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Antimicrobial activity of some Vietnamese medicinal plants extracts

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Antimicrobial activities of some Vietnamese medicinal plant extracts including *Premna integrifolia* L, *Terminalia nigrovenulosa, Pseuderanthemum palatiferum*, *Streptocaulon juventas*, *Eclipta alba*, and *Solanum hainanense* were investigated. Almost all extracts exhibited antimicrobial effects on the tested microorganisms, including *Bacillus subtilis*, *Staphylococcus aureus*, *Lactobacillus brevis*, *Escherichia coli*, *Pseudomonas aeruginose*, *Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Penicillium cyclopium*. Of these extracts, the methanol extracts exhibited higher antimicrobial activity levels compared to that of other extracts. The greatest activity was obtained with *T. nigrovenulosa* methanol extracts, followed by the extracts of *P. nigrovenulosa* and *S. juventas*. The results indicate that the methanol extracts of *T. nigrovenulosa*, *S. juventas*, and *P. nigrovenulosa* have significant potential for use as traditional therapy. Further study may lead to a novel antimicrobial compound.

Key words: Medicinal plant, antimicrobial activity, minimum inhibitory concentration (MIC).

INTRODUCTION

Medicinal plant extracts are an important reservoir for modern medicine, due to the existence of natural bioactive compounds in many plants. Secondary metabolites represent a large source of structural moieties that exhibit a wide range of biological activities. Microorganisms can change their genetics and become resistant to antibiotics, which is a major global healthcare problem (Alanis, 2005). Therefore, the number of natural antibacterial drugs in clinical use is increasing (Newman et al., 2003). Plant-derived antimicrobial compounds inhibit bacteria through different mechanisms and treat infections caused by resistant microbes (Stein et al., 2005). Many studies have reported the antimicrobial activity of crude plant extracts and have fractionated them to yield active compounds (Kyung et al., 2007).

According to Wilson (1988), > 250,000 plants exist in the world, but only 5 to 15% have been researched for their potential therapeutic value (Baladrin et al., 1985; Kinghorn, 1992). A large number remain to be evaluated for their antimicrobial activity against the antibiotic-resistant microorganisms to develop complementary phytochemical strategies (Simoes et al., 2009). The following Vietnam medicinal plants were selected for investigation of antimicrobial activity: *Premna integrifolia* L., *Terminalia nigrovenulosa* Pierre ex Laness, *Pseuderanthemum palatiferum* (Nees) Radlk, *Streptocaulon juventas* (Lour.) Merr., *Eclipta alba* (L.) Hassk, *and Solanum hainanense* Hance.

T. nigrovenulosa Pierre ex Laness belongs to the Combretaceae family widely distributed in tropical and subtropical regions of the world. There are about 250 species of *Terminalia* genus and some species have

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been used as folk medicine in Asia. In Vietnam, *T. nigrovenulosa* are used as an anti-diarrhea in the treatment of chronic dysentery, sore throat, laryngitis, and hemorrhoids. In Philippines, *Terminalia calamansanai* (Blanco) Rolf. Is used as a lithontriptic (Tanaka et al., 1991). Many researches showed that the extracts of *Terminalia* species possessed a variety of biological activities. For instance, the solvent extracts of *T. nigrovenulosa* bark and leaf showed strongly antioxidant activity (Nguyen and Eun, 2011); methanol extracts of *Terminalia chebula* fruits have effect on antioxidant (Lee et al., 2007); antimicrobial (Malekzadeh et al., 2001); antidiabetic (Gao et al., 2008) and anticancer (Saleem et al., 2002) activities. The extract of *Terminalia catappa* L. leaves has hepatoprotective activity (Tang et al., 2004).

Premna integrifolia L. is a garden shrub, belonging to the Verbenaceae family, widely spreads in tropical and subtropical regions throughout the world. In Vietnam, it is cultivated as an ornamental and shade tree. It is one of the important constituents among ten herb formulations called ‘‘Dashmula’’, a favorite decoction of ten plants used in India. The plant possessed antirheumatic, carminative, galactogenic, bechic, febrifuge, stomachic and anti-inflammatory activities. The solvent extracts of *P. integrifolia* L. leaf showed antioxidant activity (Nguyen and Eun, 2011). Rajendran and Basha (2010) have recently described significant broad-spectrum antimicrobial activity in different extracts (n-hexane, chloroform, ethylacetate and ethanol) of the roots of *P. integrifolia*. Bark and wood extracts of *P. integrifolia* possessed cardiac stimulant activity (Rajendran et al., 2008). Previous data showed that root of *P. integrifolia* contained bioactive compounds including alkaloids, flavonoids, glycosides, tannins, phenolic compounds and diterpenoids (Mali and Bhadane, 2010; Yadav et al., 2010, 2011).

Solanum hainanense Hance (synonym: *Solanum procumbens* Lour.) belongs to the Solanaceae family and is a valuable medicinal plant found in many areas of China and Vietnam. It has been used in anti-inflammation, anti-histaminemia, and vernomous snake-bite treatment (Thai et al., 1998). The main constituents presented in *S. hainanense* are proline, hydroxyproline, methionine, flavonoid and saponin steroids (Man et al., 1990). Recently, it has been reported to be effective in protection of liver; for example, *S. hainanense* extract could protect the liver of white mice poisoned-related with prolonged exposure to trinitrotoluene (TNT) and small oral doses of TNT (Thai et al., 1998). “HAINA” drug which prepared from *S. hainanense* have positive effect on hepatitis B patients (Hoa et al., 2005). Moreover, S. *hainanense* Hance extracts also have an effect on preventing experimental inflammation and cirrhosis in sewer rats poisoned by CCl_4 (Khai, 1988).

