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# Antimicrobial activity of Thai medicinal plants (*Murraya paniculata, Azadirachta indica* var. *Siamensis, Chromolaena odorata*) against periodontopathic bacteria

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## Abstract

**Objective:** The purpose of this study was to evaluate the antimicrobial activity of three Thai medicinal plants on three periodontopathic bacteria.

**Materials and methods:** Parts of *Murraya paniculata, Azadirachta indica* var. *Siamensis* and *Chromolaena odorata* were extracted using various organic solvents of different polarity; i.e. n-hexane, ethyl acetate, dichloromethane, chloroform and methanol.The agar diffusion technique was used to screen the crude extracts for their antimicrobial activity against *Porphyromonas gingivalis* (ATCC33277, FDC381), *Prevotella intermedia* (ATCC25611), and *Aggrigatibactor actinomycetemcomitans* (ATCC43718,Y4).

**Results:** All extracts demonstrated growth-inhibition effect against all strains of tested bacteria but were different in the spectrum of inhibition. The ethyl acetate extracts of *Murraya paniculata* leaves and *Murraya paniculata* branches exhibited comparable activity to that of the standard drug chlorhexidine when tested against *Porphyromonas gingivalis* FDC 381.

**Conclusion:** Crude extracts from leaves and branches of Murraya paniculata, leaves of *Azadirachta indica* var. *Siamensis*, and leaves of *Chromolaena odorata* using appropriate organic solvent demonstrated antimicrobial activity to *Porphyromonas gingivalis*, *Prevotella intermedia* and *Actinobacillus Actinomycetemcomitans*.

**Key words:** antimicrobial activity, Thai medicinal plants, periodontopathic bacteria, crude extracts

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*บทวิทยาการ*

# ฤทธิ์ต้านเชื้อจุลินทรีย์ของสมุนไพรไทย (แก้ว, สะเดาไทย, สาบเสือ) ต่อเชื้อก่อโรคปริทันต์อักเสบ

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## บทคัดย่อ

**วัตถุประสงค์:** เพื่อศึกษาประสิทธิภาพของสารสกัดหยาบของใบแก้ว, กิ่งต้นแก้ว, ใบสะเดาไทยและใบสาบเสือในการยับยั้งการเจริญของเชื้อก่อโรคปริทันต์อักเสบสามชนิด

**วัสดุอุปกรณ์และวิธีศึกษา:** ส่วนต่างๆของแก้ว (*Murraya paniculata*), สะเดาไทย (*Azadirachta indica* var. *Siamensis*) และสาบเสือ (*Chromolaena odorata*) ได้ถูกนำมาสกัดด้วยตัวทำละลายอินทรีย์ที่มีขั้วที่แตกต่างกันได้แก่ เฮกเซน, เอทิลอะซีเตต, ไดคลอโรมีเทน, คลอโรฟอร์มและเมทานอล นำสารสกัดที่ได้มาทำการทดสอบฤทธิ์ต้านเชื้อจุลินทรีย์ต่อเชื้อพอร์ไฟโรโมแนส จิงจิวาลิส (ATCC33277, FDC381), เชื้อพรีโวเทลลา อินเทอะมิเดีย (ATCC25611) และเชื้อแอกกริเกติแบคเทอร์ แอกทิโนมัยซิเตมโคมิแทนส์ (ATCC43718,Y4). โดยวิธีอะการ์ดิฟฟิวชั่น

**ผลการศึกษา:** สารสกัดสมุนไพรทุกชนิดแสดงฤทธิ์ในการยับยั้งการเจริญเติบโตของแบคทีเรียทุกสายพันธุ์แต่มีความแตกต่างในประสิทธิภาพของการยับยั้ง ทั้งนี้สารสกัดหยาบของใบแก้วและกิ่งต้นแก้วที่ใช้เอทิลอะซิเตตเป็นตัวทำละลายแสดงประสิทธิภาพที่ใกล้เคียงกับคลอร์เฮกซิดีน 2% ในการยับยั้งการเจริญของเชื้อพอร์ไฟโรโมแนส จิงจิวาลิส สายพันธุ์ FDC 381

