Journal of Multidisciplinary Sciences
www.multidisciplines.com

Simultaneous determination of common phenolic acid in leaves of *Avicennia officinalis*

Tuyet-Ngan Duong, Ngoc-Van Thi Nguyen*, To-Lien Thi Pham, Gia-Ngan Mai Le, Anh-Thu Chau, Phi-Long Ha, and Van-Cuong Nguyen
Can Tho University of Medicine and Pharmacy, 179 Nguyen Van Cu Street, An Khanh Ward, Ninh Kieu District, Can Tho City, Vietnam.
**Corresponding authors' email address: ntnvan@ctump.edu.vn (N.-V.T.N.)*

Received: 20 September 2022; Accepted: 14 November 2022; Published online: 17 November 2022

Abstract. The mangrove plant, *Avicennia officinalis* (L.), has long been utilized as an antioxidant, anti-inflammatory, and anti-cancer agent. In this study, *p*-coumaric acid and cinnamic acid were employed to simultaneously quantify common phenolic acids from leaf extract using a high-performance liquid chromatography method and a diode array detector. With a gradient elution of 0.1% formic acid in acetonitrile, 0.2% ammonium acetate/0.1% formic acid in the water, and methanol at a flow rate of 1.0 mL/min, chromatographic separation was performed on a Kromasil C_{18} column (150 mm × 4.6 mm i.d., 5 µm); detection was carried out at 280 nm. The limits of detection (LOD) and quantification (LOQ), respectively, were 0.025 mg/mL and 0.05 mg/mL. The recovery varied between 92.16 and 104.69%, whereas the relative standard deviations of intra- and inter-day analyses ranged from 1.71 to 3.22%. The validated approach was effectively used to analyze the bioactive ingredients in *A. officinalis*, and it could be a helpful process for maintaining the quality of this plant. Additionally, the bioactive substances found in the plant's leaves have a great deal of potential to become a source of traditional Vietnamese medicine.

Keywords: *Avicennia officinalis, mangrove plant*, *phenolic acid, and HPLC-DAD*

Cite this as: Duong, T.-N., Nguyen, N.-V.T., Pham, T.-L.T., Le, G.-N.M., Chau, A.-T., Ha, P.-L. & Nguyen, V.-C. (2022). Simultaneous determination of common phenolic acid in leaves of *Avicennia officinalis*. J. Multidiscip. Sci. 4(2), 10-16.

1. Introduction

Mangroves are salt-tolerant plants that can withstand adverse environmental conditions such as high salinity, toxic metals, floods, UV rays, and waterlogging (Mehta, 2021; Roy, 2021). Due to the presence of phytochemicals, mangrove plants have been used traditionally to treat several ailments (Abeysinghe, 2010; Mahmud, 2021). Mangroves are now thought to have nutritional advantages and medicinal uses due to their potent antioxidant, antibacterial, and antifungal activity (Hamzah, 2018; Mateos, 2020; Yassien, 2021; Okla, 2021). Mangrove trees may include chemical compounds such as phenolic acid, flavonoids, quinones, coumarins, lignans, stilbenes, and tannins as molecules that scavenge free radicals (Mateos, 2020; Roy, 2021). *Avicennia officinalis* (L.), a medium-sized mangrove plant, is common throughout the subtropical coast and does well in brackish water (Assaw, 2020). Pharmacological studies have revealed that several *Avicennia officinalis* leaf and bark extracts possess antibacterial, antiulcer, anti-inflammatory, anti-cancer, and antioxidant activities (Thirunavukkarasu, 2010; Sahoo, 2012; Das, 2019). The majority of this plant's distribution is in Malaysia, Bangladesh, India, and Indonesia. In Vietnam, this species is widely distributed in mangrove areas in coastal provinces such as Ca Mau, Kien Giang, and Tra Vinh.

