
Cytotoxicity and Antimicrobial Activities of Leaf Extracts from *Barleria strigosa*

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Abstract Our preliminary phytochemical screening demonstrated that the methanolic extract from leaves of *Barleria strigosa* had in vitro cytotoxicity against the P-388 murine leukemia cell line with CC₅₀ (50% cytotoxicity concentration) of 413.89 µg/ml and exhibited strong antibacterial activity against *Bacillus subtilis* with an inhibition zones of 14 mm at 10 mg/disc. Therefore, this present study attempts to evaluate the cytotoxicity and antimicrobial activity. The crude methanol extract was subjected to a liquid-liquid partition yielding hexane, dichloromethane, ethyl acetate and butanol extracts described by Beedessee *et al.* (2012). The cytotoxicity of all extracts were analyzed using MTT colorimetric assay described by Mosmann (1983) against six cancer cell lines (HepG-2, MCF-7, KB, HT-29, P388 and Hela cells) and two normal cell lines (Vero and L929 cells) at concentration range of 62.5 to 1000 µg/ml. By the MTT assay, the strongest cytotoxic activity was obtained from the butanol extract followed by the ethyl acetate, dichloromethane and hexane extract, respectively. Highest cytotoxicity of butanolic extract was found against P-388 cell line (CC₅₀ = 127.42 µg/ml) and found cytotoxic against L-929, KB, Hela, MCF-7, HT-29, Vero and HepG-2 cells at the CC₅₀ levels of 283.00, 287.22, 566.83, 574.19, 666.78, 835.92 and >1,000 µg/ml, respectively. The extracts were tested for their antimicrobial activity against five pathogenic bacteria (*Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) at concentration range of 0.125 to 2 mg/disc using paper disc diffusion method described by Ansari *et al.* (2005). The butanol extract exhibited more interesting antimicrobial activity than other extracts, being especially active against *M. luteus* and *S. aureus* at 2 mg/disc with diameters of the inhibition zones was 8.8 and 8.22 mm, respectively. The results indicated that butanol extract exhibited cytotoxicity and antimicrobial activities. Therefore, the fractions from butanolic extract were also appreciating for further investigations in future.

Keywords: Cytotoxicity, Antimicrobial activity, *Barleria strigosa*, MTT colorimetric assay, Paper disc diffusion method

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Introduction

Thailand is one of the countries that have a variety of biological resources. Many of the members of the Acanthaceae family are used as medicinal plants and have been reported in the scientific literature to possess promising bioactive compound such as *Thunbergia laurifolia* (Tangpong and Satarug, 2010), *Andrographis paniculata* (Kumar *et al.*, 2004) and *Rhinacanthus nasutus* (Puttarak *et al.*, 2010). Bioactive compound screening is very important in identifying new sources phytochemicals that might lead to drug development. Genus *Barleria* is member of Acanthaceae which are herbs and widely distributed throughout tropical Asia. The bioactive compounds and biologically active extracts have been reported from this genus. Especially in *Barleria lupulina*, leaves, stem, root and flower of *B. prionitis* possess antibacterial and anti-inflammatory activities (Singh *et al.*, 2003; Amoo *et al.*, 2009; Jaiswal *et al.*, 2010; Chavan *et al.*, 2010; Shukal *et al.*, 2011). The methanol extract of leaves and stems had a pronounced blood-glucose-lowering potential in diabetic animals (Suba *et al.*, 2004; Dher and Bhatnagar, 2010). Verma *et al.* (2005) found methanolic extract of this plant to produce anti-spermatogenic effect without affecting the general body metabolism. Iridoid enriched fraction of aerial parts was demonstrated for hepatoprotective activity in various acute and chronic animal (Singh *et al.*, 2005). Bark extract is effective in controlling candidiasis and other oral fungal infections (Aneja *et al.*, 2010) and antioxidant property (Ata *et al.*, 2009). In *B. cristata*, the ethanol extract from seeds of *B. cristata* had the effect to reduce the blood sugar of mice (Singh *et al.*, 2012). In others, the extracts from various parts of *B. prionitis*, *B. greenii*, *B. albostellata* had the antibacterial, antifungal and anti-inflammatory (Amoo *et al.*, 2009; 2011). Dichloromethane extract of *B. argillicola* had a stronger antibacterial activity against the gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) than gram-positive bacteria (*Staphylococcus aureus*) (Amoo *et al.*, 2013).

