

Characterization of antimicrobial, antioxidant, anticancer properties and chemical composition of *Sauropus androgynus* stem extract

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Background. This study describes antimicrobial, anticancer and antioxidant properties of *Sauropus androgynus* stem extract. The main objective of the present study was to reveal the potential of *S. androgynus* stem to be used as a medicinal drug.

Materials and methods. The antimicrobial properties of *S. androgynus* stem extract against *Aeromonas hydrophila*, *Escherichia coli*, *Edwardsiella tarda*, *Flavobacterium* sp., *Klebsiella* sp., *Salmonella* sp., *Vibrio alginolyticus*, *V. parahaemolyticus*, *V. cholerae* and *Pseudomonas aeruginosa* were revealed by using the broth micro-dilution method, whereas the anticancer effects of the extract was determined with a colorimetric MTT (tetrazolium) assay against human breast adenocarcinoma (MCF-7). The antioxidant activity of the plant extract was characterized also by using a α , α -diphenyl- β -picrylhydrazyl (DPPH) radical scavenging method. Finally, chemical compounds of the plant extract were screened and identified by using gas chromatography – mass spectrometry (GC–MS).

Results. The minimum inhibitory concentration (MIC) values ranged from 7.81 to 62.5 mg/l in which the plant extract was found to inhibit the growth of *Edwardsiella tarda*, *Escherichia coli*, *Flavobacterium* sp., *Pseudomonas aeruginosa* and *Vibrio cholerae* at 7.81 mg/l, *Klebsiella* sp., *Aeromonas hydrophila* and *Vibrio alginolyticus* at 15.6 mg/l, and it was able to control the growth of *Salmonella* sp. and *Vibrio parahaemolyticus* at 62.5 mg/l. The results of the present study has shown *S. androgynus* stem extract to possess a high antimicrobial activity and a moderate antioxidant activity (inhibition concentration 50%; IC₅₀ of DPPH is 8 ppt), but no anticancer activity. A total of 34 compounds were identified in the plant extract in which the major compounds were 9, 12, 15-octadecatrienoic acid, methyl ester, (Z, Z, Z)- (14.48%) and phytol (13.08%).

Conclusions. *S. androgynus* stem extract can be used as an antimicrobial and antioxidant agents.

Key words: antimicrobial, antioxidant, anticancer, chemical compound, *Sauropus androgynus* stem

INTRODUCTION

Sauropus androgynus is a member of the family Phyllanthaceae. This plant is spread in India, Sri Lanka, Thailand,

Laos, Malaysia, Indonesia and almost in all countries of Southeast Asia. In Malaysia, it is widely used in cooking and treated as a weed. However, in Thailand, this plant was commercially cultivated for medicine use (1). Different parts of this plant have different usefulness. For instance, its leaves and roots can be used to relieve fever and urinary problems, whereas the juice of *S. androgynus* leaves can serve as a remedy for earache (1). In Taiwan, *S. androgynus* has been used as a slimming agent since

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1994. However, this plant was reported to cause constrictive bronchiolitis obliterans after consuming it (2). Another study reported that overconsumption of *S. androgynus* leaves may lead to drowsiness and constipation due to the alkaloid compound papaverine present in *S. androgynus* leaves (3, 4). Although there are a few studies that have documented the medicinal properties of *S. androgynus* reported in the literature, studies of the antimicrobial, antioxidant and anticancer properties of *S. androgynus* are still rare. Therefore, in this study we evaluated its medicinal potential in terms of antimicrobial, antioxidant and anticancer effects on human health.

MATERIALS AND METHODS

Plant material

The plant preparation method was as described elsewhere (5). The plant sample was purchased from a herbal nursery located at Pasir Puteh, Kelantan, Malaysia. The fresh plant sample was oven-dried at 37 °C for 4 days. Next, the plant sample was freeze-dried prior to extraction with 70% methanol and concentrated at 1 g/ml. Finally, the plant extraction was kept at -20 °C until further use.

Bacterial isolates

All bacterial isolates, namely *Aeromonas hydrophila*, *Escherichia coli*, *Edwardsiella tarda*, *Flavobacterium* sp., *Klebsiella* sp., *Salmonella* sp., *Vibrio alginolyticus*, *V. parahaemolyticus*, *V. cholerae* and *Pseudomonas aeruginosa*, were provided by Universiti Malaysia Kelantan. These bacteria were isolated from various aquatic animals and kept in tryptic soy agar (TSA) for further uses. These isolates were selected due to their pathogenic activity against human blood cells.

