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Phytochemical Analysis and Antibacterial Activity of Ethanolic Leaf Extract of *Solanum torvum* Sw. Against Pathogenic Bacteria

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ABSTRACT

Solanum torvum Sw. is one of the important medicinal plants belonging to the family Solanaceae. Different parts of the plant are reported to possess a number of medicinal properties. This study determined the phytochemical constituents and antibacterial activity of the ethanolic extract of *S. torvum* leaves using gas chromatography-mass spectrometry and broth microdilution assays, respectively. Phytochemical analysis demonstrated the presence of 32 chemical constituents which were mainly phenolic compounds, terpenoids, palmitic acid, palmitic acid ester, linoleic acid, linolenyl alcohol, linolenic acid ester and stearic acid. The antibacterial activity of the extract was tested against five pathogenic bacteria (*Staphylococcus aureus, Staphylococcus intermedius, Staphylococcus epidermidis, Bacillus cereus* and *Pseudomonas aeruginosa*) and exhibited growth inhibition against all tested bacteria with minimum inhibitory concentration and minimum bactericidal concentration values that ranged between 1.95 and 31.25 mg.mL^{-1}. The strongest antibacterial activity of the extract was found against *Bacillus cereus*. This corresponded to the efficacies of the phenolic compounds and terpenoids detected in the extract. The study suggested that the ethanolic extract of *S. torvum* leaves is promising for development in the treatment of various infectious diseases in the future.

Keywords: *Solanum torvum* Sw., ethanolic extract, phytochemical analysis, antibacterial activity

INTRODUCTION

Solanum torvum Sw. (family Solanaceae) is a spiny shrub 2-3 m tall, having broadly oval to elliptic leaves with a shallow, indented edge and an acute-to-obtuse apex, white bell-shaped flowers and edible fruits (Jaiswal, 2012). It is found and cultivated throughout tropical areas (Little *et al*., 1974) and also is distributed widely in Thailand where it is known as turkey berry or Thai eggplant (Agrawal *et al*., 2010). Fruits are used as a vegetable and ingredient for cooking while different parts of the plant are widely used in folk medicine (Jaiswal, 2012). The whole plant is traditionally used as a digestant, diuretic and sedative (Kala, 2005). Fruit and leaf decoctions are used to treat liver and spleen enlargement and coughing (Siemonsma and Piluek, 1994). Leaves are used for haemostasis and anti-inflammation (Rastogi and Mehrotra, 1990; Ndebia *et al*., 2007; Yuanyuan *et al*., 2009). Extracts and metabolites of the plant, especially those from fruits and leaves have been studied

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extensively using different kinds of solvents and extraction procedures. They contains diverse phytochemical constituents such as steroids, glycosides, saponins, flavonoid, phenols, vitamin C, vitamin E, iron salt and steroidal alkaloids (Jaiswal, 2012; Thenmozhi and Mahadeva Rao, 2012). Many pharmacological studies have shown that this plant possesses antioxidant (Sivapriya and Srinivas, 2007; Thenmozhi and Mahadeva Rao, 2012), antiplatelet aggregation, cardiovascular (Nguelefack *et al*., 2008; Mohan *et al*., 2009), analgesic, anti-inflammation (Ndebia *et al*., 2007), antiviral (Arthan *et al*., 2002) and anti-microbial activities (Ajaiyeoba, 1999; Chah *et al*., 2000), and many more that are related with the phytochemical constituents (Agrawal *et al*., 2010). The antibacterial potency of *S. torvum* leaves against some human and plant pathogenic bacteria has already been investigated (Bari *et al*., 2010; John de Britto *et al*., 2011). However, these studies mainly used water, chloroform and methanol as extraction solvents. Medical plants have been used to treat human diseases since the plants contain components of therapeutic value (Nostro *et al*., 2000). They are now popular in developing countries due to improved knowledge of the efficacy, quality and safety assurance of ethano medicine (Jaiswal, 2012). In addition, the phytochemical constituents of the plants have been extensively investigated as sources of medicinal agents (Agrawal *et al*., 2010). In view of the increasingly difficult problem of microbial resistance to most antibiotics (Chen *et al*., 2005), medicinal plants are now being considered as alternatives for the treatment of diverse infections (Tanaka *et al*., 2002). For this reason, the current study aimed to determine the phytochemical constituents of the ethanol extract of *S. torvum* leaves using gas chromatography-mass spectrometry (GC-MS) assay and to investigate the antimicrobial efficacy of the extract against five common pathogenic bacteria.