**บทสรุป:** สารสกัดหยาบของใบแก้ว, กิ่งต้นแก้ว, ใบสะเดาไทยและใบสาบเสือที่ใช้ตัวทำละลายอินทรีย์ที่เหมาะสมในการสกัดจะแสดงประสิทธิภาพในการยับยั้งการเจริญของเชื้อก่อโรคปริทันต์อักเสบพอร์ไฟโรโมแนส จิงจิวาลิส, พรีโว-เทลลา อินเทอะมีเดียและแอกกริเกทิแบคเทอร์ แอกทิโนไมซีเท็มคอมิแทนส์

**รหัสคำ:** ฤทธิ์ต้านเชื้อจุลินทรีย์, สมุนไพรไทย, เชื้อก่อโรคปริทันต์อักเสบ, สารสกัดหยาบ

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## Introduction

Plants have been used in folk medicines for thousands of years. Previous studies revealed that phytochemical constituents in plants from Rutaceae, Meliaceae and Asteraceae families exhibited antimicrobial activity against various bacterial species both Gram-positive and Gram-negative.[1-4] *Murraya paniculata* (L.) Jack (Rutaceae) known in Thai as "Kaew" is a shrub or small sized tree, which grows up to 10 meters, distributed widely in tropical and subtropical Asia. Its leaves and roots have traditionally found wide medicinal uses in southeast Asia and China.[5] From previous chemical investigations, the isolation of isoflavonoids,[6] sesquiterpenoids,[7] flavonoids[8,9] and coumarins[10] from its dried parts were reported. *Azadirachta indica* A. Juss. var. *Siamensis* Valeton, Thai name "Sa-dao Thai", also known as Neem, belongs to the Meliaceae family. It is a subtropical tree native to the drier regions of Asia and Africa. Some parts of the plant, especially its leaves, exhibit a wide range of pharmacological activities and medicinal applications.[3] The antibacterial activity against a wide variety of pathogenic bacteria has been evaluated and known from ancient times.[11] triterpenoids,[12,13] tetranortriterpenoids,[14] isoprenylated flavonoids,[15] diterpenoids[16] and limonoids[17] are among active phytochemicals produced from this plant. *Chromolaena odorata* (L.) R. M. King & H. Rob. (Asteraceae), called "Sarb-sua", is a diffuse, scrambling shrub that grows in southern Asia and western Africa. It forms a tangle of bush from 3 to 7 meters in height when growing in the open environment. The investigations of the chemical constituents of this plant have identified various compounds that demonstrated antimicrobial activity. These chemicals include flavonoids,[18,19] tannins[20] and terpenoids.[21] The extract of the leaves of *C. odorata* has shownto inhibit the growth of bacteria.[1,2]

Even though extracts from these plants show antibacterial property, there is scarce evidence on their killing efficacy on bacterial species causing periodontitis, the disease that 10-15% of the global population suffered from its severe form.[22] Gram negative anaerobic, black-pigmented bacteria, *Porphyromonas gingivalis* and *Prevotella intermedia*, are periodontitis causative bacteria usually identified in the subgingival biofilms harvested from active periodontal pockets or in inflamed gingival tissue.[23] *Aggregatibactor actinomycetemcomitans* is another Gram-negative bacterium associated with various infectious disease including aggressive periodontitis.[24] Though the benefit of antibiotics in the treatment of periodontitis,[25] the growing problem of antibiotic drug resistance might hinder the use of these medications. It is interesting to verify the antibacterial properties of extracts from these three plant species against periodontopathic bacteria. The aim of this study was to evaluate plant extracts from *M. paniculata*, *A. indica* var. *Siamensis* and *C. odorata* for their antibacterial efficacy on the periodontopathogens, i.e. *A. actinomycetemcomitans, P. gingivalis and P. intermedia*.

## Materials and Methods

### 1. Plant materials

The leaves and branches of *M. paniculata* were collected from Hua Mark District, Bangkok, leaves of *A. indica* var. *Siamensis* were collected from Minburi District, Bangkok and leaves of *C. odorata* were collected from Bang Na District, Bangkok. Voucher specimens were deposited at the Faculty of Science, Ramkhamhaeng University.

