E-ISSN: 2378-654X

Recent Advances in Biology and Medicine

Original Research Article

Identification of Flavonoids from the Leaves Extract of Mangrove (*Rhizophora apiculata*)

HATASO, USA

Identification of Flavonoids from the Leaves Extract of Mangrove (*Rhizophora apiculata*)

Asma Nisar[1]*, Awang Bono[1], Hina Ahmad[2], Ambreen Lateef[3], Maham Mushtaq[4]
[1]Faculty of Engineering, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia.
[2]Jinnah University for Women Karachi, Karachi, Pakistan.
[3]College of Earth and Environmental Sciences, University of the Punjab, Lahore, Pakistan.
[4]Lahore College of Pharmaceutical Sciences, Lahore, Pakistan.

***Correspondence:** asmanisar1234@gmail.com

Received: Apr 30, 2019; Accepted: May 27, 2019

Citation: Nisar A, Bono A, Ahmad H, Lateef A, Mushtaq M. Identification of flavonoids from the leaves extract of mangrove (*Rhizophora apiculata*). Recent Adv Biol Med. 2019; Vol. 5, Article ID 869903, 7 pages. https://doi.org/10.18639/RABM.2019.869903

Abstract
A fast and specific reversed-phase high-performance liquid chromatography (HPLC) method was used for the immediate identification of flavonoids (gallic acid, rutin, quercetin, ascorbic acid, and kaempferol) in the leaves extract of Mangrove (*Rhizophora apiculata*). The *R. apiculata* has lots of valuable medicinal properties including antiallergic, anti-inflammatory, antimicrobial, antiviral, antioxidant, vascular antitumor activity, and enzyme inhibition; however, the activity of antioxidant is perhaps the greatest studied property attributed to flavonoids. Magnetic stirrer was used for the pretreatment process of sample with methanol by using a temperature of 50°C for 40 min, followed by separation on column size 250 mm × 4.6 mm (5 µm) Hypersil Gold C18 (Thermo Electron Corporation) with water–methanol–acetonitrile (45:40:15 v/v/v) containing acetic acid 1.0% as a mobile phase. Moreover, 254-nm wavelength was used to detect the extract. The standard retention times (Rt) of gallic acid, rutin, ascorbic acid, quercetin, and kaempferol were found to be 2.610, 2.875, 3.150, 5.789, and 8.983, respectively. The existence of gallic acid, rutin, ascorbic acid, kaempferol, and quercetin in Mangrove *leaves* extract was found matching according to the standard retention time. In Mangrove *leaves*, gallic acid was found to have the retention time at 2.538, rutin at 2.873, quercetin at 5.796, and kaempferol at 8.976. However, the ascorbic acid was not identified. The amount of rutin, gallic acid, quercetin, and kaempferol was calculated by using the assay formula. In Mangrove *leaves*, the amount of gallic acid, rutin, quercetin, and kaempferol is 3.024, 5.485, 5.144, and 8.361%, respectively.

Keywords: Mangrove (*Rhizophora apiculata*); Flavonoids; Rutin; Gallic acid; Ascorbic acid; Quercetin; Kaempferol; HPLC.

1. INTRODUCTION

The mangrove name is a mixture of a Portuguese word "Mangue," which means "tree," and an English word "grove," which means garden. Interestingly, mangrove comprises approximately 12 families and more than 50 species. Further terms suggested for synonymous include Mangrove swamp, Mangrove community, coastal woodland, and Mangrove ecosystem. Mangrove normally refers to a group of evergreen and salt-tolerant woody plants that have morphological adaptations. Extracts of mangrove plants have been used for eras for the numerous health disorder treatments. The substances that have been derived from the plants have attracted excessive attention because of their useful applications in treatment of different diseases [1]. Mangrove plants produce many secondary metabolites [2]. Several mangrove plants are used in traditional remedy, and mangrove plants' extracts have proven activity against animal, plant, and human pathogens; however, only limited research have been conducted to detect the metabolites responsible for their bioactivities. *Rhizophora apiculata*, which is one of the traditional medicinal mangrove species, is distributed along the southeast coast of India. Organic extracts of *R. apiculata* exhibited antimicrobial, antioxidant, anticancer, and antimalarial effect on experimental animal models [3].