Phenolic acids are one of the other main phenolic groups in plants, and they are typically discovered as esters, glycosides, or amides rather than in their free form. Among phenolic acids, there can be variations in the number and location of hydroxyl groups on the aromatic ring. The two main chemical structures of phenolic acids are hydroxycinnamic acid and hydroxybenzoic acid. Gallic, vanillic, syringic, and protocatechuic acids are hydroxybenzoic acid derivatives, whereas ferulic, caffeic, *p*-coumaric, and sinapic acids are hydroxycinnamic acid derivatives (Khoddami, 2013). Secondary metabolites known as phenolic compounds are produced and stored in all plant tissues. Previous *in vitro* studies on *Avicennia officinalis* have shown the

antioxidative and free radical scavenging effects of the plant's leaf extracts (Thirunavukkarasu, 2011; Hossain, 2016; Khushi, 2016; Nguyen, 2022). These possibilities could be brought on by the polyphenols in the extract. Plant extract is incredibly beneficial and efficient, and it has the potential to include new bioactive chemicals.

HPLC is the method that is advised for the separation and measurement of phenolic compounds. Among the factors that affect the HPLC analysis of phenols are sample purification, mobile phases, column types, and detectors. In an HPLC system, pure phenols are frequently applied using a reversed-phase C_{18} column (RP-C_{18}), a photodiode array detector (PDA), and polar acidified organic solvents. The basis for HPLC sensitivity and detection is the purification of phenolics and pre-concentration of complex matrices of crude plant extracts (Khoddami, 2013). To the best of our knowledge, no validated process has yet been published for analyzing the characteristic phenolic chemicals found in *A. officinalis* leaves. Using ultrasound-assisted extraction (UAE) and high-performance liquid chromatography (HPLC) with a diode-array detector, this study aims to develop and validate a straightforward, sensitive, and reliable analytical procedure for the identification of major phenolic compounds accumulated in the leaves of *A. officinalis*. The leaves came from six provinces in Vietnam's Mekong Delta. The validated process was used to measure how much of the two phenolic acids were in the extract.

2. Materials and Methods

2.1. Chemicals and solvents

Sigma-Aldrich was used to acquire all standards of *p*-coumaric acid (>99%) and cinnamic acid (>98%) (St. Louis, MO, USA). Honeywell provided methanol, acetone, acetonitrile, n-hexane, and HPLC-grade water (North Carolina, USA). The other solvents, such as formic acid and ammonium acetate, were purchased from Merck (Darmstadt, Germany).

2.2. Plant materials

Ten kilograms of fresh *Avicennia officinalis* leaves were gathered in Ca Mau Province, Vietnam. Small pieces of the newly collected leaves were lyophilized for 48 hours. The ground-up, dry sample was then sieved to produce a 40-mesh powder. The dried sample's moisture content was less than 13%. At room temperature (20 °C), all samples were housed in dark glass containers. At Can Tho University in Vietnam's Department of Biology, the plants were identified using PCR techniques.

2.3. Standard and sample preparation

Cinnamic acid and *p*-coumaric acid were created as individual standard stock solutions in methanol (1000 µg/mL) and were stable for about a year. Working standard solutions were made by adding methanol to stock solutions to dilute them and create varied concentrations every day. Standard stock and working solutions were kept at 4 °C and shielded from light.

Three times, for a total of 15 minutes, one gram of the powdered leaves was extracted in an ultrasonic bath with 20 mL of acetone. The extracted solutions were mixed and diluted with acetone in a 50 mL volumetric flask. 5 mL of the extract was transferred to a 15 mL centrifuge tube for the clean-up process, where it was dried at 40 °C under a nitrogen stream. To remove non-polar matrix interferences, the residue was partitioned with 1 mL n-hexane and reconstituted with 0.5 mL each of acetonitrile and methanol and water (50:50, v/v). The lower layer was transferred to a vial after being filtered through a 0.22 m PTFE filter.