MATERIALS AND METHODS

Plant materials

The fresh leaves of *S. torvum* Sw. were collected from the forests in Thung Chang District, Nan province, Thailand during November and December, 2011. The plants were authenticated by the Office of the Forest Herbarium Department of National Parks, Wildlife and Plant Conservation, Thailand (code: BKF 186114).

Preparation of plant extract

The leaves of *S. torvum* were cleaned and air dried in the shade for 1–2 wk and subsequently ground into fine powder. The powder was mixed with 95% ethanol at a ratio of 1:10, respectively, and kept in a closed container at room temperature for 1 wk. The mixture was stirred every 2–3 d during immersion. Then, the crude extract was collected using filtering with gauze, cotton and No.1 Whatman grade filter paper (Whatman, 1001-150), respectively. The filtrate was rotary evaporated (N-1001, EYELA; Tokyo, Japan) set at 45 °C to evaporate the solvent (Sriwiroch *et al*., 2010). The concentrated ethanolic extract was stored in vial at -20 °C until use. The extract was dissolved in ethyl acetate or 1% phosphate buffer saline (PBS) both at ratios of 1:1 before performing GC-MS and antibacterial analysis, respectively.

Gas chromatography-mass spectrometry analysis

The ethanolic extract of *S. torvum* leaves was sent to the Central Laboratory (Thailand) Co. Ltd. for CG-MS analysis to explore the phytochemical constituents. Briefly, the Agilent 6980 GC system (Agilent Technologies; Santa Clara, CA, USA) with a programmed split/splitless injector coupled to an Agilent 5973N quadrupole mass spectrometer (Agilent Technologies; Santa Clara, CA, USA) were used for the analysis with a capillary column (5% phenyl-methylpolysiloxane, 30 m × 0.25 mm internal diameter, film thickness 0.25 µm) and the components were separated

using helium as the carrier gas at a constant flow of 2 mL.min^{-1}. A 1 µL sample extract injected into the instrument was detected using an Agilent 5973N mass selective detector (Agilent Technologies; Santa Clara, CA, USA) at 550 °C. The oven temperature regime was: hold at 75 °C, 2 °C.min^{-1} to 100 °C, 3 °C.min^{-1} to 120 °C, 10 min, 2 °C.min^{-1} to 134 °C, 10 min, 5 °C.min^{-1} to 240 °C, 15 min. Mass spectra were taken at 70 eV using the El mode. The mass spectrum of the unknown component was compared with those stored in the Wiley7n.1 library (Adams, 2007). The relative percentage of each component (%Area) was calculated by comparing its average peak area to the total area. The name, molecular formula and compound nature of the component in the tested material were ascertained.

Antimicrobial analysis

Bacterial strain

The microorganisms used for testing antimicrobial sensitivity were four types of Gram-positive (*Staphylococcus aureus* ATCC 25923*, S. intermedius* DMST 11465*, S. epidermidis* ATCC 12228 and *Bacillus cereus* ATTCC 11778) and one type of Gram-negative (*Pseudomonas aeruginosa* ATCC 11778) pathogenic bacteria. They were provided by the Department of Medical Sciences (DMST), Ministry of Public Health, Bangkok, Thailand and were prepared for antimicrobial assay following the method described by Hetru and Bulet (1997). Briefly, one colony of each microorganism was cultured overnight in Luria Bertani (LB) rich medium at 37 °C. Then, the cultures were measured for absorbance at 600 nm and inoculated in Poor Broth (PB) medium at 25 °C to obtain exponential phase culture. The starting OD_{600} (optical density) of diluted bacteria in the PB medium was 0.001.

Determination of minimum inhibitory concentration and minimum bactericidal concentration

A serial broth micro-dilution method made in flat bottom 96-well microtiter plates (Eloff, 1998) was used for determination of the minimum inhibitory concentration (MIC) of the *S. torvum* leaf extract. Briefly, 100 µL of the reconstituted extract solution at a concentration of 500 mg.mL^{-1} was added into the well containing 100 µL of PB to obtain a concentration of 250 mg.mL^{-1}. Then, 100 µL of this solution was transferred to mix with 100 µL of PB in the next well. The transfer was carried on sequentially until the tenth well in order to get ten, two-fold serial dilutions. The wells of positive and negative controls did not contain any extract, but a solution of the bacterial inoculum and solvent (PBS), respectively. Then, 100 µL of the bacterial inoculum at the starting OD_{600} was added in all the wells and mixed thoroughly to give final concentrations ranging from 250 to 0.49 mg.mL^{-1}. The microplates were incubated at 37 °C on an orbital shaker (OS-10, BIOSAN®; Riga, Latvia) at 180 revolutions per minute for 24 hr. The MIC of the extract was detected after adding 40 µL of 0.2 mg.mL^{-1} p-iodonitrotetrazolium chloride (INT; Sigma-Aldrich; St. Louis, MO USA) into all wells and incubated at 37 °C for 30 min. Microbial growth was determined by observing the INT color in the microplate wells being pinkish-red formazan (if the bacterium had grown) or a clear solution (if there was no growth). The MIC was defined as the lowest concentration of extract which completely inhibited the bacterial growth showing a clear color of solution.

