

Biological Activities of *Pereskia bleo* Extracts

S.I.A. Wahab, A.B. Abdul, S.M. Mohan, A.S. Al-Zubairi, M.M. Elhassan and M.Y. Ibrahim
UPM-MAKNA Cancer Research Laboratory, Institute of Bioscience,
Universiti Putra Malaysia, Serdang, 43400, Selangor, Malaysia

Abstract: The aim of this study is to screen the hexane, dichloromethane, ethyl acetate and methanol extracts of *Pereskia bleo* (PB) for their antibacterial, anti-oxidant and anti-cancer properties using disc diffusion method, DPPH assay and MTT cytotoxicity test, respectively. It is found that hexane and methanol extracts showed highly and moderately, respectively, considerable antibacterial activity towards two Gram-negative bacteria, *P. aeruginosa* 60690 and *S. choleraesuis*. Ethyl acetate extract showed a weak narrow spectrum activity ($\approx 35\%$ of streptomycin activity). The highest antibacterial activity on MRSA is obtained by DCM extract. Hexane extract was the most effective DPPH radical scavenger (37.55%). Ethyl acetate and DCM extracts were less effective free radical scavenger (16.1%). None of the extracts were cytotoxic significantly towards MCF-7, HT-29 and CEM-SS cell lines after 72 h incubation time ($IC_{50} > 30 \mu\text{g mL}^{-1}$). It could be concluded that antibacterial activity of *P. bleo* is the most promising biological activity attributed to this plant.

Key words: *Pereskia bleo*, antibacterial, anti-oxidant, anti-tumor

INTRODUCTION

Plants produce a great deal of secondary metabolites, many of them with versatile pharmacological activities. Well-known examples of these compounds include flavonoids, phenols and phenolic glycosides, saponins, cyanogenic glycosides, unsaturated lactones and glucosinolates (Calis *et al.*, 1997; Emma *et al.*, 2001; Podolak *et al.*, 2007; Kappel *et al.*, 2008). *Pereskia bleo*, commonly known as Jarum Tujuh Bilah in Malay or Cak Sing Cam in Chinese by the locals, belongs to the botanical family Cactaceae. It is a spiny shrub which grows to a height of 2 to 8 m. It has orange-coloured flowers. It is originated from South America and cultivated in many tropical countries. The leaves of this plant are simple, spiral, glossy and succulent (Wiert, 2006). It is believed to have anti-cancer, anti-tumour, anti-rheumatic, anti-ulcer and anti-inflammatory properties. It is also used to treat diabetes and hypertension in certain ethnic groups (Tan *et al.*, 2004). The earlier study showed that the methanolic extract of *Pereskia bleo* may contain bioactive compound that kill breast carcinoma cell, T-47D by the activation of caspase-3 and c-myc pathways in apoptosis mechanism (Tan *et al.*, 2004). The later study showed that the aqueous and methanol extracts of the leaves of *Pereskia bleo* do not have significant anti-proliferative effects against the mouse mammary cancer cells (4T1) or the normal mouse fibroblast cells (NIH/3T3).

But the aqueous extract of *Pereskia bleo* generated mutagenic substances following metabolism by the liver enzymes. A more recent investigation showed the ethyl acetate fraction produced a remarkable inhibitory activity against human nasopharyngeal epidermoid carcinoma cell line (KB) (Tan *et al.*, 2004). Nevertheless, the later study which carried out by Er *et al.* (2007) showed the aqueous and methanol extracts of the leaves of *Pereskia bleo* do not have significant anti-proliferative effect towards either the mouse mammary cancer cell lines (4T1) or the normal mouse fibroblast cell lines (NIH/3T3) under optimal culture condition (when 10% FBS was used). Moreover, upon the metabolism by liver enzymes, the aqueous extract may form mutagenic compounds. A more recent investigation showed the ethyl acetate fraction produced a remarkable inhibitory activity against human nasopharyngeal epidermoid carcinoma cell line (KB). However, the extraction method used was not the same as the earlier two studies.

However, scientific investigation in order to determine the therapeutic potential of these plants is limited. Therefore, the aim of this study is to evaluate some biological activities of hexane, dichloromethane, ethyl acetate and methanol extracts of *P. bleo* using disc diffusion method and DPPH assay and MTT cytotoxicity test to assay antibacterial, anti-oxidant and anti-cancer activities, respectively.

