under the genus *Solanum* and family *Solanaceae* [13], it is used commonly for dishes and traditional medicine. This species, which is also known as turkey berry, is claimed to have anti-infective agent. *S. torvum* contains high amount of vitamin C and occasionally being used for antinematodal and antihypertension. Research on the therapeutic potential of *Solanum torvum* had been established [14,15]. The root from *S. torvum* has been reported to possess antinematodal activity against *Meloidogyne incognita* [16]. In another study, isolation of isoflavonoid sulphate and steroidal glycosides from *S. torvum* as an antiviral agent has been extensively evaluated [17]. Ajaiyeoba [18] compared the tannin content between the leaves of *S. torvum* and *S. macrocarpum* as antimicrobial agent and reported that high amount of tannin were found in *S. torvum* leaves. Chah *et al* [19] demonstrated that methanolic extract of *S. torvum* fruit showed interesting growth-inhibiting activity against bacteria commonly associated with pyogenic infections.

The present paper investigates the potential of the crude extracts *from Garcinia atroviridis* and *Solanum torvum* in the preliminary screening as antimicrobial agents.

MATERIALS AND METHODS

Plant Material

Garcinia atroviridis were collected in Taiping, Perak whereas *Solanum torvum* were collected in Puchong, Selangor. Both specimens were identified and deposited at the Herbarium Universiti Kebangsaan Malaysia. The fruits were cut to small pieces, dried and ground into powdered material.

Extraction of *G. atroviridis* and *S. torvum*

The powder of the fruits of *G.atroviridis* and *S. torvum* were soaked successively in two different solvents of different polarity namely, ethyl acetate (Searle Company Hopkin & Williams) followed by ethanol (Chemical Industries, Malaysia) each for three days at room temperature. Each of the mixtures were then filtered and evaporated using a rotary evaporator (Buchi rotavapor R-114) at 45°C under reduced pressure to evaporate the solvent from the extracts. The resulting pellet was then subjected under a vacuum in a freeze-dryer (Heto LyoLab 3000) and the dry powdery extract was kept at 4°C in an air-tight jar prior to bioassay activity.

Preparations for extract solutions

The extracts were dissolved in Phosphate Buffer Saline (PBS) solution to a final concentration of 10 mg/ml and sterilized by passing through a 0.45 μm membrane filter (UNIFLO 25/0, 45RC Dassel, Germany).

Microorganisms

Test bacterial strains used (*Staphylococcus aureus* ATCC 25923, *Pseudomonas aeroginosa* ATCC 27853; local clinical isolates of *Staphylococcus epidermidis* and *Bacillus subtilis*) and the yeast strains used (*Candida glabrata* and *Candida parapsilosis)* were obtained from the laboratory stock of the Department of Biomedical Science, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia. Three bacterial strains *Escherichia coli* (NCTC 12079 serotype O157:H7), *Salmonella typhimurium* NCTC 74 and *Salmonella enteritidis* NCTC 5188 were kindly supplied by Dr.

Noraziah Mohamed Zin from the Department of Biomedical Science, Faculty of Allied Health Sciences, UKM. All the bacterial strains were maintained on nutrient agar slants (MERCK, Germany) and the yeast strains on Sabouraud Dextrose agar slants (Beeton-Dickinson and Company, USA).

Preparation of inoculum

The concentration of the cultures were adjusted turbidometrically to a wavelength of 620 nm to 10^8 colony forming units (CFU) per ml. Approximately, 4-5 colonies from agar slants were subcultured onto plates and left overnight in the incubator. The colonies from subcultured agar were then inoculated into the tryptic soy broth (Beeton-Dickinson and Company, USA) for the bacteria and Sabouraud dextrose broth (MERCK, Germany) for the yeast strains. The spectrophotometer (UV-160A Visible Recording Spectrophotometer) was used to standardized the size of the bacteria and yeast inoculums. The inoculum size of each test strain was standardized by adjusting the optical density of the bacterial suspension to a turbidity corresponding to about absorbance = 0.08 (OD_{620} = 0.08) at 620 nm using spectrophotometer.