1.1. Vernacular Name

The vernacular name of this plant is bakau minyak in Malaysia, and pyoo in Burma. In Indonesia, the name of this plant is bukan minyak, bako, and babakoan laut, and kongkang-bailek in Thailand. Table 1 illustrates the name of *R. apiculata* in different countries.

Figure 1: *Rhizophora apiculata*.

Figure 2: Leaves of *Rhizophora apiculata*.

Table 1: Vernacular names of *Rhizophora apiculata* [4].

Countries	Vernacular name
Brunei	Bakau minyak, bakau
Burma	Pyoo
Cambodia	Kaông ka:ng nhi:
Malaysia	Bakau minyak, bakau tandok, bakau akik
Indonesia	Bako (Javanese), bakau minyak (general), babakoan laut (Sundanese)
Papua New Guinea	Bahkweh (Northern Province), abia (Gulf Province), pana (Central Province)
Philippines	Uakatan (Tagalog), bakauan (lalaki), bakhau (Samar)
Singapore	Red-tree, bakau minyak
Thailand	Kongkang-bailek, kongkang
Vietnam	Cây du'ó'c.

1.2. Geographical Distribution/Ecology

R. apiculata is mostly grown in the tropical mudflats. This species is known as the fast growing tree species that is suitable for planting in coastal landward zones where natural regeneration is often inadequate after clear-felling (FAO 1994). Generally, *R. apiculata* is located in the coastal areas of the Asia Pacific region, including Thailand, Indonesia, Malaysia, Singapore, Pakistan, India, Sri Lanka, Taiwan, the Maldives, Papua New Guinea, Vanuatu, Vietnam, Micronesia, Australia (Queensland and the Northern Territory), and New Caledonia [5].

1.3. *Rhizophora apiculata* Classification

Table 2 shows the classification of *R. apiculate*

Table 2: *Rhizophora apiculata* classification [6].

Scientific classification	
Kingdom	**Plantae**
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Order	Malpighiales
Family	Rhizophoraceae
Genus	Rhizophora
Species	*R. apiculata*

1.4. Medicinal Uses

R. apiculata belongs to the Rhizophoraceae family. *R. apiculata* is a significant plant used in folk medicines in the Africa and Asia. A polysaccharide extracted from the leaf of *R. apiculata* inhibited HIV-1 or HIV-2 or SIV strains in various cell cultures [7]. Moreover, organic extracts of *R. apiculata* exhibited antimicrobial, antioxidant, anticancer, and antimalarial effect on experimental animal models [3].

2. METHOD(S)

2.1. Chemicals

The chemicals used for analysis purposes in this research are kaempferol, rutin, quercetin, gallic acid, ascorbic acid, acetonitrile, and methanol, and glacial acetic acid that were purchased by the Sigma-Aldrich made in the United States.

2.2. Extraction of Plant

In this study, 10 g of dried leaves powder of *R. apiculata* was added into a flask with 150 ml of methanol. The flask was placed onto a magnetic stirrer at 50°C for 40 min. After that, the solution was filtered with the help of filter paper. Furthermore, the filtered solution was concentrated using a rotary evaporator; then, the concentrate was collected and kept in a refrigerator at 4°C until the time of the experiment.

2.3. Isolation of Flavonoids from *R. apiculata* Using HPLC

Samples were analyzed by using Agilent 1200 high-performance liquid chromatography (HPLC) system, and the UV detector was set at 254 nm with a column size of 250 mm × 4.6 mm (5 µm) Hypersil Gold C18 (Thermo Electron Corporation). Furthermore, the combination of water, methanol and acetonitrile (45/40/15 v/v/v) has been used as a mobile phase [8]. The injection volume was 10 µl, and the flow rate was set at 0.7 ml/min.

