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# FRACTIONATION OF PHENOLIC COMPOUNDS FROM *Sonneratia apetala* PNEUMATOPHORES AND THEIR BIOACTIVITIES

**Nguyen Sao Mai, Dao Van Tan***

Hanoi National University of Education, Hanoi

***ABSTRACT***: *Sonneratia apetala* pneumatophore samples were collected from Xuan Thuy National Park, and then 80% methanol extract was fractionated through Sephadex LH-20 column chromatography. After elution with 95% ethanol and 50% acetone, two phenolic fractions were obtained from the crude extract of of *S. apetala* pneumatophores. The acetone fraction consisted of tannins having high antioxidant activity, 1.14 times higher than that of ascorbic acid. This fraction also had higher activity of α-amylase inhibition ($IC_{50}$ 0.83 mg/mL) and *R. sativus* root growth inhibition ($IC_{50}$ 6.4 mg/mL) than did the ethanol fraction. In contrast, ethanol fraction consisted of low-molecular phenolic compounds having inhibitory effects on *Samonella tiphimurium* growth. Such antibacterial activity was not observed in the acetone extract.

*Keywords*: *Sonneratia apetala*, tannin, low-molecular phenolic, antioxidant, α-amylase inhibition, root growth inhibition, anti-biotic activity

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**Corresponding author:* daotanvn@yahoo.com

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## INTRODUCTION

Recently mangrove plants are considered as a rich source of natural bioactive compounds. Mangrove is attractive primarily because it can survive and grow in extremely stressful environments, such as severe salinity, extreme tides, strong winds, high temperatures and muddy anaerobic soils. In addition to unique morphological features as the survival strategy, mangrove plants acquired various other strategies including stimulation of the synthesis of secondary metabolites protective against destructive environmental elements (Crozier et al, 2006). Polyphenols, as plant secondary metabolites, have multiple biological effects such as anti-aging, anti-inflammatory, antioxidant, antiproliferative activity, especially the property of inhibiting α-amylase, an important enzyme in transformation process from carbohydrates into glucose (Lin et al, 2016). Tannins, which are distinguished from other phenolic compounds by their capability to bind to and precipitate protein, have been used in the tanning industry for many years (Crozier et al, 2006).

*Sonneratia apetala* Buch. Ham was first imported from Myanmar in August 2003 and grew in Xuan Thuy National Park, Nam Dinh Province, Vietnam (Tinh, 2006). This species belongs to the family Sonneratiaceae, which is reported as a rich source of tannins (Das et al, 2016). Several studies revealed that *S. apetala* is a potential plant to exploit bioactive compounds. For example, Hossein et al (2013) reported that *S. apetala* fruit extracts have antioxidant, antidiabetic and antibacterial activities. Jaimini et al. (2011) determined the antibacterial potential of *S. apetala* leave extracts. Also, Patra et al. (2015) focused on both *S. apetala* leaf and bark extracts and they concluded that the extracts have potent antibacterial, antioxidant, antidiabetic and anticancer properties, which can be further explored for their pharmaceutical applications. After fractionation of *S. apetala* bark extract using Sep-pak plus cartridges, various bioactivities in the fractions were reported (Tan & Thuy, 2014). In the present study, towards searching a new medical resource, we focused on bioactivity separation and characterization of phenolic

compounds isolated *S. apetala* pneumatophore using Sephadex LH-20 column chromatography.

## MATERIALS AND METHODS

Pneumatophores of *Sonneratia apetala* Buch. Ham were collected in the garden of the Mangrove Ecosystem Research Station, Giao Lac commune, Giao Thuy District, Nam Dinh Province, Vietnam. The samples were washed with tap water, separated into stellar and cortical parts, dried at room temperate, and ground into powder.

