

Full Length Research Paper

Energy-dispersive X-ray microanalysis of elements' content and antimicrobial properties of *Pereskia bleo* and *Goniothalamus umbrosus*

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This study was designed to investigate elements' content and anti-microbial effects of two Malaysian plants, *Pereskia bleo* and *Goniothalamus umbrosus*. Elements' analysis was carried out using Energy Dispersive X-ray Microanalysis combined with Variable Pressure Scanning Electron Microscope (EDX, VPSEM). Anti-microbial screening was performed using disc diffusion method using two gram-positive bacteria, methicillin resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis* B29, and other two gram-negative bacteria, *Pseudomonas aeruginosa* 60690 and *Salmonella choleraesuis*. The elemental distribution revealed the presence of C, O, Mg, P, S, Cl, K, Ca, Al, Si and Fe, in these medicinal plants and their relative weight percentages were estimated. A variable susceptibility of microbial strains of this study towards *Pereskia bleo* and *Goniothalamus umbrosus* was noticeably observed. From this study, it is shown that the analysis of elements of anticancer plants can be considered as potential sources for providing information regarding the medicinal usages of plants.

Key words: *Pereskia bleo*, *Goniothalamus umbrosus*, energy-dispersive X-ray, antimicrobial property.

INTRODUCTION

Drug discovery from medicinal plants continues to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria, infections and pain (Balunas and Kinghorn, 2005). Therefore, research on understanding the metabolism of several pathways in plant tissues, and their main elements' content is needed (de-Oliveira et al., 2006). These medicinal plants are known to be rich in one or more element which might have a possible link to the therapeutic action that possessed by these plants. The active principles and the mechanism of action have not been fully elucidated (Ibrahim, 2006).

The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with re-

duced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (Borris, 1996). Many efforts have been made to discover new anti-microbial compounds from various kinds of sources such as micro-organisms, animals, and plants. One of such resources is folk medicines (Mulholland, 2005). Systematic screening of them may result in the discovery of novel effective compounds (Janovska et al., 2003).

Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques. In traditional methods, medicinal plants are being used, which contain both organic and inorganic constituents (Aliannis et al., 2001). Therefore, to correlate, the chemical and biological properties of medicinal plants, many of these techniques have been applied. In this respect, the current study was designed to investigate the elemental contents (C, O, Mg, P, S, Cl, K,

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Al, Ca, Si and Fe) of *Pereskia bleo* and *Goniothalamus* using variable pressure scanning electron microscope microanalysis (EDX). On the other hand, the antimicrobial activity of these two plants has been tested using disc diffusion method co-cultured concurrently with gram positive and gram negative bacteria.

MATERIALS AND METHODS

Energy dispersive X-ray microanalysis

Energy dispersive X-ray microanalysis has been applied before to determine elements' content of plants (Obiajunwa et al., 2002; Slavica et al., 2005). Fresh leaves of *P. bleo* and *Goniothalamus umbrosus*, were collected from the Biodiversity Unit, Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM). Samples were cut into 1 X 1 mm, and mounted on Aluminium stub specimen holders and viewed under a variable pressure scanning electron microscope (VPSEM, EDX) model LEO1455 with an Oxford INCA EDX 300 microanalysis attachment. Samples were examined at an accelerating voltage of 20 kV. For such analysis, three spectra from each sample were acquired for 120 s with process time number 5. This experiment was conducted at the microscopy unit, IBS, UPM, Malaysia.

Antimicrobial activity

Preparation of extracts

The fresh leaves of *P. bleo* and *G. umbrosus* were dried under the shade and ground into a fine powder. Three hundred gram (300 g) of this powder for each plant was then extracted sequentially with hexane, and dichloromethane using the soxhlet extractor. The extracts were stored in the refrigerator. The antibacterial activity of plant extracts was evaluated using two gram-positive bacteria, methicillin resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis* B29, and other two gram-negative bacteria, *Pseudomonas aeruginosa* 60690 and *Salmonella choleraesuis*. All the bacterial strains were obtained from Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Malaysia.

Anti-microbial assay

The screening of the extracts antimicrobial effect was carried out by determining the zone of inhibition using paper disc (6 mm in diameter, Whatman No. 1) diffusion method (Sahoo et al., 2006). The extracts were dissolved in dimethyl sulphoxide which was previously tested for antibacterial activity against all test bacteria and found to have no antibacterial activity. The extracts were diluted to concentration of 100 mg/ml and finally sterilized by filtration using 0.45 µm millipore filters. The sterile discs were impregnated with extract solution (0.05 ml from 100 mg/ml extract) to achieve desired concentration and placed in inoculated agar. Streptomycin (10 µg/disc) was used as standards. The controls were prepared using the same solvents without extracts. The inoculated plates that contained the test and standard discs were incubated at 37 °C for 24 h.

RESULTS AND DISCUSSION

This study was designed to investigate elements' content

and anti-bacterial effects of two Malaysian plants, namely *P. bleo* and *Goniothalamus*. Elements analysis was carried out using Energy Dispersive X-ray Microanalysis combined with Variable Pressure Scanning Electron Microscope microanalysis (EDX). The results showed that plants' variable elements' contents could possibly explain their difference in terms of medicinal properties as demonstrated by the variability of *P. bleo* and *Goniothalamus* in antimicrobial results obtained in this study.