2.4. Preparation of Standard and Sample Solutions

Methanol has been used as a solvent for the preparation of sample and standard. Moreover, 10 mg of standard kaempferol, rutin, gallic acid, ascorbic acid, and quercetin were dissolved in 25 ml of the solvent. For sample preparation, 10 mg of leaves extract of *R. apiculata* was dissolved in 10 ml of the solvent same as standard preparation [9].

The amount of standard kaempferol, rutin, gallic acid, ascorbic acid, and quercetin in *R. apiculata* leaves was calculated by using the following formula.

$$\%\ \text{Assay} = \frac{\textit{Sample Area}}{\textit{Standard Area}} \times \frac{\textit{Standard Weight}}{\textit{Standard Dilution}} \times \frac{\textit{Sample Dilution}}{\textit{Sample Weight}} \times \textit{Standard Purity}$$

3. RESULTS AND DISCUSSION

The identification of antioxidant compounds such as flavonoids, the secondary metabolites, is carried out because they are an important class of phytochemicals. Therefore, *R. apiculata* leaves were analyzed for the possible presence of these flavonoids. For this purpose, rutin, gallic acid, ascorbic acid, quercetin, and kaempferol were selected. The standard retention times (Rt) of gallic acid, rutin, ascorbic acid, quercetin, and kaempferol were found to be 2.610, 2.875, 3.150, 5.789,

and 8.983. The existence of gallic acid, rutin, quercetin, ascorbic acid, and kaempferol in *R. apiculata* leaves extract was found matching according to the standard retention time. Figures 3–8 illustrate the comparison between standard and *R. apiculata* leaves. And tables 3 and 4 shows the retention time, area, height, and % area of standard and sample.

Figure 3: Chromatogram of gallic acid.

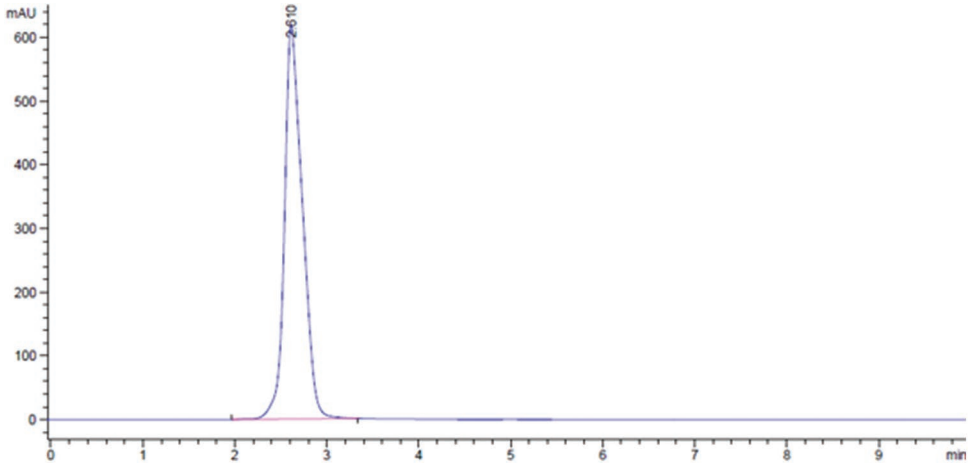


Figure 4: Chromatogram of rutin.