*Raphanus sativus* L. seeds; *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 13709), *Samonella typhimurium*, and *Pseudomonas aeruginosa w*ere produced or maintained in the Institute of Natural Product Chemistry and the Department of Microbiology and Biotechnology, Faculty of Biology, Hanoi National University of Education. Sephadex LH-20, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, gallic acid, and agar were purchased from Sigma-Aldrich (USA). Folin-Ciocalteu and Iodine were purchased from MERCK (Germany). Other chemicals were purchased from HiMedia (India).

*Extraction*

Samples were extracted and separated as description by Karamac (2007). Two grams (2 g) of stellar powder was soaked in 50 mL of 80% methanol. After 72 hr, the suspension was filtered through Whatman No.1 filter paper to obtain the first filtrate. The residues on the filter were soaked again in 25 mL of 80% methanol for 3 hr, and filtered to obtain the second filtrate. Two filtrates were combined and evaporated to produce a solid crude extract, which was then dissolved in 5 ml of 95% methanol to run on Sephadex LH-20 column chromatography (15 mm diameter 500 mm in height). As the first eluent, 250 mL of 95% ethanol was run through the column to obtain low-molecular phenolics, and then 250 mL of 50% acetone was applied to elute tannins. The flow rate was 4 mL/min. Eluates were pooled every 50 mL into fraction I-V for both ethanol and acetone solvents. Both ethanol and acetone fractions were separately pooled and evaporated using a rotary evaporator.

*Determination of total phenolic content*

Total phenolics were measured using the spectrophotometric analysis with Folin-Ciocalteu (FC) reagent (Kim et al., 2003). The extracts were diluted with corresponding solvents into different concentrations. Briefly, 10 µL of FC was added to 100 µL of the extracts, and 100 µL of 7% $Na_2CO_3$ was added after 5 min. The mixture was allowed to stand for 90 min in a dark condition and room temperature. The standard curve was established using gallic acid as the standard phenolic compound. The absorbance of the mixture was measured at 750 nm using UV-VIS spectrophotometer (Biotech, USA). Total phenolic content was expressed as mg gallic acid equivalent (GAE)/mL sample.

*Determination of antioxidant activity*

Antioxidant activity was evaluated using DPPH assay (Kim et al., 2003). The absorbance was measured at 517 nm using a spectrophotometer (Biotech, USA).

*Determination of α - amylase-inhibition activity*

Alpha-amylase from Gastrozym probiotic (HDPHARMA, Vietnam) was used in this experiment. α-amylase-inhibition activity was evaluated using soluble starch as a substrate according to the method described by Mui (2001) with a slight modification (Tan & Thuy, 2014). Briefly, 20 µL of 10 IU enzyme was mixed with 20 µL of extracts, and incubated at 30°C for 10 min. Then, 160 µL of 1% (w/v) starch in phosphate buffer pH 6.0 was added and incubated at 30°C for 15 min. After incubation, 50 µL of the mixture was mixed with 950 µL of iodine solution and mixed well. Starch content was determined at 656 nm using a UV-VIS Biotech spectrophotometer. As positive and negative controls, water and solvents were used, respectively, instead of the extracts

*Determination of Raphanus sativus root-growth inhibition*

*Raphanus sativus* root growth inhibition was evaluated using the method described by Tan et al. (2014). *Raphanus sativus* seeds were

germinated at 30°C for 24 hr. The uniformly germinated seedlings were selected for the experiment. Each group of 10-20 seedlings was soaked in different concentrations of the phenolic extracts for 1 hr. Water and solvents were used for positive and negative controls, respectively. At 24 hr after treatment, the length of the roots of seedlings was measured.

*Antibacterial activity*

Antibacterial activity was tested against *B. subtilis*, *E. coli*, *S. aureus*, *S. typhimurium* and *P. aeruginosa* using a well diffusion method (Murray et al., 1995). Briefly, after adding 0.1 mL of bacterial suspension into Petri dishes containing agar, use sterile sticks to well spread the suspension on the agar surface. Five wells were drilled on the agar plate. Two hundred microliters (200 µL) of phenolic extracts were added to wells and allowed to diffuse at 4°C for 5-6 hr and then incubated at 32˚C for 24 hr. The positive control was prepared with 200 µL of 0.1 mg/mL kanamycin and negative control was done with 200 µL of corresponding solvents alone.

