Antioxidant and Antimicrobial Activities of *Vitex scabra* and *Syzygiumgratum*

Wirinratch Sue-aok[1],Suwanna Jankana[1*]

[1]Department of Chemistry, Faculty of Science, Buriram Rajabhat University
[*]Corresponding Author: suwanna.cn@bru.ac.th

Abstract
The study examined the efficacy of ethanol extracts obtained from *Vitex scabra* and *Syzygium gratum* in terms of their antioxidant and antimicrobial activities. The Folin-Ciocalteu reaction was utilized to determine the total phenolic content of the extracts. The antioxidant capacity of the extracts was measured using the DPPH and ABTS assays.The findings indicated that *S. gratum* extract had higher antioxidant activity than *V. scabra* extract as demonstrated by both DPPH and ABTS assays. The total phenolic content of the extracts had a significant correlation with DPPH and ABTS assays, with *S. gratum* extract possessing higher amounts of phenolic compounds than *V. scabra* extract. The antimicrobial activity of the extracts was measured using the broth dilution method against *Candida albicans*, *Clostridium butyricum*, and *Pseudomonas aeruginosa*. The *V. scabra* extract showed antimicrobial activity against *Clostridium butyricum* with a MIC value of 1.37 mg/ml. These findings suggest that both plants could serve as natural sources of antioxidant and antimicrobial agents.

Keywords: *Vitex scabra*, *Syzygium gratum*, antioxidant activity, antimicrobial activity

Introduction

Therapeutic plants can yield a multitude of secondary metabolites that exhibit biological activity with potential medical uses. These medicinal properties enable the use of such plants in treating a variety of conditions, such as anti-inflammatory, neuroprotective, and antidiabetic, as well as antioxidant (Bursal et al., 2020).

Thailand, a tropical country known for its plentiful rainfall and natural diversity, is the birthplace of various medicinal plants that have been utilized for both medicinal and culinary purposes (Junsongduang et al., 2020). *Vitex scabra* and *Syzygium gratum* are common Thai plants and indigenous to the province of Buriram in the Northeast of Thailand.

Vitex scabra Wall. ex Schauer (Lamiaceae) is commonly found in dipterocarp, mixed deciduous, and dry evergreen forests and is characterized by its flaky leaves. this species was misidentified as Vitex quinata for a long time (Chantaranothai, 2011). According to the relevant study, two new forms of ecdysteroids, namely 24-*epi*-pinnatasterone and scabrasterone, were extracted from the stem bark. Both ecdysteroids exhibited only slight molting activity in the Musca bioassay (Suksamrarn et al., 2002).

Syzygium gratum (Wight) S.N. Mitra (Myrtaceae) is an herb and common food plant native to Southeast Asia. Research has shown that both the aqueous and ethanolic extracts of *S. gratum* leaves have potent antioxidant and intracellular oxygen radical scavenging properties (Senggunprai et al., 2010). Additionally, the leaf extract of *S. gratum* exhibits a significant inhibitory effect on MDA-MB-231 breast cancer cells (Rocchetti et al., 2019) and

the aqueous extract has antihypertensive properties (Pakdeechote et al., 2020). The ethanolic extract of *S. gratum* leaves, on the other hand, is non-toxic to normal human lymphocytes and significantly suppresses the proliferation of MCF7 breast cancer cell lines (Woraratphoka et al., 2012).

Despite a literature search for previous research on *V. scabra* and *S. gratum*, few studies have explored the therapeutic potential of these plants, with *V. scabra* receiving the least attention. The objective of this investigation was to evaluate the antioxidant and antimicrobial characteristics of extracts derived from *V. scabra* and *S. gratum*. The findings of this research can serve as a guide for the future utilization of both plants as medicinal plants for health promotion and treatment.

Materials and Methods

Chemicals and Instrument

Gallic acid and DPPH (1,1-diphenyl-2-picryl-hydrazyl) were obtained from Aldrich (Germany), while Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) was obtained from Aldrich (Switzerland). ABTS (2,2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid) was obtained from Aldrich (USA), and Folin-Ciocalteu reagent was obtained from Fisher Chemical (Leicestershire, UK). All other chemicals used were of analytical grade. The UV-Vis Spectrophotometer instrument employed in this study was Lambda 365 (Perkin-Elmer, USA).