Streptocaulon juventas (Lour.) Merr. is a plant of the Asclepiadaceae family and is native to Indochina. The Vietnamese name of *S. juventas* is “Ha thu o trang”. In Vietnam, the root of this plant is used as a tonic for various treatments such as anemia, chronic malaria, rheumatism, menstrual disorders, neurasthenia, and dyspepsia. The extracts of different parts of *S. juventas* possessed various biological activities for instances, the methanol extract of *S. juventas* roots showed inhibition of proliferation of HT1080 cell lines and human lung A549 adenocarcinoma cells (Ueda et al., 2002). The solvent extracts of leaf also showed antioxidant activity (Nguyen and Eun, 2011).

Pseuderanthemum palatiferum (Nees) Radlk is a new medicinal plant belonging to the Acanthacea family (Ho, 2000), found in Cuc Phuong forest of northern Vietnam in the latter half of the 1990’s. After that, this plant has cultivated throughout the country including the Mekong Delta region (Cuong and Quynh, 1999). The leaves of this plant have been used as a folk medicine in Vietnam and Thailand for promoting and treating various diseases including hypertension, diarrhea, arthritis, hemorrhoids, stomachache, tumors, colitis, bleeding, wounds, constipation, flu, colon cancer, nephritis, and diabetes (Padee and Nualkeaw, 2009). Some reports showed that *P. palatiferum* possesses a variety of biological activities, such as antifungal activity (Giang et al., 2005); anti-diarrhea (Dieu and Hoa, 2003); inhibition of the synthesis acetylcholinesterase reduction in the rat’s brain (Buncharoen, 2010).

Eclipta alba (L.) belongs to the family of Asteraceae, widely spread in tropical and subtropical regions of the world. *E. alba* has been used as traditional medicinal resources in Vietnam for many purposes such as treatment of internal and external haemorrhages, metrorrhagia, menorrhagia, epistaxis, haemorrhoids, haematuria, bloody stools, haemoptysis, haematemesis and hypodermic haemorrhage (Medicinal Plants in Viet Nam, 1990). Previous reports showed that the alcoholic extract of the plant possessed anti-inflammation (Singh et al., 2008) and anticancer activity (Chaudhary et al., 2011). The fresh juice of leaves is used for increasing appetite, improving digestion (Cheryl Lans, 2007). The water extract (whole plant) exhibited a potent inhibitory activity against human immunodeficiency virus (HIV)-1 integrase (HIV-1 IN) (Tewtrakul et al., 2007).

However, there have been few data published on the antimicrobial properties of these plants. The aim of this study is to investigate the antimicrobial effect of extracts from some Vietnamese medicinal plants that possess antioxidant activity (Nguyen and Eun, 2011) and to determine whether they are good sources for new antimicrobial agents and standardized phytomedicines.

MATERIALS AND METHODS

Chemicals and culture media

Dimethyl sulfoxide (DMSO) was obtained from Kanto Chemical Company (Nibonbashi, Chuo-ku, Japan), phenol red from Sigma-Aldrich (St Louis, MO, USA) and ampicillin and cycloheximide from Biosesang (Bundang-gu, Korea). Other chemicals and reagents

used were of analytical grade. The culture media were purchased from Becton, Dickinson and Co. (Sparks, MD, USA), including yeast mold (YM) agar, YM broth, potato dextrose broth, potato dextrose agar, Difco nutrient broth, and Bacto agar.

Plant

The medicinal plants were harvested from different locations in Vietnam. The names of the plants and their parts used in this study are as follows:

Premna integrifolia L. (*P. integrifilia*) (leaves);
Terminalia nigrovenulosa Pierre ex Laness (*T. nigrovenulosa*) (leaves and trunk bark);
Pseuderanthemum palatiferum (Nees) Radlk (*P. palatiferum*) (leaves);
Streptocaulon juventas (Lour.) Merr**.** (*S. juventas*) (whole plant);
Eclipta alba (L) Hassk (*E. alba*) (whole plant);
Solanum hainanense Hance (*S. hainanense*) (whole plant);

The fresh parts of plants were cut and dried at ambient temperature in a room with active ventilation, packed in polyethylene bags, and stored at -80°C before use.

Extraction

Dried powder of plants (10 g) was extracted with 100 ml of various solvents (methanol, acetone, and ethanol) for 24 h in a glass conical flask on a shaker at room temperature, followed by filtration through filter paper (No. 1, Whatman International LTD, Maidstone, England). The residue was then extracted twice more with 100 ml of solvent. The combined solvent extracts were concentrated in a rotary evaporator (VV 2011-Antrieb, Heidolph, Germany) at 40°C under vacuum to obtain dry extracts. The extracts were stored at -80°C until use.

Microorganisms

The microorganisms used were *Bacillus subtilis* subsp. subtilis KCTC 1021, *Staphylococcus aureus* subsp. aureus KCTC 1928, *Lactobacillus brevis* KCTC3102, *Escherichia coli* KCTC 2593, *Pseudomonas aeruginose* KCTC 2513, *Candida albicans* KCTC 7965, *Saccharomyces cerevisiae* KCTC7904, *Aspergillus niger* KCTC 1231, and *Penicillium cyclopium* KCTC 6256. These microorganisms were obtained from the Biological Resource Center of the Korean Collection for Type Cultures.

