IJPT
1735-2657/11/101-1-5
IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS

IJPT 10: 1-5, 2011

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RESEARCH ARTICLE

Antibacterial Activity and Cytotoxicity Screening of Sumatran Kaduk (*Piper sarmentosum Roxb.*)

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Received December 15, 2009; Revised July 3, 2010; Accepted September 28, 2010

This paper is available online at *http://ijpt.iums.ac.ir*

ABSTRACT

Phytochemical investigations of *Piper sarmentosum* Roxb., yielded four compounds; three amides, identified as 3-(3',4',5'-trimethoxyphenylpropanoyl) pyrrolidine, 3-(4'-methoxyphenylpropanoyl) pyrrole, *N*-(3-phenylpropanoyl) pyrrole and a sterol namely *β*-sitosterol. 3-(4'-Methoxyphenylpropanoyl) pyrrole was found for the first time in this *Piper* species. All chemical constituents were tested for their antibacterial activity using disk diffusion method and cytotoxicity screening using sul-forhodamine B (SRB) assay. All of the compounds were found only active towards gram-positive bacteria except 3-(4'-methoxyphenylpropanoyl) pyrrole with no activity against both gram-positive and gram-negative bacteria. Meanwhile, the cytotoxicity screening using SRB assay indicated that none of these compounds was active as an anticancer agent.

Keywords: *Piperaceae, P. sarmentosum, Amides, Antibacterial, Cytotoxicity*

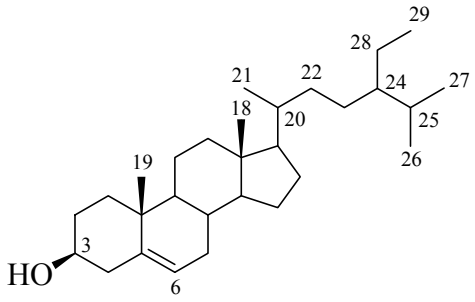
The study of medicinal plants opened the door to the development of purified and defined chemical compounds as dose-controlled medicines. Natural compounds can become central players in the treatment of disease and in the understanding of disease mechanisms. Compounds that emerged from the study of ethnobotanic extracts became important as medicines and were enabling as pharmacologic tools in the elucidation of disease mechanisms [1]. Piperaceae family has provided many past and present civilizations with a source of diverse medicines and food grade spice [2]. This plant is distributed pantropically. The earliest classification of the Piperaceae family recognized between 7 to 15 genera and five of them such as *Piper*, *Peperomia*, *Lepianthes*, *Macropiper*, and *Trianopiper* are only accepted as the principle genera of Piperaceae. This genus contains over 1000 species in the world [3]. This plant can be recognized by three main features: articulate stem, asymmetrical or cordate leaves, and axillary spikes of little round berry-like fruits [4]. According to Jaramilo [3], Asian tropic has 340 species of *Piper*, including Sumatra tropical rainforests. This species takes the form of shrubs, herbs, lianas, and mostly woody climbers. They are common in the warm, humid region and in the lowland of wet forests. The leaves are typically aromatic or have pungent smell. This genus consists of a large family of plants indigenous to the tropic and native people have used them for a long time in a variety of medicinal capacities [5].

Piper sarmentosum Roxb. is one of the *Piper* genus which is known as kaduk, sirih duduk or mengkadak in Indonesia. Traditionally, it was used as a remedy for tooth-ache and for fungoid dermatitis on the feet, for treatment of coughs, influenza and rheumatism [6]. A decoction of the leaves is drunk to treat malarial fever [7] and the crushed leaves are mixed with water and used for bathing to treat kidney stones and difficulty in urination [8]. Previous chemical constituents on this plant have resulted in the isolation of a number of compounds [9-13]. We now describe the isolation of an additional amide from the aerial part of this plant which was collected from Sumatra, Indonesia and also its antibacterial and cytotoxicity activities.

