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Extraction and Basic Testing for Antibacterial Activity of the Chemical Constituents in *Suregada multiflorum*

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ABSTRACT

Extractions of biologically active chemical constituents from the leaves, bark and stem of *Suregada multiflorum* with a polarity sequence of hexane, dichloromethane and methanol were conducted. Antibacterial activities of the crude extracts were examined by paper disc agar diffusion and spread plate methods with *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Mycobacterium lacticola, Pseudomonas aeruginosa* and *Xanthomonas campestris*. The crude extracts of the greatest inhibition activities, i.e. dichloromethane extracts, were chromatographed over a silica gel column using a single solvent and solvent mixtures of increasing polarity as the eluents. The bacterial growth inhibitions of the pure fractions were also examined to find out the most active fractions.

The chemical constituents extracted from various parts of *Suregada multiflorum* provided high yields of polar fractions. The crude and pure fractions, particularly the polar compounds, of each part of the plant inhibited several tested bacteria. The purified fractions exhibited stronger inhibition than the crude extracts. The inhibition of *Xanthomonas campestris* growth indicated the potential of chemical constituents from *Suregada multiflorum* to inhibit plant bacterial diseases such as citrus canker and that they would probably be useful for canker prevention in replacing chemical pesticides.

Key words: extraction, *Suregada multiflorum*, antibacterial activity, chemical constitutents, column chromatography

INTRODUCTION

Suregada multiflorum is a plant belonging to the *Euphorbiacae* family found in the tropical rain forests (Daubenfeld *et al*., 2005) around central, eastern and southern Thailand. Isolation of chemical constituents from *Suregada multiflorum* has provided various compounds of alkaloids, cardiac glycosides, flavanoid, saponin, terpenoids, lactone and gelonin. The structures of the terpenoids and related compounds (bauerenol and multiflorenol) isolated from its bark were identified by Sengupta and Khastgir (1963). Two flavone diglycosides along with 7, 4'-O-dimethylscuttelarein 6-O-beta-D-glucopyranocides were extracted from seeds of *Suregada multiflorum* and the chemical structures were also described (Das and Chakravarty, 1993). The minor constituents, i.e., diterpine, lactones, and gelomulides G-J, were isolated from the leaves. The stereostructures and absolute configurations have been identified (Talapatra *et al*., 1998).

Barbieri *et al*. (1987) purified ribosome-inactivating protein by large scale chromatography

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from seeds of *Suregada multiflorum*. The gelonin located in the vacuole of *Suregada multiflorum* seeds consisted of the amino acid sequence and the glycosylation motif of the ribosome inactivation proteins. The glycosylation patterns of $GlcNAc_2Man_{3-5}Xyl$ and N189 were identified by Daubenfeld *et al*. (2005). The MAP30 (Momordica anti-HIV protein of 30 kDa) and GAP31 (Gelonium anti-HIV protein of 31 kDa) from *Suregada multiflorum* potentially inhibited the infection of the human immuno-deficiency virus type I (HIV-1) in T lymphocytes and monocytes. MAP30 and GAP31 posed an N-glycosidase activity on 28s-ribosomal RNA and a topological activity on plasmid and viral DNAs including HIV-1 long repeats with no toxicity to normal cells (Lee-Huang *et al*., 1995). MAP30 and GAP31 also inhibited the infection of Herpes Simplex Virus (HSV) (Bourinbaiar and Lee-Huang, 1996). The antiviral agents, MAP30 and GAP31, also had no effect on the motility and vitality of human sperm cells (Schreiber *et al*., 1999).

Due to the fact that stems of *Suregada multiflorum* are straight, the plants are evergreen and are resistant to plant diseases, attention was paid to the isolation of some biologically active chemical compounds from different parts of the plant with expectations that these chemicals could be biologically active in controlling plant pathogens such as citrus canker and that they could probably be useful for canker prevention by replacing chemical pesticides.

MATERIALS AND METHODS

Suregada multiflorum trees were collected from the botanical garden at Kasetsart University, Si Racha Campus. The trees had already been identified at the botanical garden and confirmed by comparison with Vecsommai and Kavduengtain (2004). The tree, fruits and leaves of *Suregada multiflorum* are shown in Figure 1. Different parts of the plant were air-dried at room temperature and ground up.

Extraction of *Suregada multiflorum*

Leaf: Three samples of leaf powder (340 g) were each steeped in 6 l of hexane for seven days and filtered isolated. The three filtrates were dehydrated under vacuum using a rotary evaporator. The leaf residues were consecutively extracted two more times with hexane. The dried crude extracts were then combined and weighed. The leaf residues from the hexane extraction were further extracted with dichloromethane followed by methanol.

