Antioxidant and Antimicrobial Activity of *Bauhinia strychnifolia Craib* Stem Extract Against Oral Pathogens

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Abstract:

Background: *Bauhinia strychnifolia* Craib *(B. strychnifolia)* is used for treatment of poisoning and various illnesses in Thai traditional medicine. To investigate antioxidant and antimicrobial activity of extracts from *B. strychnifolia* stem (aqueous and ethanol crude extracts) against oral pathogens *Streptococcus mutans* (*S. mutans*) and *Candida albicans* (*C. albicans*).

Materials & Methods: The aqueous and ethanol crude extracts of *B. strychnifolia* stem were used to evaluate the antioxidant property, the content of phenolic compounds, flavonoids and alkaloids, and the antimicrobial activity.

Results: The ethanol extract possessed stronger antioxidant and antimicrobial activity than the aqueous one. Extracts of *B. strychnifolia* at various concentration were investigated for their antioxidant potency. The lowest concentration of the extracts of 31.25 µg/ml, the DPPH radical-scavenging of the ethanol and the water extracts was 87.67 ± 1.46% and 35.60 ± 9.52%, respectively. The antimicrobial activity of the ethanol and the aqueous extracts against *S. mutans* DMST 18777 showed the MIC and the MBC of 0.25 mg/ml and 0.50 mg/ml, respectively. The antifungal activity of both extracts against *C. albicans* revealed the same levels of MIC and the MFC of 1.0 mg/ml.

Conclusions: This study, *B. strychnifolia* was found to have antioxidant and antimicrobial activity against oral pathogens causing dental caries and oral candidiasis.

Keywords: Antioxidant, Antimicrobial, *Bauhinia strychnifolia* Craib, *Streptococcus mutans*, *Candida albicans*

ฤทธิ์ต้านอนุมูลอิสระและฤทธิ์ต้านจุลชีพของสารสกัดเถาย่านางแดงต่อเชื้อก่อโรคในช่องปาก

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บทคัดย่อ

บทนำ: ย่านางแดงเคยใช้ถอนพิษและรักษาการเจ็บป่วยที่หลากหลายในแพทย์แผนไทย เพื่อศึกษาฤทธิ์ต้านอนุมูลอิสระและฤทธิ์ต้านจุลชีพของสารสกัดเถาย่านางแดงอย่างหยาบชั้นน้ำและเอทานอล ต้านเชื้อก่อโรคในช่องปาก สเตร็ปโตคอคคัส มิวแทนส์ และแคนดิดา อัลบิแคนส์

วิธีการศึกษา: นำสารสกัดเถาอย่างหยาบชั้นน้ำและเอทานอลมาศึกษาฤทธิ์ต้านอนุมูลอิสระ ปริมาณสารประกอบฟีนอลิค ฟลาโวนอยด์ และ อัลคาลอยด์ และฤทธิ์ต้านจุลชีพ

ผลการศึกษา: สารสกัดชั้นเอทานอล มีฤทธิ์ต้านอนุมูลอิสระและฤทธิ์ต้านจุลชีพดีกว่าสารสกัดชั้นน้ำ สารสกัดมีฤทธิ์ต้านอนุมูลอิสระที่ความเข้มข้นหลากหลาย ความเข้มข้นต่ำที่สุดของสารสกัดคือ 31.25 µg/ml ให้ค่า DPPH radical-scavenging ของสารสกัดชั้นเอทานอลและน้ำ เท่ากับ 87.67 ± 1.46% และ 35.60 ± 9.52% ตามลำดับ ฤทธิ์ต้านจุลชีพของสารสกัดชั้นเอทานอลต้านเชื้อ สเตร็ปโตคอคคัส มิวแทนส์ ให้ค่า MIC 0.25 mg/ml และค่า MBC 0.50 mg/ml ตามลำดับ ฤทธิ์ต้านเชื้อราของสารสกัดทั้งสองชั้นต่อเชื้อรา แคนดิดา อัลบิแคนส์แสดงให้เห็นระดับที่เท่ากันของค่า MIC และ MFC มากกว่า 1.0 mg/ml