Antimicrobial activity

Disc diffusion test

The antimicrobial activities of the medicinal plant extracts were evaluated by the agar disc diffusion assay (Bagamboula et al., 2003). Plant extracts were dissolved in 10% dimethyl sulfoxide (DMSO) to a final concentration of 20 and 50 mg/ml and then filtered with 0.45 µm membrane filters (Whatman International) for sterilization. Suspension (100 µl) containing 10^8 CFU/ml bacteria, 10^6 CFU/ml yeast, and 10^4 spores/ml fungi was spread on nutrient agar, yeast malt extract agar, and potato dextrose agar, respectively. After 20 min, a 6 mm diameter sterile filter disc (no. 3, Whatman International) was placed on the agar seeded with microorganisms, and then 10 µl of several extract concentrations were dropped onto each paper disc. The treated Petri dishes were kept at 4°C for 1 h, followed by a 24 h incubation at 37°C for bacteria and a 36 h incubation at 30°C for yeasts and molds. Antimicrobial activity was assessed by measuring the growth inhibition zone surrounding the disc. Under the same conditions, a solution of ampicillin (antibiotic) and cycloheximide (antifungal) were used as positive standards, and discs with 10 µl of 10% DMSO were used as negative controls. Each experiment was carried out in triplicate, and the mean diameter of the inhibition zone was recorded.

Minimum inhibitory concentration (MIC)

The MIC values of the plant extracts and the reference were determined according to the method of Mbaveng et al. (2008). Plant extracts were dissolved in 10% DMSO, and the solution was added to nutrient broth containing 0.05% phenol red supplemented with 10% glucose (NBGP) to a final concentration of 5,000 µg/ml extract. This solution was then diluted two-fold in NBGP to obtain a concentration range of 9.7 to 5,000 µg/ml. One hundred µl of each concentration was added to a well (96-wells) containing 95 µl NBGP and 5 µl inoculum (10^8 CFU/ml bacteria). Wells containing 195 µl NBGP and 5 µl inoculum were used as a negative control. The plate was covered with a sterilized plate sealer, shaken slightly, and incubated at 37°C for 24 h. Microbial growth was determined by observing the change in well color (yellow when there was growth and red when there was no growth). The assays were performed in triplicate. The lowest concentration indicating no color change was considered as the MIC.

Statistical analysis

Results are given as means ± standard deviation (STD) of three replicated determinations. A one way analysis of variance (ANOVA) test (using STATGRAPHICS Centurion XV statistical software) was used to compare the mean values of each treatment. Significant differences between the means of parameters were determined by least significant difference (LSD) test ($p < 0.05$).

RESULTS AND DISCUSSION

Preliminary screening of activity (agar-diffusion method)

The antimicrobial activities of the different plant extracts and the control using the agar disc diffusion method are shown in Tables 1 to 6. Almost all plant extracts showed antimicrobial activity against the tested microorganisms. In general, the methanol extracts exhibited the highest level of antimicrobial activity compared to that of the other solvents. This result agreed with previous studies that found the highest antibacterial activities in methanol extracts of medicinal plants (Mothana et al., 2005; Askun et al., 2009) such as *Satureja hortensis* L (Shahin et al., 2003). These results may depend on the compounds being extracted by each solvent, and their different bio-activities in plants. Moreover, increasing the plant extract concentration tended to increase the diameter of the inhibition zone, indicating that active antimicrobial compounds are present in these extracts.

The antifungal capacity of the extracts depended on both extraction solvent and plant type (Tables 1 and 2). A stronger effect was found in methanol extracts of *T. nigrovenulosa* bark and leaf , which had inhibition zone

Table 1. Antimicrobial activity of the extracts against fungal strains at a concentration of 20 mg/mL (0.2 mg/disc).

Plant	Growth inhibition zone diameter (mm)					
	Aspergillus niger			*Penicillin cyclopium*		
	MeOH	EtOH	Acetone	MeOH	EtOH	Acetone
T. nigrovenulosa (bark)	$^{A}10.00\pm0.340^{a}$	$^{B}7.00\pm0.60^{a}$	$^{C}8.83\pm0.83^{a}$	$^{A}12.33\pm0.83^{ab}$	$^{B}7.58\pm0.77^{ad}$	$^{C}10.25\pm0.96^{bc}$
T. nigrovenulosa (leaves)	$^{A}10.44\pm0.51^{a}$	$^{B}7.00\pm0.74^{a}$	$^{C}8.75\pm1.06^{a}$	$^{A}13.50\pm0.00^{a}$	$^{B}7.92\pm0.99^{a}$	$^{C}11.50\pm0.67^{a}$
P. integrifolia L	$^{A}9.17\pm0.38^{b}$	$^{B}6.50\pm0.52^{a}$	$^{C}8.33\pm0.49^{ab}$	$^{A}11.33\pm0.38^{bc}$	$^{B}6.92\pm0.67^{b}$	$^{C}10.58\pm0.67^{b}$
*P. palatiferum (*Nees) Radlk	$^{A}8.08\pm0.14^{cd}$	$^{B}6.50\pm0.67^{a}$	$^{A}7.75\pm0.75^{bc}$	$^{A}10.42\pm0.38^{c}$	$^{B}6.92\pm0.51^{b}$	$^{A}9.75\pm1.14^{c}$
E. alba (L) Hassk	$^{A}8.50\pm0.66^{bc}$	$^{B}6.58\pm0.67^{a}$	$^{A}8.25\pm0.75^{ab}$	$^{A}11.22\pm1.02^{bc}$	$^{B}7.00\pm0.60^{bd}$	$^{A}10.75\pm0.87^{b}$
S. juventas (Lour.) Merr	$^{A}7.10\pm0.20^{e}$	-	$^{A}7.17\pm0.83^{c}$	$^{A}10.61\pm0.63^{c}$	$^{B}7.33\pm0.98^{abc}$	$^{A}10.42\pm1.08^{cd}$
S. hainanense Hance	$^{A}7.59\pm0.70^{de}$	-	$^{A}7.33\pm0.89^{c}$	$^{A}10.11\pm0.19^{c}$	$^{B}6.92\pm0.79^{b}$	$^{A}10.17\pm0.72^{cd}$