As previously discussed found the plants in *Barleria* had demonstrated for the biological activity. Despite the popular use *B. strigosa* as a medicinal plant, there are no data about the biological activity. The aim of the present study was to evaluate the potential cytotoxicity and antimicrobial activity of different extracts obtains hexane, dichloromethane, ethyl acetate and butanol derived from *B. strigosa* using MTT colorimetric assay and paper disc diffusion method, respectively. Including, the chemical composition were analyzed by the GC-MS.

Materials and methods

Plant material

Characteristics of *B. strigosa* are shown in Fig. 1. The leaves of *B. strigosa* were collected in June 2013 from the Eastern Botanical Garden (Khao Hin Son), Chachoengsao, Thailand. The samples were rinsed thoroughly in running tap water to remove soil particles and adhered debris. The leaves were dried in an oven at 45 °C for 3-4 days and ground in coarse powder as well as stored in a plastic bag.



Figure 1. Plant of *Barleria strigosa*

Preparation of extract

1 kg powder of dried leaves was macerated with 95% methanol at room temperature on shaker for 3 times of 7 days. After maceration, the extracts was filtered using Whatman filter paper No.1 and evaporated to dryness on a rotary evaporator under reduced pressure at 40°C and removed the last tract solvent in desiccator. This constituted the crude extract, which was dissolved in distilled water to be partitioned subsequently with hexane, dichloromethane, ethyl acetate and butanol to afford of the level of polarity described by Beedessee *et al.* (2012) Finally, the extracts were weighed and stored in universal bottles and refrigerated at 4 °C prior to use used.

Cell cultures

The six cancer cell lines including human hepatocellular carcinoma (HepG2), human breast adenocarcinoma (MCF7), human oral epidermoid carcinoma (KB), human colon adenocarcinoma (HT29), murine lymphocytic leukemia (P388) and human cervical carcinoma (HeLa) as well as two normal

cell lines including african green monkey kidney (Vero) and mouse subcutaneous connective tissue (L929) were used for cytotoxicity assays (Fig.2). The cells were cultured in RPMI-1640 medium with 10% (v/v) heat-inactivated fetal bovine serum (FBS) and 0.1% gentamycin and were grown at 37 °C in 5% CO₂-humidified atmosphere in 25 cm² flasks. When the cells reached 80% confluence, they were washed with phosphate-buffered saline (PBS) and harvested with 1 ml of 0.25% trypsin-EDTA solution (except P388).

MTT colorimetric assay

In vitro cytotoxic activity has been measured using modified MTT assay described by Mosmann (1983). Briefly, viable cells were counted by trypan blue exclusion using a haemocytometer (Freshny, 1994). The cell lines (100 µl/ well) were seeded at seeding densities about 1-1.5×10⁵ cells/ml into 96-well plates and incubated for 24 h at 37 °C. After this incubation period, the cells were treated with different extracts (hexane, dichloromethane, ethyl acetate and butanol) at various concentrations. The highest concentration of the extracts (2000 µg/ml) was added to row 1 of the 96-well plates and several 2-fold serial dilutions were made until row 5. The 0.2% (v/v) dimethyl sulphoxide (DMSO) and 0.5% anticancer drug mitomycin C (MMC) were used as negative and positive control, respectively. After incubated for 24 h, MTT (2mg/ml in PBS) were added and incubated for 4 h at 37 °C. The media was carefully removed with a needle and syringe. The formazan crystals were dissolved in 100 µl DMSO: ethanol (1: 1) and the absorbance was measured at 570 nm using a microplate reader. All the concentrations were tested in triplicated on the same cell batch.

Microbial strains and culture condition

Five microbial strains: *B. subtilis* ATCC6633, *E. coli* DMST4212, *M.luteus* TISTR9341, *Psu.aeruginosa* ATCC27853 and *S.aureus* TISTR1466 were used for antimicrobial activity. All microorganisms were cultured in Mueller-Hinton Broth (MHB) and incubated at 37 °C for 24 h. To adjust the concentration of suspensions was O.D. 0.5-0.8 at 600 nm.