Antimicrobial assay

An even spread of microorganism was prepared by transferring 50 µl of microbial suspension to Mueller-Hinton agar plates (MERCK, Germany) for bacteria and Sabouraud dextrose agar plates for yeast. Sterile cotton wool swab was used to spread the inoculum over the entire surface of the plates. 10 mg/ml of each extract was loaded onto Whatman No. 1 filter paper discs and placed on the previously inoculated agar. Gentamycin and Nystatin (both at 10 µg/disc) were used as positive control and PBS solution as negative control. Each extract was tested in triplicates. The plates were then incubated at 37°C for 24 hrs. The assessment of antimicrobial activity was determined by measuring the diameter of a clear inhibition zone around the disc using a vernier caliper. The mean diameter of inhibition zone was measured to the nearest millimeter (mm). The results were expressed as mean ± S. D.

RESULTS AND DISCUSSION

The results of the ethyl acetate (EtOAc) and ethanol (EtOH) extracts from *Garcinia atroviridis* and *Solanum torvum* for antimicrobial activity are presented in Table 1. Figure 1 showed the antibacterial activity of both the extracts from *G. atroviridis* and *S. torvum* whereas Figure 2 illustrated the effect of both extracts from *G. atroviridis* against 2 yeast strains. The EtOAc and EtOH extracts from *S. torvum* did not inhibit the growth of the yeasts studied.

As determined from disc diffusion assays, both the EtOAc and EtOH extracts from *Garcinia atroviridis* and *Solanum torvum* showed antimicrobial activity against all the Gram-positive bacteria (*Staphylococcus aureus ATCC* 25923, *S. epidermidis* and *Bacillus subtilis*) tested. As far as the Gram-negative organisms (*Escherichia coli* O157:H7, *Salmonella typhimurium* NCTC 74, *S. enteritidis* NCTC 5188 and *Pseudomonas aeroginosa* ATCC 27853) and yeasts (*Candida glabrata* and *Candida parapsilosis)* are concerned, only the EtOAc and EtOH extracts from *G. atroviridis* displayed potential activity against these microorganisms. However, the antimicrobial activity of the EtOAc extract from *G. atroviridis* was much stronger than those of the EtOH extract from *G. atroviridis*. It seems likely that the active compound in *G. atroviridis* is probably more soluble in EtOAc than in EtOH. The most susceptible microorganisms towards the EtOAc extract from *G. atroviridis* was *Staphylococcus epidermidis* with a maximum inhibitory zone of 25.27 ± 1.30 mm followed by *S. aureus* (23.87 ± 1.10 mm) (Table 1). Interestingly, the effect of the extract against these two strains of Gram-positive organisms are even higher compared to the positive control used (Gentamycin at 10 µg/disc) with an inhibition zone of 20.7 mm and 17.8 mm, respectively (Figure 1).

The EtOH extract from *G. atroviridis* displayed moderate activity with inhibition diameters ranging from 13.00 ± 1.00 mm to 17.40 ± 0.56 mm against all the microorganisms tested except for *Candida glabrata* which is weakly inhibited by the extract (Table 1). EtOAc extract from *G. atroviridis* appeared to exhibit a broad antibacterial spectrum to both Gram-positive bacteria and Gram-negative bacteria studied.

The strong antimicrobial activity of the extracts from *G. atroviridis* may be attributed to the presence of phenylpropanes and related metabolites that have been implicated for this property in other species of *Garcinia* [16]. In fact, *G. atroviridis* has proven to contain flavonoids as one of its components [17]. However, further work to confirm the presence of flavonoids in the fraction used in our study are underway. It is known that flavonoids are synthesized by plants in response to microbial infections [20] and as such, it is of no surprise that plants are effective sources of antimicrobial substances. It has been postulated that some *Garcinia* benzophenone derivatives exert their antimicrobial effect through their ability to complex with bacterial cell walls [21].

The EtOAc and EtOH extracts from *Solanum torvum* showed weak inhibitory effect against all the Gram-positive bacteria tested and only 2 Gram-negative organisms; *Salmonella typhimurium* NCTC 74 and *Pseudomonas aeroginosa* ATCC 27853 (Table 1). It is noteworthy that Gram-negative organisms are in general terms, more resistant than Gram-positive ones to most antimicrobial agents from plant sources [22]. Among the most resistant microorganisms to the effects of EtOAc and EtOH extracts from *S. torvum* are *Salmonella enteritidis* NCTC 5188 and the two yeasts strains tested, *Candida glabrata* and *C. parapsilosis*. This is in contradiction with [19] in which the methanol extract from *S. torvum* exhibited an interesting growth-inhibiting activity against the bacteria and yeast. Among the classes of compounds isolated from *S. torvum* are steroidal alkaloids, saponins and tannins [14]. Tannins were found to be an excellent source for antimicrobial compound [23,24]. It is therefore, postulated that tannins in *S. torvum* is responsible for the antibacterial activity. Condensed tannins have been determined to bind cell walls of ruminal bacteria, preventing growth and protease activity [25].