Plant materials

Fresh leaves of *Vitex scabra* and *Syzygium gratum* were harvested from Buriram province in the northeast region of Thailand. *V. scabra* was identified botanically following the research conducted by Chantaranothai (2011). The leaves were cleaned with tap water and then cut into small pieces before being dried at 50°C in a hot air oven. Once dried, the leaves were ground into a fine powder and stored at 4°C until they were ready for extraction.

Extraction

The dried leaves were macerated in ethanol at room temperature for 24 hours. The procedure was repeated three times before collecting all the solutions together. The resulting extract was filtered through Whatman No.1 and concentrated using a rotary evaporator. The crude extract was then kept at 4ºC until analysis.

Assessment of Antioxidant Activity Using the DPPH Radical Scavenging Assay

The method used for the DPPH radical scavenging assay was adapted from a previous study by Hammi et al. (Hanato et al., 1988; Hammi et al., 2015). 250 µl of 0.2 mM DPPH solution in ethanol was blended with 1 ml of the extract in ethanol at different concentrations. The mixture was then incubated in the dark at room temperature for 30 minutes, after which the absorbance was measured at 517 nm using a UV-Vis Spectrometer.The experiment was performed in triplicate for each concentration, and the average value was computed. The radical scavenging activity, expressed as percent inhibition, was calculated from the absorbance values using the following equation:

$$\% \text{ Inhibition} = [1-\frac{A_{sample}}{A_{control}}] \times 100$$

The value of $A_{control}$ refers to the absorbance of the control, which is the DPPH solution without any sample, while A_{sample} indicates the absorbance of the test samples added to the DPPH solution. The IC_{50} value, which indicates the concentration that results in 50%

inhibition, was obtained by plotting the percent inhibition against the extract concentration. Trolox, a reference antioxidant, was used in the experiment.

Methodology for ABTS Radical Scavenging Activity

To perform the ABTS radical scavenging assay, the procedure from Atere et al. was followed with some modifications (Re et al., 1999; Atere et al., 2018). The ABTS stock solution was created by mixing 7.4 mM ABTS solution with 2.45 mM potassium persulphate in a 1:1 ratio and was left to sit in the dark at room temperature for 16 hours. The stock solution was diluted to achieve an absorbance of 0.70 ± 0.05 at 734 nm. Then, 2 ml of the ABTS working solution was combined with 50 µl of different extract concentrations or Trolox (used as the standard antioxidant) and kept in the dark for 30 minutes at room temperature. The absorbance was measured at 734 nm, and the experiment was conducted in triplicate for each concentration. The percent inhibition was computed, and the ABTS radical scavenging activity was stated as mg of Trolox equivalent per gram of extract (mg TE/g extract).

Total phenolic content

To determine the total phenolic content, the authors utilized the Folin-Ciocalteu method with slight modifications from a previous study by Derakhshan et al. (Parsaei et al., 2013; Derakhshan et al., 2018). The procedure involved mixing 500 µl of Folin-Ciocalteu's reagent (10% v/v) with 100 µl of the extract, followed by a 3-minute incubation at room temperature. Next, 400 µl of sodium carbonate (7.5% w/v) was added to the mixture and allowed to stand for 30 minutes at room temperature. The absorbance was then measured at 765 nm, with triplicate experiments conducted for each sample. The calibration curve was created using gallic acid, and the total phenolic content was expressed as mg of gallic acid equivalent per gram of extract (mg GAE/g extract).

Microorganisms

In this study, three microorganisms were employed: *Candida albicans*, *Clostridium butyricum*, and *Pseudomonas aeruginosa*. These microorganisms were provided by the Biodiversity Research Centre at the Thailand Institute of Scientific and Technological Research.

Antimicrobial activity

To evaluate the antimicrobial activities, the minimum inhibitory concentration (MIC) broth dilution method was employed against three different pathogenic microbial strains: *Candida albicans*, *Clostridium butyricum*, and *Pseudomonas aeruginosa*. A stock solution of *V. scabra* and *S. gratum* extracts was prepared at a concentration of 21.9 mg/ml and 25.9 mg/ml, respectively, in 10% dimethyl sulfoxide (DMSO). During the testing, both extracts were further diluted for 10 levels. To obtain inoculums, the microorganisms were cultured from the frozen stock 100 µl in a culture medium and incubated under optimum conditions (as shown in Table 1). The turbidity of the microorganisms was set based on the McFarland 0.5 scale, yielding a final concentration of 10^8 CFU/ml. Subsequently, 1 ml of the extract at different concentrations was combined with 1 ml of the culture medium and 100 µL of the working inoculum solution, and then the mixture was incubated under appropriate conditions for 24-48 hours. Following incubation, the growth of the pathogenic microorganisms was examined after 24 and 48 hours, and the outcome was reported as the MIC (Minimum Inhibitory Concentration) value.DMSO was used as a negative control.