Bark: Two samples of dried bark (1000 g) were each initially steeped in 4 l of hexane and the same procedures as that used for the leaf-extract

Figure 1 The tree, fruits and leaves of *Suregada multiflorum*. (http://wanakorn.com/upload/news/Suregada-multiflorum-Bail.jpg http://www.fisheries.go.th/cf-kung_krabaen/picture/kkb_23.jpg)

preparation was followed.

Stem: Two dried, stem samples of 1000 and 2000 g were steeped in 4 and 6 l of hexane respectively, and then followed the same procedure that was applied in the leaf extract preparation.

Antibacterial activities of the crude extracts

The antibacterial activities as indicated by inhibition zones were determined for all crude extracts by paper disc agar diffusion and spread plate methods (Nester *et al*., 2004). The crude extracts (0.1 ml of 10% extracted solution) were tested against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Mycobacterium lacticola, Pseudomonas aeruginosa*, and *Xanthomonas campestris* in nutrient agar and the clear inhibition zones were determined within 48 h. The crude extracts with the greatest inhibiting activities were selected for column chromatographic purification.

Thin-layer chromatography for preliminary separation

The crude extracts were spotted on to TLC plates impregnated with silica gel and eluted using solvents and solvent mixtures of increasing polarity. The position of each separated fraction was visualized under UV light at 240 and 254 nm.

Purification of extracts using column chromatography

The dichloromethane extracts of leaf (L), bark (B) and stem (S) were chromatographed over silica gel column using solvents and solvent mixtures of increasing polarity as the eluents. Every 50-ml of eluted fraction was collected and dehydrated under vacuum by a rotary evaporator. The dried fractions were spotted on to TLC plates impregnated with silica gel and eluted with the mixture of chloroform and petroleum ether (30:70). The gel from the positions having the same retention time based on the TLC plates were combined and tested for antibacterial activities.

Antibacterial activities of purified fractions

The antibacterial activities in the inhibition zones using the spread plate technique with the pure fractions were also tested to obtain the right biological active fractions.

RESULTS AND DISCUSSION

Each part of the plant was extracted using a solvent of increasing polarity. The yields of crude extracts are presented in Table 1. The leaf crude extracts had higher yields than those from other parts of plant (except for the dichloromethane extract) due to the presence of chlorophyll in the leaves. Comparing the effect of solvents on crude extract weights, methanol extracts gave the highest yield. This indicated that crude extracts from the three parts of the plant contained a higher amount of polar fractions than non-polar fractions.

Bacterial growth inhibition of crude extracts

The antibacterial activities of the crude extracts from the three parts of *Suregada multiflorum* using paper disc agar diffusion and spread plate methods are shown in Table 2. The leaf-hexane extracts partially inhibited the growth of *Staphylococcus aureus* and *Mycobacterium lacticola* (10 to 11 mm inhibition zones). The bark-

Table 1 Yield of crude extracts.

Solvent	Extract yield (g/kg dried weight)		
	Leaf	Bark	Stem
Hexane	0.1022	0.0061	0.0023
Dichloromethane	0.0032	0.0050	0.0038
Methanol	0.0883	0.0170	0.0155

hexane extract partially inhibited *Staphylococcus aureus* (10 mm inhibition zone). The leaf-dichloromethane extract partially inhibited *Staphylococcus aureus* (10 mm inhibition zone), *Escherichia coli* (0.5 mm inhibition zone and *Mycobacterium lacticola* (11 mm inhibition zone) and moderately inhibited *Bacillus subtilis* (16 mm inhibition zone). The bark-dichloromethane extracts moderately inhibited *Staphylococcus aureus* (12 to 13 mm inhibition zone), *Bacillus subtilis* (15 mm inhibition zone), *Mycobacterium lacticola* (13 to 14 mm inhibition zone) and *Xanthomonas campestris* (12 to 13 mm inhibition zone). The stem-hexane extract partially inhibited *Mycobacterium lacticola* (11 mm inhibition zone). The stem-dichloromethane extracts partially inhibited *Escherichia coli* (0.3 mm inhibition zone) and *Xanthomonas campestris* (3 mm inhibition zone) and moderately inhibited *Staphylococcus aureus* (13 mm inhibition zone), *Bacillus subtilis* (13 mm inhibition zone) and *Mycobacterium lacticola* (18 mm inhibition zone). It was clearly noticed that the polar fractions of the crude extracts were mostly able to inhibit microorganism activity. Among the investigated extracts, the dichloromethane extracts exhibited the highest antibacterial effect followed by hexane and methanol, respectively. Therefore, dichloromethane extracts were considered to be the most active constituents and those were chromatographed over a silica gel column using the solvent mixture of 30:70 v/v chloroform: petroleum ether as an eluent.