### 2. Extraction process

The air-dried powdered plant materials were extracted successively with the following organic

solvents: n-hexane, ethyl acetate (EtOAc) and methanol (MeOH) for the leaves and branches of *M. paniculata;* n-hexane, chloroform ($CHCl_3$) and MeOH for the leaves of *A. indica* var. *Siamensis*; n-hexane, dichloromethane ($CH_2Cl_2$) and MeOH for the leaves of *C. odorata* using maceration method. The solutions were filtered and the solvents were removed under vacuum at ca. 40°C using a rotary evaporator to give crude extracts. The quantities and percentage yields of the extracts obtained from the above plant materials are presented in table 1. Each of the plant extracts was dissolved in dimethyl sulfoxide (DMSO, Sigma, St. Louis, USA) to obtain the solutions of 100 mg/ml (w/v) concentration.The stock solutions were kept in a refrigerator at the temperature of 4-8°C.

**3. Microorganisms**

The antibacterial activities of all plant extracts were determined against *Porphyromonas gingivalis* ATCC 33277 (Pg ATCC 33277), *Porphyromonas gingivalis* FDC 381 (*Pg* FDC 381), *Prevotella intermedia* ATCC 25611 (Pi ATCC 25611) and *Aggregatibactor actinomycetemcomitans* ATCC 43718 (Y4) (*Aa* ATCC 43718,Y4).

**3.1 Culture media**

The medium used for the growth of the microorganisms was Brain heart infusion broth (BHI). The following agar media were used for the antimicrobial test: Brucella (Oxoid®) supplemented with hemin and vitamin K for *Pg* and *Pi*. Trypticase soy agar with bacitracin and vancomycin (TSBV) for *Aa*

**3.2 Inoculum**

*Pg* and *Pi* were inoculated into prereduced BHI broth and incubated in Anaerobic chamber (Anaerobic system, Forma Scientific, Inc.) at 37°C for 5-7 days. *Aa* was inoculated into prereduced BHI broth and incubated at 37°C in 5% $CO_2$ atmosphere for 3-4 days. The bacterial suspension was then diluted with BHI broth medium to obtain approximately $1x10^8$ cfu/ml of bacteria, comparable to 0.5 Mc-Farland turbidity standard.

**4. Antibacterial activity**

The growth inhibition tests were performed using agar diffusion technique. One hundred microlitre (μl) of each BHI-suspended microorganism was distributed on the agar medium (25 ml/plate) using small-size glass beads. Once the agar surface was dried, it was punched to create a 5 millimeters diameter well which was filled with 4 μl of the plant extracts solution. The positive control and the negative control were filled with chlorhexidine 2% and DMSO respectively. Each plate cultured with *Pg* or *Pi* was incubated in anaerobic chamber at 37°C for 5-7 days, whereas plates cultured with *Aa* were incubated at 37°C in 5% $CO_2$ atmosphere for 3-4 days. All tests were performed three times and the antibacterial activity was expressed as the mean diameters (mm) of inhibition zone produced by the plant extracts.

## Results

The yields of the plant extracts presented in table 1. The results of the agar diffusion test of plant extracts against periodontal pathogens were shown in Table 2. The negative control (DMSO) showed no inhibitory effect. The positive control (chlorhexidine 2%) showed average inhibition diameters of 18.5, 30.5, 17.5, 19.5 mm against *Pi*, *Aa*, *Pg* FDC381 and *Pg* ATCC 33277, respectively.

**Inhibitory effects of plant extracts against *Porphyromonas gingivalis (Pg)***

The leaf and branch extracts of *M. paniculata* demonstrated inhibitory effect against both strains of *Pg* (Table 2). The EtOAc extract of leaves and branches

of this plant species exerted high degree of inhibition on *Pg* FDC 381 and *Pg* ATCC 33277 respectively. The $CHCl_3$ extract of *A. indica* var. *Siamensis* leaves showed inhibition effect only against *Pg* FDC 381. All hexane extracts did not exert inhibitory effect on these bacterial strains except that of *M. paniculata* leaves which exerted inhibition effect against *Pg* ATCC 33277. All extracts of *C. odorata* and all methanol extracts showed no inhibitory effect.

**Inhibitory effects of plant extracts against *Prevotella intermedia (Pi)***

The EtOAc and hexane extracts of leaves of *M. paniculata* showed high degree of antimicrobial activity against *Pi* (Table 2). The $CHCl_3$ extract of *A. indica* var. *Siamensis* leaves and the $CH_2Cl_2$ extract of *C. odorata* leaves also demonstrated inhibitory effect on *Pi*. The MeOH extracts of all plant species did not give inhibition zone on the agar except that of branches of *M. paniculata*. The hexane extracts of *A. indica* var. Siamensis leaves and *C. odorata* leaves did not have effect on microbial growth.