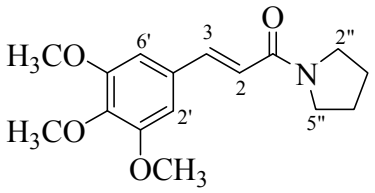
MATERIALS AND METHODS

General Experimental Procedures

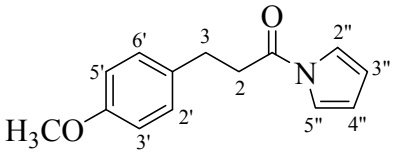
Mps. (uncorr.) were determined using the Leica Gallen III apparatus. IR spectra were recorded on a Perkin-Elmer 1650 FTIR spectrophotometer. NMR spectra were recorded on a Bruker Avance 300 Spectrometer, ^{1}H NMR spectra were measured at 300 MHz and ^{13}C NMR spectra were measured at 75 MHz. Deuterated solvent of chloroform ($CDCl_3$) was used as



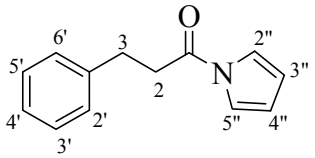
(Compound 1)
β-sitosterol



(Compound 2)
3-(3',4',5'-trimethoxy-phenylpropanoyl)pyrrolidine



(Compound 3)
3-(4'-methoxyphenylpropanoyl) pyrrole



(Compound 4)
N-(3-phenylpropanoyl) pyrrole

Fig 1. Chemical structure of the isolated compounds of *P. sarmentosum*

solvents. Mass spectra data were obtained from Kent Mass Spectrometry Services, United Kingdom. CC: silica gel (Merck 70-230 mesh and 230-400 mesh). Spots on TLC were visualized by UV (254 and 365 nm) and vanillin-sulphuric acid reagent. Streptomycin sulphate standard was purchased from Oxoid (Hampshire, UK).

Plant Material

The aerial parts of *P. sarmentosum* were collected from Desa Sariak, Sungai Pua, about 11 km from Bukittinggi, West Sumatra, Indonesia in 2005. The sample (EM-01/1205) was identified by Mr. Rusdi Tamin and Ms. Nurainas and specimen was deposited at the Andalas Herbarium (ANDA), University of Andalas, Padang, Indonesia.

Extraction and Isolation

The powdered of aerial plant parts of *P. sarmentosum* (1.6 kg) was soxhlet-extracted successively with 3.5 L of each hexane and ethyl acetate for 18 hours. The solvent of each extract was evaporated *in vacuo* to afford the crude hexane, PSH (28.9 g, 1.81 %) and ethyl acetate, PSE (24.9 g, 1.55 %). The crude PSH extract (10 g) was fractionated by VLC over silica gel (230-400 mesh, 250 g) and eluted with gradient solvent system of hexane, hexane- CH_2Cl_2 and CH_2Cl_2 to afford 10 fractions (PSHA-PSHJ).

Fraction PSHD and PSHF were combined (421.5 mg) and subjected to CC over SiO_2 (70-230 mesh, 40 g) to yield nine fractions. The seventh fraction was concentrated and recrystallized from CH_2Cl_2-hexane to give *β*-sitosterol (Fig 1- Compound 1) (95.1 mg, 0.0059%) as white crystalline needles with melting point (mp) 133-134^{o}C (lit. [14] 138-139^{o}C).

Fraction PSH I (248.5 mg) was chromatographed over SiO_2 (30 g) CC with CH_2Cl_2 (100%) as eluent to give 156 fractions. The combined fractions 17-39 was concentrated and further purified by recrystallization from hexane to yield 3-(3',4',5'-trimethoxy-phenylpropanoyl)pyrrolidine (Fig 1- Compound 2) (135.6 mg, 8.5 x 10^{-3}%) as colourless crystalline solids with mp 158-159^{o}C (lit. [15] 156-157^{o}C).

The crude EtOAc extract (10 g) was fractionated by VLC using hexane, mixture of hexane-CH_2Cl_2 and CH_2Cl_2 by step gradient polarity technique to yield seven fractions (PSEA-PSE G). Fraction PSEB (1.033 g) was purified by CC over SiO_2 (70-230 mesh, 75 g) with hexane-CH_2Cl_2 (50: 50) as eluent to give four fractions. Fractions 25-124 were combined and concentrated to yield 3-(4'-methoxyphenylpropanoyl) pyrrole (Fig 1- Compound 3) (18.9 mg, 0.0012%) as yellow crystalline solids with mp 83-84^{o}C (lit. [16] 86-87^{o}C).

Fraction PSE D (481.7 mg) was subjected to CC over SiO_2 (70-230 mesh, 40 g) with hexane-CH_2Cl_2 (10:90) as eluent to afford 102 fractions (PSED1-PSE D9). Fraction PSE D4 (83.6 mg) was further rechromatographed by CC over SiO2 with CH_2Cl_2 as eluent to yield *N*-(3-phenylpropanoyl) pyrrole (Fig 1- Compound 4) (34.5 mg, 0.0022%) as colourless liquid.