*Data access*

Each assay in this study was repeated at least three times. Data was analyzed by t-test and Tukey test.

## RESULTS AND DISCUSSION

### Total phenolic content

Total phenolic content in the 95% ethanol and 50% acetone fractions I-V were measured. No signals of phenolics were detected in the fractions V of both 95% ethanol and 50% acetone eluents. The total phenolic content was highest in fraction I (104.1 µgGAE/mL), and then the content decreased 57% and 73% in fraction II and III of ethanol eluent, respectively, in comparison to that of fraction I. Similarly, the fraction I of acetone eluent also exhibited highest total phenolic content (189.8 µgGAE/mL) and then decreased in the following fractions (fig. 1). Since the phenolic content was not detected in the fraction V, 250 mL of eluent is sufficient enough to elute maximum phenolic compounds from the column.

In ethanol solution, Sephadex LH-20, a hydroxypropyl derivative of Sephadex G-25 shows a high affinity to aromatic materials such as tannin. Hence, non-tannin phenolic compounds (low-molecular compounds) are recovered from the gel using ethanol as eluent, and the bound tannins are recovered in the subsequent elution with acetone-water (1:1; v/v) (Hagerman & Butler, 1980).

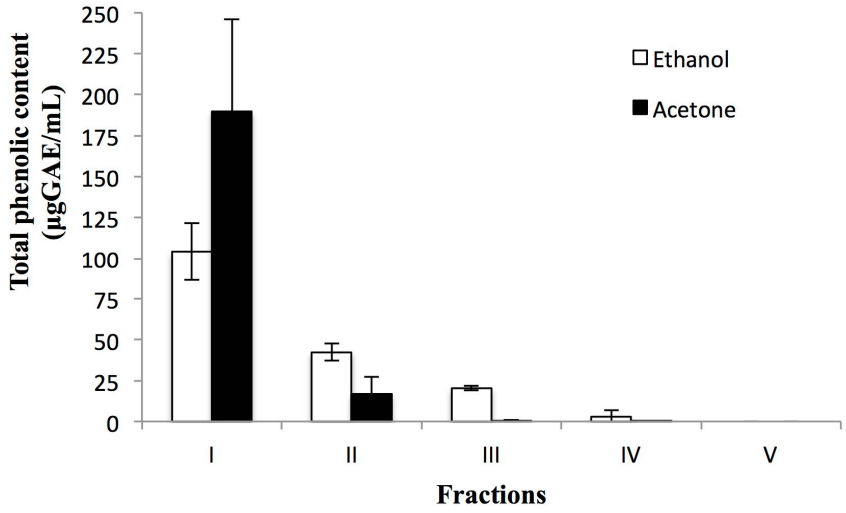


*Figure 1.* Total phenolic content of the fractions eluted with 95% ethanol and 50% acetone

The bars indicate the standard deviation (n = 3). Eluates were pooled every 50 mL into fraction I - V for each solvent.