Paper disc diffusion

The antimicrobial activity of plant extracts were tested by using agar disc diffusion described by Ansari *et al.* (2005). Each bacterial suspension was swab over the surface of Mueller Hinton Agar (MHA) with a sterile cotton swab. Various concentrations of extracts (0.125, 0.25, 0.5, 1 and 2 mg/disc) were

dissolved in methanol and loaded into the 6 mm sterile paper discs, then were placed on the agar. The same volume of methanol was used a negative control and gentamicin was used as positive control due to its broad spectrum of activity against various organisms. After being kept at room temperature for 30 min, they were incubated at 37 °C for 24 h. The diameters (mm) of the inhibition zone were measured and the presented values are average of three separate experiments.

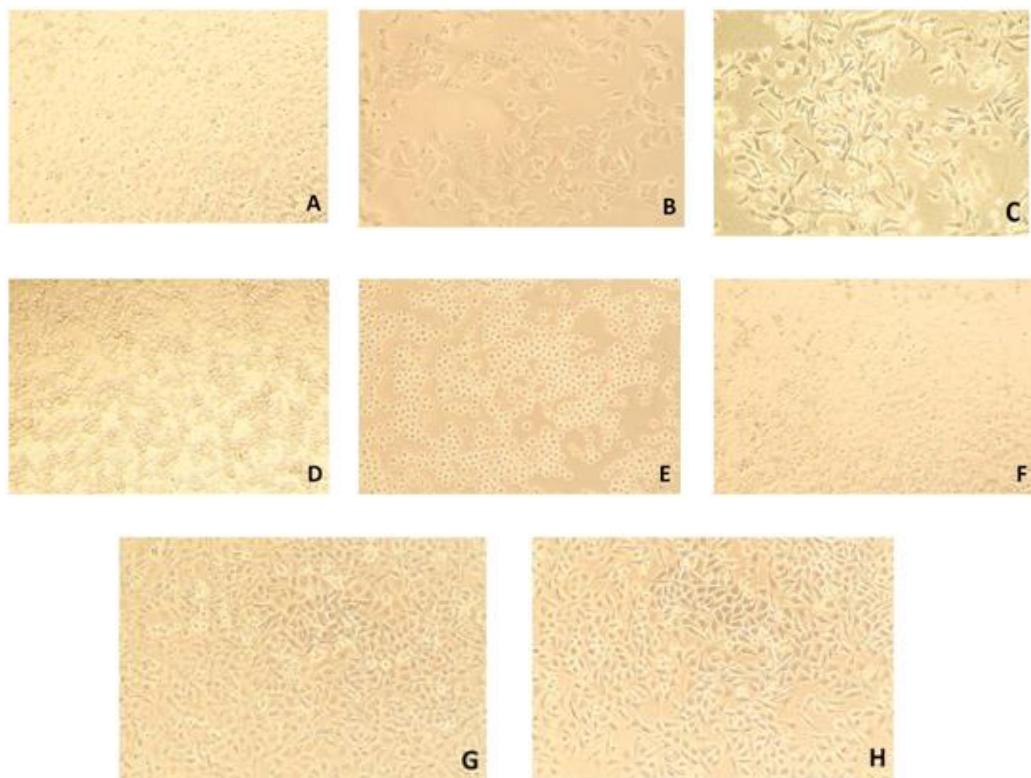


Figure 2. Morphological appearance of the cell lines: (A) human hepatocellular carcinoma (HepG2), (B) human breast adenocarcinoma (MCF7), (C) human oral epidermoid carcinoma (KB), (D) human colon adenocarcinoma (HT29), (E) murine lymphocytic leukemia (P388), (F) human cervical carcinoma (HeLa), (G) african green monkey kidney (Vero) and (H) mouse subcutaneous connective tissue (L929)

Gas chromatography-mass spectrometry (GC-MS)

The extracts were sent to analyzed by the GC-MS using Acq method at Scientific Instruments Center, KMITL. An aliquot of 10:1 was injected (split) into the GC-MS . The data were obtain on a HP-5 column (30 m x 0.25 mm, 0.25 μ m). The carrier gas was helium 99.99% and the GC oven temperattrue program was as follows: 70 °C hold for 5 min, rate of 8 °C/min to 290 °C and hold for 12 min. The injector and detector temperatures were set at 250 °C and the pressure were 8.73 psi. The mass range was scanned from 50 to 500 amu.

Statistical analysis

Experiments were performed in triplicate and the results were expressed by using GraphPad Prism5 and Microsoft Excel 2007.