Table1 Microorganisms used in this study

Microorganism	Culture Medium	Conditions
*Candida albicans*TISTR5554	Sabouraud Dextrose Broth (SDB)	30°C Aerobic condition 48 hr.
Clostridiumbutyricum TISTR1032	Reinforced Clostridial Medium (RCM)	37°C Anaerobic condition 48 hr.
Pseudomonas aeruginosa TISTR1995	Tryptic Soy Broth (TSB)	37°C Aerobic condition 24 hr.

Statistical Analysis

The data were collected in triplicate and recorded as mean±standard deviation. One-way analysis of variance (ANOVA) was used for statistical analysis, and a significant difference was considered when the *p*-value was less than 0.05.

Results and Discussion

Antioxidant activity

The evaluation of the antioxidant activity of plants and foods commonly utilizes radical scavenging methods (Köksal et al., 2017; Bursal et al., 2020). In this study, the antioxidant activity of *V. scabra* and *S. gratum* extracts was determined through the use of DPPH radical scavenging and ABTS radical scavenging assays, with the results summarized in Table 2. Figure 1 displays a comparison of the different antioxidant assays and the total phenolic content of *V. scabra* and *S. gratum* extracts.

Table 2 Antioxidant activity and total phenolic content of *V. scabra* and *S. gratum* extracts

Extract	DPPH IC_{50} (µg/ml)	ABTS (mg TE/g extract)	Total phenolics (mg GAE/g extract)
V. scabra	84.1613 (R^2 0.9990)	841.8750	95.2690
S. gratum	33.5549 (R^2 0.9860)	1,177.0282	152.2263
Trolox	10.0977 (R^2 0.9990)		

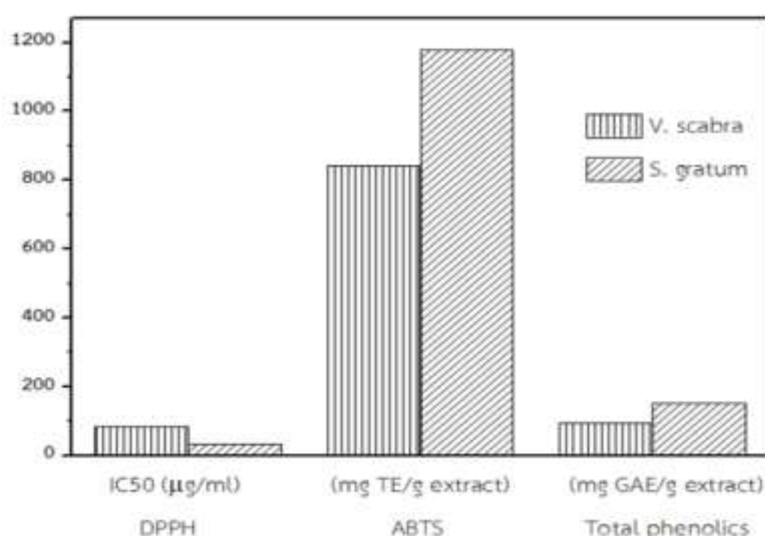

Figure 1 showed a comparison of different antioxidant assays and total phenolic content of *V. scabra* and *S. gratum* extracts

The DPPH assay is a widely used spectrophotometric method for determining radical scavenging activity. DPPH is a stable free radical that reacts with antioxidant substances, accepting an electron or hydrogen radical and becoming a stable molecule. A lower IC_{50} value indicates higher antioxidant activity. In this study, the results showed that *V. scabra* and *S. gratum* extracts displayed antioxidant activity. The DPPH radical scavenging capacity of *S. gratum* extract was higher than that of *V. scabra* extract. However, both extracts had lower antioxidant activity than the standard compound, Trolox.

The ABTS method functions similarly to the DPPH method in assessing antioxidant activity. However, the ABTS method is deemed more dependable than the DPPH method because the ABTS reagent can dissolve in both aqueous and organic solvents, and it rapidly reacts with lipophilic and hydrophilic antioxidant species (Teow et al., 2007; Iqbal et al., 2013; Atere et al., 2018).The antioxidant activity of *V. scabra* and *S. gratum* extracts was measured using the ABTS radical scavenging assay, and the results were expressed in mg GAE/g extract, with higher values indicating stronger antioxidant activity. The findings indicated that the antioxidant activity of both extracts was similar to that observed in the DPPH assay, with the *S. gratum* extract exhibiting higher ABTS radical scavenging capacity compared to the *V. scabra* extract.