Results are means ± SD of triplicate measurements. Different letters (a to e) in the same column and (A to C) in the same row indicate a significant difference at $P < 0.05$; (-) no inhibition.

Table 2. Antimicrobial activity of the extracts against fungal strains at 50 mg/mL (0.5 mg/disc).

Plant	Growth inhibition zone diameter (mm)					
	Aspergillus niger			*Penicillin cyclopium*		
	MeOH	EtOH	Acetone	MeOH	EtOH	Acetone
T. nigrovenulosa (bark)	$^{A}11.44\pm0.51^{a}$	$^{B}8.66\pm0.98^{a}$	$^{C}10.33\pm0.89^{ab}$	$^{A}14.43\pm0.40^{a}$	$^{B}8.75\pm0.75^{ab}$	$^{C}12.50\pm1.09^{b}$
T. nigrovenulosa (leaves)	$^{A}11.44\pm0.51^{aA}$	$^{B}8.08\pm0.99^{a}$	$^{A}10.83\pm0.83^{a}$	$^{A}15.92\pm0.80^{b}$	$^{B}9.33\pm1.49^{a}$	$^{C}14.08\pm1.24^{a}$
P. integrifolia L	$^{A}9.75\pm0.66^{bA}$	$^{B}7.25\pm0.45^{bB}$	$^{A}9.25\pm0.62^{cd}$	$^{A}12.75\pm0.75^{c}$	$^{B}8.58\pm1.31^{abc}$	$^{A}11.67\pm0.65^{c}$
*P. palatiferum (*Nees) Radlk	$^{A}9.83\pm0.38^{b}$	$^{B}7.08\pm0.52^{b}$	$^{C}8.67\pm0.49^{de}$	$^{A}11.42\pm0.29^{d}$	$^{B}8.08\pm0.90^{bc}$	$^{A}11.67\pm0.89^{c}$
E. alba (L) Hassk	$^{A}9.80\pm0.38^{b}$	$^{B}7.00\pm0.60^{b}$	$^{A}9.75\pm0.75^{bc}$	$^{A}12.11\pm1.9^{c}$	$^{B}8.25\pm0.62^{bc}$	$^{A}12.42\pm1.16^{bc}$
S. juventas (Lour.) Merr	$^{A}8.00\pm0.6^{c}$	-	$^{A}8.00\pm1.04^{f}$	$^{A}11.44\pm0.38^{c}$	$^{B}8.00\pm1.04^{bc}$	$^{A}12.00\pm0.74^{bc}$
S. hainanense Hance	$^{A}8.60\pm0.2^{c}$	-	$^{A}8.42\pm0.67^{ef}$	$^{A}12.89\pm0.69^{cde}$	$^{A}7.75\pm0.75^{c}$	$^{A}12.08\pm0.90^{bc}$
Cycloheximide (2μg/ml)	16.92 ± 2.63^{e}	-	-	15.3 ± 0.12^{c}	-	-

Results are means ± SD of triplicate measurements. Different letters (a to f) in the same column and (A to C) in the same row indicate a significant difference at $P < 0.05$; (-) no inhibition.

diameters of 10 to 11.44 mm (bark) and 10.44 to 11.44 mm (leaf) against *Aspergillus niger* and 12.33 to 14.43 mm (bark) and 13.50 to 15.92 mm (leaf) against *P. cyclopium* at concentrations of 20 to 50 mg/ml (0.2 to 0.5 mg/disc). Previous studies have reported that *Terminalia* species possess substantial antifungal properties (Masoko et al., 2005; Liu et al., 2009). The methanol extract of *P. integrifolia* also had relatively high antifungal activity with inhibition zone diameters of 9.17 to 9.75 mm (against *A. niger)* and 11.33 to 12.75 (against *P. cyclopium*) at the same concentrations mentioned above.

However, the results in Tables 3 and 4 showed that the most effective extracts against *S. cerevisiae* were the methanol extracts of *P. palatiferum, S. juventas*, and *S. hainanense* with inhibition zone diameters of 8.17 to 9.08, 8.17 to 9.08, and 7.75 to 9.00 mm at 20 to 50 mg/ml, respectively. A higher effect against *Candida albicans* was observed in the methanol extracts of

Table 3. Antimicrobial activity of the extracts against yeast strains at 20 mg/ml (0.2 mg/disc).

