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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *Antheroporum harmandii* Gagnep. COLLECTED FROM QUANG TRI PROVINCE

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ABSTRACT

Antheroporum harmandii Gagnep. is a native plant species of Vietnam, belonging to a small genus of the Fabaceae family. In this study, the bioactivities of different extracts from twigs and seeds of *A. harmandii* plants collected from Dakrong, Quang Tri province were evaluated. Antimicrobial and antioxidant activities of plant extracts had been done using an agar well diffusion method and DPPH assay. The results showed that methanol, dichloromethane and ethyl acetate extracts from twigs and methanol extract from seeds exerted activities to significantly inhibit two out of nine tested microorganisms. Particularly, the dichloromethane extract from the twigs sample exhibited the highest activity to *Micrococcus luteus* and *Staphylococcus aureus* strains with inhibition zones of 4.7 ± 0.3 mm and 2.3 ± 0.2 mm, respectively, at concentrations of 50 mg/mL. The lowest MIC values (500 µg/mL) and 50% inhibition concentration values (IC_{50}) against *M. luteus* and *S. aureus* were recorded for the methanol and dichloromethane extracts of twigs. Among tested extracts, the ethyl acetate extract of twigs was the strongest antioxidant, showing radical scavenging potential with IC_{50} of 117.24 µg/mL. Therefore, *A. harmandii* species can be suggested as a new source for the isolation of valuable natural compounds.

Keywords: *Antheroporum harmandii*, anti-microbial activity, anti-bacterial activity, natural compounds.

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INTRODUCTION

Plants play an important role in sustaining life on Earth and provide human with food, fiber, shelter, medicine, and fuel. Because of rich in bioactive compounds, plants have physiological effects such as anticancer, antioxidant, anti-inflammatory, and antimicrobial activities. Nowadays, 25% of modern medicine come from trees, shrubs or herbs, which are either directly obtained from the plant extracts or synthesized to mimic compounds derived from plants (Prasad & Tyagi, 2015).

Antheroporum is a genus of the Fabaceae family, which is ranked the third largest family of blossoming plants with approximately 650 genera and 19,000 different species, and a big source of bioactive compounds (Tungmunnithum et al., 2021). In Vietnam, the genus distributes across the country and predominantly in the Central region. The genus only contains 6 species (*Antheroporum banaense* L. K. Phan & J. E. Vidal; *Antheroporum glaucum* Z. Wei; *Antheroporum harmandii* Gagnep., *Antheroporum pierrei* Gagnep., *Antheroporum vidalii* L. K. Phan and *Antheroporum puudjaae* Mattapha & Tetsana), which are native to China, Thailand, and Vietnam. The genus was established by Gagnepain (1915) on the basis of a combination of the following characters: evergreen trees with stipules and stipels absence, imparipinnate leaves, basally thickened petiole and petiolules, sub-opposite leaflet blades, inflorescences with racemes in group of 2-5 in axils of leaves at tips of branches, small flowers with cup-shaped calyx and markedly long clawed petals, monadelphous stamens, 1 to 6-ovuled ovary with trichomes, dehiscent fruits dilated and not winged (Mattapha & Tetsana, 2021; Gagnepain, 1915). The specialized characteristics of *A. harmandii* include branchlets, leaves, and inflorescences with yellowish trichomes; petiolules with grayish trichomes; leaflet blades oblong, abaxially densely- appressed sericeous, base rounded, asymmetric (Gagnepain, 1915).

Until now, there are only a few biological and/or chemical investigations had been done for this genus. There were reports of the non-protein amino acids arginine and homoarginine in seeds of *A. pierrei* (Evans et al., 1985). In the selective extract of the leaves and twigs of *A. pierrei*, pyranoi soflavones including rotenone, 12a-hydroxyrotenone, and tephrosin have been isolated and exhibited strong biological activity to solid tumor cell lines (Gao et al., 2011). For *A. harmandii* plants, their seeds were used as a poison for fish and insect by local people. In practice, the seeds were ground and mixed with ash to catch fish or mixed with water and sprayed to kill lice.

In order to explore the importance of the *Antheroporum* genus in pharmaceutical applications, two bioactivities of *A. harmandii* plant extracts have been evaluated in this study. Antimicrobial and antioxidant activities of *A. harmandii* extracts will provide evidences for further chemical composition studies to detect new bioactive compounds from this species.