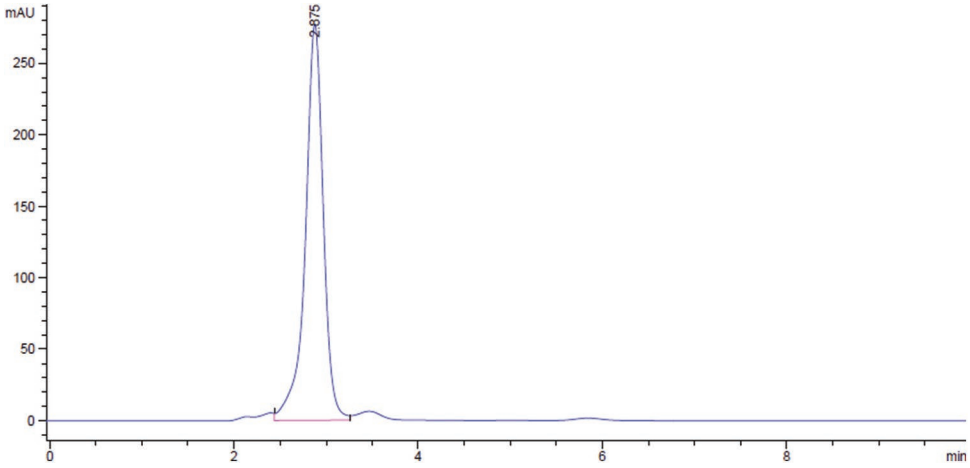


Figure 5: Chromatogram of ascorbic acid.

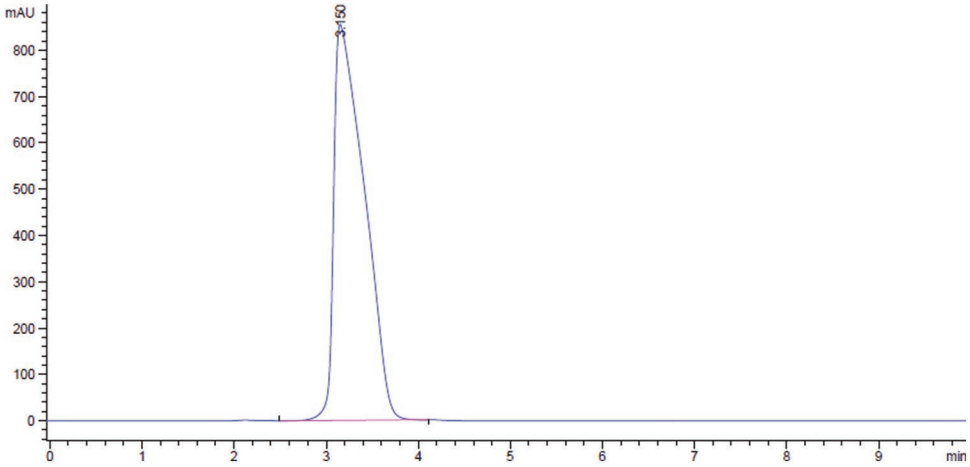


Figure 6: Chromatogram of quercetin.

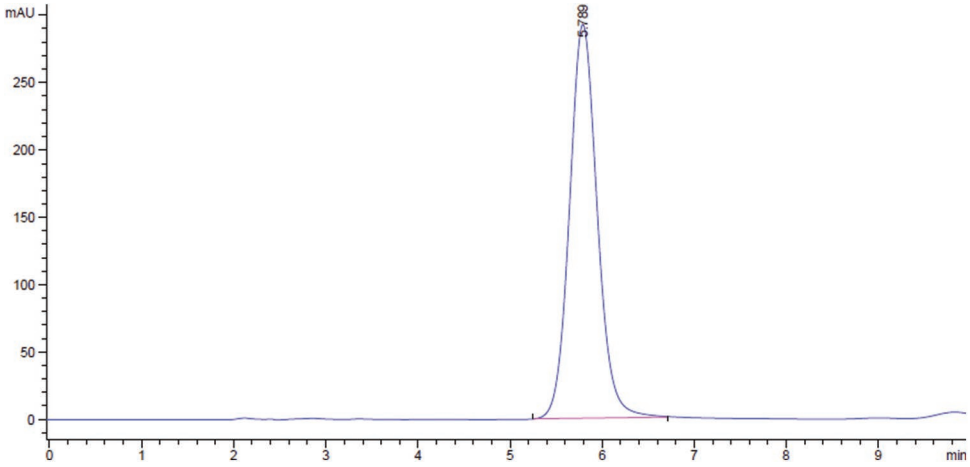


Figure 7: Chromatogram of kaempferol.