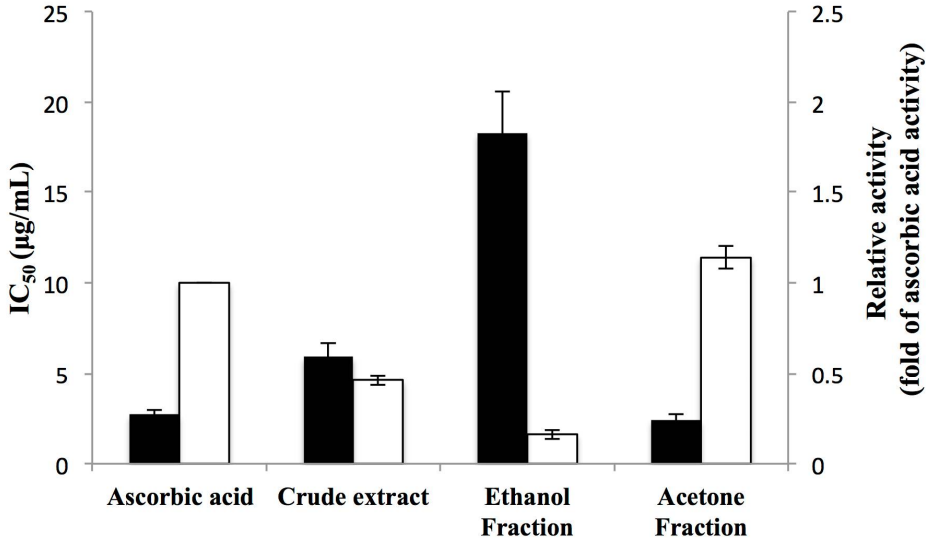


*Figure 2.* DPPH radical scavenging activity of crude extract and the fractions of ethanol and acetone

The solid bars (■) indicate DPPH radical scavenging activity in $IC_{50}$ and the open bars (□) indicate relative activity; the bars indicate the standard deviation (n = 3).

**Antioxidant activity**

Figure 2 displayed antioxidant activities of crude extract, ethanol and acetone fractions. Acetone fraction exhibited the highest antioxidant activity ($IC_{50}$ 2.4 µg/mL), 1.14 times higher than that of ascorbic acid. In contrast, ethanol fraction, which contains mainly low-molecular phenolic compounds, showed extremely low antioxidant activity, equal to 0.16 times activity of ascorbic acid. In general, natural compounds exhibiting $IC_{50}$ < 20 µg/mL are considered high antioxidant compounds (Qusti et al., 2010). In comparison with the results of our previous study of the antioxidant activity of bark extracts of *S. apetala*, the crude extract from pneumatophore showed lower antioxidant activity. However, after Sepadex LH-20 column chromatography, acetone fraction has antioxidant activity comparable or higher than ascorbic acid, suggesting its potential application (Tan & Thuy, 2014).

**Antibacterial activity**

The crude extract showed no effects on gram-negative (*E. coli, P. aeruginosa*) and gram-positive (*B. subtilis, S. aureus*) bacteria. However, both crude extract and ethanol fraction exhibited inhibitory effects (inhibition zones of 3.3 ± 0.6 mm; 4.3 ± 0.5 mm, respectively) on the growth of *S. typhimurium*. Acetone fraction showed no antibacterial activity. *S. apetala* leaf extracts is known to have antibacterial activity against both gram-negative and gram-positive bacteria (Jaimini et al., 2011). Patra et al. (2015) focused on the antibacterial activity of *S. apetala*, and suggested that methanol extracts of leaves and bark possess inhibition activity on all 9 and 7 (except *E. coli* and *S. epidermidis*) test species, respectively. We also reported previously that methanol extracts of leaves, fruit, bark, and pneumatophore of *S. apetala by* Soxhlet extractor showed antibacterial activity against *E. coli* (Tan et al., 2014). The present results suggested that depending on different fraction solvents or extraction methods, a variety of compounds could be extracted from various parts of *S. apetala*.