MATERIALS AND METHODS

Plant materials

The plant samples (twigs, fruits and seeds) of *A. harmandii* were collected at Dakrong, Quang Tri province in April 2021 and taxonomically identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST. The voucher specimen (TNSV-QT-13) was deposited at the Institute of Marine Biochemistry, VAST. Twigs chopped into small pieces and seeds separated from fruits were dried in an oven (EYELA WFO-451SD) at 50 ^{o}C before grounding into powder (Fig. 1).

Extraction and fractionation

The sequential extraction of samples was conducted with different solvents. Firstly, a dried and finely powdered twigs sample (1.0 kg) was extracted twice with 4 L methanol in a sonicator (Emasonic Easy 180H, Germany) for 1 hour at 40–50 ^{o}C. The extractions were filtered through filter paper, pooled, and

evaporated in a vacuo to obtain crude methanol extract. A total of 65.28 g of methanol extract was obtained from 1 kg of dried twigs. Then, the crude methanol extract (50 g) was fractioned. About 5.3 g of hexane, 4.2 g of dichloromethane, 1.2 g of ethyl acetate and 2.5 g of water extracts were generated. From 85 g of dried seeds, about 2.6 g of methanol extract was obtained. All extracts were stored at 4 ^{o}C.

Figure 1. *Antheroporum harmandii* samples collected from Dakrong, Quang Tri province. a: twig sample; b: fruit sample; c: seed sample

Microbial strains

The antimicrobial activity of plant extracts was tested against five standard Gram-negative bacteria strains (*Escherichia coli* ATCC® 25922™, *Salmonella typhi* ATCC®19430™, *Proteus mirabilis* ATCC®29245™, *Proteus vulgaris* ATCC® 33420™, *Pseudomonas aeruginosa* ATCC® 9027™), three Gram-positive (*Staphylococcus aureus* ATCC® 25923™, *Bacillus subtilis* ATCC® 6633™, *Micrococcus luteus* ATCC 13377) and one fungal strain (*Candida albicans* ATCC®24433™), which were provided by Microbiologics (USA). The Gram-positive and Gram-negative bacteria were pre-cultured in 5 mL of Luria Bertani Broth (LB) in a rotary shaker at 37 ^{o}C overnight. Afterwards, each strain was recovered for further 2–4 hours until reaching a concentration of about 4–5 × 10^8 cells/mL (OD_{600} ≈ 0.5–0.6). The fungal inoculum was cultured in Hansen's Medium, 30 ^{o}C until reaching a concentration of about 10^6 spores/mL.

Agar well diffusion method

The agar well diffusion method according to Hadacek & Greger (2000) with minor modifications was applied to evaluate the antibacterial and antifungal activities of extracts. One hundred µL of each microbial strain culture was spread on a Petri dish surface containing a suitable agar medium. Upon solidification, 9 mm in diameter wells were made in agar plates containing inoculums. Then, 50 µL of tested concentrations of each extract (dissolved in DMSO) was added to respective wells. The plates were incubated at 37 ^{o}C, 16 hours for bacteria or 30 ^{o}C, 48 hours for fungus. DMSO was employed as a negative control and gentamicin 200–400 µg/mL (for bacteria) or nystatin 100 µg/mL (for fungus) were the positive controls. Antimicrobial activity was detected by measuring the inhibition zones and calculated by the formula: D = D1 – D2**.** In which, D exhibits the microbial resistant ability (mm), D1 is the diameter (mm) of the inhibition zone around a certain well and D2 is the agar well diameter (mm).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MIC) of extracts were determined by the broth dilution method (Wiegand et al., 2008). Different concentrations of extracts were