Antibacterial Assay (disk diffusion method)

The chemical constituents from *P. sarmentosum* were tested against gram-negative; *Escherichia coli*, *Pseudomonas aeruginosa* and gram positive bacteria; *Bacillus subtilis*, and *Staphylococcus aureus*. Agar cultures of the test microorganisms were prepared according to Mackeen *et al*. [17]. Samples were dissolved in MeOH (1 mL). The test samples (10 µL) were loaded onto each Whatman filter paper disks (0.6 mm) and evenly placed on the agar surface previously inoculated with the suspensions of microorganism to be tested. Standard disk of streptomycin sulphate (10 µg/disk) was used as the positive control and DMSO was used as the negative control. The plates were

Table 1. Antibacterial activity of the isolated compounds of *P. sarmentosum*

Compounds	Zone of Inhibition (mm)			
	Gram-Positive Bacteria		Gram-Negative Bacteria	
	B. s	*S. a*	*P. a*	*E. c*
(1)	9.7 ± 0.52	10.3 ± 0.82	-	-
(2)	12.3 ± 0.52	-	-	-
(3)	-	-	-	-
(4)	-	10.2 ± 0.41	-	-
SS	19.3 ± 1.21	21.3 ± 0.81	19.1 ± 0.54	18.8 ± 0.74

Data represent mean ± standard deviation of three independent experiments performed in duplicate. (-): no activity; *B.s*: *Bacillus subtilis*; *S.a*: *Staphylococcus aureus*; *E.c*: *Escherichia coli*; *P.a*: *Pseudomonas aeruginosa*.

Table 2. MIC and MBC value of the isolated compounds of *P. sarmentosum*

Compounds	MIC (μg/mL)				MBC (μg/mL)			
	Gram-Positive bacteria		Gram-Negative Bacteria		Gram-Positive Bacteria		Gram-Negative Bacteria	
	B. s	*S. a*	*P. a*	*E. c*	*B. s*	*S. a*	*P. a*	*E. c*
(1)	-	500	-	-	-	500	-	-
(2)	500	-	-	-	1000	-	-	-
(3)	-	-	-	-	-	-	-	-
(4)	-	125	-	-	-	125	-	-
SS	3.91	3.91	3.91	3.91	7.81	7.81	7.81	7.81

MIC: Minimal inhibitory concentration, MBC: minimal bacterial concentration, B.:, Bacillus subtilis; S.a: Staphylococcus aureus; E.c: Escherichia coli; P.a: Pseudomonas aeruginosa.

inverted and incubated for 18 hours at 37°C. Clear inhibition zones around the discs indicated the presence of antimicrobial activity.

The positive results then followed by the determination of Minimum Inhibitory Concentration (MIC) by the micro-titer broth dilution method [18]. This test was performed in a sterile 96-well micro titer plates. The test samples (1 mg) were dissolved in methanol to obtain 1000 μg/mL stock solution. Each methanolic stock samples (10 μL) was transferred to micro titer plate well in duplicate at row A. A number of wells were reserved in each plate for positive and negative controls. Sterile broth (100 μL) was added to each micro-titer plate well from row B to row H. Then, the suspensions of microorganisms (200 μL) were added to the samples at row A. Mixture from row A (100 μL) was transferred to each micro titer plate well in order to obtain a twofold serial dilution of stock samples (concentration of 500 μg/mL to 3.9 μg/mL) plates were then incubated for 18 hours at 37°C. Bacterial growth was indicated by the presence of turbidity and a pellet at the bottom of the well. The lowest concentrations, which did not show any growth of tested microorganisms after macroscopic evaluation were determined as MIC values.

The MIC values were confirmed by the determination of Minimal Bactericidal Concentration (MBC) values according to method developed by Arias *et al*. [19]. All wells in the MIC study, which did not show any growth of bacteria after incubation period were first diluted in fresh nutrient broth (1:4) and then sub-cultured onto the surface of freshly prepared nutrient agar plates (θ,15 mm). The plates were incubated for 18 hours at 37°C. The MBC were recorded as the lowest concentration of the sample that did not permit any visible bacteria colony growth on the appropriate agar plate after the incubation period.