Determination of minimum inhibitory concentration (MIC)

The values of minimum inhibitory concentration (MIC) of *S. androgynus* stem extract against bacterial isolates were determined through a two-fold broth microdilution method as described in (5-7). The bacterial isolates were cultured in tryptic soy broth for 24 h at room temperature, and the concentration of these cultures was adjusted to 10⁹ CFU mL⁻¹ by using physiological saline. The concentration was cross-checked with a biophotometer (Eppendorf, Germany). The bacterial suspensions were then inoculated into a microtiter plate which contained a serial dilution of *S. androgynus* stem extract. The microplate was then incubated at room temperature for 24 h. The MIC values were defined as the lowest concentration of the *S. androgynus* stem extract in the wells of the microtiter plate that showed no visible turbidity after 24 h incubation. Methanol solvent was used as a negative control.

Determination of antioxidant activity by the α, α -diphenyl- β -picrylhydrazyl (DPPH) radical scavenging method

The DPPH radical scavenging method was used as described in other studies (8-11), with some modifications. The assay was carried in a 96-well ELISA plate with three replicates; 5 μ l of the sample (0.5 mg/ml) solution was added into the well, followed by 200 μ l of DPPH. The absorbance of the sample was recorded with an ELISA reader for each 6 s. The percentage inhibition of the DPPH radical was calculated based on the absorbance. Methanol solvent was used as a negative control.

Cancer cell lines

The human breast adenocarcinoma (MCF-7) cell line was derived from Institute of Marine Biotechnology, Universiti Malaysia Terengganu. All the cells were grown in a standard cell medium (RPMI 1640) supplemented with 5% fetal bovine serum in a 5% CO₂ atmosphere. The cells were then transferred into a microplate at a concentration of 1 \times 10² cells per well for the plant extract cytotoxicity test. At 48 h, proliferation was measured by the MTT colorimetric assay. Methanol solvent was used as a negative control. The IC₅₀ value was calculated from the following formula as described in (12):

$$\log_{10}(IC_{50}) = \frac{\log_{10} C_L (I_H - 50) + \log_{10} C_H (50 - I_L)}{I_H - I_L}$$

$$IC_{50} = 10^{\log_{10}(IC_{50})}$$

where I_H: 1% above 50% I_L: 1% below 50%, C_H: high drug concentration, C_L: low drug concentration.

Colorimetric MTT (tetrazolium) assay

Colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma, USA) assay was carried out as described in (13); 10 μ l of MTT solution (5 mg/ml) was added to all wells of a 96-well microplate, followed by 4 h of incubation at 37 °C. Acid isopropanol was added to all wells to dissolve the dark-blue crystals. The microplate was then read on an ELISA reader at a wavelength 570 nm within 1 h after adding isopropanol.

Bioactive compound characterization

The chromatographic procedure was carried out using a Shimadzu QP2010-GC-MS with an autosampler. The sample was diluted 25 times with acetone, and 1 μ l of sample was injected into a column. A fused silica capillary column HP5-MS (30 m \times 0.32 mm, film thickness 0.25 μ m) was used. Helium was the carrier gas, and a split ratio of 1 / 100 was used. The oven temperature was maintained at 60 °C for 8 min. The temperature was then gradually raised at a rate of 3 °C per min to 180 °C and maintained at 180 °C for 5 min. The temperature at the injection port was 250 °C. The components of the test solution were identified by comparing the spectra with those of known compounds stored in the internal library.

RESULTS

The MIC values ranged from 7.81 to 62.5 mg/l in which the plant extract was found to inhibit the growth of *Edwardsiella tarda*, *Escherichia coli*, *Flavobacterium* sp., *Pseudomonas aeruginosa* and *Vibrio cholerae* at 7.81 mg/l, *Klebsiella* sp., *Aeromonas hydrophila* and *Vibrio alginolyticus* at 15.6 mg/l and was able to control the growth of *Salmonella* sp. and *Vibrio parahaemolyticus* at 62.5 mg/l (Table 1). The 50% inhibition concentration (IC₅₀) of the DPPH of the plant extract was recorded as 8.34 ± 0.32 ppt, whereas the plant extract failed to show anticancer activity. A total of 34 compounds were identified in the plant extract (Table 2). The major compounds in the plant extract that were detected in the present study were 9, 12, 15-octadecatrienoic acid, methyl ester, (Z, Z, Z)- (14.48%) and phytol (13.08%). They are followed by glycerin (2.52%), 1-methyl-2-pyrrolidineethanol (2.27%), acetic acid (1.81%), pent-1-en-3-one, 1-(2-furyl)-5-dimethylamino (1.69%), benzofuran, 2, 3-dihydro- (1.65%), 2-acetylpyrrolidine (1.51%), 4-O-methylmannose (1.46%), N-ethyl-2-carbomethoxyazetidine (1.43%), 9-ethoxy-10-oxatricyclo [7.2.1.0 (1, 6)] dodecan-11-one, (1.36%), 1H-indole, 5-fluoro- (1.30%), hexadecanoic acid (1.18%), oleic acid (1.18%), heptaethylene glycol (1.12%), monododecyl ether N, N-dimethyl-2-aminoethanol (1.05%), 2-methoxy-4-vinylphenol (0.97%), L-phenylalanine (0.95%), pentaethylene glycol (0.95%), 4, 6-di-O-methyl- α -d-galactose (0.94%), octadecanoic acid (0.85%), thiophene, tetrahydro-2-methyl (0.82%), 3-hexanol, 2, 5-dimethyl- (0.79%), phenol (0.76%), tetradecanoic acid (0.75%), benzophenone, 3-methoxy-4'-methyl- (0.75%), ethylidenecycloheptane (0.75%), β -sitosterol (0.68%), 9, 12-octadecadienoic acid, methyl ester, (E, E)- (0.63%), 2-pyrrolidinone (0.50%), morpholine (0.48%), N-chloroacetyl-d-phenylalanine (0.47%), 1-butanol, 2-ethyl- (0.44%), 4, 6-di-O-methyl- α -d-galactose (0.40%) and unidentified compounds (38.03%).