prepared by serial dilutions in LB or Hansen's medium. Firstly, 200 µL of each dilution extract was added to the 96-well culture plate, separately. Following, 5 µL of microorganism culture (4–5 × 10^4–10^5 cells/mL) were added to each well. The wells containing only extracts and without microbial culture solution were used as the negative controls. The wells containing antibiotic (gentamicin) instead of diluted extracts were the positive controls. The plates were incubated at 37 ^oC for 16 hours (bacteria) or 30 ^oC for 48 hours (fungus). The microbial concentration was measured at 600 nm (OD) using a microplate spectrophotometer (Epoch). The MIC was considered the lowest concentration of the tested sample, which shows the growth inhibition of the respective microorganism. Inhibition (%) was calculated by the following formula: [(OD blank- ODsample)/OD blank]*100. Inhibition activity was also represented by the IC_{50} value, which is the concentration of the sample inhibiting 50% of the microorganism growth. This IC_{50} value was calculated by IC_{50} Calculator (https://www.aatbio.com/tools/ic50-calculator). The experiment was done in triplicate and data were analyzed using Microsoft Excel 2010 software. The data were analyzed using one-way ANOVA followed by Tukey Test. Differences between the means were considered significant at $p < 0.05$.

DPPH assay method

The antioxidant activity of extracts was measured by 1,1-diphenyl-2-picryl- hydrazyl (α,α-diphenyl-β-picrylhydrazyl) (DPPH) method as described by Mensor et al. (2001).

The tested extracts were diluted by DMSO to the final concentrations from 100, 50, 25, 12.5, 6.25 to 3.125 µg/mL. Ascorbic acid was used as the positive control and diluted in DMSO to the concentrations at 100, 50, 25, 10, 2 and 1.25 µg/mL. DPPH at a concentration of 100 µM was prepared in methanol. Each diluted extract (100 µL) was transferred to the 96-well plate in triplicate. After that, 100 µL of 100 µM DPPH was added to each well. The plates were incubated at 37 °C for 30 min on a shaker. The absorbance of samples at λ = 517 nm was measured in a microplate spectrophotometer (Epoch). DPPH radical scavenging activity was calculated by the following equation: % DPPH radical scavenging activity = {(A0−A1)/A0}×100, in which, A0 is the absorbance of the control, and A1 is the absorbance of the extract.

The IC_{50} value, which is the concentration of the sample at which 50% of DPPH free radicals were reduced under specified conditions, was calculated by IC_{50} Calculator (https://www.aatbio.com/tools/ic50-calculator).

RESULTS AND DISCUSSION

Antimicrobial activities of Antheroporum harmandii extracts

Based on the agar well diffusion method, the antimicrobial activity of methanol extracts at three concentrations: 50, 100 and 200 mg/mL was tested against eight bacterial species and one fungus which are common pathogens of human. Due to their low amounts, hexane, dichrolomethane, ethyl acetate and water extracts were only tested at two concentrations: 20 and 50 mg/mL.

Among the tested strains, only *M. luteus* and *S. aureus* strains showed sensitive effects with methanol, dichloromethane, and ethyl acetate extracts of *A. harmandii* (Fig. 2). The growth of the remaining strains did not influence by any tested extracts (data not shown). For *M. luteus*, both methanol extracts from twigs and seeds at 200 mg/mL showed the maximum zone of inhibition, with D values of 6.8 ± 0.3 mm and 5.0 ± 0.5 mm, respectively (Table 1). These values are comparable to the antibiotic control at 0.4 mg/mL (6.3 ± 0.6 mm). The other fraction extracts exhibited significant inhibitory activity at 20 and 50 mg/mL concentrations. Indeed, dichloromethane and ethyl acetate extracts showed a zone of bacterial clearance of 2.7 mm and 2 mm at a low concentration of 20 mg/mL. In the case of *S. aureus* strain, the methanol extract from seeds at all tested concentrations showed moderate antibacterial activity with the D value ranging from 0.5 ±

0.1 mm to 4.7 ± 0.3 mm. Notably, methanol extract from twigs showed better inhibition activity with *S. aureus* (D value from 1.3 ± 0.3 to 6.2 ± 0.3 mm) than the methanol extract from seeds. The dichloromethane and ethyl acetate extracts at the concentration of 50 mg/mL showed better antibacterial activity against the *M. luteus* strain than *S. aureus*. These fractions are expected to have a better effect at higher concentrations.