Table 1. Gradient elution program

Time (min)	Flow rate (mL/min)	Mobile phase ratio		
		A (%)	B (%)	D (%)
0.0	1.0	0	5	95
1.5	1.0	13	5	82
5	1.0	13	15	72
10	1.0	11	21	68
11	1.0	14	28	58
12	1.0	24	28	48
20	1.0	0	100	0
25	1.0	0	5	95
30	1.0	0	5	95

2.4. Chromatographic conditions

UFLC Shimadzu (LC-20AD), detector DAD, and SPD-M20A investigations were conducted for method development, quantification, and validation. An Agilent C_{18} column (250 mm x 4.6 mm; 5 µm) was used to accomplish chromatographic separation. In Table 1, the gradient elution program was displayed. Acetonitrile (A), methanol (B), and 0.2% ammonium acetate/0.1% formic acid in water made up the mobile phase (C). All chromatographic separations were carried out at a flow rate of 1.0 mL/min with an injection volume of 20 µL. Analytical identification was carried out using a 280 nm detection wavelength.

2.5. Method validation

The proposed method was tested for selectivity, linearity, the limit of detection (LOD), the limit of quantification (LOQ), precision, and accuracy using the Association of Official Analytical Chemists (AOAC) and ICH criteria (Association of Analytical Communities International, 2013).

3. Results and Discussion

3.1. Optimization of sample preparation

The extraction of phenolic compounds from plants has recently been carried out using novel methods such as supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE). As a result of the excellent extraction efficiencies that may be attained at very low temperatures, UAE was one of these methods that was frequently used to extract bioactive chemicals from plant materials (González-de-Peredo, 2021). Plant cell walls were damaged by ultrasound waves, which also facilitated solvent absorption and mass transfer across cell membranes. The use of moderate temperatures and more effective extraction, which will help heat-sensitive compounds and conserve energy, will be the main benefits. The applied ultrasonic power, extraction temperature, extraction frequency, how the solvent and sample interact, and the parameters of the reactor are some of the most important process factors that must be looked at to use the UAE correctly.

The recovery of phenolic contents in samples is influenced by the polarity of the extraction solvents and the solubility of each component in the solvents. As a result, it might be challenging to choose a solvent that would work well for extracting phenol from various plants. High-polarity solvents can produce extracts with greater yields of polyphenol content and antioxidant activity, which helps us choose the best solvents for screening several compounds. A component's ability to be extracted is generally influenced by the extraction polarity and the solute-to-solvent ratio. Moreover, the extraction solvents, their polarity index (PI), and the solubility of phenolic compounds in the extraction solvents all appear to have an impact on the recovery of phenolic compounds. The presence and placement of hydroxyl groups as well as the molecular weight and length of the component hydrocarbon chains were the main determinants of polyphenol solubility. Highly polar solvents are routinely used to extract phenolic chemicals in higher concentrations (Iloki-Assanga, 2015). In a study by Nguyen et al. (2022) on the effects of solvents on antioxidant compounds, it was found that acetone was the best solvent for extracting the *A. officinalis* leaves. Based on the result, the extraction solvent for the leaf *A. officinalis* sample quantification procedure was acetone (Figure 1).