Plant	Growth inhibition zone diameter (mm)					
	S. cerevisiae			*Candida albicans*		
	MeOH	EtOH	Acetone	MeOH	EtOH	Acetone
T. nigrovenulosa (bark)	$^{A}7.42\pm0.29^{ab}$	$^{B}6.92\pm0.51^{b}$	$^{B}6.67\pm0.49^{a}$	$^{A}6.67\pm0.14^{cd}$	-	$^{A}6.58\pm0.51^{bc}$
T. nigrovenulosa (leaves)	$^{A}7.08\pm0.14^{a}$	$^{B}6.58\pm0.67^{ab}$	$^{AB}6.83\pm0.39^{a}$	$^{A}6.42\pm0.14^{de}$	$^{A}6.33\pm0.50^{a}$	$^{A}6.58\pm0.67^{bc}$
P. integrifolia L	$^{A}8.42\pm0.29^{d}$	$^{B}6.25\pm0.45^{a}$	$^{B}6.83\pm0.39^{a}$	$^{A}7.17\pm0.14^{b}$	$^{A}6.58\pm0.50^{a}$	$^{A}7.00\pm0.60^{a}$
*P. palatiferum (*Nees) Radlk	$^{A}8.17\pm0.14^{cd}$	$^{B}6.50\pm0.52^{ab}$	$^{B}6.75\pm0.62^{a}$	$^{A}6.75\pm0.25^{c}$	-	$^{A}6.25\pm0.45^{c}$
E. alba (L) Hassk	$^{A}7.25\pm0.00^{ab}$	$^{B}6.67\pm0.65^{ab}$	$^{B}6.83\pm0.39^{a}$	6.58 ± 0.14^{cde}	-	-
S. juventas (Lour.) Merr	$^{A}8.17\pm0.14^{cd}$	$^{B}6.67\pm0.52^{ab}$	$^{B}6.75\pm0.45^{a}$	6.33 ± 0.29^{e}	-	-
S. hainanense Hance	$^{A}7.75\pm0.66^{bc}$	$^{B}6.75\pm0.75^{b}$	$^{B}6.80\pm0.46^{a}$	$^{A}7.20\pm0.14^{a}$	$^{A}6.83\pm0.40^{a}$	$^{A}6.83\pm0.58^{ab}$

Results are means ± SD of triplicate measurements. Different letters (a to e) in the same column and (A to B) in the same row indicate a significant difference at $P < 0.05$; (-) no inhibition.

Table 4. Antimicrobial activity of the extracts against yeast strains at 50 mg/ml (0.5 mg/disc).

Plants and reference	Growth inhibition zone diameter (mm)					
	S. cerevisiae			*Candida albicans*		
	MeOH	EtOH	Acetone	MeOH	EtOH	Acetone
T. nigrovenulosa (bark)	$^{A}8.25\pm0.43^{ad}$	$^{B}7.16\pm0.58^{b}$	$^{AB}7.67\pm0.49^{a}$	$^{A}7.08\pm0.38^{a}$	-	$^{A}6.92\pm0.67^{ab}$
T. nigrovenulosa (leaves)	$^{A}7.92\pm0.58^{ac}$	$^{B}7.00\pm0.43^{ab}$	$^{A}7.50\pm0.52^{a}$	$^{A}7.08\pm0.14^{a}$	$^{A}6.75\pm0.62^{a}$	$^{A}6.92\pm0.51^{ab}$
P. integrifolia L	$^{A}8.58\pm0.14^{ab}$	$^{B}7.08\pm0.51^{ab}$	$^{C}7.75\pm0.45^{a}$	$^{A}7.75\pm0.14^{b}$	$^{B}7.00\pm0.60^{a}$	$^{AB}7.33\pm0.49^{a}$
*P. palatiferum (*Nees) Radlk	$^{A}9.08\pm0.38^{b}$	$^{B}7.08\pm0.90^{ab}$	$^{B}7.75\pm0.62^{a}$	$^{A}6.83\pm0.29^{ac}$	-	$^{A}6.50\pm0.52^{bc}$
E. alba (L) Hassk	$^{A}7.42\pm0.29^{c}$	$^{B}6.67\pm0.49^{a}$	$^{A}7.92\pm0.79^{a}$	$^{A}6.58\pm0.14^{cd}$	-	$^{A}6.33\pm0.49^{c}$
S. juventas (Lour.) Merr	$^{A}9.08\pm0.38^{b}$	$^{B}7.17\pm0.72^{b}$	$^{C}7.91\pm0.79^{a}$	$^{A}6.33\pm0.29^{d}$	-	$^{A}6.25\pm0.45^{c}$
S. hainanense Hance	$^{A}9.00\pm0.66^{bd}$	$^{B}7.08\pm0.28^{ab}$	$^{C}7.75\pm0.87^{a}$	$^{A}8.17\pm0.14^{e}$	$^{B}7.17\pm0.57^{a}$	$^{B}7.25\pm0.75^{a}$
Cycloheximide (2μg/ml)	28.80 ± 0.69^{d}	-	-	8.67 ± 0.38^{e}	-	-

Results are means ± SD of triplicate measurements. Different letters (a to e) in the same column (A to C) and in the same row indicate a significant difference at $P < 0.05$; (-) no inhibition.

S. hainanense with an inhibition zone of *7.2* to 8.17 mm, and *P. integrifolia* with an inhibition zone of 7.17 to 7.75 mm at 20 to 50 mg/ml.