Correlation between total phenolic content and antioxidant activity

Phenolic compounds derived from plants are recognized for their antioxidant characteristics and are known to be capable of scavenging free radicals, inhibiting lipid peroxidation, and chelating metal ions (Shahidi et al., 1997; Tachakittirungrod et al., 2007).Table 2 shows the total phenolic content of the extracts., with *S. gratum* extract exhibiting a higher phenolic content than *V. scabra* extract. The correlation between total phenolic content and antioxidant activity was shown in Figures 2 and 3, revealing a strong positive correlation between the two factors in both extracts. The results indicate that the high amount of phenolic compounds present in the extracts may play a crucial role in their antioxidant activity. Similar results have been reported by Oktay et al (2003), Yan and Asmah (2010), and Basma et al (2011), who also observed a significant association between phenolic content and antioxidant capacity. The hydroxyl groups in phenolic compounds play a crucial role in scavenging free radicals, stabilizing lipid peroxidation, and exhibiting direct antioxidative activity (Yen and Wu, 1993; Basma et al, 2011; Ochuko et al, 2011; Paul et al, 2011; Patel et al, 2011; Ravikumar and Gnanadesigan, 2011; Thirumalai et al, 2011).

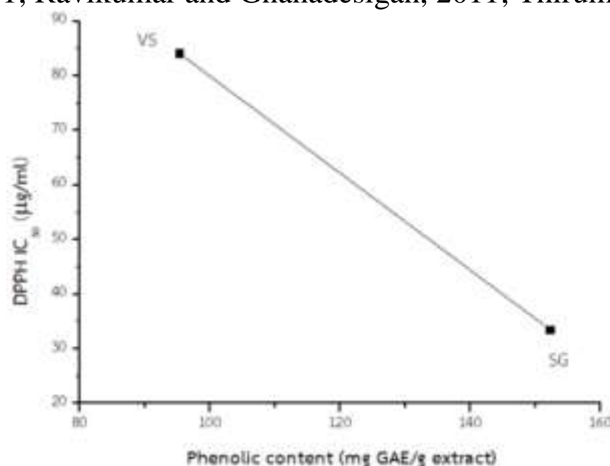

Figure 2 Correlation of phenolic contents and DPPH IC_{50} values of extracts from *V. scabra* (VS) and *S. gratum* (SG)

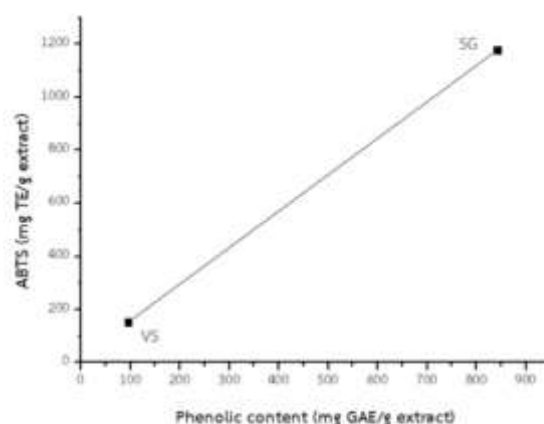

Figure 3 Correlation of phenolic contents and ABTS values of extracts from *V. scabra* (VS) and *S. gratum* (SG)

Antimicrobial activity

Table 3 and Figure 4 show the results of the antimicrobial activity of *V. scabra* and *S. gratum* extracts. The extract of *V. scabra* exhibited antimicrobial activity against only *Clostridium butyricum* with a MIC value of 1.37 mg/ml, while the *S. gratum* extract did not show inhibition against any of the three microorganisms tested. It is noteworthy that *Clostridium butyricum*, which produces type E botulinum toxin (BoNT/E), was first isolated in Italy in 1984 (Aureli et al, 1986; McCroskey et al, 1986; Wang et al, 2000), and can cause type E food-borne botulism (Wang et al, 2000). Our study suggests that *V. scabra* may contain an antimicrobial agent that exhibits activity against *C. butyricum*, which is the pathogen responsible for botulism.