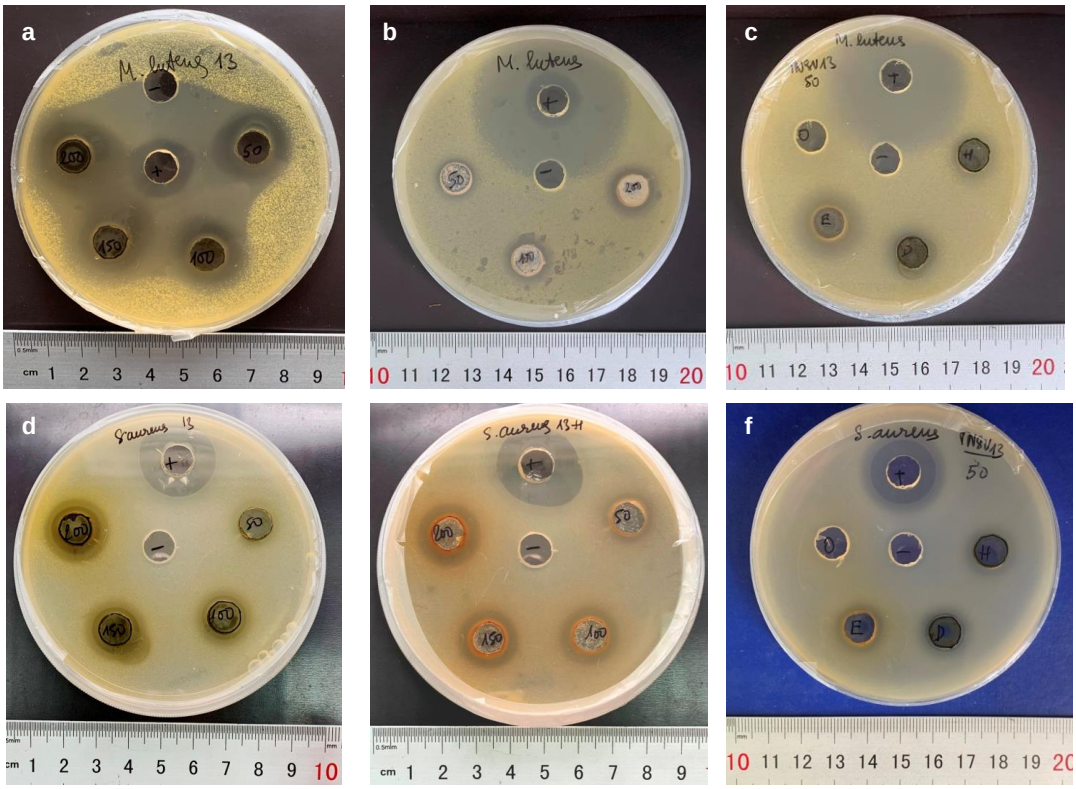

Figure 2. The inhibition zone of different extracts against *Micrococcus luteus* or *Staphylococcus aureus*. Methanol extract of twigs (a) or seeds (b) against *Micrococcus luteus.* Methanol extract of twigs (d) or seeds (e) against *Staphylococcus aureus*. Hexane (H), dichrolomethane (D), ethyl acetate (E) or water (O) extracts against *Micrococcus luteus* (c) or *Staphylococcus aureus* (f). The signs (50), (100), (150), (200) on the plate represent the extract concentrations in mg/mL; (+): positive control using gentamicin (0.4 or 0.2 mg/mL for *Micrococcus luteus* or *Staphylococcus aureus*, respectively); (-): negative control with DMSO

MIC evaluation

The microbial sensitivity to crude methanol extracts and different fractions was represented by MIC values ranging from 0.5 mg/mL to 4 mg/mL. The result showed that each extract presented different inhibitory concentrations for a given bacterial strain.

The growth of *M. luteus* and *S. aureus* strains were then observed in the culture media supplemented with plant extracts. The results revealed that *M. luteus* was totally inhibited by the methanol extract of seeds or the methanol, dichloromethane extracts of twigs at concentrations of 0.5 mg/mL to 4 mg/mL (Table 2). The ethyl acetate extract at 4 mg/mL exhibited the growth inhibition of *M. Luteus.*

For the *S. aureus* strain, the methanol and dichloromethane extracts of twigs showed the best antibacterial activity with a MIC value at 0.5 mg/mL (Table 3). The methanol

extract of seeds showed lower antibacterial activity (MIC value at 1 mg/mL). Ethyl acetate extract exhibited the lowest antibacterial activity.