MATERIALS AND METHODS

Preparation of extracts: The fresh leaves of *Pereskia bleo* were collected from Puchong, Selangor, Malaysia in October 2007. The plant was identified by Mr. Tajuddin Abd Manap, Assistant Agriculture Officer, Unit of Biodiversity, Institute of Biosains, Universiti Putra Malaysia, Malaysia. Leaves were dried under the shade and ground into a fine powder. 238.5213g of this powder was then extracted sequentially with hexane, dichloromethane, ethyl acetate and methanol by using the soxhlet extractor. The recovery weight was about 3.42% for hexane extract, 1.54% for dichloromethane extract, 1.87% for ethyl acetate extract and 7.61% for methanol extract from dried material. The extracts were stored in the refrigerator.

Antibacterial activity of *Pereskia bleo*

Bacterial strains: 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-bacterial assay: The 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 (Sahoo *et al.*, 2006). The obtained microorganism strains were inoculated in a Petri dish containing nutrient broth at 37°C for 24 h and were referred as seeded broth. The density of the bacterial suspension was standardized by standard method and the concentrations of the cultures were adjusted turbidometrically at wavelength of 600 nm to 500,000-1000, 000 colony forming unit per mL (cfu mL⁻¹) (McDermott *et al.*, 2001). 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⁻¹ 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⁻¹ extract) to achieve desired concentration and placed in inoculated agar. Streptomycin (10 µg disc⁻¹) was used as standards. The controls were prepared using the same solvents without extracts. The inoculated plates contain the test and standard discs were incubated at 37° for 24 h.

Antioxidant assay

DPPH radical scavenging assay: Radical scavenging activity of plant extracts against stable DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate, Sigma-Aldrich Chemie, Steinheim, Germany) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep-violet to light-yellow) were measured at 517 nm wavelength.

Radical scavenging activity of extracts was measured by slightly modified method of Changwei *et al.* (2008), as described below. Extract stock solutions were prepared in 100 mg mL⁻¹ in ethanol. Methanol extract was not fully soluble in ethanol (even after treating solutions for 5 min in an ultrasonic bath), therefore it was dissolved in dimethylsulphoxide. The working solution was prepared using methanol in a concentration of 500 µg mL⁻¹ (Labsystems iEMS Reader MF). The solution of DPPH in methanol (2.5 mg mL⁻¹) was prepared daily, before UV measurements. Five microliter of this solution were mixed with 100 µL extract solution 96 well plate. The samples were kept in the dark for 30 min at ambient temperature and then the decrease in absorption was measured. Absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured daily. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula:

$$\text{Inhibition (\%)} = ((A_B - A_A) / A_B) \times 100$$

Where:

A_B = Absorption of blank sample (t = 0 min)

A_A = Absorption of tested extract solution (t = 30 min)

Commercial standard antioxidant butylated hydroxytoluene (BHT) was also tested against DPPH and used as a reference.

Anti-cancer activity of *Pereskia bleo*

Preparation of extracts: To screen the extracts of PB, dried extracts of the plant were dissolved in 1 mL of DMSO to give stock solution of extract (10 mg mL⁻¹). All the extracts were stored in 4°C till the end of experiments. During the experiment, stock solutions were diluted with the complete media (RPIM1640) to obtain original concentrations of 0.469, 0.938, 1.875, 3.75, 7.5, 15 and 30 µg mL⁻¹.

Cell culture condition: The MCF-7 human breast cancer cell lines and HT-29 human colon carcinoma cells were purchased from American Type Culture Collection (ATCC), USA. Human T4-lymphoblastoid cell

line CEM-ss were obtained from NIH AIDS Reagent and Reference Reagent Program, USA and used in this study. The cell lines were grown at 37°C at humidified CO₂ incubator with 5% CO₂ in RPMI-1640 media (Sigma, MO, USA) supplemented with 10% fetal bovine serum (Invitrogen Corp., Auckland, New Zealand).

Cell growth inhibition assay: The cell suspension (0.1 million cells mL⁻¹) was plated out into 96-well microplates. Plant extracts were dissolved with dimethylsulfoxide (DMSO) and the final concentration of DMSO was 0.1% (v/v). Different concentrations of the sample were prepared with serial dilution. Dimethylsulfoxide (0.1%) was used as a control. The toxicity profiles of the extracts were assessed using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) microculture tetrazolium viability assay as described by Mosmann (1983). Thereafter, the various concentrations of plant samples were plated out in triplicate. Each plate included untreated cell controls and a blank cell-free control. After 68 h of incubation, MTT (5 µg mL⁻¹) was added to each well and the plates incubated for a further 4 h and the media removed. DMSO was later added into each well to solubilize the formazan crystals. The absorbance was read at wavelength of 595 nm using a microtitre plate reader (Labsystems iEMS Reader MF). The percentage cellular viability was calculated with the appropriate controls taken into account. The concentration which inhibited 50% of cellular growth (IC₅₀ value) was determined. The inhibitory rate of cell proliferation was calculated by the following formula:

$$\text{Growth inhibition (\%)} = \frac{\text{OD control} - \text{OD treated}}{\text{OD control}} \times 100$$

The cytotoxicity of sample on cancer cells was expressed as IC₅₀ values (the drug concentration reducing the absorbance of treated cells by 50% with respect to untreated cells).