Results and discussion

MTT colorimetric assay

The cytotoxicity of all extracts were analyzed using MTT colorimetric assay in eight cell lines (L929, KB, Vero, HeLa, HepG-2, HT-29, P-388 and MCF-7) at concentration ranging from 62.5-1000 μ g/ml. The result in the hexane extract was found the CC₅₀ against to Vero and L929 cell lines were 763.1 and 934.7 μ g/ml, respectively. The dichloromethane extract was found the CC₅₀ against to L929, P-388 and MCF-7 were 687.68, 728.07 and 960.14 μ g/ml, respectively. The ethyl acetate extract was found the CC₅₀ against to P-388, L929, HeLa, KB and HT-29 were 654.27, 932.09, 944.83, 985.41 and 981.73 μ g/ml, respectively. The butanol extract was found the CC₅₀ against to P-388, L929, KB, HeLa, MCF-7, HT-29 and Vero were 127.42, 283.00, 287.22, 566.83, 574.19, 666.78 and 835.92 μ g/ml, respectively (Fig.3). The research of the our preliminary of methanolic extract from leaves of *B. strigosa* had in vitro cytotoxicity against the P-388 murine leukemia cell line with CC₅₀ of 413.89 μ g/ml (Manapradit *et al.*, 2013) and not found the previous report on cytotoxicity about this plant.

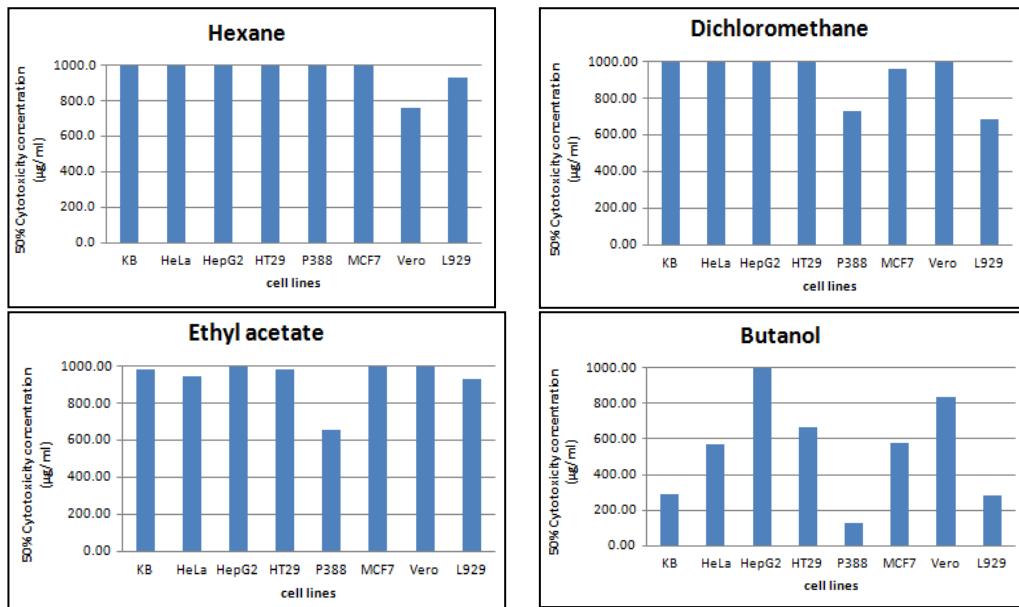


Figure 3. The 50% cytotoxicity concentration (CC_{50}) of eight cell lines after the addition of the hexane, dichloromethane, ethyl acetate and butanol extract at various concentrations were analyzed using MTT colorimetric assay.

Paper disc diffusion

All extracts were tested for their antimicrobial activity against five pathogenic bacteria strains (*Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) at concentrations ranging from 0.125-2 mg/disc using paper disc diffusion method. The results was found the butanol extract had active against for *M. luteus* and *S. aureus* at 2 mg/disc and the diameters of the inhibition zones was 8.8 and 8.22 mm. respectively (fig.4). The hexane, dichloromethane and ethyl acetate extracts was not found to be active against any of the test strains at this various concentrations. The researches of the other plant in Barleria genus were showed antibacterial activity as the petroleum ether extract from *B. prionitis* at concentration 10 mg/ml had maximum antibacterial activity against *Klebsiella pneumonia* (Chavan *et al.*, 2010). Including, Salib *et al.* (2013) found the ethanolic extract from *B. cristata* at concentration 10 mg/ml was strong inhibitory activity against *S. aureus*, *B. subtilis* and *S. mutans*.