Cytotoxicity Screening

The cytotoxicity screening was carried out according to sul-forhodamine B (SRB) method described by Houghton *et al*. [20]. This method relies on the uptake of the negatively charge pink aminoxanthine dye, sulphorhodamine B by basic amino acids in the cells. The greater the number of cells, the greater the amount of dye is taken up and, after fixing, when the cells are lysed, the released dye will give a more intense colour and greater absorbance.

The screening of cytotoxicity test for isolated compounds from this *Piper* species against four cancerous cell lines i.e. human breast carcinoma cell lines (MCF-7 and MDA-MB-231), human intestine epithelial cell line (HT-29) and human ovarian carcinoma cell line (SKOV-3), was carried out by Mr. Cheah Yew Hong from the Institute for Medical Research (IMR), Malaysia.

Statistical Analysis

Statistical analyses were performed using Sigma plot 8.0. Data is presented as means standard error of triplicate samples.

RESULTS

Two chemical constituents have been isolated from the crude hexane extract of *P. sarmentosum* identified as *β*-sitosterol (Compound 1) and 3-(3',4',5'-trimethoxyphenylpropanoyl)-pyrrolidine (Compound 2) and two amides have also been isolated from the crude EtOAc extract namely as 3-(4'-methoxyphenylpropanoyl)pyrrole (Compound 3) and *N*-(3-phenylpropanoyl)pyrrole (Compound 4).

The antibacterial activity using disk diffusion method, followed by the determination of MIC and MBC were presented in Table 1 and Table 2. The

Table 3. Percentage of cells survival on cytotoxicity assay of isolated compounds of *P. sarmentosum* by SRB assay

Compounds	Percentage of cell survival (%) at 20 µg/ml of samples			
	Cell lines			
	MCF-7	SKOV3	HT-29	MDA-MB-231
(1)	100.16	128.59	102.54	110.43
(2)	97.39	110.26	133.71	113.79
(3)	93.67	105.23	102.49	100.46
(4)	69.00	122.31	91.53	94.28

SRB = sulphorhodamin B, MCF-7 and MDA-MB-231 (human breast cancer cell lines), SKOV3 (human ovarian carcinoma cell lines), HT-29 (human colon/intestinal carcinoma cell lines).

isolated compounds were also screened for their cytotoxic assay using SRB assay. Their activities are given in Table 3.

DISCUSSION

The isolated compounds were identified based on the physical, chemical and spectroscopic properties and comparison with data of the literatures. This is the first reported of the isolation of 3-(4'-methoxyphenylpropanoyl) pyrrole from *P. sarmentosum*. It was reported previously from *Piper lolot* C.DC., from Vietnam [16]. Occurrence of chemical constituents of a plant species depends on several factors, such as location or environment that will probably give variation in constituents. Geographical distribution, seasons, different plant parts and morphology, climate as well as ecological conditions may also influence the biosynthesis of the secondary metabolites of the plants. This is may be the reason why the chemical constituents of this species are different from the same species which were reported previously.

As shown in Tables 1 and 2, all isolated compounds were found active towards Gram positive bacteria except 3-(4'-methoxyphenylpropanoyl)pyrrole that shown no activity against both Gram negative and positive bacteria. 3-(3',4',5'-Trimethoxyphenyl-propanoyl)pyrrolidine showed significant activity against *B. subtilis* (MIC 500 µg/ml, MBC 1000 µg/ml) followed by *β*-sitosterol (MIC and MBC 500 µg/ml) and *N*-(3-phenylpropanoyl)pyrrole (MIC and MBC 125 µg/ml) against *S. aureus*. But, activity of these compounds is not as good as the activity of positive control streptomycin sulphate (MIC 3.91 µg/ml and MBC 7.81 µg/ml). All isolated compounds exhibited no activity towards Gram negative bacteria.

In the toxicity screening using SRB assay showed that all isolated compounds have the percentage of the cell survival was higher than 50%. Thus, indicated that none of these compounds was active as anticancer agent (Table 3).

As the conclusion, geographical distribution, location or environment, seasons, different plant parts, climate as well as ecological conditions may influence the biosynthesis of the secondary metabolites of the plants species which portray the variation in chemical constituents. Investigations on the methanolic extracts of these *Piper* species should be carried out. Different models of biological activities should be performed on the crude extracts and pure compounds to verify the mode of action of the active candidates.

