Phytochemical Screening and Antibacterial Activity of *Millettia pinnata* (L.) Panigrahi

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ABSTRACT

Phytochemical screening of lipophilic extracts from leaves, stem bark and root bark of *Millettia pinnata* (L.) Panigrahi had been investigated by using Thin Layer Chromatography (TLC) technique. Appearance of colours as well as fluorescence under UV light could be detected after spraying with Dragendorff's reagent, anisaldehyde - sulfuric acid reagent, 10% NaOH and 10% KOH in order to detect alkaloids, terpenoids and steroids, coumarins and anthraquinones respectively. The results revealed the presence of alkaloids, terpenoids, steroids and coumarins. Anthraquinones could be detected only in lipophilic extract of root bark. The lipophilic extracts were tested for antibacterial activity against *Bacillus cereus* by using disc diffusion method. The lipophilic extract of root bark could inhibit *B. cereus* growth.

Key words: *Millettia pinnata*, phytochemical screening, lipophilic extract

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INTRODUCTION

Plants can produce many different types of secondary metabolites, which have been utilized by human for their valuable characters in a diverse process of application. Nowaday, there are increased interests in natural products derived from plants with valuable medicinal properties. Thailand is rich in its plant diversity. A number of plants have been documented for their medicinal potential, which are used by traditional health and herbal folklorists.

Millettia pinnata (L.) Panigrahi [synonyms, *Pongamia pinnata* (L.) Pierre, *Pongamia pinnata* (L) Merr., *Pongamia glabra* Vent. *Cytisus pinnatus* (L.) and *Derris indica* (Lam.) Bennet)] belongs to the family Fabaceae, subfamily Papilionoideae. It is a medium size glabrous tree and widely distributed in tropical Asia, Australia, the Polynesia and Philippine Islands (Sajid *et al*., 2012), grows naturally along coasts, beaches and the edges of mangrove forest. They are interesting energy crops due to their oil from seeds used for biodiesel production. Some parts of plant have been applied in traditional medicine for the treatment of tumors, piles, skin diseases, wounds and ulcers (Tanaka *et al*., 1992). It has been reported that the extracts have the properties of potential antifungal and antibacterial activities (Dahikar *et al*., 2008).

In Thailand, this plant is locally known as “Yi nam or Yi thale”. Their habitats are in the littoral regions of the country and most distributed in the south. Although several reports have revealed medicinal and agricultural usages of this valuable plant, there are a few work which have been conducted so far on phytochemical profiles and bioactive properties from leaves, stem and root extracts of *M. pinnata* indigenous to our country. Therefore, the objectives of this study were to look for phytochemical profiles and antibacterial activity of the extracts from different plant parts. The extracts of *M. pinnata* were tested for antibacterial activity against *Bacillus cereus* which is a type of bacteria that causes food poisoning. The finding will enhance of chemotaxonomic knowledge as a basic for its useful in the future.

MATERIALS AND MEDTHODS

1. Plant materials and extraction

Plant materials were collected from Auo Khoei, Phangnga province. Fresh leaves, stem bark and root bark were dried under shade in order to protect from direct exposure of sunlight. Dried samples were powdered and macerated with methanol (MeOH) for 7 days at room temperature. The extracts were filtered through Whatman No. 1 filter paper and concentrated by using rotary evaporator at 37 ^{o}C obtained solid-crude extracts. The solid-crude extracts were portioned into two parts: hydrophilic extract and lipophilic extract with distilled water and chloroform ($CHCl_3$) in separatory funnel and the $CHCl_3$ fractions were evaporated to dryness. The dried lipophilic extracts were dissolved in MeOH and kept at -40 ^{o}C for further experiments.

2. Phytochemical screening

Phytochemical screening of secondary metabolites i.e. alkaloids, terpenoids and steroids, coumarins and anthraquinones was done by thin layer chromatography (TLC) technique. Chromatography was performed on 10 x 20 cm silica gel TLC plates (0.2 mm thickness, 60 F_{254}, Merck). The lipophilic extracts of leaves, stem bark and root bark 10 µl at concentration 10 mg/ml were spotted on TLC plates. The TLC plates were developed by solvent system n-hexane - diethylether 2:3 (v/v) with the distance 15 cm. The developed TLC plates were dried and sprayed by colour detection reagents. Dragendorff's reagent was used for alkaloids screening. Orange spot indicates the presence of alkaloids (Merck, 1980). Anisaldehyde - sulfuric acid is reagent for detection of terpenoids and steroids which violet, blue, red, grey or green spots indicate the presence of terpenoids and steroids (Merck, 1980). 10% NaOH was used in order to examine coumarins, blue or yellow-green fluorescence of spot under UV wavelength at 365 nm indicates the presence of coumarins (Farnsworth, 1966). Anthraquinones were examined by spraying with 10% KOH in ethanol which red spot indicates the presence of anthraquinones (Soontornjarernnon, 2008). The relative between distance compounds move in chromatography to solvent front calculated to become relative front (R_f) value (Muthuvelan and Raja, 2008).