CONCLUSION

Our findings suggest that the ethyl acetate from *G. atroviridis* generally exhibits a high degree of antibacterial activity and this seems to confirm the therapeutic claims of these traditional plants, especially in its uses as folklore medicines in treatment of earache, throat irritation and cough. However, further studies in view of elucidating the composition of its active metabolites are worth looking into, in order to confirm its antimicrobial properties.

Table 1. The mean diameter of inhibitory zone (mm ± S.D.) of ethyl acetate and ethanol extracts from *G. atroviridis* and *S. torvum* at 10 mg/ml against seven bacterial strains and two yeast strains. The positive control was Gentamycin (10 µg/disc) for bacteria and Nystatin (10 µg/disc) for yeast strains. The negative (–) sign indicates no inhibition.

Microorganisms	*Garcinia atroviridis*		*Solanum torvum*		Positive control
	EtOAc	EtOH	EtOAc	EtOH	
Staphylococcus aureus (ATCC 25923)	23.87 ± 1.10	14.71 ± 0.63	8.03 ± 0.19	10.01 ± 1.00	17.8
Staphylococcus epidermidis	25.27 ± 1.30	16.17 ± 1.46	7.40 ± 0.53	6.47 ± 0.31	20.7
Bacillus subtilis	17.50 ± 0.98	14.40 ± 1.45	8.50 ± 0.10	7.33 ± 0.58	19.4
Escherichia coli (0157:H7)	20.83 ± 0.54	14.50 ± 1.31	-	8.21 ± 0.54	20.6
Salmonella typhimurium (NCTC 74)	21.23 ± 1.57	16.32 ± 0.85	7.59 ± 0.36	6.67 ± 0.42	22.8
Salmonella enteritidis (NCTC 5188)	14.97 ± 0.91	13.00 ± 1.00	-	-	16.8
Pseudomonas aeroginosa (ATCC 27853)	19.61 ± 0.70	17.40 ± 0.56	12.20 ± 1.35	6.67 ± 0.58	19.7
Candida glabrata	11.87 ± 0.76	7.80 ± 0.20	-	-	24.5
Candida parapsilosis	15.33 ± 0.78	15.73 ± 1.15	-	-	17.0

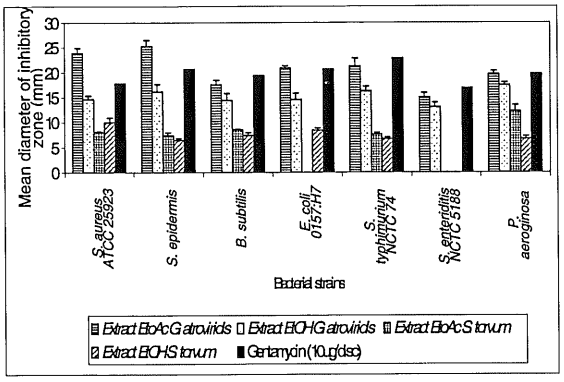

Figure 1. The mean diameter of inhibitory zone (mm ± S.D) of ethyl acetate (EtOAc) and ethanol (EtOH) extracts from *Garcinia atroviridis* and *Solanum torvum* at 1 mg/ml against seven bacterial strains. The positive control was Gentamycin (10 µg/ml).

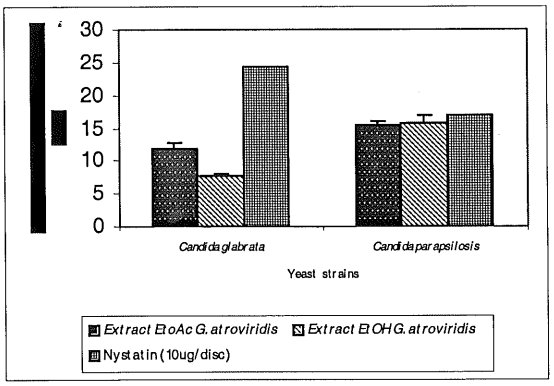

Figure 2. The mean diameter of inhibitory zone (mm ± S.D) of ethyl acetate (EtoAc) and ethanol (EtOH) extracts from *Garcinia atroviridis* at 1 mg/ml against two yeast strains. The positive control was Nystatin (10 µg/ml).

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