The diameters of the inhibition zones for antibacterial activity are shown in Tables 5 and 6. The results showed that almost all extracts displayed antimicrobial activity against both gram positive and gram negative bacteria, indicating the presence of broad spectrum antibiotic compounds in these plants. The antibacterial capacity of the extract depended on plant type. These plant extracts had a significantly stronger inhibitory effect against gram positive than gram negative bacteria. This agreed with previous studies reporting that gram negative bacteria are more resistance to antimicrobial

Table 5. Antimicrobial activity of the extracts against bacterial strains at 20 mg/ml (0.2 mg/disc).

Plant	Growth inhibition zone diameter (mm)								
	S. aureus			*B. subtilis*			*P. aeruginosa*		
	MeOH	EtOH	Acetone	MeOH	EtOH	Acetone	MeOH	EtOH	Acetone
T. nigrovenulosa (bark)	^{A}**8.58±0.52**^{ae}	^{B}7.00±0.18^{a}	^{A}8.17±0.72^{b}	^{A}7.33±0.47^{ad}	^{A}7.56±0.77^{a}	^{A}7.17±0.39^{b}	^{A}6.58±0.52^{a}	^{A}6.42±0.47^{a}	^{A}6.58±0.67
T. nigrovenulosa (leaves)	^{A}**9.25±0.98**^{b}	^{B}7.75±0.18^{b}	^{A}8.75±0.75^{a}	^{A}10.58±0.93^{b}	^{B}8.33±0.50^{a}	^{C}9.33±0.65^{a}	^{A}6.67±0.65^{a}	^{A}6.33±0.50^{a}	^{A}6.58±0.67
P. integrifolia L	^{A}7.33±0.47^{c}	^{B}6.58±0.18^{a}	^{AB}6.92±0.51^{c}	^{A}6.50±0.52^{c}	^{A}6.42±0.52^{b}	^{A}6.58±0.67^{c}	^{A}6.25±0.47^{a}	^{A}6.17±0.40^{b}	^{A}6.25±0.45
*P. palatiferum (*Nees) Radlk	^{A}7.08±0.78^{c}	^{A}6.58±0.18^{a}	^{A}7.08±0.79^{c}	^{A}7.33±0.47^{d}	^{B}6.67±0.50^{b}	^{AB}7.00±0.43^{b}	-	-	6.33±0.49
E. alba (L) Hassk	^{A}6.33±0.47^{df}	-	^{A}6.08±0.29^{d}	^{A}7.17±0.41^{d}	^{B}6.67±0.50^{b}	^{A}7.33±0.49^{b}	-	-	-
S. juventas (Lour.) Merr	^{A}**8.68±0.81**^{e}	^{B}6.67±0.18^{a}	^{A}8.33±0.65^{ab}	^{A}**8.00±0.63**^{e}	^{B}6.92±0.54^{c}	^{B}7.08±0.51^{b}	-	-	-
S. hainanense Hance	^{A}6.17±0.41^{f}	-	^{A}6.58±0.51^{cd}	^{A}6.33±0.51^{fc}	^{A}6.33±0.50^{b}	-	-	-	-

Plant	Growth inhibition zone diameter (mm)					
	E. coli			*L. brevis*		
	MeOH	EtOH	Acetone	MeOH	EtOH	Acetone
T. nigrovenulosa (bark)	^{A}7.83 ± 0.60^{ab}	^{A}7.83±0.58^{a}	^{A}7.75±0.62^{a}	^{A}8.08±1.04^{a}	^{AB}7.58±0.67^{a}	^{B}7.42±0.51^{a}
T. nigrovenulosa (leaves)	^{A}8.08 ± 0.78^{a}	^{A}7.92±0.67^{a}	^{A}7.83±0.72^{a}	^{A}8.17±1.19^{a}	^{A}7.67±0.78^{a}	^{A}7.42±0.67^{b}
P. integrifolia L	^{A}7.33 ± 0.67^{b}	^{A}7.17±0.39^{b}	^{A}7.08±0.51^{b}	^{A}8.42±0.82^{b}	^{A}8.00±0.74^{a}	^{A}7.83±0.58^{c}
*P. palatiferum (*Nees) Radlk	^{A}6.75 ± 0.65^{c}	^{A}6.75±0.62^{bc}	^{A}6.92±0.51^{bc}	^{A}6.67±0.65^{c}	^{A}6.50±0.52^{b}	^{A}6.58±0.51^{b}
E. alba (L) Hassk	^{A}6.92 ± 0.54^{c}	^{B}6.50±0.52^{cd}	^{A}7.08±0.29^{b}	-	-	-
S. juventas (Lour.) Merr	^{A}6.42 ± 0.52^{c}	^{A}6.12±0.39^{d}	^{A}6.50±0.52^{c}	-	-	-
S. hainanense Hance	-	-		-	-	-

Results are means ± SD of triplicate measurements. Different letters (a–f) in the same column and (A–C) in the same row indicate a significant difference at $P < 0.05$; (-) no inhibition.

agents than gram positive bacteria due to their outer lipopolysaccharide membrane (Negi et al., 2003; Naz et al., 2007). Among the tested bacteria, *S. aureus, B. subtilis, L. brevis,* and *E. coli* were relatively susceptible to the methanol extracts, whereas *P. aeruginosa* displayed a significantly stronger resistance. Among gram negative bacteria, *E. coli* was more sensitive to the extracts than *P. aeruginosa.* The most effective of these extracts on *E. coli* was the methanol extract of *T. nigrovenulosa* (bark and leaf). The highest inhibition zone diameters were 7.83 to 9.17 mm and 8.08 to 10.25 mm at 20 to 50 mg/ml for bark and leaf, respectively. The inhibition by these extracts against gram positive bacteria was in the order of *S. aureus* > *B. subtilis* > *L. brevis*. *S. aureus*, which causes serious disease in hospital patients (Curran et al., 1980) and was the most susceptible microorganism to the methanol extract of *T. nigrovenulosa* leaf, followed by *T. nigrovenulosa* bark, and the *S. juventas* extracts. Inhibition zone diameters at 20 to 50 mg/ml were 9.25 to 17.58, 8.58 to 11.83, and 8.68 to 11.33 mm, respectively.