$$R_f = \frac{\text{Distance moved by the solute (cm)}}{\text{Distance moved by the solvent (cm)}}$$

3. Evaluation of bacterial activity

Bacillus cereus was obtained from Microbiology Laboratory, Faculty of Natural Resources and Agro-industry, Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus. The bacteria were cultured on Tryptic Soy Agar (TSA) and incubated at 37 $^{\circ}$C overnight. Single colony bacterium was isolated into a flask containing Tryptic Soy Broth (TSB) and incubated at 37 $^{\circ}$C for 24 hours. Turbidity of culture was adjusted with sterile saline normal solution to match McFarland No.0.5 standard (approximately 10^8 CFU/ml). 100 µl of aqueous bacterial culture was spread on surface TSA plates. Using disc diffusion method, at the concentrations 5, 10, 50 and 100 mg/ml of each extracts were loaded on each sterile paper discs with 6 mm diameter (20 µl/disc), MeOH was used as the control and dried paper discs. The paper discs were placed on surface TSA plate which was spread *B. cereus* and incubated at 37 $^{\circ}$C for 24 hours. Antibacterial activity was observed via presence of clear inhibitory zone devoid of bacterial growth around paper disc and the diameter of inhibition zone was measured.

RESULTS AND DISCUSSION

1. Phytochemical screening

Preliminary phytochemical investigation of lipophilic leaves, stem bark and root bark extracts of *M. pinnata* revealed the presence of alkaloids, terpenoids, steroids and coumarins in all plant parts except anthraquinones, which was presented uniquely in root bark extract with R_f value 0.09 (Figure 1D). In the extracts of three plant parts, four different R_f values of alkaloids appeared in the range of 0.10 to 0.72 (Figure 1A), sixteen different R_f values of terpenoids and steroids presented in the range of 0.04 to 0.90 (Figure 1B). There were nine different R_f values of coumarins in the range of 0.16 to 0.69 (Figure 1C). The phytochemical profiles of lipophilic leaves, stem bark and root bark extracts in this investigation are presented in Table 1. The presence of alkaloids, terpenoids and steriods conform with the previous works (Dahikar *et al.,* 2008; Badole and Bodhankar, 2009; Gupta and Sharma, 2011), whereas the coumarins and anthraquinones have never been reported yet.

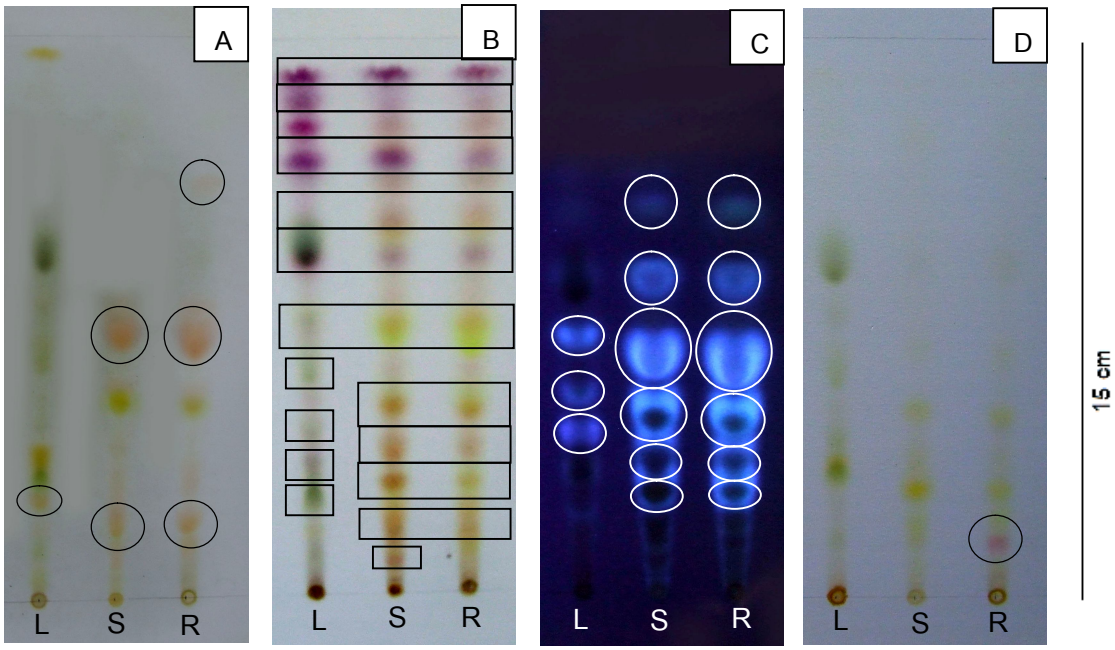

Figure 1 Investigation of alkaloids, terpenoids, steroids, coumarins and anthraquinones from leaves (L), stem bark (S) and root bark (R) extracts of *M. pinnata* on the developed TLC plate which were sprayed with colour detection reagent. A: positive test of alkaloids are orange spots; B: positive test of terpenoids and steroids are violet, blue, grey or green spots; C: positive test of coumarins are fluorescent under UV light at 356 nm; D: positive test of anthraquinones are red spot.