Table 3 Antimicrobial activity of *V. scabra* and *S. gratum* extracts

Extract	***Candida albicans***	***Clostridium butyricum***	***Pseudomonas aeruginosa***
V. scabra	–	+ (MIC 1.37 mg/ml)	–
S. gratum	–	–	–

– was used as ineffective
+ was used as effective

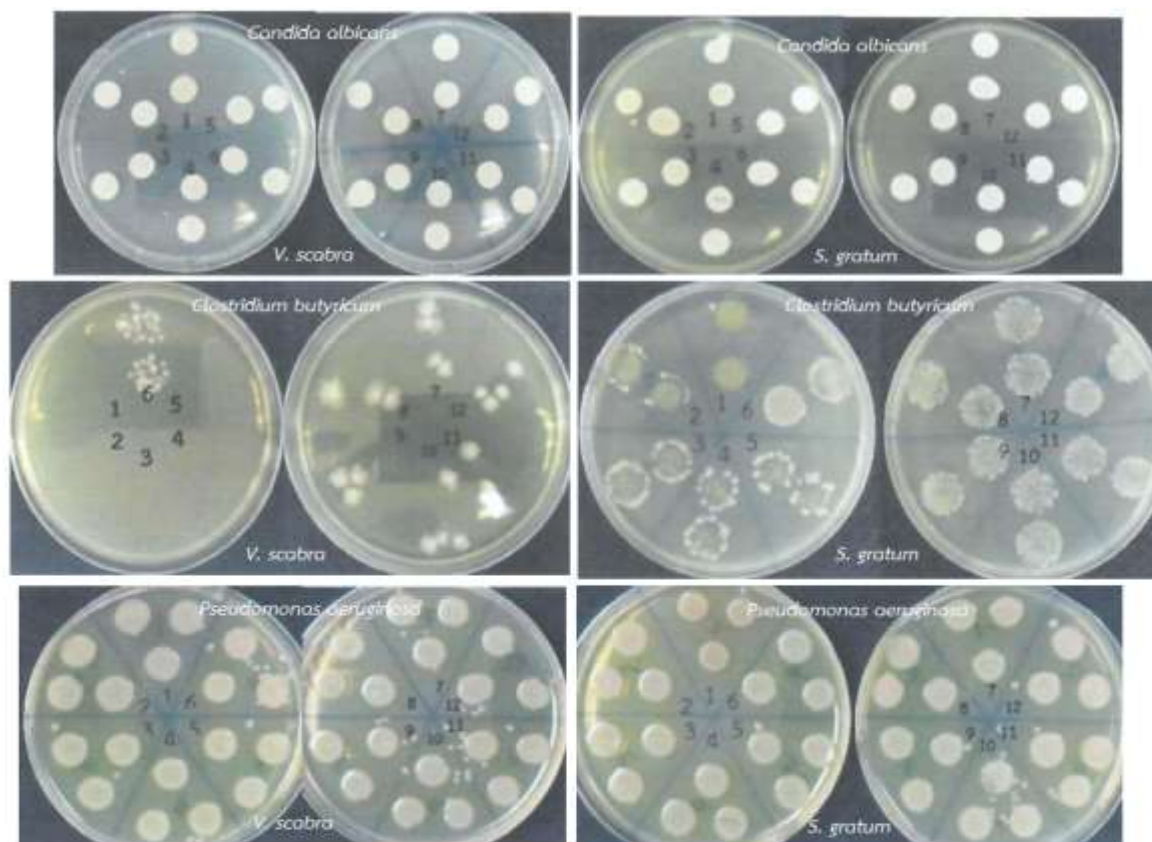

Figure 4 Growth inhibition of microbial strains caused by extract

Conclusion

This study provided a comprehensive analysis of the antioxidant and antimicrobial properties of *V. scabra* and *S. gratum*, including the total phenolic content of both plants. The results showed that both plants exhibited antioxidant activity as measured by the DPPH and ABTS radical scavenging assays, which could be attributed to the presence of phenolic compounds. In addition, *V. scabra* showed antimicrobial activity against *Clostridium butyricum*, a pathogen that can cause botulism. To our knowledge, this is the first report on the antioxidant and antimicrobial activities of *V. scabra*. The findings of this study have important implications for the potential use of these plants as a source of antioxidant agents and for further research on their health benefits.

Acknowledgment

The authors would like to acknowledge the financial support provided by Buriram Rajabhat University's Plant Genetic Conservation Project, which is under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn (RSPG).

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