Research article Rapports De Pharmacie Vol.4 (2), 2018, 457-461
ISSN: 2455-0507

ANTIOXIDANT, ANTIMICROBIAL AND SUN PROTECTION FACTOR DETERMINATION OF *PHYLLANTHUS ACIDUS*

Lim Chia Chia, Fazlina Mustaffa*, Geethaa Sahgal
Faculty of Pharmacy, AIMST University, Jalan Bedong-Semeling, 08100 Bedong, Kedah.

Objective: To evaluate the antioxidant, antimicrobial and sun protection factor properties of *P. acidus.*
Method: The extraction of the *P. acidus* fruits was done by using maceration. The Sun protection factor (SPF) was determined by using Mansur simple mathematical equation. Few antioxidant tests were carried out such as 2, 2-diphenyl -1- picrylhydrazyl (DPPH) test, total phenolic test and total flavonoid test. Antimicrobial test was carried out by well diffusion test by using six different bacterial strains namely *Bacillus subtilis, Enterococcus faecalis, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia* .
Results: SPF value of *P. acidus* was found to be even higher than the SPF of well-known medicinal plant, *Aloe vera*. The SPF value for *P. acidus* extract was 2.5 for dilution of 50 mL and 0.54 for 25 mL. The percentage of scavenging activity of *P. acidus* extract was considered good but its activity is lower than standard ascorbic acid. The total phenolic content of extract was 62.14 ug/mL and flavonoid content was 364.71 ug/mL. *P. acidus* extract showed antimicrobial activity against the *Pseudomonas aeruginosa, Klebsiella pneumonia, Enterococcus faecalis, Bacillus subtilis* except *Escherichia coli* and *Streptococcus pyogenes.*
Conclusion: *P. acidus* has promising antimicrobial, antioxidant and sun protective activity, thus may be used as alternative treatment for the infectious diseases and as natural sunscreen product with anti-ageing property.
Keywords: Phenolic, Flavonoid, Scavenging activity

INTRODUCTION

The use of plants to cure diseases started from the earliest times of mankind's history. Although traditional healers around the world use many plants to treat various diseases but lack of documentation may cause loss of ancient wisdom [1]. Therefore, the efficacy of these plants still needs more investigation to provide usefulness in treatment. Due to the antimicrobial resistance, there are emerging infectious diseases. The microorganisms acquire resistance to certain drugs cause development of new disease [2]. Therefore, continuous effort in identification of new antimicrobial compound from plant extract is required to overcome microbial resistance. Furthermore, plants acts as dietary supplements which is a good source for scavenging free radicals in human body [3]. Besides, over exposure to UV radiation being the risk of skin cancer. The natural herbal sunscreen can avoid risk effects of synthetic chemical sunscreens like aminobenzoic acids, titanium oxide, zinc oxide and others, therefore present work was planned to study the photo-absorptive property of extract *P. acidus. P. acidus* grows widely in the tropics at low and medium altitudes during a short or prolonged dry season. The tree prefers hot and humid tropical lowlands. This species was originated in Madagascar and commonly grown in Indonesia, South Vietnam, Laos and northern Malaya [4]. *P. acidus* demonstrates antidiabetic and antitussive effect. Chakraboty et al reported that *P. acidus* leaves as possessing anti-inflammatory, antinociceptive, and antioxidant activity [5]. The antihyperlipidemic and hepatoprotective activity of *P. acidus* fruit acetone and methanol extract were also reported [6]. The root of this plant is used as purgative. The leaves can be used to control fever. The peppered leaves are used for the treatment of sciatica, lumbago and rheumatism [5]. Despite of the numerous researches on this plant, there is lack of study on antimicrobial activity and sun protective activity of this plant. Hence, this study is anticipated to evaluate the antimicrobial, antioxidant and sun protective effect of *P.acidus* fruit extract.

Address for correspondence:
Dr. Fazlina Mustaffa,
Faculty of Pharmacy,
AIMST University, Bedong- Semeling, Kedah,
Malaysia 08100
Email: fazlina_harris@yahoo.com

MATERIALS AND METHODS

Extraction:

Fruits of *P. acidus* were collected in Aimst University. Then, the fruits were thoroughly washed with water to remove dirt's. They were left for drying in hot air oven for two days at 37°C to remove the moisture. Thereafter, 300 g of fruits were macerated using 1 L of ethanol in conical flask. It was then placed into the shaker evaporator at room temperature for three days. The extracts obtained were then filtered using whatman filter paper (No 2) to obtain ethanol and aqueous extract. The filtrate was then dried in the water bath at 65°C for three days [6].