สรุปผล: การศึกษาพบว่าย่านางแดงมีฤทธิ์ต้านอนุมูลอิสระและฤทธิ์ต้านจุลชีพของเชื้อก่อโรคในช่องปาก ซึ่งเป็นสาเหตุของโรคฟันผุและเชื้อราในช่องปาก

คำสำคัญ: ฤทธิ์ต้านอนุมูลอิสระ ฤทธิ์ต้านจุลชีพ ย่านางแดง สเตร็ปโตคอคคัส มิวแทนส์ แคนดิดา อัลบิแคนส์

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1. Introduction

Streptococcus mutans is a Gram-positive bacteria commonly found in the human oral cavity, is an etiological agent for dental caries and is also involved in dental plaque formation and accumulation on tooth surfaces. *Candida albicans* is an opportunistic fungal pathogen that is responsible for oral candidiasis or thrush. It usually occurs in

immunocompromised individuals, such as HIV-infected patients, transplant recipients, chemotherapy patients, and low birth-weight babies. Thrush appears as creamy-white patches on the tongue, on the lining of the mouth or in the throat. Recent evidence indicated the interaction between *S. mutans and C. albicans* might mediate cariogenic development[1].

Previous studies reported extracts of garlic, lime, mangosteen and tea leaves inhibited growth of *S. mutans*. Tea leaves possessed high content of antioxidant (polyphenols)[2] and antibacterial effects to oral cavity infections causing dental caries and periodontal diseases. Antioxidants play important roles in cellular function and have been implicated in processes associated with aging, including vascular, inflammatory damage and cancer such as ascorbic acid or vitamin C[3].

B. strychnifolia is a Thai herbal plant commonly known as Ya-nang-dang and is used for treatment of poisoning and various illnesses in Thai traditional medicine. *B. strychnifolia* belong to the family of Leguminosae-Caesalpiniaceae. It is a common plant in Thailand known as Ya-nang-dang. The leaves, stems and roots have medicinal properties. *B. strychnifolia* stem extract was reported to possess anticancer property [4] and the leaf extract has been used for the treatment of diarrhea[5]. The objectives of this study are to investigate antioxidant and antimicrobial activity of *B. strychnifolia* stem extracts against *S. mutans* causing dental caries and *C. albicans* causing oral candidiasis. The findings will be useful for further study to develop products containing *B. strychnifolia* extract for oral care.

2. Materials and Methods

2.1 Collection of *B. strychnifolia* sample

Dry stems of *B. strychnifolia* were collected from Suan Ya Thai herbal garden in Noppitum District, *Nakhon Si Thammarat* Province, South Thailand.

2.2 Preparation of *B. strychnifolia* extracts

Dried *B. strychnifolia* stem was ground into powder and extracted using distilled water and ethanol at 1:10 ratio. The mixture was heated at 50°C for 1 hour and then filtered through Whatman no. 1 filter paper. The residue was re-extracted with the same volume of solvents. Combined filtrates were evaporated to dryness using vacuum rotary evaporator. The obtained extracts were kept in sterile containers and stored in a refrigerator at 4° C and used for further studies. Percentage yield of *B. strychnifolia* extracts was calculated using the following equation

% yield (w/w) = (Extract weight/Powdered material weight) x 100

2.3 Estimation of phenolic compounds

The phenolic compounds content in *B. strychnifolia* extracts was determined using Folin-Ciocalteu method. The concentration of 1.0 mg/ml in methanol of each crude extract was used in the analysis. The reaction mixture was prepared by mixing 250 µl of 1 mg/ml of the extract solution, 1.25 ml of distilled water, 250 µl of ethanol and 125 µl of 50% Folin-Ciocalteu's reagent. After 5 minutes 250 µl of 5% sodium carbonate was added. After 1 hour of incubation in the dark at room temperature, the absorbance was read at 725 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration curve was constructed. The concentration of phenolic compounds was read from the calibration curve and expressed in terms of gallic acid equivalent (GAE) [6].