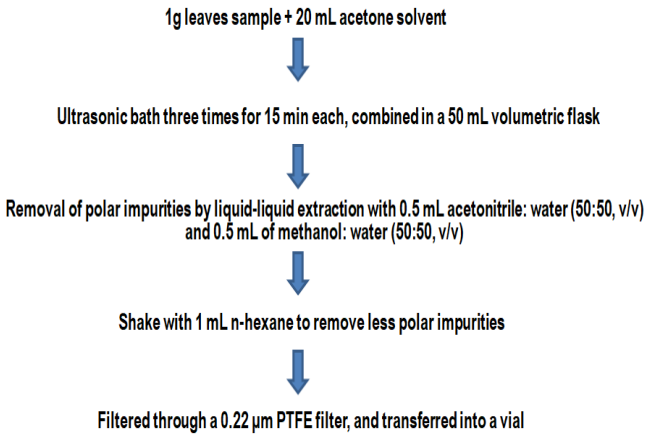

Figure 1. Optimization of sample preparation

3.2. Optimization of chromatographic conditions

Ultrafast liquid chromatography (UFLC) shows a great improvement in speed, resolution, and sensitivity of the separation. The UFLC system is operated under low pressure (300 bars), but this does not affect the analytical column or other system components. Outstanding speed and separation are achieved even at normal pressure. Although UFLC provides extremely fast analysis capabilities, it still maintains high accuracy and reliability. In addition to shortening time and improving separation, which helps save solvents, UFLC is also a reliable aid in the separation and detection of trace substances in many fields. The operating cost for the UFLC system is also not too expensive.

As a distribution separation mechanism, most current liquid chromatography methods employ reverse phase chromatography techniques with a less polar stationary phase and a polar mobile phase. In reverse phase chromatography, separation is affected by many things, such as the nature of the stationary phase, the amount of mobile phase, the pH of the mobile phase, the flow rate, the temperature of the column, and the analytical wavelength. In our research, we used chromatographic conditions to look at things like the mobile phase and the analytical wavelength, as well as other things that have stayed the same.

Because pH has a significant impact on solute retention in most types of HPLC, particularly reversed-phase chromatography, choosing the ideal mobile phase pH is a crucial decision. The pH modification alone, without the introduction of organic modifiers, can occasionally resolve a combination of acidic or basic solutes. The majority of times, factorial design experiments have been used to optimize pH. In these experiments, the pH is either changed on its own or in combination with another discrete variable, like the number of pairing ions present in a reversed-phase ion-pairing chromatographic system. Acidic modifiers are typically added to the mobile phase when phenolic acids are analyzed to reduce the ionization of polyphenolic compounds and silanols. Additionally, the inclusion lessens the interaction on the stationary phase between analytes and ionized silanols, which might lead to peak tailing. Numerous earlier studies stated that the mobile phase typically used for the separation of phenolic acids by RP-HPLC was a mixture of water and acetonitrile, water and methanol, or water, acetonitrile, and methanol.

In this investigation, the suitable mobile phase was determined by evaluating the mixture of water, methanol, or acetonitrile, as well as different amounts of formic acid in water (0.1 to 1%). After repeated tries, it was discovered that adding more formic acid at a pH lower than 2 resulted in reduced column performance, while adding more formic acid at a concentration over 0.5% did not appreciably enhance the peak shape. Another modifier, namely ammonium acetate, was added to the water to lessen the peak tailing at low formic acid concentrations. These quantities of ammonium acetate and formic acid were chosen for all future tests since it was demonstrated that 0.2% ammonium acetate and 0.1% formic acid in water resulted in a separation efficiency as acceptable as that obtained with 1% formic acid. Even at 5% acetonitrile, phenolic acids were not well preserved; nevertheless, the combination of water and methanol improved phenolic acid resolution. As a result, a mixture of methanol, acetonitrile, and water (Table 1) was taken into consideration (Nguyen, 2021).

3.3. Method validation

System suitability. The system's stability (Rs) was measured by giving a mixed standard solution (50 g/mL) six times and figuring out the theoretical plate number (N) and resolution. All analytes had %RSD values for the peak area and a RT that was less than 2.0%. As a result, the suggested approach satisfied this condition (Table 2).

Table 2. System suitability of the HPLC-PDA method

		t_R	S	As	Rs	N
***p*-coumaric acid**	Mean	16.71	5654010	1.5	--	35659
	RSD%	0.6	0.5	0.5	--	1.3
Cinnamic acid	Mean	23.67	6074674	1.5	18.8	61410
	RSD%	0.05	1.0	0.8	0.9	1.7

Specificity. By using the UFLC technique to examine the leaf extracts of *A. officinalis*, specificity was put to the test. It was assessed by contrasting the peaks discovered after extract analysis with those of each component's RT and UV absorption spectra in reference solutions. Figure 2 illustrates how the UFLC technique may separate *p*-coumaric acid and cinnamic acid from other elements of the leaf matrix. When the spectra of the two chemicals were put on top of the graphs of three-point purity detection, the peak purity of the two chemicals was found to be more than 99.9%.