MIC values of the extracts

A quantitative evaluation of antimicrobial activity of the methanol extracts was carried out against tested bacteria according to Mbaveng et al. (2008). The MIC values of the most efficient extracts (methanol extracts) are given in Table 7. Nearly all extracts of the tested plants possessed antibacterial activity but showed different selectivity and MICs for each bacteria. For gram negative bacteria, the most effective of the extracts on *E. coli* was the methanol extracts of *T.*

Table 6. Antimicrobial activity of the extracts against bacteria at 50 mg/ml (0.5 mg/disc).

Plant	Growth inhibition zone diameter (mm)								
	S. aureus			***B. subtilis***			***L. brevis***		
	MeOH	**EtOH**	**Acetone**	**MeOH**	**EtOH**	**Acetone**	**MeOH**	**EtOH**	**Acetone**
T. nigrovenulosa (bark)	$^{A}11.83\pm0.75^{ae}$	$^{B}9.58\pm0.9^{a}$	$^{C}10.50\pm0.67^{b}$	$^{A}13.58\pm1.12^{a}$	$^{B}11.17\pm0.75^{a}$	$^{A}12.92\pm0.79^{a}$	$^{A}11.17\pm1.64^{a}$	$^{A}9.75\pm0.75^{a}$	$^{AB}10.42\pm0.90^{b}$
T. nigrovenulosa (leaves)	$^{A}17.58\pm1.70^{b}$	$^{B}11.17\pm0.87^{b}$	$^{C}13.67\pm1.30^{a}$	$^{A}13.67\pm0.87^{a}$	$^{B}10.83\pm0.77^{a}$	$^{C}12.83\pm1.03^{a}$	$^{A}11.92\pm1.83^{a}$	$^{AB}10.83\pm1.33^{b}$	$^{B}10.58\pm0.90^{b}$
P. integrifolia L	$^{A}8.17\pm0.60^{c}$	$^{B}7.58\pm0.69^{c}$	$^{B}7.67\pm0.49^{c}$	$^{A}8.50\pm0.82^{c}$	$^{B}7.83\pm0.60^{b}$	$^{AB}8.33\pm0.65^{b}$	$^{A}13.00\pm1.79^{b}$	$^{B}11.17\pm1.11^{b}$	$^{B}11.58\pm0.99^{c}$
*P. palatiferum (*Nees) Radlk	$^{A}7.92\pm0.83^{c}$	$^{A}7.67\pm0.79^{c}$	$^{A}7.50\pm0.52^{c}$	$^{A}8.92\pm0.70^{c}$	$^{B}7.67\pm0.79^{b}$	$^{B}8.00\pm0.60^{b}$	$^{A}8.25\pm0.65^{c}$	$^{B}7.50\pm0.52^{c}$	$^{AB}7.75\pm0.75^{a}$
E. alba (L) Hassk	$^{A}6.25\pm0.47^{df}$	$^{B}6.25\pm0.47^{d}$	$^{B}6.08\pm0.29^{e}$	$^{A}7.50\pm0.52^{d}$	$^{A}7.50\pm0.52^{b}$	$^{B}8.08\pm0.67^{b}$	-	-	-
S. juventas (Lour.) Merr	$^{A}11.33\pm1.12^{e}$	$^{B}7.83\pm0.75^{c}$	$^{A}10.83\pm0.83^{b}$	$^{A}8.83\pm0.94^{ce}$	$^{B}7.92\pm0.70^{b}$	$^{AB}8.50\pm0.67^{b}$	-	-	-
S. hainanense Hance	$^{A}6.58\pm0.69^{f}$	$^{A}6.50\pm0.52^{d}$	$^{A}6.83\pm0.58^{d}$	$^{A}6.96\pm0.47^{fd}$	$^{A}6.96\pm0.47^{c}$	-	-	-	-
T. nigrovenulosa (bark)	23.67 ± 1.12^{g}	-	-	29.50 ± 0.69^{g}	-	-	13.00±1.45	-	-