2.4 Qualitative analysis of flavonoid

Flavonoid in *B. strychnifolia* extracts was analyzed by aluminium chloride method. The reaction mixture was prepared by mixing 500 µl of 1 mg/ml of each plant extract, 1.5 ml methanol, 100 µl 10% aluminium chloride solution, 100 µl 1 M potassium acetate solution and 2.8 ml of distilled water. After 30 minutes of incubation at room temperature, the color of the mixture was observed. Positive result for flavonoid showed orange to dark red color [7].

2.5 Alkaloid Test

Alkaloid in *B. strychnifolia* extract was tested by Wagner method. The reaction mixture was prepared by mixing 0.01 g of the concentrated plant extract, 1 ml of 2 N HCl, and filtered. After adding 1.0 ml of Wagner's reagent to the filtrate the color of the precipitate was observed. Positive result for alkaloids showed red precipitate, and negative result showed no precipitate.

2.6 Determination of antioxidant activity by DPPH Assay

The antioxidant activity of *B. strychnifolia* stem extracts was measured by DPPH assay using 2,2-diphenyl-1-picrylhydrazyl in methanol as stable radical. 500 µl of various concentrations of *B. strychnifolia* extracts (31.25 – 1000 µg/ml in methanol) were mixed with 1.5 ml of 0.1 mM DPPH solution and incubated in dark at room temperature for 30 minutes. After incubation, the absorbance was read at 517 nm using methanol as blank, DPPH solution as control[8], and 0.1% (W/V) ascorbic acid as standard[9]. The assays were performed in triplicates, values were expressed as percent inhibition of the DPPH by the samples and calculated using the following equation:

$$\% \text{ inhibition} = [(OD \text{ control} - OD \text{ sample}) / OD \text{ control}] \times 100$$

2.7 Preparation of microbial culture

S. mutans was cultured in Todd Hewitt broth (THB) in 5% CO_2 at 37° C for 18 hours. The test suspension was prepared by adjusting the bacterial culture to 0.5 McFarland Standard with THB to obtain the bacterial density of 6.75 x 10^6 CFU/ml. *C. albicans* was cultured in Sabouraud Dextrose broth (SDB) at 37° C for 24 hours. The test suspension was prepared by adjusting the fungal culture to 0.5 McFarland Standard with SDB to obtain the fungal density of 5 x 10^4 CFU/ml.

2.8 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal and fungicidal concentration (MBC/MFC)

The minimum inhibitory concentration (MIC) was determined by broth microdilution method. Suspensions *of S. mutans* and *C. albicans* were placed in 96-well microtiter plates, 1 mg/ml of *B. strychnifolia* extracts were added in the wells and subjected to two-fold serial dilutions to obtain concentrations from 0.25 mg/ml to 1.0 mg/ml at the total volume of 100 µl per well. The mixtures of *S. mutans* were incubated in 5% CO_2 at 37°C for 18 hours, and 1.95 µg/ml of oxytetracycline and 100% dimethyl sulfoxide (DMSO) were served as positive and negative controls, respectively. *C. albicans* was incubated at 37°C for 24 hours, and 3.125 µg/ml fluconazole and 100% dimethyl sulfoxide (DMSO) were served as positive and negative controls, respectively. Growth was observed by adding 10 µl of 0.18% resazurin solution in the test mixtures and incubated for 4 hours. Color change of the test mixtures was taken as indication of growth, the mixtures with no growth showed blue color and those with growth showed pink color or colorless. MIC was defined as the lowest concentration of the extract showing no growth.

In order to determine minimum bactericidal and fungicidal concentration (MBC/MFC), 10 µl of the well contents with no growth was cultured on blood agar plates and colony counted. MBC/MFC was defined as the lowest concentration of the extract that prevent colony formation or with colony formation not exceeded 99.9% of the total volume tested of 110 µl.