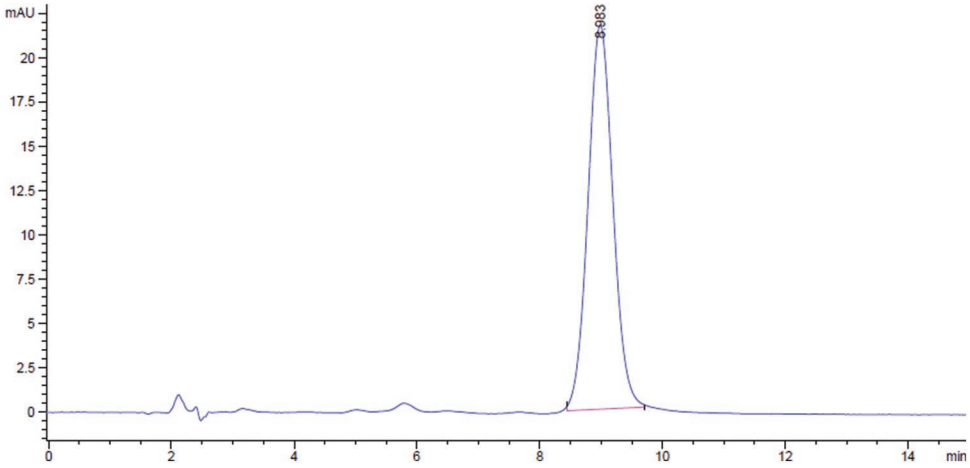
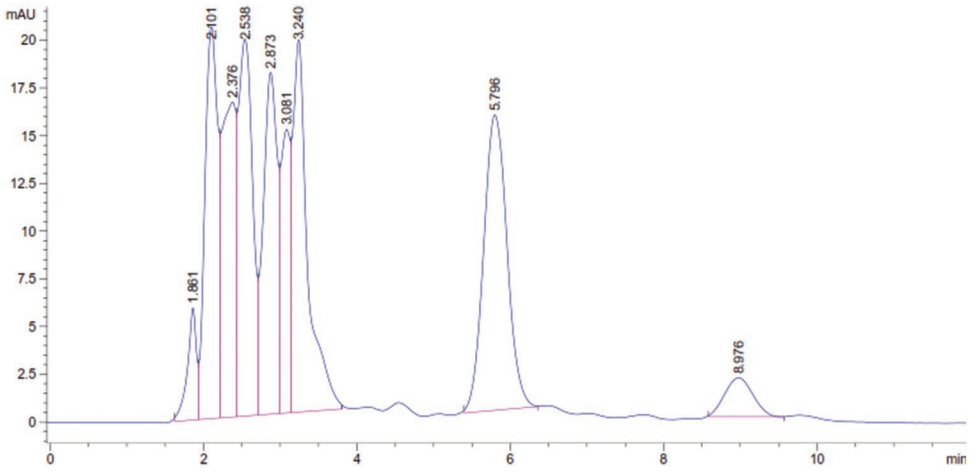


Figure 8: Chromatogram of *Rhizophora apiculata*.



 https://doi.org/10.18639/RABM.2019.869903

Table 3: Retention time, area, height, and % area of standards gallic acid, rutin, ascorbic acid, quercetin, and kaempferol.

Standards	Retention time (min)	Area (m V.s)	Height (mV)	Area (%)
Gallic acid	2.610	8416.88379	619.74945	100.00
Rutin	2.875	3771.66162	277.45462	100.00
Ascorbic acid	3.150	19602.3	855.53058	100.00
Quercetin	5.789	6126.98340	292.44537	100.00
Kaempferol	8.983	611.26239	21.69806	100.00

Table 4: Retention time, area, height, and % area of standards gallic acid, rutin, quercetin, and kaempferol in the leaves extract of *Rhizophora apiculata*.