The minimum bactericidal concentration (MBC) values were determined by removing 100 µL of bacterial suspension from the MIC wells and also two of the more concentrated dilution wells, and subculturing into LB rich agar and incubating at 37 °C. After 24 hr incubation, the concentration which showed no visible growth of bacteria was recorded as the MBC (Hetru and Bulet, 1997). The solutions from the positive and negative controls were also subcultured into the same agar plate. The MIC and MBC experiments were carried out in triplicate and repeated three times.

RESULTS AND DISCUSSION

The yield percentage of the *S. torvum* ethanolic leaf extract was 8.13%. Ethanol was used as an extraction solvent since previous studies by Thenmozhi and Mahadeva Roa (2012) and Lu *et al.* (2009) had shown that some phytochemical constituents and biological activity of *S. torvum* were also detected in the ethanolic extracts. Lalitha *et al.* (2010) reported the antimicrobial activity of *S. torvum* leaf extract against seed-borne pathogens of rice by using aqueous and different solvents including ethanol. In addition, ethanol is relatively safe and cheap for herbal medicine extraction compared to other organic solvents (Phrompittayarat *et al*., 2007; Lolita *et al*., 2012). Moreover, ethanol possessed an antiseptic property which helped to prevent microbial contamination during the extraction procedure.

Gas chromatography-mass spectrometry analysis

The GC-MS analysis resulted in the identification of a number of volatile organic compounds from the GC fractions of the ethanolic extract of the *S. torvum* leaves. In total, 32 compounds were detected and identified by their mass spectra compared with the library scan. The GC-MS spectrum confirmed the presence of various components with different amounts (%Area) and retention times (RT). The major compounds identified in the ethanolic leaf extract of *S. torvum* are shown in Table 1. They were divided into three major groups—phenolic compounds, terpenoids and fatty acids including their ester and alcohol. This finding corresponded to the study by Thenmozhi and Mahadeva Roa (2012) which revealed the presence of phenols, alkaloids, saponin glycosides, cardioactive aglycons, sterol and flavonoids in the ethanolic leaf extract. However, they performed a different phytochemical assay using the standard methods for alkaloids, saponin, glycosides, flavonoids, phenols, steroids, glycosides-cardio active aglycons, proteins and reducing sugars. The presence of these compounds in the extract

Table 1 Major compounds identified in the ethanolic leaf extract of *Solanum torvum* Sw. using gas chromatography-mass spectrometry analysis.

Compound	Molecular formula	Reaction time (min)	%Area	Compound nature
4-Vinyl phenol	C_8H_8O	14.37	0.79	Phenolic compound
2-Methoxy-4-vinyl phenol	$C_9H_{10}O_2$	18.60	1.22	Phenolic compound
Neophytadiene	$C_{20}H_{38}$	47.46	3.55	Diterpene
Hexadecanoic acid	$C_{16}H_{32}O_2$	50.78	19.54	Palmitic acid
Hexadecanoic acid, ethyl ester (Ethyl palmitate)	$C_{18}H_{36}O_2$	51.12	4.46	Palmitic acid ester
Phytol	$C_{20}H_{40}O$	53.46	14.09	Diterpene
9,12-Octadecadienoic acid (z,z)	$C_{18}H_{32}O_2$	54.12	10.72	Linoleic acid
9,12,15-Octadecatrien-1-ol (z,z,z)-	$C_{18}H_{32}O$	54.22	10.80	Linolenyl alcohol
9,12-Octadecadienoic acid (z,z), Ethyl ester	$C_{20}H_{36}O_2$	54.40	3.46	Linolenic acid ester
9,12,15-Octadecatrienonic acid, Ethyl ester (z,z,z)	$C_{20}H_{34}O_2$	54.51	2.77	Linolenic acid ester
Octadecanoic acid	$C_{18}H_{36}O_2$	54.64	8.11	Stearic acid
Squalene	$C_{30}H_{50}$	72.30	1.46	Triterpene

probably explains its various uses in traditional medicine.