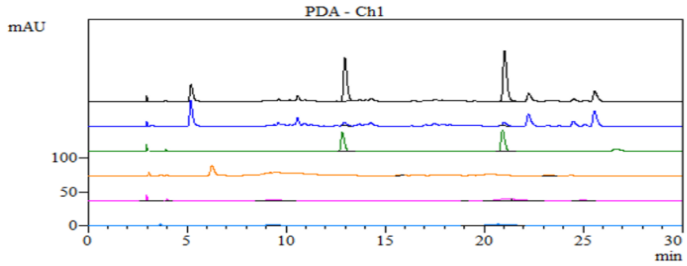

Figure 2. HPLC chromatograms for specificity (a: mobile phase solvent; b: extraction solvent; c: blank leaf sample; d: *p*-coumaric acid and cinnamic acid standard solution mix; e: leaf sample extract; f: leaf sample extract with standard added)

Linearity, the limit of detection, and the limit of quantification. The stock solutions were diluted and combined into eight different *p*-coumaric acid and cinnamic acid concentrations, ranging from 0.05 to 25 g/mL. Each mixed standard sample was injected into the UFLC system in triplicates, and calibration curves were created by plotting the average of the peak area responses versus concentration for each sample to assess the linearity. The square correlation values (R^2) for *p*-coumaric acid and cinnamic acid were greater than 0.999 (Table 3). The LOD and LOQ were 0.025 mg/mL and 0.05 mg/mL, respectively.

Table 3. Precision, recovery, calibration parameters, LOD, and LOQ

Substance	Calibration curve		Precision		Recovery (%)		
	Regression equation	R^2	Intra-day RSD (%) (n=6)	Inter-day RSD (%) (n=18)	Low-level	Mid-level	High-level
***p*-coumaric acid**	y =148929x - 49424	0.9997	1.17	2.29	101.32	98.97	92.16
Cinnamic acid	y =169689x + 63640	0.9991	3.03	3.22	98.31	98.73	104.69

Precision. By comparing the intra-day and inter-day precisions, the method's accuracy was confirmed. As an accuracy indicator, the relative standard deviation (%RSD) was used. Six samples of each drug were analyzed to determine the intra-day precision, and six samples were analyzed daily for three days to establish the inter-day accuracy. According to the accuracy findings in Table 4, the total intra- and inter-day fluctuations (%RSD) were less than 6%, in conformity with AOAC recommendations.

Table 4. The amount of *p*-coumaric acid and cinnamic acid in the leaves of *A. officinalis* that were taken from the Mekong Delta in Vietnam

No.	Location	*p*-coumaric acid (%)*	Cinnamic acid (%)*
1	Bac Lieu province	0.0034±0.00050	0.0198±0.00204
2	Ben Tre province	0.0003±0.00003	0.0005±0.00003
3	Soc Trang province	0.0057±0.00049	0.0025±0.00398
4	Tra Vinh province	0.0010±0.00003	0.0026±0.00030
5	Kien Giang province	0.0010±0.00003	0.0141±0.00070
6	Ca Mau province	0.0007±0.00004	0.0016±.0.00021

(*) mg/100 g dried leaf sample

Accuracy. Recovery experiments were carried out to look at the method's accuracy. The blank samples were treated with three quantities of reference compounds: low (80%), medium (100%), and high (120%). The spiked samples were then included and measured using the aforementioned techniques. The findings showed that the developed method had a good level of accuracy, with a total recovery falling between 92.16 and 104.69%. The method was thought to be accurate in light of the recovery test's outcomes.