Table 1. Antibacterial activity of *Antheroporum harmandii* extracts with agar well diffusion method

Samples	Concentration (mg/mL) of the extract							
	20	50	100	200	20	50	100	200
	Micrococcus luteus				*Staphylococcus aureus*			
Methanol extract (from seeds)	-	2.0 ± 0.5	3.5	5.0 ± 0.5	-	0.5 ± 0.1	2.0	4.7 ± 0.3
Methanol extract (from twigs)	-	1.0 ± 0.2	3.3 ± 0.3	6.8 ± 0.3	-	1.3 ± 0.3	3.2± 0.3	6.2 ± 0.3
Dichloromethane fraction	2.7 ± 0.5	4.7 ± 0.3	-	-	0.5	2.3 ± 0.2	-	-
Ethyl acetate fraction	2.0	3.0 ± 0.5	-	-	0	0.7 ± 0.3	-	-
Gentamicin	6.3 ± 0.6				11.5 ± 0.5			
DMSO	0				0			

Notes: Data are average values of D (mm) from three independent experiments. Gentamicin (0.2 mg/mL for *Staphylococcus aureus* and 0.4 mg/mL for *Micrococcus luteus*): positive control; DMSO: negative control; (-): not applicable.

Table 2. Inhibition and minimum inhibitory concentrations (MIC) of *Antheroporum harmandii* extracts against *Micrococcus luteus*

Concentration (mg/mL)	Inhibition (%)				
	Gentamicin	Methanol extract (twigs)	Methanol extract (seeds)	Ethyl acetate fraction	Dichloromethane fraction
$0.5*10^{-3}$	19.10 ± 0.90	-	-	-	-
$1*10^{-3}$	21.41 ± 0.43	-	-	-	-
$2*10^{-3}$	27.35 ± 1.09	-	-	-	-
$4*10^{-3}$	46.30 ± 1.75	-	-	-	-
$8*10^{-3}$	89.43 ± 0.66	-	-	-	-
$16*10^{-3}$	100.53 ± 0.43	-	-	-	-
0.125	-	62.08 ± 2.22	51.08 ± 0.25	16.93 ± 1.75	73.08 ± 4.89
0.25	-	91.17 ± 4.80	88.85 ± 2.22	44.86 ± 1.15	90.88 ± 2.85
0.5	-	111.72 ± 3.78	106.51 ± 3.71	44.13 ± 3.69	100.57 ± 1.00
1	-	-	-	60.63 ± 3.31	-
2	-	-	-	96.38 ± 2.47	-
4	-	-	-	105.20 ± 1.50	-
MIC (mg/mL)	$16*10^{-3}$	0.5	0.5	4	0.5

Notes: Data are the means from three independent experiments. Gentamicin was used as a positive control; (-): not applicable.

Table 3. Inhibition and Minimum inhibitory concentrations (MIC) of *Antheroporum harmandii* extracts against *Staphylococcus aureus*

Concentration (mg/mL)	Inhibition (%)				
	Gentamicin	Methanol extract (twigs)	Methanol extract (seeds)	Ethyl acetate fraction	Dichloromethane fraction
$0.0625*10^{-3}$	2.49 ± 1.53	-	-	-	-
$0.125*10^{-3}$	6.79 ± 2.28	-	-	-	-
$0.25*10^{-3}$	14.32 ± 2.13	-	-	-	-
$0.5*10^{-3}$	16.58 ± 1.13	-	-	-	-
$0.6*10^{-3}$	18.45 ± 0.68	-	-	-	-
$0.7*10^{-3}$	50.31 ± 1.54	-	-	-	-
$0.8*10^{-3}$	54.10 ± 0.70	-	-	-	-
$0.9*10^{-3}$	100.28 ± 0.87	-	-	-	-
0.125	-	-		14.77 ± 0.45	29.42 ± 1.44
0.25	-	45.84 ± 2.50	41.54 ± 1.57	23.88 ± 1.09	92.69 ± 0.34
0.3	-	59.82 ± 1.29	-	-	-
0.4	-	71.25 ± 1.02	-	-	-
0.5	-	100.11 ± 0.80	73.34 ± 1.28	33.10 ± 0.34	105.66 ± 3.90
1	-	-	113.81 ± 2.72	38.65 ± 2.63	-
2	-	-	-	46.63 ± 2.34	-
4	-	-	-	90.83 ± 3.85	-
MIC (mg/mL)	$0.9*10^{-3}$	0.5	1	> 4	0.5

Notes: Data are the means from three independent experiments. Gentamicin was used as a positive control; (-): not applicable.