2.9 Statistical analysis

The data were presented as mean ± S.E.M for n = 3. Statistical analysis between experimental results was based on student's t-test. Significant difference was statistically considered at the level of *$p < 0.05$ and **$p < 0.01$.

3. Results and Discussion

3.1 Percent yield of extracts (w/w)

Preparation of *B. strychnifolia* extracts in aqueous and ethanol had the %yield of 9.29 and 5.04, respectively. This indicated that ethanol extract had higher % yield compared to the aqueous extract.

3.2 Phenolic compounds content

The concentration of phenolic compounds determined using Folin-Ciocalteu method were expressed as mg of gallic acid/g of extract and was directly proportional to the antioxidant activity. The results indicated that *B. strychnifolia* had a significant amount of phenolic compounds in both aqueous and ethanol extracts at the concentration of 1,607 mg GAE/g and 1,640 mg GAE/g, respectively.

3.3 Flavonoids test

The flavonoids test of *B. strychnifolia* extracts by aluminium chloride method showed the presence of flavonoids in both aqueous and ethanol extracts.

3.4 Alkaloid Test

Wagner's test of aqueous and ethanol extracts of *B. strychnifolia* showed positive results in alkaloid test.

3.5 Antioxidant activity by DPPH assay

The antioxidant activity of *B. strychnifolia* extracts is expressed in terms of percentage of DPPH inhibition and 0.1% (W/V) ascorbic acid as standard. The ethanol extract had significantly higher antioxidant activity than the aqueous extract in all tested concentration from 31.25 – 1,000 µg/ml ($p<0.05$ and $p<0.01$), more details were described in Table 1. The highest % DPPH inhibition of 87.67 ± 1.46 was found in the ethanol extract at the concentration of 31.25 µg/ml. The ethanol extract at the concentration of 31.25 - 500 µg/ml was found to have higher % DPPH inhibition than at the concentration of 1,000 µg/ml. Each value is the average of three analyses ± standard error of the mean.

3.6 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal and fungicidal concentration (MBC/MFC)

The extracts of *B. strychnifolia* and possessed antibacterial activity against *S. mutans* comparing with 1.95 µg/ml oxytetracycline and and *C. albicans* comparing 3.125 µg/ml fluconazole as positive controls. The aqueous extract showed the same values of MIC and MBC/MFC at 1.0 mg/ml, and the ethanol extract showed the MIC and MBC ranged from 0.25 mg/ml to 0.50 mg/ml, respectively (Table 2). The ethanol extract with MBC value of 0.50

mg/ml tested for bactericidal property against *S. mutans* giving the colony count of 106 colonies which was within the calculated 310 colonies (99.9% of 110 µl of extract). The aqueous extract with MBC value at 1.0 mg/ml was found to have more than 310 colonies growth.

4. Discussion

The biological activity of a Thai herb *B. strychnifolia* was studied. The plant stem was used for extraction using aqueous and ethanol as solvent. The results indicated ethanol is a better solvent than aqueous for the extraction of *B. strychnifolia* as shown by higher antioxidant and antimicrobial activity. The ethanol extract at the lowest concentration (31.25 µg/ml) was found to have higher % DPPH inhibition than at the highest concentration (1,000 µg/ml). This indicated the ethanol extract of *B. strychnifolia* exhibited stronger antibacterial activity at the reduce MIC and MBC values and prevent *S. mutans* colony formation. Analysis of *B. strychnifolia* stem extracts showed the presence of phenolics, flavonoids, alkaloids, and antioxidant activity expressed as %DPPH inhibition in all extracts.

5. Conclusions

The extracts of *B. strychnifolia* stem showed antibacterial activity against oral bacterial pathogens *S. mutans* and *C. albicans*. The results suggested that *B. strychnifolia* stem extract is a potential source of natural antioxidant and antimicrobial properties. Therefore further studies are required to provide a better understanding of the antioxidant properties and cytotoxicity for the development into natural oral care products.

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