**Inhibitory effects of plant extracts against *Aggregatibacter actinomycetemcomitans (Aa)***

The MeOH extract of *A. indica* var. *Siamensis*, the $CH_2Cl_2$ extract of *C. odorata* and the MeOH extract of leaves of *M. paniculata* were the best three extracts exhibiting antimicrobial activity against *Aa* (Table 2). The extracts which have no effect on microbial growth was the hexane and methanol extract of *C. odorata.*

**Table 1** The quantities and percentage yields of the extracts from the plant materials used in this study

| Plant extracts | Dry weight (g.) | Quantities yield (g.) | Percentage yield (%) |
|---|---|---|---|
| *Murraya paniculata* (leaves) | 1,640 | | |
| Hexane extract | | 193.04 | 11.7 |
| Ethyl acetate extract | | 103.75 | 6.3 |
| Methanol extract | | 283.65 | 17.3 |
| *Murraya paniculata* (branches) | 2,338 | | |
| Hexane extract | | 2.42 | 0.1 |
| Ethyl acetate extract | | 10.00 | 0.4 |
| Methanol extract | | 127.20 | 5.4 |
| *Azadirachta indica* (leaves) | 7,000 | | |
| Hexane extract | | 135.08 | 1.9 |
| $CHCl_3$ extract | | 88.67 | 1.2 |
| Methanol extract | | 212.86 | 3.0 |
| *Chromolaena odorata* (leaves) | 15,000 | | |
| Hexane extract | | 41.10 | 0.2 |
| $CH_2Cl_2$ extract | | 147.63 | 0.9 |
| Methanol extract | | 427.10 | 2.8 |

$CHCl_3$ = Chloroform, $CH_2Cl_2$ = Dichloromethane

**Table 2** Zone of growth inhibition (mm) showing antimicrobial activity of plant extracts against periodontal pathogens

| Plant extracts | Diameter of inhibition zone (Mean±SD) | | | |
|---|---|---|---|---|
| | ***Pi*** | ***Aa*** | ***Pg 381*** | ***Pg 33277*** |
| *Murraya paniculata* (leaf) | | | | |
| Hexane extract | 13±5.29 | 6±0.71 | 0 | 9.5±3.92 |
| Ethyl acetate extract | 14±3.46 | 7±1 | 19±4 | 11±1.22 |
| Methanol extract | 0 | 8±2 | 0 | 0 |
| *Murraya paniculata* (branch) | | | | |
| Hexane extract | 11±2.76 | 7±0 | 0 | 0 |
| Ethyl acetate extract | 0 | 6.5±0.61 | 17.5±2.52 | 11.75±0.31 |
| Methanol extract | 9±2 | 6.5±0.61 | 0 | 0 |
| *Azadirachta indica* (leaf) | | | | |
| Hexane extract | 0 | 6.5?±0.61 | 0 | 0 |
| $CHCl_3$ extract | 9.5±1.12 | 7±1.06 | 12.5±1.17 | 0 |
| Methanol extract | 0 | 9±2.47 | 0 | 0 |
| *Chromolaena odorata* (leaf) | | | | |
| Hexane extract | 0 | 0 | 0 | 0 |
| $CH_2Cl_2$ extract | 10.5±2.6 | 8.5±5.52 | 0 | 0 |
| Methanol extract | 0 | 0 | 0 | 0 |
| Chlorhexidine 2% | 18.5±1.54 | 30.5±0.61 | 17.5±0.5 | 19.5±2.52 |
| DMSO | 0 | 0 | 0 | 0 |

*Pi = Prevotella intermedia* ATCC 25611

*Aa = Aggregatibactor actinomycetemcomitans* ATCC 43718(Y4)