Among tested extracts, the dichloromethane extract showed the best IC_{50} value against *M. luteus* (58.74 ± 12.61 µg/mL), followed by the methanol extract of twigs (94.75 ± 5.59 µg/mL) (Table 2). In the case of *S. aureus*, the lowest IC_{50} value also belongs to the dichloromethane extract (153.21 ± 2.86 µg/mL) (Table 3).

The investigation of the bioactivity of six extracts of the *A. harmandii* plant against tested microorganisms indicates that the four extracts produced significant activity against two Gram-positive bacteria strains, *S. aureus* and *M. luteus* (Table 2, Table 3 and Fig. 3). In this study, the dichloromethane extract from twigs showed the strongest antibacterial activity against *S. aureus* and *M. luteus*. The methanol extracts had moderate inhibition activity and the ethyl acetate extract showed very weak activity. To date, this is the first study to examine the antimicrobial activity of *A. harmandii* species.

The microbial growth inhibition of the *A. harmandii* extracts may be related to the presence of secondary metabolism compounds such as prenylated isoflavones, chalcone, and pterocarpan, which once were reported from *A. pierrei* (Gao et al., 2011) and other species of the closer genus *Mellitia (*Marco et al., 2017), These compounds are known to carry the prenyl groups having a function to prevent the adhesion of bacterial cells to the outer membrane. As a result, our findings provide evidence for further analyzing the phytochemicals of *A. harmandii* for practical uses.

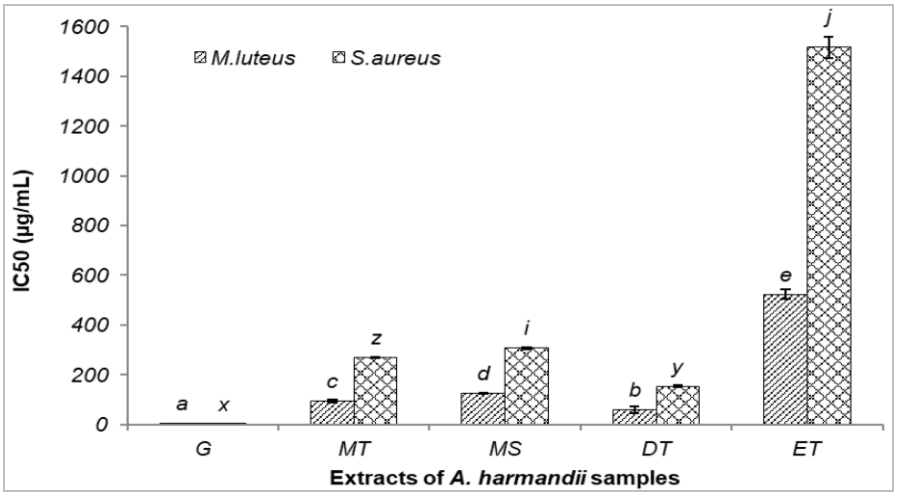

Figure 3. The concentration of extracts inhibiting 50% of microorganism growth. Data are means of three replicate samples and bars indicate standard errors. Means with different letters are significant different (Tukey'HSD, $p \leq 0.05$). G: Gentamicin; MT: Methanol extract (twigs); MS: Methanol extract (seeds); DT: Dichloromethane fraction; ET: Ethyl acetate fraction

***In vitro* antioxidant capacity of *Antheroporum harmandii* extracts**

Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical, hydroperoxy radical, lipid peroxide, nitric oxide and peroxynitrite, are produced during aerobic cellular metabolism. Antioxidants can directly react with active free radicals to produce inactive ones, which in turn can prevent the chain reaction initiated by free radicals (Stańczyk et al., 2005). Therefore, discovering natural compounds with antioxidant activity is attractive for practical uses. The DPPH method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis (Kedare et al., 2011). In this study, the antioxidant activity of *A. harmandii* extracts was performed.

The extracts of *A. harmandii* generally showed weak antioxidant activities. Methanol extracts from twigs and seeds, hexane, dichloromethane and water fractions from twigs did not show antioxidant activity. Only ethyl acetate extract from twigs exhibited moderate activity with an IC_{50} value of 117.24 ± 10.01 µg/mL.