Standards	Retention time (min)	Area (m V.s)	Height (mV)	Area (%)
Gallic acid	2.538	254.86652	19.77791	14.5173
Rutin	2.873	228.80684	17.93952	13.0329
Quercetin	5.796	328.67783	15.4909	18.7216
Kaempferol	8.976	52.19215	2.03947	2.0729

In *R. apiculata* leaves, gallic acid was found to have the retention time at 2.538, rutin at 2.873, quercetin at 5.796, and kaempferol at 8.976. However, the ascorbic acid was not identified.

The amount of gallic acid, quercetin, rutin, and kaempferol was calculated using the assay formula. In the Mangrove *leaves*, the amount of gallic acid, rutin, quercetin, and kaempferol is 3.024, 5.485, 5.144, and 8.361%, respectively.

In 2015 [3], Satyavani *et al.* identified rutin, quercetin, and kaempferol from the ethanolic leaves extract of *R. apiculata* using HPLC. They used the mixture of alcohol, water and hydrochloric acid as a extraction solvent and mixture of methanol, water and phosphoric acid as a mobile phase. HPLC is equipped with a 270 nm detector and a 4.6 mm $\times$ 25 cm column with a flow rate of about 1.5 ml per minute. Injected volume was 20 µl. The amount of rutin, quercetin, and kaempferol was 4.5, 5.6, and 7.6 w/v. In 2012 [2], Asha *et al.* also identified the rutin, quercetin, and gallic acid from the ethanolic root extract of *R. apiculata*.

4. CONCLUSION

The drug development field researchers worldwide used floral populations for cost-effective, low side-effect, and nontoxic medicinal product development. Mangrove-derived metabolites, especially phenolic compounds, are largest and ubiquitous groups that could make the plant material useful for potential antioxidant and antidiabetic activities, and some of them are involved in the drug development process. HPLC results evidenced that the *R. apiculata* contains flavonoids. This investigation concludes that *R. apiculata* has flavonoids. *R. apiculata*-derived flavonoids have antioxidant, antidiabetic, and antibacterial activity.

Acknowledgment
No financial and material support.

Author Contributions
All authors contributed equally to this study.

Conflict of Interest
There is no conflict of interest.

References

1. Shelar P, Reddy S, Shelar G, Reddy V. Medicinal value of mangroves and its antimicrobial properties—a review. Cont J Fish Aquat Sci. 2012; 6:26-37.
2. Asha KK, Mathew S, Lakshmanan PT. Flavonoids and phenolic compounds in two mangrove species and their antioxidant property. Indian J Mar Sci. 2012; 41(3):259-64.
3. Satyavani K, Gurudeeban S, Ramanathan T, Muthusankar A. Influence of *Rhizophora apiculata* flavonoids on chemical and thermal induced nociceptive models. Br J Pharm Res. 2015; 7(2):102-9

4. *Rhizophora apiculata* (PROSEA) – PlantUse English. [Online]. Retrieved January 5, 2019. https://uses.plantnet-project.org/en/Rhizophora_apiculata_(PROSEA)
5. Hassan MK, Jintana V, Kuittinen S, Pappinen A. Management practices and aboveground biomass production patterns of *Rhizophora apiculata* plantation: study from a Mangrove Area in Samut Songkhram Province, Thailand. BioResources. 2018; 13(4):7826-50.
6. *Rhizophora apiculata* : tall-stilted mangrove. https://bie.ala.org.au/species/http://id.biodiversity.org.au/node/apni/2886167
7. Vinod Prabhu V, Guruvayoorappan C. Anti-inflammatory and anti-tumor activity of the marine mangrove *Rhizophora apiculata*. J Immunotoxicol. 2012; 9(4):341-52.
8. Zu Y, Li C, Fu Y, Zhao C. Simultaneous determination of catechin, rutin, quercetin kaempferol and isorhamnetin in the extract of sea buckthorn (*Hippophae rhamnoides* L.) leaves by RP-HPLC with DAD. J Pharm Biomed Anal. 2006; 41(3):714-19.
9. Srinivasa Rao A, Ahmed MF. Simultaneous determination of phenolic compounds in *Catharanthus roseus* leaves by. Int J Pharm Sci Res. 2014; 5(3):976-81.