**α-amylase inhibition test**

Tannins have an ability to bind strongly to proteins to form insoluble and indigestible complexes, and therefore probably it is the

action mechanism to cause inhibition of α-amylase (Sales et al, 2012). Our results show that acetone fraction containing tannins has the highest inhibition activity on α-amylase with $IC_{50}$ 0.83 ± 0.27 µg/mL. In contrast, ethanol fraction showed an inhibition of less than 50% of the control α-amylase activity (table 1). Recent studies showed inhibitory activity of plant extract on α-amylase. For example, *Juglans regia* extracts exhibited α-amylase inhibitor activity with an IC50 = 0.32± 0.07 mg/mL (Rahimzadeh et al., 2014). The methanol-HCl fraction of *S. apetala* bark exhibited the highest inhibition ($IC_{50}$=6.9 µg/ml) followed by the ethyl acetate fraction ($IC_{50}$=16.9 µg/mL) and the crude extract ($IC_{50}$=300.4 µg/mL) (Tan & Thuy, 2014).

*Table 1.* α - amylase inhibition by *S. apetala* fractions

| | $IC_{50}$ (mg/mL) |
|---|---|
| Crude extract | 2.14 ± 0.27[a] |
| Ethanol fraction | - |
| Acetone fraction | 0.83 ± 0.22[b] |

*Table 2. R. sativus* seedling-Inhibition by different fractions of *S. apetala*

| | $IC_{50}$ (mg/mL) |
|---|---|
| Crude extract | 30.6 ± 4.9 [a] |
| Ethanol fraction | - |
| Acetone fraction | 6.4 ± 3.3 [b] |

The data are the mean of triplicate (mean ± standard division). Different letters (a,b) indicate differences of values in the same column at $P < 0.05$ using t-test.

**Inhibition of *Raphanus sativus* root-growth**

α-amylase plays an important role in hydrolysis of starch into glucose and therefore relates closely to the germination process. In this study, the inhibitory activity of the phenolic extracts on *R. sativus* root growth was examined. Acetone fraction exhibited 50% root growth inhibition at a concentration 6.4 ± 3.3 mg/ml while ethanol fraction showed root growth inhibition of less than 50% (table 2). This result suggests the necessity of further studies on the effects of allelochemicals from *S. apetala.*

In terms of plant root growth inhibition, both aqueous and ethanol extracts of *Derris trifoliate* seeds inhibited more than 25% of *R. sativus* root growth (Tan et al., 2012). Similarly, *R. sativus* root growth was inhibited by extracts from various parts of *Ancathus ilicifolius*, these inhibitory activities ranged from 65-72% (Tan et al., 2013). Our results also show that the fractions of *S. apetala* pneumatophores have high potential of root growth inhibition.

**CONCLUSION**

Crude extract, ethanol and acetone fractions of *S. apetala* pneumatophores exhibited high antioxidant activities, especially acetone fraction consisting of tannins and other high molecular phenolic compounds showed high antioxidant potential comparable with that of ascorbic acid. Root growth inhibition and α-amylase inhibition were highest in acetone fraction while an effective antibacterial activity against *Samonella typhimurium* was observed in crude alcoholic extract and ethanol fractions. Further research with high-pressure liquid chromatography is recommended to carry out detailed chemical compounds resulting in these bioactivities. Our study suggested *S. apetala* pneumatophore as a potential pharmaceutical material for diabetes and antiaging. This material might be interested in allelopathy as well.

*REFERENCES*

Crozier A., Clifford M. N., Ashihara H., 2006. Plant Secondary Metabolites: Occurence, Structure and Role in the Human Diet. Blackwell publishing, pp.1-22.

Das S. K., Samantaray D., Patra J. K., Samanta L.,Thatoi H., 2016. Antidiabetic potential of mangrove plants: a review. Frontiers in Life Science, 9(1): 75-88.

Hagerman A. E., Butler L. G., 1980. Condensed tannin purification and characterization of tannin-associated proteins. Journal of Agricultural and Food Chemistry, 28(5): 947-952.

Hossain S. J., Basar M. H., Rokeya B., Arif K. M. T., Sultana M. S., Rahman M. H., 2013. Evaluation of antioxidant, antidiabetic and

antibacterial activities of the fruit of *Sonneratia apetala* (Buch.-Ham.). Oriental Pharmacy and Experimental Medicine, 13(2): 95-102.