3.4. Application

Quantitative results of 18 samples of *A. officinalis* leaves were collected in 6 provinces, including Bac Lieu, Ben Tre, Soc Trang, Tra Vinh, Kien Giang, and Ca Mau Province in the Mekong Delta, Vietnam (Table 4). All leaf samples detected *p*-coumaric acid and cinnamic acid. The highest concentration of *p*-coumaric acid was found in the Soc Trang province and the lowest in the Ben Tre province, at 0.057% and 0.0003%, respectively. The cinnamic acid content was significant in Bac Lieu

province (0.0198%). The provinces of Bac Lieu and Soc Trang had significantly higher phenolic acid content in *A. officinalis*. This difference can be explained by the collection location's different geographical and climatic conditions.

4. Conclusions

The best extraction method used in this investigation produced high extraction yields and efficiency. The UFLC methodology further allowed for the quantitative separation of the common phenolic acids (*p*-coumaric acid and cinnamic acid), and it was effective, precise, and accurate. The Mekong delta's six provinces were used for the experimental fresh sample collection, and the results showed that leaves were a significant source of phenolic acids. Through this study, *A. officinalis* in Vietnam has many potential compounds to help the plant become a source of medicinal herbs in the future.

Acknowledgment. This research received no external funding. The authors would like to express their gratitude to the Can Tho University of Medicine and Pharmacy. We also thank all of our colleagues for their excellent assistance.

Conflicts of interest. The authors declare that there are no conflicts of interest regarding the publication of this paper.

ORCID

Tuyet-Ngan Duong: https://orcid.org/0000-0002-7373-2873
Ngoc-Van Thi Nguyen: https://orcid.org/0000-0002-7397-4071
To-Lien Thi Pham: https://orcid.org/0000-0001-8811-0930

References

Abeysinghe, P.D. (2010). Antibacterial activity of some medicinal mangroves against antibiotic resistant pathogenic bacteria. Indian Journal of Pharmaceutical Sciences, 72(2), 167–172.

Assaw, S., Mohd-Amir, M.I.H., Khaw, T.T., Bakar, K., Mohd-Radzi, S.A. & Mazlan, N.W. (2020). Antibacterial and antioxidant activity of naphthofuranquinones from the twigs of tropical mangrove *Avicennia officinalis*. Natural Product Research, 34(16), 2403–2406.

Association of Analytical Communities International (2013). Guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals. AOAC International, 1–38.

Das, S.K., Samantaray, D., Sahoo, S.K., Patra, J.K., Samanta, L. & Thatoi, H. (2019). Bioactivity guided isolation and structural characterization of the antidiabetic and antioxidant compound from bark extract of *Avicennia officinalis* L. South African Journal of Botany, 125, 109–115.

González-de-Peredo, A.V., Vázquez-Espinosa, M., Espada-Bellido, E., Ferreiro-González, M., Carrera, C., Barbero, G.F. & Palma, M. (2021). Development of optimized ultrasound-assisted extraction methods for the recovery of total phenolic compounds and anthocyanins from onion bulbs. Antioxidants (Basel), 10(11), 1755.

Hamzah, T.N.T., Lee, S.Y., Hidayat, A., Terhem, R., Faridah-Hanum, I. & Mohamed, R. (2018). Diversity and characterization of endophytic fungi isolated from the tropical mangrove species, *Rhizophora mucronata*, and identification of potential antagonists against the soil-borne fungus, *Fusarium solani*. Frontiers in Microbiology, 9, 1707.

Hossain, M.L. (2016). Medicinal activity of *Avicennia officinalis*: Evaluation of phytochemical and pharmacological properties. Saudi Journal of Medical and Pharmaceutical Sciences, 2, 250–255.

Iloki-Assanga, S.B., Lewis-Luján, L.M., Lara-Espinoza, C.L., Gil-Salido, A.A., Fernandez-Angulo, D., Rubio-Pino, J.L. & Haines, D.D. (2015). Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*. BMC Research Notes, 8, 396.