Table 1 Phytochemical profiles of *M. pinnata* according to lipophilic leaves, stem bark and root bark extracts.

R_f	Leaves	Stem bark	Root bark
0.04		+2	
0.09			+4
0.10		+1, 2	+1, 2
0.16	+1,2	+3	+3
0.19		+2	+2
0.21	+2	+3	+3
0.25		+2	+2
0.27	+2, 3		
0.32		+2, 3	+2, 3
0.34	+3		
0.38	+2		
0.42		+3	+3
0.46	+2, 3	+1, 2	+1, 2
0.56		+3	+3
0.59	+2	+2	+2
0.65	+2	+2	+2
0.69		+3	+3
0.72			+1
0.75	+2	+2	+2
0.81	+2	+2	+2
0.86	+2	+2	+2
0.90	+2	+2	+2

+ = present; 1= alkaloids; 2= terpenoids and steroids; 3= coumarins; 4= anthraquinones

2. Evaluation of bacterial activity

The lipophilic extracts of leaves, stem bark and root bark of *M. pinnata* were investigated for antibacterial activity against *B. cereus* by disc diffusion method. The results showed that the lipophilic root bark extract exhibited clear zone of inhibition of the *B. cereus* growth in all treatments. The average diameters of the inhibition zones were 7.6, 8.1, 9.4 and 11.8 mm at concentration 5, 10, 50 and 100 mg/ml, respectively. While all treatments of leaves and stem bark extracts could not inhibit of bacteria growth as well as the control (methanol) (Figure 2).

However, Bajpai *et al.* (2009) found that methanolic leaves extract of *M. pinnata* could inhibit *Bacillus subtilis* which is the same genus. The root bark extract showed the effective growth inhibition of *B. cureas*, but active compounds are still unknown. Although the anthraquinones presented only in root bark extract. It does not indicate that antibacterial effect caused by anthraquinones solely because some secondary metabolites such as flavonoids and tannins those showed antimicrobial activity (Sandhar *et al.,* 2011; Tomczyk and Latte, 2009) had not been detected. Moreover, antimicrobial effect might cause by high concentrations of the other detected compounds (alkaloids, terpenoids, steroids and coumarins). Thus the separation of pure compounds and test for their bioactivities will be performed in further study.

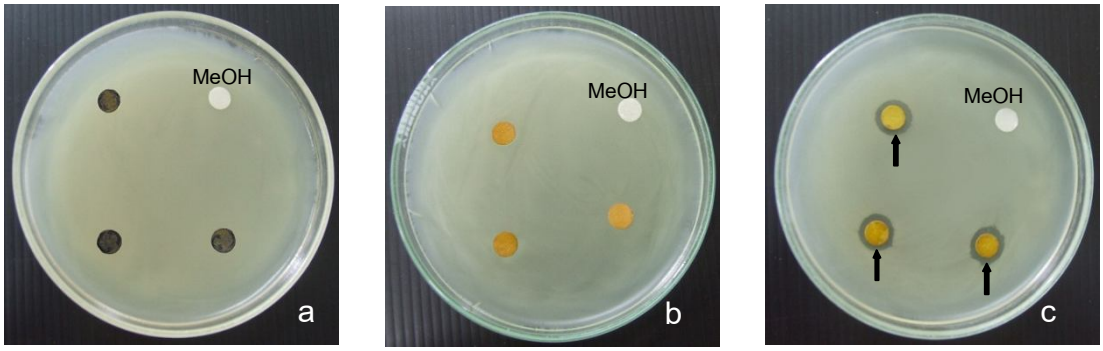

Figure 2 The effect of lipophillic extracts of leaves (a), stem bark (b) and root bark (c) of *M. pinnata* on inhibition of *B. cereus* growth at 100 mg/ml; black arrows indicate inhibition zone of bacterial growth.

CONCLUSION

The phytochemical analysis of lipophilic extracts from leaves, stem bark and root bark of *M. pinnata* showed the presence of alkaloids, terpenoids, steroids and coumarins. The anthraquinones existed only in root bark extract. Moreover, the lipophilic extract of root bark exhibited clear zone which could be specified the inhibition of *B. cereus* growth, while this ability was not found in leaves and stem bark extracts.

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