Jaimini D., Sarkar C., Shabnam A. A.,Jadhav B. L., 2011. Evaluation of antibacterial properties of mangrove plant *Sonneratia apetala* Buch. Ham leaf. World Applied Sciences Journal, 14(11): 1683-1686.

Karamac´ M., Kosin´ska A., Rybarczyk A., Amarowicz R., 2007. Extraction and chromatographic separation of tannin fractions from tannin-rich plant material. Polish

Kim D. O., Chun O. K., Kim Y. J., Moon H. Y., Lee C. Y., 2003. Quantification of Polyphenolics and Their Antioxidant Capacity in Fresh Plums. Journal of Agricultural and Food Chemistry, 51(22): 6509-6515.

Lin D., Xiao M., Zhao J., Li Z., Xing B., Li X., Chen, S., 2016. An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. Molecules, 21(1374):1-19.

Nguyen Van Mui, 2001. Biochemical Practice. Publisher of Vietnam National University: pp 173.

Murray P. R., Baron E. J., Pfaller M. A, Tenover F. C and Yolken H. R., 1995. Manual of clinical microbiology. 6th. The American Society for Microbiology Press: pp. 1482.

Patra J. K., Das S. K., Thatoi H., 2015. Phytochemical profiling and bioactivity of a mangrove plant, *Sonneratia apetala*, from Odisha Coast of India. Chinese Journal of Integrative Medicine, 21(4): 274-285.

Qusti S. Y., Abo-khatwa A. N., Lahwa, M. a Bin., 2010. Screening of Antioxidant Activity and Phenolic Content of Selected Food Items Cited in the Holly Quran. European Journal of Biological Sciences, 2(1): 40-51.

Rahimzadeh M., Jahanshahi S., Moein S., Moein M. R., 2014. Evaluation of alpha-amylase inhibition by *Urtica dioica* and *Juglans regia* extracts. Iranian Journal of Basic Medical Sciences, 17(6): 465-469.

Sales P. M., Souza P. M., Simeoni L. A., Magalhães P. O., Silveira D., 2012. α-Amylase inhibitors: a review of raw material and isolated compounds from plant source. Journal of Pharmacy & Pharmaceutical Sciences, 15(1): 141-183.

Dao Van Tan, Dao Thi Sen Thi, Vu Thi Hien, 2012. Bioactivities of *Derris trifoliata* seed extracts in Xuan Thuy National Park, Nam Dinh province. Proceedings of the First national sientific conference on biological research and teaching in Vietnam. Agricultural publishing house, Hanoi, pp.670-677. (Vietnamese summary in English)

Dao Van Tan, Dao Thi Sen, Nguyen Thi Thu Huyen, 2014. Potential bioactivities of *Sonneratia apetala* extracted from the mangrove forest of Xuan Thuy National Park, Viet Nam. Journal of Science and Technology, 52: 313-320.

Dao Van Tan, Dao Thi Sen, Nguyen Thi Thu Huyen, Hoang Van Chinh, 2013. Antibacterial and antioxidative activities and plant-growth inhibition by methanolic extracts from various parts of the mangrove plant *Acanthus ilicifolius* L.. National Biotechnology conference, pp.701-704. (Vietnamese summary in English)

Dao Van Tan, Mai Nhat Thuy, 2014. Antioxidant, antibacterial and alpha-amylase inhibitory activities of different fractions of *Sonneratia apetala* bark extract. Tap chi Sinh hoc, 37(1se): 54-60.

Le Thanh Tinh, Dao Van Tan, Nguyen Hoang Tri, Tran Mai Sen, 2006. Research on the growth and some biological characteristics of *Sonneratia apetala*, introduced and growth in Giao Thuy district, Nam Dinh province. The role of mangrove and coral reef ecosystems in National disaster mitigation and coastal life environment (ed) Phan Nguyen Hong. Agricultural publishing house, Hanoi: 305-316.