Bacterial growth inhibition of purified fractions

The antibacterial activities of pure dichloromethane extract from the three parts of *Suregada multiflorum* are shown in Table 3. It was found that *Mycobacterium lacticola* and *Xanthomonas campestri* were inhibited by most fractions from all parts of the plant. *Staphylococcus aureus* and *Escherichia coli* were inhibited by some fractions from the bark and stem, while *Bacillus subtilis* and *Pseudomonas aeruginosa* were resistant to all fractions. These results are in agreement with previous studies in which *Suregada multiflorum* was reported to yield pentacylic triterpenoids from its bark and a

Table 2 Bacterial growth inhibition of various parts of *Suregada multiflorum*.

Extracts	Part of plant	Extraction	Diameter of inhibition zone (mm)					
			Sa	*Ec*	*Bs*	*Ml*	*Pa*	*Xc*
LH1	Leaf	Hexane 1	11	0	0	11	0	0
LH2	Leaf	Hexane 2	10	0	0	11	0	0
LH3	Leaf	Hexane 3	10	0	0	10	0	0.3
LD1	Leaf	Dichloromethane 1	10	0.5	16	11	0	0
LM1	Leaf	Methanol 1	10	0	0	0	0	0.5
BH1	Bark	Hexane 1	10	0	0	0	0	0
BD1	Bark	Dichloromethane 1	12	0	15	13	0	12
BD2	Bark	Dichloromethane 2	13	0	15	14	0	13
BM1	Bark	Methanol 1	0	0	0	0	0	3
BM2	Bark	Methanol 2	0	0	0	0	0	0
SH1	Stem	Hexane 1	0	0	0	11	0	0
SD1	Stem	Dichloromethane 1	13	0.3	13	18	0	3
SM1	Stem	Methanol 1	9	0	17	0	0	0

L - leaf, B - bark, S - stem; H - hexane, D - dichloromethane, M - methanol

Sa - *Staphylococcus aureus*; Ec - *Escherichia coli*; Bs - *Bacillus subtilis*; Ml - *Mycobacterium lacticola*; Pa - P*seudomonas aeruginosa*; Xc - *Xanthomonas campestris*

flavanoid glucoside from its leaves (Talapatra *et al*., 1998). Most of those compounds generally showed biological activity including antimicrobial and antioxidant activity (Katerere *et al*., 2003; Subcharoen, 2007).

It was interesting to note that a pure dicholoromethane extract from all plant parts had a higher potential to inhibit the tested bacteria, especially *Xanthomonas campestris,* than crude extracts. *Xanthomonas campestris* is a plant pathogenic bacteria found in many plant diseases such as of cabbage black rot, bacterial leaf spot of peppers and tomatoes, bacterial leaf bright disease, black rot of crucifers (Williams, 1980) and bacterial citrus canker (Schubert and Sun, 2003). Antibacterial activity against those pathogenic plant diseases should be further examined using the pure fractions.

CONCLUSION

Extraction of chemical constituents from the leaf, bark and stem of *Suregada multiflorum* provided high yields of polar fractions. The crude

Table 3 Bacterial growth inhibition of pure dichloromethane extracts from the leaf, bark and stem of *Suregada multiflorum*.

Fraction	Diameter of inhibition zone (mm)					
	Sa	*Ec*	*Bs*	*Ml*	*Pa*	*Xc*
LD1	0	0	0	25	0	23
LD2	0	0	0	0	0	15
LD3	0	0	0	19	0	13
LD4	0	0	0	21	0	40
LD5	0	0	0	22	0	27
BD1	0	0	0	12	0	25
BD2	0	0	0	0	0	9
BD3	0	0	0	12	0	0
BD4	11	0	10	19	0	19
BD5	0	0	0	14	0	10
BD6	0	0	0	13	0	0
BD7	9	9	11	11	0	9
BD8	0	0	0	17	0	12
BD9	0	0	0	0	0	0
BD10	10	9	0	10	0	9
SD1	0	0	0	0	0	0
SD2	0	0	0	16	0	9
SD3	12	0	0	19	0	16
SD4	0	0	0	15	0	9
SD5	0	0	0	14	0	11
SD6	0	0	0	13	0	0
SD7	0	0	0	25	0	15
SD8	10	10	0	10	0	18
SD9	9	10	0	10	0	10

L - leaf, B - bark, S - stem; H - hexane, D - dichloromethane, M - methanol

Sa - *Staphylococcus aureus*; Ec - *Escherichia coli*; Bs - *Bacillus subtilis*; Ml - *Mycobacterium lacticola*; Pa - *Pseudomonas aeruginosa*; Xc - *Xanthomonas campestris*

and pure fractions, particularly the polar compounds, of each part of the plant generally provided some inhibition activity against several microorganisms. Purified fractions exhibited higher bacterial growth inhibition than crude extracts. Bacterial growth inhibition against *Xanthomonas campestris* indicated the potential of chemical constituents from *Suregada multiflorum* to inhibit plant bacterial diseases such as citrus canker and that they could probably be useful for canker prevention by replacing chemical pesticides.

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