Table 1. Minimum inhibition concentration (MIC) of *Sauropus androgynus* stem extract against bacterial isolates

Bacterial isolates	MIC (mg/l)
<i>Aeromonas hydrophila</i>	15.6
<i>Edwardsiella tarda</i>	7.8
<i>Escherichia coli</i>	7.8
<i>Flavobacterium</i> sp.	7.8
<i>Klebsiella</i> sp.	15.6
<i>Pseudomonas aeruginosa</i>	7.8
<i>Salmonella</i> sp.	62.5
<i>Vibrio alginolyticus</i>	15.6
<i>Vibrio cholerae</i>	7.8
<i>Vibrio parahaemolyticus</i>	62.5

Table 2. Compound composition of *Sauropus androgynus* stem extract

Compound	Compound composition (%)
9, 12, 15-octadecatrienoic acid, ethyl ester, (Z, Z, Z)-	14.48
Phytol	13.08
Glycerin	2.52
1-methyl-2-pyrrolidineethanol	2.27
Acetic acid	1.81
Pent-1-en-3-one, 1-(2-furyl)-5-dimethylamino	1.69
Benzofuran, 2, 3-dihydro-	1.65
2-Acetylpyrrolidine	1.51
4-O-methylmannose	1.46
N-Ethyl-2-carbomethoxyazetidine	1.43
9-Ethoxy-10-oxatricyclo [7.2.1.0 (1, 6)] dodecan-11-one	1.36
1H-Indole, 5-fluoro-	1.30
Hexadecanoic acid	1.18
Oleic acid	1.18
Heptaethylene glycol monododecyl ether	1.12
N, N-Dimethyl-2-aminoethanol	1.05
2-Methoxy-4-vinylphenol	0.97
L-Phenylalanine	0.95
Pentaethylene glycol	0.95
4, 6-Di-O-methyl- α -d-galactose	0.94
Octadecanoic acid	0.85
Thiophene, tetrahydro-2-methyl	0.82
3-Hexanol, 2, 5-dimethyl-	0.79
Phenol	0.76
Tetradecanoic acid	0.75
Benzophenone, 3-methoxy-4'-methyl-	0.75
Ethylidenecycloheptane	0.75
β -sitosterol	0.68
9, 12-Octadecadienoic acid, methyl ester, (E, E)-	0.63
2-pyrrolidinone	0.50
Morpholine	0.48
N-Chloroacetyl-d-phenylalanine	0.47
1-butanol, 2-ethyl-	0.44
4, 6-Di-O-methyl- α -d-galactose	0.40
Unidentified compounds	38.03
Total	100

DISCUSSION

The antimicrobial test results have revealed *S. androgynus* stem extract to possess a high antimicrobial activity against the tested bacteria. The antimicrobial properties of *S. androgynus* investigated in a previously study have shown that methanol extract of the leaves of this plant can inhibit the growth of both gram-positive and gram-negative bacteria such as *Bacillus cereus*, *Enterobacter aerogenes* and *Salmonella typhimurium* (14). However, this

study did not detail on the MIC value of the plant extract against the tested bacteria (14). Till present, no study has documented the antimicrobial properties of *S. androgynus* stem extract. Therefore, this is the first report on the antimicrobial property of *S. androgynus* stem extract. In the present study, several bioactive compounds were also successfully detected in the plant extract. They were 9, 12, 15-octadecatrienoic acid, ethyl ester, (Z, Z, Z)- (the major compound in the plant extract), acetic acid, hexadecanoic acid, oleic acid and many more.