Biological, chemical and geological materials have been extensively analyzed for their trace element content using different techniques by a lot of authors (Ekinci et al., 2004). It is significant to quantify the element content of remedial plants (Balunas and Kinghorn, 2005). For this reason, reliable analyses will help to clarify and define the effective. The most important advantages of energy dispersive X-ray for the quantitative and qualitative analysis are: (1) simultaneous determination of many elements, (2) determination in a wide concentration range, (3) simple and fast sample preparation, and (4) much lower equipment cost than that of a conventional wavelength X-ray fluorescence spectrometer, especially when a radioisotope is used instead of X-ray tube (Ekinci et al., 2004).

The macrocosmic and microcosmic elements in relation to their philosophical and scientifically defined role in health and diseases and being catalysts in nature they activate certain enzymes and are essential components of vitamins and hormones and thus play a vital role in the medicinal value of plant therapy in health and diseases (Simon et al., 1990). As depicted in Table 1, the elements' contents of these two medicinal plants were analyzed. Apart from carbon and oxygen, both of the plants contain potassium and chlorine. Aluminum and silicon were not detected in these two plants while percentages of magnesium and potassium in *P. bleo* were higher. But calcium content in *G. umbrosus* is observed to be higher compared to *P. bleo*. Moreover, it was previously reported that containing rich amount of calcium may support the medicinal uses of plants (Jawhar et al., 2004). The high concentrations of Ca are very significant because Ca is known to enhance the qualities of bones and teeth and also of neuromuscular systemic and cardiac functions (Obiajunwa et al., 2002).

Studies on the antibacterial activities of medicinal plants have clearly become a progressive trend using different screening method. Disc diffusion method was the first method of choice, possibly due to its simplicity and capability to analyze a large number of test samples. Many earlier publications used this method as a means of determining activity (Van-Vuuren, 2008). The antibacterial activity of PB extracts was evaluated using Gram-positive and Gram-negative bacteria. The solvents used for control and all extracts did not show any activity (the results not shown). In Table 2, the screening of the extracts antibacterial effect was summarized. From these

Table 1. Energy dispersive X-ray microanalysis of elements in the fresh leaves of *P. bleo* and *G. umbrosus*.

Element	<i>P. bleo</i>	<i>G. umbrosus</i>
C	50.60 ± 5.2%	77.17 ± 12.5%
O	35.37 ± 5.35%	18.15 ± 2.6%
Mg	0.427 ± 0.15%	0.19 ± 0.06%
P	0.38 ± 0.12%	ND
S	1.51 ± 0.49%	0.11 ± 0.3%
Cl	1.24 ± 0.36%	0.87 ± 0.05%
K	10.16 ± 2.52%	1.43 ± 0.35%
Al	ND	ND
Ca	0.31 ± 0.09%	1.72 ± 0.5%
Si	ND	ND
Fe	ND	0.2 ± 0.05%

Table 2. Antimicrobial analysis of hexane and dichloromethane extracts of the plants of this study.

Plant extract	Diameter of inhibition (millimetre)							
	MRSA		PA		SC		BS	
	<i>G. umbrosus</i>	<i>P. bleo</i>	<i>G. umbrosus</i>	<i>P. bleo</i>	<i>G. umbrosus</i>	<i>P. bleo</i>	<i>G. umbrosus</i>	<i>P. bleo</i>
Hexane	-	-	-	+++	-	+++	-	-
Dichloromethane	++	++	++	+	+	-	+++	-

^aThe screening of the extracts antibacterial effect was carried out by determining the zone of inhibition using paper disc (6 mm in diameter, Whatman No. 1) diffusion method (n = 2). MRSA: Methicillin Resistant *Staphylococcus aureus*, PA: *Pseudomonas aeruginosa*, SC: *Salmonella choleraesuis* and BS: *Bacillus subtilis*. Streptomycin showed 20, 20, 23 and 23 mm inhibition towards MRSA, PA, SC and BS respectively.

+ stands for activity between 6 – 9 mm; ++ stands for activity between 9 – 14 mm; +++ stands for activity more than 14.

results it was concluded that hexane extract showed considerable antibacterial activity towards two gram-negative bacteria, *P. aeruginosa* 60690 and *S. choleraesuis*. The highest antibacterial activity on MRSA observed in this investigation is obtained by dichloromethane extracts. However MRSA has shown bacterial resistance to the rest of the extract. Both MRSA and *P. aeruginosa*, well noted for their insusceptibility to most antibiotics, that were inhibited by hexane extract of *P. bleo* with a remarkable activity. *P. aeruginosa* is known to have a high level of intrinsic resistance to virtually all known antimicrobials and antibiotics, due to a very restrictive outer membrane barrier (Mann et al., 2000).

Conclusion

In conclusion, EDX has been fruitfully utilized for the determination of elements in medicinal plants. This technique requires only small amounts of sample materials and it is multitalented in comparison with other techniques. Many of other available methods require difficult sample preparation techniques, big amounts of sample material and time consuming separation procedures before the elemental composition can be

determined. In this study, the elements' content of these two plants, has shown a good indicator and supportive explanation for the variability in their antimicrobial properties. Therefore, this correlation could be a good basis for further research using supportive biotechnological techniques such as bioinformatics and genetic markers.

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