In-vitro sun protection factor (SPF) determination:

SPF determination was evaluated according to the Sayre et al., 1979 [7]. 1.0 g of sample was weighed and diluted with ethanol (40 µg/ml and 80 µg/ml), followed by ultrasonification for 5 minutes. Then, it was filtered by using whatman filter paper. Then, 5.0 mL of aliquot was transferred to a 50 mL volumetric flask and diluted with ethanol. The absorption spectra of sample were obtained in the range of 290 to 450 nm using Shimadzu UV-Spectrophotometer. Ethanol was used as a blank. The data were obtained in the range of 290-320, every 5 nm.

$$SPF = CF \sum\_290\text{^}320 \; [EE(\lambda) \times I(\lambda) \times Abs(\lambda)]$$

The Mansur equation was applied for the SPF calculation; where: EE = Erythemal effect spectrum; I = Solar intensity spectrum; Abs = Absorbance of sunscreen product; CF = Correction factor =10; the values of EE × I are constants.

In-vitro antioxidant determination by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method:

Free radical scavenging activity of *P. acidus* extract against DPPH radical was evaluated according to the Choi et al., 2000 [8]. The stock solution of the extract was prepared for a concentration of 1000 µg/mL in 10 mL of 95% ethanol. From this stock solution, various dilution of extract was prepared such as 80, 60, 40 and 20 µg/mL respectively. For the DPPH solution preparation, 11.83 mg of DPPH reagent was prepared in 120 mL of 95% ethanol. The assay was performed by adding 4.0 mL of the various concentrations of the extracts to 2.0 mL of DPPH reagent. The mixture was then incubated in the dark at room temperature for 30 minutes. Next, the absorbance was measured at 517 nm. Ethanol serve as blank and DPPH alone served as control.

AA = [(Absorbance of the control-Absorbance of the sample))/ (Absorbance of the control)] × 100

AA; Antioxidant activity

Total phenolic content determination:

Total phenolic content determination was evaluated according to the Prieto et al 1999 [9]. The phenolic content in extracts was determined by using Folin-Ciocalteu procedure. Firstly, extract or gallic acid was introduced into the test tube. It was then mixed with 5 mL of Folin-Ciocalteu's reagent (diluted to 10 folds) and 7.5% w/v of 4 mL of sodium carbonate in each tube. The mixture was allowed to stand for 30 minutes at room temperature. Then, the absorption was measured by using UV-Visible spectrophotometer at 765nm.

Determination of total flavonoid content

Total flavonoid content determination was evaluated according to the Rahman et al., 2011[10]. Firstly, 1 mL of sample (200 ug /ml) or rutin at different concentration was introduced into test tube. Then, it was mixed with 3 ml of methanol, 10% of 0.2 mL of aluminum chloride, 0.2 ml of potassium acetate solution (1 M) and 5.6 mL of distilled water. Then, it was incubated at room temperature for 30 minutes. Finally, the absorbance at 415nm was measured.

Antimicrobial test

The bacterial strains used in this study consist of gram-positive (*Bacillus subtilis, Enterococcus faecalis, Streptococcus pyogenes*) and gram-negative organisms (*Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia*). Microorganisms were exposed to the different extract concentration (2:5 and 4:5) using an agar based assay [6, 7]. 100 µl of each isolate was spread uniformly over Mueller Hinton Agar (MHA) medium by using a cotton swab. Five mm wells were formed on the agar plates using a sterile cork borer and aliquots (100µL) of extracts each at different concentrations were deposited into the well. The well at the center was placed with tap water which acts as a negative control. Inhibition activities against microbial strains were determined by measuring the zones of inhibition formed around the well in millimeter (mm) after 24 h of incubation at 37°C. Standard ciprofloxacin was used as positive control to ensure the activity of standard antibiotic against the test organisms. Distilled water was used as negative control.

RESULT AND DISCUSSION

SPF determination:

The SPF is a quantitative measurement of the effectiveness of a sunscreen formulation. The higher the SPF value, the higher is the protection activity. A sunscreen product should have a wide range of absorbance in wavelength between 290 and 420 nm to provide maximum protection. In- vitro SPF is useful as a screening tool for sunscreen agent development. In this study, *P. acidus* extract was evaluated for sunscreen protective activity by UV spectrophotometry applying Mansur mathematical equation. The SPF value is compared to the *A. vera* and marketed sunscreen product. SPF value of *P. acidus* was found to be even higher than the SPF of well-known medicinal plant which commonly used in cosmetic product, *A. vera* (Figure 1). This study also revealed that the exact SPF value of marketed sunscreen product was found to be lower than its labelled SPF. The sunscreen activity of the extract is due to the presence of phenols, flavonoids and other constituents of the fruits. Flavonoids and phenols have been reported to protect the skin from the inflammation and erythema. The antioxidant property of the *P. acidus* potentiates the UV protection and photo-protection activity [8].This study suggest that *P.acidus* may be used topically as alternative for synthetic sunscreen product to protect skin against ultraviolet, that provides antioxidant benefit as well. Furthermore, the use of synthetic sunscreen is related to the side effect of various chemical presences such as amino benzoic acids, titanium oxide and zinc oxide.