REFERENCES

1. Rishton JM. Natural Products as a Robust Source of New Drugs and Drug Leads: Past Successes and Present Day Issues. *Am J Cardiol* 2008; 101: 43D-49D.
2. Scott IA, Jensen HR, Philogene BJR, Arnoson JTA. Review of *Piper* spp. (Piperaceae) Phytochemistry, Insecticidal Activity and Modes of Action. *Phytochem Rev* 2008; 7:65-75.
3. Jaramillo MA, Manos PS. Phylogeny and Pattern of Floral Diversity in the Genus *Piper* (Piperaceae). *Am J Bot* 2001; 88:706-16.
4. Wiart C. *Medicinal Plants of Asia and the Pacific*. Boca Raton, London, New York: Taylor & Francis. 2006.
5. Parmar VS, Jain SC, Bisht KS, Jain R, Taneja P, Jha A, Tyagi OD, Prasad AK, Wengel J, Olsen CE, Boll PM. Phytochemistry of the Genus *Piper*. *Phytochemistry* 1997; 46:597-503.
6. Rahman NNNA, Furuta T, Kojima S, Takane K, Mohd MA. Anti-malarial Activity of Extracts of Malaysian Medicinal Plants. *J Ethnopharmacol* 1999; 64:249-54.
7. Ong HC, Nordiana M. Malay Ethno-medico Botany in Machang, Kelantan, Malaysia. *Fitoterapia* 1999; 70: 502-13.
8. Ong HC, Norzalina J. Malay Herbal Medicine in Gemenceh, Negeri Sembilan, Malaysia. *Fitoterapia* 1999; 70:10-4.
9. Masuda T, Inazumi A, Yamada Y, Padolina WG, Kikuzaki H, Nakatani N. Antimicrobial Phenylpropanoids from *Piper sarmentosum*. *Phytochemistry* 1991; 30:3227-8.
10. Likhitwitayawuid K, Ruangrusi N. Structural Elucidation and Synthesis of New Components Isolated from *Piper sarmentosum* (Piperaceae). *Tetrahedron* 1987; 43:3689-94.
11. Stohr JR, Xiao PG, Bauer R. Isobutylamides and a new methylbutylamide from *Piper sarmentosum*. *Planta Med* 1999; 65:175–7.
12. Rukachaisirikul T, Siriwattanakit P, Sukcharoenphol K, Wongvein C, Rattanaweang P, Wongwattanavuch P. Suksamrarn A. Chemical Constituents and Bioactivity of *Piper sarmentosum*. *J Ethnopharmacol* 2004; 93:173-6.
13. Tuntiwachwuttikul P, Phansa P, Pootaengon Y, and Taylor WC. Chemical Constituents of the Roots of *Piper sarmentosum*. *Chem Pharm Bull* 2006; 54:149-51.
14. Gunston FD. *Fatty Acid and Lipid Chemistry*. London, Glasgow, New York: Blacky Academic & Professional 1996.
15. Achenbach H, Fietz W, Woerth J, Waibel R, Portecop J. Constituents of Tropical Medicinal Plants IXX GC/MS-investigations of the Constituents of *Piper amalago*-30, New Amides of the Piperine-type. *Planta Med* 1986; 1:12-7.
16. Luger P, Weber M, Dung NX, Luu VT, Rang DD, Tuong DT, Ngoc PH. The Crystal Structure of 3-(4'-Methoxyphenylpropanoyl)pyrrole of *Piper lolot* C.DC., from Vietnam. *Crystal Res Tehnol* 2002; 37:627-33.
17. Mackeen MM, Ali AM, El-Sharkawy SH, Manap MY, Salleh KM, Lajis NH, Kawazu K. Antimicrobial and Cytotoxic Properties of Some Malaysian Traditional Vegetables. *Int J Pharmacog* 1997; 35:174-8.
18. Zavala MA, Perez GS, Perez GRM. Antimicrobial Screening of Some Medicinal Plants. *Phytother Res* 1997; 11:368-71.
19. Arias ME, Gomez JD, Cudmani NM, Isla MI. Antibacterial Activity of Ethanolic and Aqueous Extracts of *Acacia aroma* Gill. Ex Hook et Arn. *Life Sciences*, 2004; 75:191-202.

20. Houghton P, Fang R, Techatanawat I, Steventon G, Hylands PJ, Lee CC. The sulphorhodamine (SRB) Assay and other Approaches of Testing Plant Extracts and Derived Compounds for Activities Related to Reputed Anticancer Activity. *Cancer Methods* 2007; 42:377-87.

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