*Pg* 381 = *Porphyraomonas gingivalis* FDC 381

*Pg 33277 = Porphyromonas gingivalis* ATCC 33277

$CHCl_3$ = Chloroform, $CH_2Cl_2$ = Dichloromethane, DMSO = Dimethyl sulfoxide

## Discussion

Traditional use of plants in treating various infectious ailments might indicate the usefulness of plant extracts in specific medical conditions. Evidently, the antimicrobial properties of many plant extracts have been reported.[26,27] As the emergence of popularities in natural therapies, the quantitative and qualitative data are required to confirm the effectiveness and safety of plant extracts. Agar diffusion technique used in this study showed effectiveness of tested plants in growth-inhibition effect against all strains of tested bacteria but were different in the specificity of the spectrum of inhibition. *C. odorata* showed the narrowest spectrum in that it could only inhibit the growth of *Pi* and *Aa*, *A. indica* var. *Siamensis* demonstrated inhibiting effects on all strains except *Pg* ATCC 33277 whereas *M. paniculata* exerted its activity against all tested bacterial strains. This might be explained by the difference in the chemical constituents within each plant.[3,5,13,14,15,23] It was worth noting that the ethyl acetate extracts of *M. paniculata* leaves and *M. paniculata* branches exhibited comparable

activity against *Pg* FDC 381 to that of the standard drug, chlorhexidine 2% (Table 2).

Within each plant, the results of this study showed that the selection of the extracting solvents might be important in the exploitation of the plant activity. It was found that the different use of solvents affected the effectiveness of plant extracts on bacterial growth-inhibition demonstrating in the difference in the diameter of inhibition zone. Among the organic solvents used, the moderately polar organic solvents ($CHCl_3$, $CH_2Cl_2$) seem to be the most suitable solvents for the extraction of compounds that demonstrated the best inhibiting effect on tested bacterial strain. It was noted that the less polar organic solvent (n-hexane) and highly polar organic solvent (MeOH) could not extract the compounds essentially benefit for inhibiting black-pigmented bacteria (Pg). Thus, it could extrapolate that the polarity of the organic solvent is an important factor in extraction of active chemicals from the plants.[28]

Regarding bacterial susceptibility, all extracts (except the hexane and methanol extracts of *C. odorata*) inhibit growth of *Aa*. It was postulated that this bacterial strain might be easily eradicated and the active compounds could be found in all studied plants extracted with organic solvents of different polarity.

Though most of the extracts of the studied plants have less effectiveness against periodontopathic bacteria comparing to chlorhexidine 2%, these plants might have a significant advantage concerning adverse effects. Chlorhexidine has many adverse effects, for example, brown staining on teeth and oral tissues, taste disturbance, allergic reaction,[29] the use of plants might be a good alternative since these plants have been used to treat oral disease for century which could be postulated that there is no harmful adverse reaction.[1,3,5]

The agar diffusion technique is a useful method in screening *in vitro* antimicrobial activity of substances including plant extracts but is limited to preliminary data only since the hydrophobicity of plant extract solutions might prevent their diffusion through the agar medium.[30,31] The results obtained from this *in vitro* test must be interpreted with care as this assay may not demonstrate the full clinical potential of these plants. As the studied plant extracts exerted antimicrobial activity against these putative periodontopathic bacteria, this could interprete that there might be chemical constituents with microbial inhibitory effect in these plant extracts. The study conducts to purify compounds and reveals chemical structure of substance that have antimicrobial potential within plants might be useful in the development of new products for human use in treating periodontal disease.

## Conclusion

Our study indicated that three Thai medicinal plants: leaves and branches of *Murraya paniculata* (Kaew), leaves of *Azadirachta indica* var. siamensis (Sa-dao Thai) and leaves of *Chromolaena odorata* (Sarb-sua), demonstrated antimicrobial activities against the periodontopathic bacteria: *Porphyromonas gingivalis* ATCC 33277, *Porphyromonas gingivalis* FDC 381, *Prevotella intermedia* ATCC 25611 and *Aggregatibactor actinomycetemcomitans* ATCC 43718 (Y4). The long time use of these plants as a method of curing many ailments causing by bacteria may be a reliable evidence showing their safety and economy. Accordingly, the chemical constituents in these plants might be potentially active enough to be used as substances in manufacturing effective antimicrobial medication for preventing and treating periodontal infection. This achievement might not only be helpful in treating the disease but also minimize the de-

velopment of bacterial resistance. Further investigation leading to a better understanding of Thai traditional medicine is noteworthy.

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