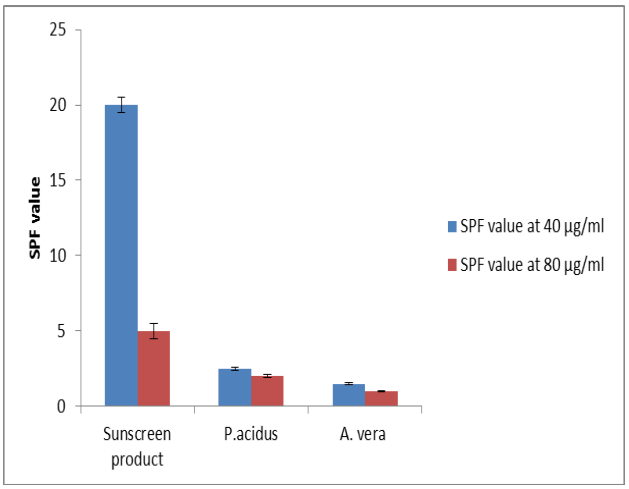

Figure 1: Comparison of SPF value of *P. acidus* extract, marketed sunscreen product and *A. vera* extract.

Antioxidant activity:

In this research, total phenolic and total flavonoid content which is correlated with the antioxidant potential were found to be 620.14 ± 0.85 mg gallic acid equivalent/g plant material and 36.71 ± 0.1 mg rutin equivalent/g plant material respectively. The antioxidant potential of extract was determined by in-vitro antioxidant activity using DPPH assay. The DPPH assay is a rapid and sensitive method which has been widely used for the screening of scavenging activity of antioxidant. DPPH antioxidant assay is based on the ability of antioxidants presence in the plant extracts to scavenge DPPH free radical and causing decolourization of DPPH.The ability to scavenge DPPH free radical indicate hydrogen donating capability of plant extracts[13].Radical scavenging activities can prevent the deleterious effects of free radicals in Alzheimer's disease, cardiovascular disease and cancer [14]. This study revealed that the antioxidant activity of *P.acidus* is directly proportional to the concentration of the extract as depicted in figure 2. The IC_{50} value of *P.acidus* was found to be 40.25µg/ml. The DPPH scavenging activity of this plant is better than as other reported plant with good antioxidant activity (*Centella asiatica* , 0.2 mg/ ml; *Pisonia alba*, 0.18 mg/ ml; *Orthosiphon stamineus*, 0.21 mg mL; *Mentha arvensis*, 0.22 mg/ml; *Ocimum basilicum*, 0.19 mg/mL) (Subhasree et al., 2009; Zakaria et al., 2008).The percentage of scavenging activity of *P. acidus* extract was considered good but its activity is lower than standard ascorbic acid (Figure 2).

Antimicrobial test:

Antibacterial activity of *P. acidus* extract was evaluated *in-vitro* against *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterococcus faecalis*, *Bacillus subtilis*, *Escherichia coli* and *Streptococcus pyogenes* using well diffusion antimicrobial assay. The well diffusion antimicrobial assay served as an established method to determine antimicrobial activity of herbs, commercial oral product and cosmetic product [17]. Extract showed antimicrobial activity against all the tested microorganisms except *Escherichia coli* and *Streptococcus pyogenes* (Table 1). Standard antibiotics, ciprofloxacin showed greater zone of inhibition for all the strains while distilled water showed no inhibition activity against all the strains. The antimicrobial activity of *P. acidus* might be via disruption of microorganism's cell wall, inhibition of microbial enzyme activity or by slowing multiplication process of microbial species [18]. The antimicrobial activity of *P. acidus* might

be due to the high phenolic and flavonoid compound present in this plant which is 620.14 ± 0.85mg gallic acid equivalent/g plant material and 36.71 ± 0.1mg rutin equivalent/g plant material respectively. The Presences of phenolic and flavonoid compound are Associated with the antimicrobial activity [19].

Table 1: Zone of inhibition (mm) of *P. acidus* aqueous extract against different strains of Microorganisms

Plant extract	Strains Conc.	*P. aeruginosa* (mm)	*K. pneumonia* (mm)	*E. feacalis* (mm)	*B. subtilis* (mm)	*E. coli* (mm)	*S. pyogenes* (mm)
Aqueous *P. acidus*	1.00 g /ml	5.6±0.5	3.3±0.6	2.3±0.5	9.1±0.3	0.0±0.0	0.0±0.0
	0.50 g/ ml	3.6±0.5	0.0±0.0	0.0±0.0	8.3±0.6	0.0±0.0	0.0±0.0
	0.25 g/ml	3.0±0.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Ciprofloxacin	30.00 µg/ml	5.2±0.3	4.6±0.5	3.5±0.4	4.1±0.2	2.4±0.5	3.2±0.7

CONCLUSION

This present study gathered experimental evidence that the *P. acidus* fruits extract possesses antioxidant property that might contribute to its antibacterial and sun protective activity. Therefore, it can be used as potential agent for the treatment of infectious disease and protection against oxidative damage due to free radicals. *P. acidus* also can be used as natural agent topically to provide sun protective activity. Further studies can be done for this plant for isolation and identification of more constituents from *P. acidus*.