Plant	Growth inhibition zone diameter (mm)					
	E. coli			***P. aeruginosa***		
	MeOH	**EtOH**	**Acetone**	**MeOH**	**EtOH**	**Acetone**
T. nigrovenulosa (bark)	$^{A}9.17\pm0.72^{a}$	$^{A}8.83\pm0.58^{a}$	$^{A}8.75\pm0.62^{b}$	$^{A}7.25\pm0.75^{a}$	$^{A}7.0\pm0.43^{a}$	$^{A}7.00\pm0.43^{a}$
T. nigrovenulosa (leaves)	$^{A}10.25\pm0.79^{b}$	$^{B}9.33\pm0.65^{b}$	$^{B}9.58\pm0.51^{a}$	$^{A}7.29\pm0.64^{a}$	$^{A}6.92\pm0.55^{a}$	$^{A}6.92\pm0.52^{a}$
P. integrifolia L	$^{A}8.50\pm0.52^{c}$	$^{A}8.33\pm0.49^{c}$	$^{A}8.42\pm0.51^{b}$	$^{A}6.92\pm0.70^{a}$	$^{A}6.67\pm0.49^{a}$	$^{A}6.75\pm0.62^{a}$
*P. palatiferum (*Nees) Radlk	$^{A}7.08\pm0.54^{d}$	$^{A}7.00\pm0.60^{d}$	$^{A}7.33\pm0.49^{c}$	$^{A}6.25\pm0.41^{b}$	-	$^{A}6.67\pm0.49^{a}$
E. alba (L) Hassk	$^{A}7.08\pm0.45^{d}$	$^{A}6.92\pm0.29^{d}$	$^{A}7.25\pm0.62^{c}$	-	6.16 ± 0.58^{b}	-
S. juventas (Lour.) Merr	$^{A}6.83\pm0.60^{d}$	$^{A}6.83\pm0.58^{d}$	$^{A}7.0\pm0.43^{c}$	$^{A}7.08\pm0.45^{ac}$	$^{B}6.08\pm0.29^{b}$	$^{AB}6.83\pm0.58^{a}$
S. hainanense Hance	-	-	-	$^{A}6.67\pm0.51^{bc}$	$^{A}6.43\pm0.57^{b}$	$^{A}6.67\pm0.49^{a}$
T. nigrovenulosa (bark)	14.08 ± 1.27^{e}	-	-	-	-	-

Results are means ± SD of triplicate measurements. Different letters (a to g) in the same column and (A to C) in the same row indicate a significant difference at $P < 0.05$; (-) no inhibition.

Table 7. Minimum inhibitory concentration of the methanol extracts against tested bacteria.

Plants and reference	Minimum inhibitory concentration (µg/ml)				
	S. aureus	***B. subtilis***	***L. brevis***	***P. aeruginosa***	***E. coli***
T. nigrovenulosa (bark)	156	78	156	1250	625
T. nigrovenulosa (leaves)	78	78	156	1250	312
P. integrifolia L	625	312	156	2500	625
*P. palatiferum (*Nees) Radlk	625	625	625	2500	1250
E. alba (L) Hassk	1,250	625	>5000	>5000	1250
S. juventas (Lour.) Merr	156	625	>5000	2500	2500
S. hainanense Hance	1,250	>5000	>5000	2500	>5000
Ampicillin	1	1	2	-	2

(-) no inhibition.

nigrovenulosa (bark and leaf), which had the lowest MIC values of 312 and 625 µg/ml, respectively. For gram positive bacteria, the *T. nigrovenulosa* extracts were the most effective against *S. aureus* and *B. subtilis* with MICs value of 78 and 156 µg/ml (leaf and bark) and 78 µg/ml (leaf and bark), respectively. Particularly, the extracts of *T. nigrovenulosa* leaf and bark had significant effects on *P. aeruginosa,* which was resistance to ampicillin, with an MIC value of 1,250 µg/ml. This result indicated that the *Terminalia* species extracts possess not only antifungal activity (Masoko et al., 2005) but also antibacterial activity. However, the MIC values of these plant extracts were higher than that of ampicillin for all microorganisms, except *P. aeruginosa*. These results suggest that the methanol extracts had a stronger and broader spectrum of antimicrobial activity against the tested microorganisms compared to that of the other extracts, indicating that the antimicrobial compounds were hydrophilic. Therefore, hydrophilic extraction solvents are better solvents for extracting antimicrobial compounds from these plants (Negi et al., 2003; Alzokery et al., 2003). This investigation corroborated evidence in previous studies reporting that methanol is the best solvent for extracting antimicrobial compounds from medicinal plants compared to other solvents (Ahameethunisa and Hopper, 2010; Sahin et al., 2003).

Phenolic and flavonoid compounds possess antioxidant, anti-inflammatory, and anticancer effects, inhibit the growth of microorganisms, and play an important role in decreasing the risk of some diseases (Lee et al., 2004; Tanabe et al., 2002). This study also showed that the anti-microbial capacity of investigated plants extracts is associated with the contents of total phenolic and flavonoid presented in our previous research (Nguyen and Eun, 2011). This observation agrees with previous studies reporting that antimicrobial activity may be contributed by a plant's phenolic compound content (Ali-Shtayeh et al., 1997). The antimicrobial and other biological activities of phenolics have been reported (Alzoreky et al., 2003; Machado et al., 2007). Phenolic compounds might interact with membrane proteins rendering deformation in structure and functionality (Rico-Munoz et al., 1987). Phenolics are also able to vary alkyls into a phenol nucleus (Dorman and Deans, 2000), or change microbial cell permeability and obstruct membrane functions including electron transport, nutrient uptake, protein and nucleic acid synthesis and enzyme activity (Bajpai et al.,2008).

Conclusion

The current study demonstrated that the type of extraction solvent and plant had a significant influence on the antimicrobial activity of the extracts against the tested microorganisms. The results showed that the methanol extracts displayed the strongest antimicrobial activity, particularly, the extracts of *T. nigrovenulosa* (leaf and bark), *P. integrifolia* L, *and S. juventas*. Further studies are required to determine the types of bioactive compounds in the plant extracts, as well as the compounds responsible for the antimicrobial activity in these plant extracts.

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