Determination of minimum inhibitory concentration and minimum bactericidal concentration

The MIC and MBC assays were used to evaluate the efficacies of antimicrobial agents in the ethanolic extract of *S. torvum* leaves. The MIC and MBC values of the extract against all tested pathogenic bacteria are summarized in Table 2 with the values ranging between 1.95 and 31.25 $mg.mL^{-1}$. The extract showed the strongest antimicrobial activity for *Bacillus cereus* compared to all other tested bacteria with both MIC and MBC values of 1.95 $mg.mL^{-1}$. The present findings were similar to the study by John de Britto *et al*., 2011) who found a remarkable antibacterial effect of methanolic extract of *S. torvum* leaves on *Xanthomonas campestris* and *Aeromonas hydrophilia* which affect plants and animals, respectively. Bari *et al.* (2010) also reported that methanolic extract from the root of *S. torvum* exhibited an antibacterial effect on all tested organisms including *B. cereus*. However, the current results showed inhibition of bacterial growth at a much higher MIC value (1.95 $mg.mL^{-1}$ versus 0.128 $mg.mL^{-1}$) than that of both these reported studies. It was possible that the antibacterial efficacy of the plant extract was dependent on the kind of solvent used in the extraction procedure. In addition, other factors such as the source of plant cultivation, harvesting season and method, processes undertaken during extract preparation and the age of the plant may influence the phytochemical constituents of the plant extract including those related to the antimicrobial activity. The current MIC results also corresponded to the study by Perumal *et al*. (2012) that investigated the antimicrobial efficacy of *Euphorbia hira* extract against 13 clinically isolated microorganisms using a tetrazolium microplate assay method. The ethanolic extracts of the aerial part of *E. hirta* exhibited the strongest antimicrobial activity against *Salmonella typhi* and *Pseudomonas aeroginosa*. However, the MIC values of *E. hira* extract were in the lower range of concentrations compared to those of the extract from the current study. In addition, the preliminary phytochemical analysis of the *E. hira* extract showed the presence of flavonoids and terpenoids that were also found in the plant extract from the current study.

The MIC value of the extract in the current study was analyzed using the serial broth micro-dilution method in 96-well micro-titer plates, using p-iodonitrotetrazolium chloride (INT) as a colorimetric indicator to evaluate the growth of bacteria. The assay enhanced the sensitivity and accuracy of the MIC determination because the formazan derivatives produced by bacteria can be quantified (Masoko *et al*., 2007). The MIC and MBC values were predictors of the efficacy of the agents. However, the MBC values, which were obtained after reculturing the bacterial suspension from selected dilutions of the extract, made the activity test more reliable. In the current study, the values of the MIC and MBC of the extract against all tested organisms were the same.

Table 2 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanolic leaf extract of *Solanum torvum* Sw.

Organism	MIC ($mg.mL^{-1}$)	MBC ($mg.mL^{-1}$)
Bacillus cereus	1.95	1.95
Staphylococcus epidermidis	15.63	15.63
Staphylococcus aureus	15.63	15.63
Staphylococcus intermedius	15.63	15.63
Pseudomonas aeruginosa	31.25	31.25

A correlation between the presence of phytochemicals and the antimicrobial activities of this extract was apparent. The antibacterial activity could be attributed to the presence of its phytochemical constituents such as phenolic compounds and terpenoids, which have been reported to possess antimicrobial activities (Gupta *et al*., 2011; Daglia, 2012). As was previously reported by Navarro *et al*. (1996), the antimicrobial effect of plant extracts was due to the presence of plant secondary metabolites which include among many others alkaloids, terpenoids and phenolic compounds (Krishnaial *et al*., 2009).

According to the results of the antibacterial assay, the ethanolic extract of *S. torvum* can be used as an antibacterial agent against all the tested pathogenic bacteria which affect humans and animals. More species of pathogenic bacteria should be tested to ascertain the spectra of activities of the present antibacterial substances in the plant preparations.

CONCLUSION

The ethanolic extract of *Solanum torvum* Sw. leaves possesses potential antimicrobial effect against all tested pathogenic bacteria which correspond to its phytochemical constituents. Further studies should be carried out to investigate clinical trials and the toxicity of the extract that should be conducted to support its therapeutic uses.

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