REFERENCES

[1] M Idrisi, KB Hemant, S Rita. Indigenous knowledge and medicinal use of plants by local communities in Rangit Valley, South Sikkim, India. 1(2):34 (2010).

[2] PD Tchakam, P Lunga, TK Kowa, HK Wabo. Antimicrobial and antioxidant activities of extracts and compounds from the leaves of *Psorospermum aurantiacum* and *Hypericum lanceolatum*. *BMC Complementery and Alternative Medicine*. 12(3):135-136 (2012).

[3] B Lamichhane, S Adhikaris. Study of phytochemical, antioxidant, antimicrobial and anticancer activity of *Bacteris aristita*. *The Journal of Tropical Life Science*. 4(1):1-7 (2014).

[4] J Morton, F Julia, FL Miami. Otaheite Gooseberry. In: Fruits of warm climates. 217-219 (1987).

[5] R Chakraboty, B De, N Devanna, S Sen. Asian Pacific Journal of Tropical Medicine. 2: S953-S961 (2012).

[6] D Andrianto, T Katayama, TJ Suzuki. Forest Biomass Utilization Society. 10: 19-25 (2015).

[7] RM Sayre, PP Agin, GJ Levee, E Marlowe. Comparison of in-vivo and in-vitro testing of sun screening formulas. Photochem. Photobiol, Oxford. 559-566 (1979).

[8] HY Choi, EJ Jhun, BO Lim. Application of flow injection-chemilumineacence to the study of radical scavenging activity in plants. Phytotherapy Research. 14:250–253 (2000).

[9] P Prieto, M Pineda, M Aguilar. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex, specific application to the determination of vitamin E. Analytical Biochemistry. 269:337–341 (1999).

[10] MM Rahman, MR Habib, SMR Hasan, MA Sayeed, MS Rana. Antibacterial, cytotoxic and antioxidant potential of methanolic extract of *Phyllanthus acidus* L. *International Journal of Drug Development and Research*. 3:154–161 (2011).

[11] JE Inetianbor, G Ehiowenmwenguan, JM Yakubu, AC Ogodo. In-Vitro antibacterial activity of commonly used toothpaste in Nigeria against dental pathogens. *Journal of Advanced Scientific Research*. 5(2):40-45 (2014).

[12] AL Barry, C Thornsberry. Susceptibility tests: Diffusion test procedures. In; manual of clinical microbiology, 5th edition.

Balows, A. (ed). American Society for Microbiology,
Washington. 1117-1125 (1991).

[13] JG Baumann, Wurn, FV Bruchlausen. Prostaglandin synthetase inhibiting O-2 radical scavenging properties of some flavonoids and related phenolic compounds. *Archives of Pharmacology*. 307: R1-R77 (1979).

[14] LC Wu, HW Hsu, YC Chen, CC Chiu, YI Lin, JA Ho. Antioxidant and antiproliferative activities of red pitaya. Food Chemistry. 95: 319-327 (2006).

[15] BR Subhasree, RL Baskar, RL Keerthana, Susan, P Rajasekaran. Evaluation of
antioxidant potential in selected green leafy vegetables. *Food Chemistry*. 115: 1213-1220 (2009).

[16] ZR Zakaria, YL Aziz, S Lachimanan, Sreenivasan, X Rathinam. Antioxidant activity of *Coleus blumei*, *Orthosiphon stamineus*, *Ocimum basilicum* and *Mentha arvensis* from Lamiaceae family. *International Journal of Natural Science*. 2: 93-95 (2008).

[17] ZD Degiam. An *in vitro* antimicrobial activity of six commercial toothpastes. *Thi-Qar Medical Journal*. 4(4):127-133 (2010).

[18] NA Bou-chacra, SS Gobi, MT Ohara, TJA Pinto. Antimicrobial activity of four different
dental gel formulas on cariogenic bacteria evaluated using the linear regression method. *Brazilian Journal of Pharmaceutical Sciences*. 41(3): 323-331 (2005).

[19] R Baharfar, R Azimi, M Mohseni. Antioxidant and antibacterial activity of flavonoid-, polyphenol- and anthocyanin-rich extracts from *Thymus kotschyanus* boiss & hohen aerial parts. Journal of Food Science and Technology. 52(10): 6777–6783 (2015).