Statistical analysis: Data is expressed as Mean±SD. ANOVA was used to analyze the difference between with 0.05 as a level of significance.

RESULTS AND DISCUSSION

In this study, some of biological activities of PB have been investigated, whereby; hexane, dichloromethane, ethyl acetate and methanol extracts of *P. bleo* were assayed for their antibacterial, anti-oxidant and anti-cancer properties using disc diffusion method, DPPH assay and MTT cytotoxicity test, respectively.

Table 1: Paper disk diffusion of plant alcoholic extracts on growth^a

	Diameter of inhibition (mm)			
	Bacterial strains			
	MRSA	PA	SC	BS
<i>Pereskia bleo</i> extracts				
Hexane	-	14	14	-
Dichloromethane	11	7	-	-
Ethyl A	-	7	-	-
Methanol	-	9	9	-
Control	20	20	23	23

^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

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 activities of PB different extracts were evaluated using Gram-positive and Gram-negative bacteria. The solvents used for control and all PB extracts did not show any activity (the results not shown). In Table 1, the screening of the extracts antibacterial effect was shown. From these results it was concluded that hexane and methanol extracts showed highly and moderately, respectively, considerable antibacterial activity towards two Gram-negative bacteria, *P. aeruginosa* 60690 and *S. choleraesuis*. Ethyl acetate extract showed a weak narrow spectrum activity (≈35% of streptomycin activity), compared to the control (Streptomycin) against *P. aeruginosa* 60690. The highest antibacterial activity on MRSA observed in this investigation is obtained by DCM extract. 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, were inhibited by hexane extract of PB 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).

The results of DPPH inhibition by different plant extracts are shown in Fig. 1. Hexane extract was the most effective DPPH radical scavenger (37.55%: low absorbance values). Ethyl acetate and DCM extracts were less effective free radical scavenger (16.1%) compared to hexane extract. Methanol extract of PB was the less effective towards the scavenging of DPPH free radicals (13.2%). In this study, the DPPH radical-scavenging assay was selected due to its straightforwardness, quickness, sensitivity and reproducibility (Sanja *et al.*, 2008). This

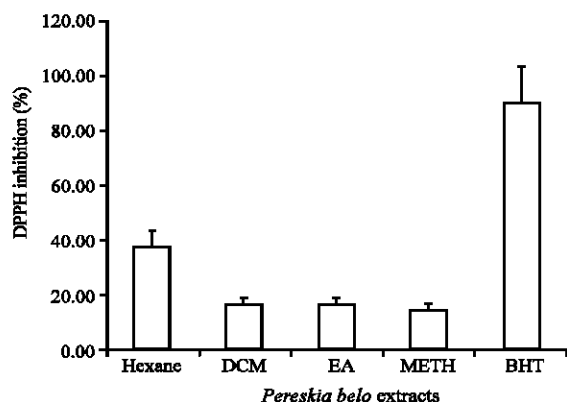


Fig. 1: DPPH absorption inhibition (%) of *Pereskia bleo* extracts isolated with methanol, ethyl acetate, hexane and DCM: dichloromethane, EA: ethyl acetate, MeTH: Methanol extracts; BHT Butyl hydroxy toluene

method is also very handy for the screening of large numbers of samples with different polarity. As also used in this study, the extraction was performed in increasing order using sequentially different solvent systems.

Screening of plant extracts on different cancer cell lines has been applied using MTT assay. After conducting the MTT assay, a dose-response curve for the percentages (0-100%) of viability cells was plotted against the concentrations of the extract (0.469, 0.938, 1.875, 3.75, 7.5, 15 and 30 $\mu\text{g mL}^{-1}$). The 50% inhibitory concentration (IC_{50}) of the extract was checked from the graph. The hexane, dichloromethane, ethyl acetate and methanol extract of *Pereskia bleo* were determined to have IC_{50} values over 30 $\mu\text{g mL}^{-1}$ for MCF-7, HT-29 and CEM-SS cell lines after 72 h incubation time (Arunporn *et al.*, 2004). This had been shown that entire four extracts of *Pereskia bleo* were conferred non-effective in inducing cell death towards MCF-7, HT-29 and CEM-ss cell lines according to the guidelines from American National Cancer Institute. The results of this study are not in consistence with previous published data (Tan *et al.*, 2004) and that might be due the different methods of extraction.