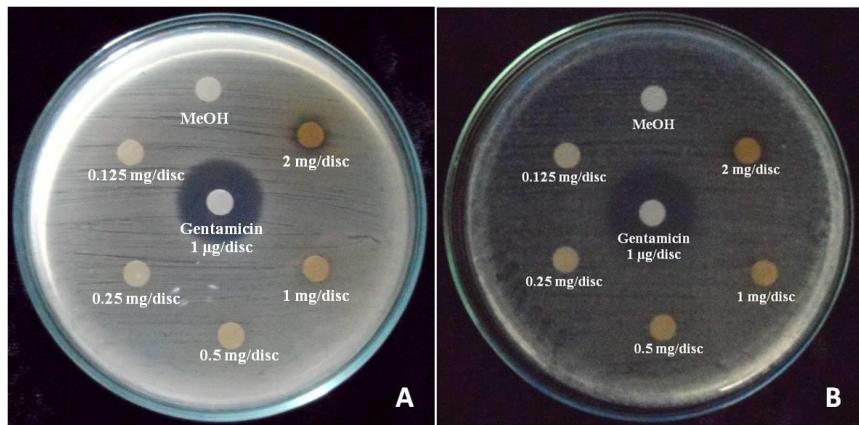


Figure 4. The diameters of the inhibition zones of butanol extract: (A) *S. aureus* and (B) *M. luteus* at concentrations ranging from 0.125-2 mg/disc using paper disc diffusion method.

Gas chromatography-mass spectrometry (GC-MS)

The extracts were analyzed by GC-MS system. The results of the hexane, dichloromethane, ethyl acetate and butanol extract found 13, 28, 26 and 22 compounds, respectively. The main chemical compositions of different extracts were presented in Table 1. The extract in each partition has a different chemical composition and not found the previous report study about the chemical composition by GC-MS in this plant. However, the other plant in *Barleria* sp. were studies about chemical constituents such as Ata *et al.* (2009) were analyzed structures of the compound from the aerial parts of *B. prionitis* by NMR spectral and found the one new phenylethanoid glycoside, barlerinoside along with six known iridoid glycosides, shanzhiside methyl ester, 6-O-trans-p-coumaroyl-8-O-acetylshanzhiside methyl ester, barlerin, acetylbarlerin, 7-methoxydiderroside, and lupulinoside. Including, Salib *et al.* (2013) were analyzed the structures of the compounds from the ethanolic extracts of the *B. cristata* bark by UV spectral and found the new flavonoid compound 6-O- α -L-rhamnopyranoside-3,7,3'-O-trimethylated-8-hydroxyquercetin and identified for the first time in nature together with the known flavonoids 6-O- α -L-rhamnopyranoside quercetagetin, 3-methoxy quercetin, gossypetin 8-methyl ether, quercetagetin, tamarixetin, gossypetin and quercetin.

Table 1. The main chemical composition from the different extracts of *B. strigosa*

Extracts	Compounds	Main chemical composition	Relative amount (%)
Hexane	13	9,12,15-octadecatrien-1-ol	18.746
		9-octadecenoic acid	14.814
		Phytol	12.795
Dichloromethane	28	2(4H)-benzofuranone	40.167
		ethyl ester	9.992
		Cyclopentane	5.856
Ethyl acetate	26	ethyl ester	17.918
		4-butylbenzonitrile	17.479
		benzoic acid	11.803
Butanol	22	m-tolunitrile	16.581
		Benzonitrile	11.970
		cyclohexane acetic acid	10.304

Conclusion

The present study demonstrated the pharmacological potential of *B. strigosa*. The cytotoxicity and antimicrobial activities of *B. strigosa* was reported for the first time. No previous report on the biological activity of this plant. The butanol extract was found the greater effect of their cytotoxicity and antimicrobial activity. The chemical composition was found the highest chemical in dichloromethane extract were 2(4H)-benzofuranone but the butanol extract were found the most of m-tolunitrile. The results provided evidence that the studied plant extracts might be potential sources of new anticancer and antibacterial drug and the butanol extract is suitable to be used to study in further and suggests the therapeutic potential of this plant when foraged by animals.

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