This is the first study to evaluate the antioxidant activity of *A. harmandii* extracts. Havyarimana et al. (2012) determined the antioxidant activity of hexane and ethyl acetate extract from *Millettia barteri*. The result revealed that both extracts showed relatively strong antioxidant activity with IC_{50} values of 62.74 and 77.23 µg/mL, respectively. In our study, the hexane, dichloromethane fraction did not show antioxidant activity, only the ethyl acetate fraction of twigs showed moderate activity with IC_{50} values of 117.24 ± 10.01 µg/mL.

CONCLUSION

In this study, crude methanol, hexane, dichloromethane, ethyl acetate and water extracts were obtained from twigs and seeds of *A. harmandii* plant samples collected from Dakrong district, Quang Tri province. The methanol, dichloromethane and ethyl acetate extracts from twigs and methanol extract from seeds possess strong antimicrobial activity against two tested bacteria (*M. luteus* and *S. aureus*). The ethyl acetate fraction showed moderate antioxidant activity. These results provide promising information for the potential use of this plant species in finding new bioactive compounds.

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REFERENCES

Evans S. V., Fellows L. E., Bell E. A., 1985. Distribution and systematic significance of basic non-protein amino acids and amines in the Tephrosieae. *Biochemical Systematics and Ecology*, 13: 271–302.

Gagnepain F., 1915. Antheroporum, gen. nov. *Notulae Systematicae* (Paris) 3: 180–182.

Gao S., Xu Y. M., Valeriote F. A., Gunatilaka A. A., 2011. Pierreiones A-D, solid tumor selective pyranoisoflavones and other cytotoxic constituents from *Antheroporum pierrei*. *Journal of Natural Products*, 74 (4): 852–856. https://doi.org/10.1021/np100763p

Hadacek F., Greger H., 2000. Testing of antifungal natural products: methodologies, comparability of results and assay choice. *Phytochemical Analysis*, 11: 137–147. https://doi.org/10.1002/(SICI)1099-1565(200005/06)11:3<137:AID-PCA514>3.0.CO;2-I

Havyarimana L., Ndendoung S. T., de Dieu Tamokou J., de Théodore Atchadé A., & Mbafor Tanyi J., 2012. Chemical constituents of *Millettia barteri* and their antimicrobial and antioxidant activities *Pharmaceutical Biology*, 50(2): 141–146. doi: 10.3109/13880209.2011.579618

Kedare S. B., Singh R. P., 2011. Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, 48: 412–22. https://doi.org/10.1007/s13197-011-0251-1

Marco M., Deyou, T. G., Holleran A., Duffy S., Heydenreich M., Firtzpatrick P. A., Landberg G., Koch A., Derese S., Pelletier J., Avery V. M., Erdélyi M., Yenesew, A., 2017. Pterocarpans and isoflavones from the root bark of *Millettia micans* and of *Millettia dura*. *Phytochemistry Letters*, 21: 216–220. https://doi.org/10.1016/j.phytol.2017.07.012

Mattapha S. & Tetsana N., 2021. *Antheroporum puudjaae* (Millettieae: Fabaceae), a new species from Thailand. *Thai Forest Bulletin Botany*, 49(1): 130–134. https://doi.org/10.20531/tfb.2021.49.1.16

Mensor L. L., Menezes F. S., Leitão G. G., Reis A. S., dos Santos T. C., Coube C. S., Leitão S. G., 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*, 15(2): 127–130. https://doi.org/10.1002/ptr.687

Prasad S. & Tyagi A. K., 2015. Traditional medicine: The goldmine for modern drugs. *Advanced Techniques in Biology & Medicine*: 03. https://doi.org/10.4172/2379-1764.1000e108

Stańczyk M., Gromadzińska J., Wasowicz, W., 2005. Roles of reactive oxygen species and selected antioxidants in regulation of cellular metabolism. *International Journal of Occupational and Environmental Health*, 18: 15–26.

Tungmunnithum D., Drouet S., Lorenzo J. M., Hano C., 2021. Characterization of bioactive phenolics and antioxidant capacity of edible bean extracts of 50 Fabaceae populations grown in Thailand. *Foods*, 10 (12): 3118.

Wiegand I., Hilpert K., Hancock R. E. W., 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*, 3(2): 163–175. https://doi:10.1038/nprot.2007.521