An antioxidant is a molecule that can eliminate free radicals from the body of an animal. Therefore, an antioxidant can maintain health and prevent diseases such as cancer, heart disease and many more. Thus, this plant can also be used to prevent cancer, although no anticancer activity was observed in the present study. The study of Benjapak et al. (2008) claimed *S. androgynus* to possess different antioxidant capacities at different harvest periods; e. g., *S. androgynus* harvested in November possesses an almost two times higher antioxidant activity ($IC_{50} = 0.7$ ppt) compared to the plant harvested in January ($IC_{50} = 1.4$ ppt). However, the antioxidant properties of *S. androgynus* in our study stem ($IC_{50} = 8.0$ ppt) were much lower compared to a previous study (1). The highly different antioxidant data between these two studies are due to different methods used to obtain a plant extract. In the previous study (1), the fresh juice of *S. androgynus* was used in the antioxidant test, whereas in the present study, plant extract was obtained through methanol extract. Using methanol extraction, the sample had to undergo the drying process which may lead to the degradation of the bioactive compounds that may be responsible for the antioxidant activity of the plant. However, several compounds that contribute to the antioxidant activity of the plant extract, such as phytol, phenol, acetic acid, β -sitosterol and many more, were detected in the present study.

CONCLUSIONS

In conclusion, the present study and information from the literature clearly show that *S. androgynus* stem extract possesses a huge potential as an antimicrobial and antioxidant agent. A further study should be carried out on the clinical test of this plant extract before introducing it to public use.

ACKNOWLEDGEMENTS

This project was funded by the University Malaysia Kelantan short-term projects (R/SGJP/A03.00/00387A/001/2009/000018 and R/SGJP/A03.00/00302A/001/2009/000019).

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Received 12 December 2010

Accepted 28 February 2011

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SAUROPUS ANDROGYNUS STIEBO EKSTRAKTO ANTIMIKROBINIS, PRIEŠVĖŽINIS POVEIKIS BEI CHEMINĖ SUDĖTIS

Santrauka

Įvadas. Šiame tyrime nagrinėjamas *Sauropus androgynus* stiebo ekstrakto antimikrobinis, priešvėžinis ir antioksidacinis poveikis. Pagrindinis darbo tikslas – atskleisti galimą *S. androgynus* stiebo ekstrakto kaip medicininio preparato panaudojimą.

Medžiaga ir metodai. *S. androgynus* stiebo ekstrakto antimikrobinės savybės prieš *Aeromonas hydrophila*, *Escherichia coli*, *Edwardsiella tarda*, *Flavobacterium* sp., *Klebsiella* sp., *Salmonella* sp., *Vibrio alginolyticus*, *V. parahaemolyticus*, *V. cholerae* ir *Pseudomonas aeruginosa* analizuojamos praskiedus nuovirą, o priešvėžinis ekstrakto aktyvumas, gydant krūties adenokarcinomą (MCF-7), buvo nustytas kolorimetriniu MTT (tetrazolio) analizatoriumi. Antioksidacinis aktyvumas charakterizuojamas naudojant α ,

α -diphenyl- β -picrylhydrazyl (DPPH) prapūtimo metodą. Cheminė augalo ekstrakto sudėtis nustatyta dujų chromatografijos-masės spektrometrijos (GC-MS) būdu.

Rezultatai. Minimali inhibitorinė koncentracija (MIC), galinti sutrikdyti bakterijų augimą, svyravo nuo 7,81 iki 62,5 mg/l: *Edwardsiella tarda*, *Escherichia coli*, *Flavobacterium* sp., *Pseudomonas aeruginosa* ir *Vibrio cholerae* – 7,81 mg/l, *Klebsiella* sp., *Aeromonas hydrophila* ir *Vibrio alginolyticus* – 15,6 mg/l ir *Salmonella* sp. ir *Vibrio parahaemolyticus* – 62,5 mg/l. Tyrimo rezultatai rodo, kad *S. androgynus* stiebo ekstraktas pasižymi dideliu antimikrobinium aktyvumu ir vidutiniu antioksidaciniu aktyvumu (inhibicijos koncentracija 50 %; IC_{50} iš DPPH 8 ppt), tačiau nepasižymi priešvėžiniu aktyvumu. Augalo ekstrakto buvo rasti 34 komponentai, iš kurių svarbiausi yra 9, 12, 15-oktadekatrienoinė rūgštis, metilo esteris (14,48 %) ir fytolis (13,08 %).

Išvados. *S. androgynus* stiebo ekstraktas gali būti naudojamas kaip antimikrobinis ir antioksidacinis preparatas.

Raktažodžiai: antimikrobinis, antioksidacinis, priešvėžinis, cheminė sudėtis, *Sauropus androgynus* stiebas