CONCLUSION

Although PB extracts did not show any potential anticancer activities towards various tested cancer cells, All extracts has shown a potential differences in the profile of their antibacterial and antioxidant activities suggesting the presence of different chemical constituents existed in this plant.

ACKNOWLEDGMENTS

The authors wish to thank gratefully Universiti Putra Malaysia (Grant No. RUGS 91143) and the Malaysian National Council for Cancer for financial support and The Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Malaysia for providing the bacterial strains.

REFERENCES

- Arunporn, I., J.H. Peter, P.J. Eno-Amooquayec, J. Burcek and R. Amala, 2004. In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. *J. Ethnopharmacol.*, 90: 33-38.
- Calis, T., M.E. Satana, A. Yürüker, P. Kelican, R. Demirdamar, R. Alaçam, N. Tanker, H. Rügger and O. Sticher, 1997. Triterpene saponins from *Cyclamen mirabile* and their biological activities. *J. Nat. Prod.*, 60: 315-318.
- Changwei, A., L. Anping, A.E. Abdelnaser, D.X. Tran and T. Shinkichi, 2008. Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. fil. *Extract. Food Control*, 19: 940-948.
- Emma, N.Q., R.S. Antonio and A.V. Marta, 2001. Screening antifungal activities of selected medicinal plants. *J. Ethnopharmacol.*, 74: 89-96.
- Er., H.M., E.H. Cheng and A.K. Radhakrishnan, 2007. Anti-proliferative and mutagenic activities of aqueous and methanol extracts of leaves from *Pereskia bleo* (Kunth) DC (Cactaceae). *J. Ethnopharmacol.*, 113: 448-456.
- Kappel, V.D., G.M. Costa, G. Scola, F.A. Silva and M.F. Landell *et al.*, 2008. Phenolic content and antioxidant and antimicrobial properties of fruits of *Capsicum baccatum* L. Var. pendulum at different maturity stages. *J. Med. Food*, 11: 267-274.
- Mann, C.M., S.D. Cox and J.L. Markham, 2000. The outer membrane of *Pseudomonas aeruginosa* NCTC6749 contributes to its tolerance to the essential oil of *Melaleuca alternifolia* (tea tree oil). *Lett. Applied Microbiol.*, 30: 294-297.
- McDermott, P.F., A.L. Barry, R.N. Jones, G.E. Stein, C. Thornsberry, C.C. Wu and R.D. Walker, 2001. Standardization of broth microdilution and disk diffusion susceptibility tests for actinobacillus pleuropneumoniae and haemophilus somnus: Quality control standards for ceftiofur, enrofloxacin, florfenicol, gentamicin, penicillin, tetracycline, tilmicosin and trimethoprim-sulfamethoxazole. *J. Clin. Microbiol.*, 39: 4283-4287.

- Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, 65: 55-63.
- Podolak, I., Z. Janeczko, A. Galanty, M. Michalik and D. Trojanowska, 2007. A triterpene saponin from *Lysimachia thyrsoflora* L. *Acta Pol. Pharm.*, 64: 39-43.
- Sahoo, S., D.M. Kar, S. Mohapatra, S.P. Rout and S.K. Dash, 2006. Antibacterial activity of *Hybanthus enneaspermus* against selected UTI pathogens. *Indian J. Pharm. Sci.*, 68: 653-655.
- Sanja, C., M. Milka, E.Š. Marija, J.M. Anesa and B. Renata, 2008. Chemical composition and antioxidant and antimicrobial activity of two *Satureja* essential oils. *Food Chem.*, 111: 648-653.
- Tan, M.L., S.F. Sulaiman, N. Najimuddin, M.R. Samian and T.S. Tengku Muhammad, 2004. Methanolic extract of *Pereskia bleo* (Kunth) DC. (Cactaceae) induces apoptosis in breast carcinoma, T47-D cell line. *J. Ethnopharmacol.*, 96: 287-294.
- Van-Vuuren, S.F., 2008. Antimicrobial activity of South African medicinal plants. *J. Ethnopharmacol.*, 119: 462-472.
- Wiart, C., 2006. Medicinal plants of the Asia-Pacific: Drugs for the Future. World Scientific Publishing, pp: 98-99.