Khoddami, A., Wilkes, M.A. & Roberts, T.H. (2013). Techniques for analysis of plant phenolic compounds. Molecules, 18(2), 2328–2375.

Khushi, S., Hasan, M.M., Monjur-Al-Hossain, A.S.M., Hossain, M.L. & Sadhu, S.K. (2016). Medicinal activity of *Avicennia officinalis*: Evaluation of phytochemical and pharmacological properties. Saudi Journal of Medical and Pharmaceutical Sciences, 2(9), 250–255.

Mahmud, S., Paul, G.K., Afroze M., Islam, S., Gupt, S.B.R., Razu, M.H., Biswas, S., Zaman, S., Uddin, M.S., Khan, M., Cacciola, N.A., Emran, T.B., Saleh, M.A., Capasso, R. & Simal-Gandara, J. (2021). Efficacy of phytochemicals derived from *Avicennia officinalis* for the management of COVID-19: A combined in silico and biochemical study. Molecules, 26(8), 2210.

Mateos, R., Pérez-Correa, J.R. & Domínguez, H. (2020). Bioactive properties of marine phenolics. Marine Drugs, 18(10), 501.

Mehta, B., Nagar, B., Patel, B., Chaklashiya, P., Shah, M., Verma, P. & Shah, M.B. (2021). A review on a lesser known Indian mangrove: *Avicennia officinalis* L. (Family: Acanthaceae). International Journal of Green Pharmacy, 15, 1–10.

Nguyen, K. N. H. & Kim, K. H. (2021). Determination of phenolic acids and flavonoids in leaves, calyces, and fruits of *Physalis angulata* L. in Viet Nam. Pharmacia, 68, 501.

Nguyen, N.V., Duong, N.T., Nguyen, K.N.H., Bui, N.T., Pham, T.L., Nguyen, K.T., Le, H.P. & Kim, K.H. (2022). Effect of extraction solvent on total phenol, flavonoid content, and antioxidant activity of *Avicennia officinalis*. Biointerface Research in Applied Chemistry, 12(2), 2678–2690.

Okla, M.K., Alatar, A.A., Al-Amri, S.S., Soufan, W.H., Ahmad, A. & Abdel-Maksoud, M.A. (2021). Antibacterial and antifungal activity of the extracts of different parts of *Avicennia marina* (Forssk.) Vierh. Plants (Basel), 10(2), 252.

Roy, M. & Dutta, T.K. (2021). Evaluation of phytochemicals and bioactive properties in mangrove associate *Suaeda monoica Forssk*. ex J.F.Gmel. of indian sundarbans. Frontiers in Pharmacology, 12, 584019.

Sahoo, G., Mulla, N.S., Ansari, Z.A. & Mohandass, C. (2012). Antibacterial activity of mangrove leaf extracts against human pathogens. Indian Journal of Pharmaceutical Sciences, 74(4), 348–351.

Thirunavukkarasu, P., Ramanathan, T., Ramkumar, L. & Shanmugapriya, R. (2010). Antiulcer effect of *Avicennia offcinalis* leaves in Albino rats. World Applied Science Journal, 9, 55–58.

Thirunavukkarasu, P., Ramanathan, T., Ramkumar, L., Shanmugapriya, R. & Renugadevi, G. (2011). The antioxidant and free radical scavenging effect of *Avicennia officinalis*. Journal of Medicinal Plants Research, 5(19), 4754–4758.

Yassien, E.E., Hamed, M.M., Abdelmohsen, U.R., Hassan, H.M. & Gazwi, H.S.S. (2021). *In vitro* antioxidant, antibacterial, and antihyperlipidemic potential of ethanolic *Avicennia marina* leaves extract supported by metabolic profiling. Environmental Science and Pollution Research, 28(21), 27207–27217.