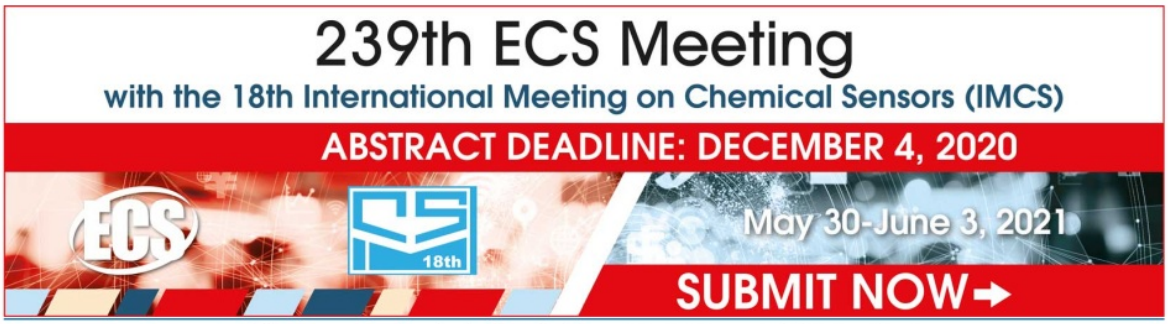
Phytochemical Analysis of Mangrove Leaves (Rhizophora sp.)

by Niken Subekti

Submission date: 03-Dec-2020 10:01AM (UTC+0700)
Submission ID: 1463104768
File name: Phytochemical_Analysis_of_Mangrove_Leaves_Rhizophora_sp.pdf (781.15K)
Word count: 3790
Character count: 20946

Phytochemical Analysis of Mangrove Leaves (*Rhizophora* sp.)



IOP Conf. Series: Materials Science and Engineering **593** (2019) 012007 doi:10.1088/1757-899X/593/1/012007

Phytochemical Analysis of Mangrove Leaves (*Rhizophora* sp.)

Syahidah[1*], N Subekti[2]

[1]Forestry Faculty, Hasanuddin University Jl. Perintis Kemerdekaan Km. 10 Tamalanrea, Makassar 90245, Indonesia
[2]Faculty of Mathematics and Natural Science, State University of Semarang, Central Java, Indonesia

*Email : syahidah@unhas.ac.id

Abstract. Mangrove plants have been reported as resources of many traditional folk of medicine and contain several kinds of trace elements which are being used to treat diseases. The aim of this study was to analyzed the phytochemical compounds of mangrove leaves. Our previous studies showed that the extract of mangrove leaves tend to be polar. The acetone and methanol extract of the mangrove leaves had high anti termite and antifungal activity. Based on this information, phytochemical content of mangrove leaves have been analyzed to find out the chemical substances that responsible for the bioactivity properties. Folin-Ciocalteu method was used to quantify the total phenolic content using a calibration curve of gallic acid, while for phytochemical were analyzed using spectrofotometric method. The results showed that the acetone extract of mangrove leaves containing alkaloid, polyphenolic, flavonoid, and total tannin higher than the methanol extract. This data will be further analyzed to obtain the compounds that may responsible for the biological performance of mangrove leaves.

1. Introduction

Mangroves are one of the most productive and unexplored ecosystem that approximately covers one fourth of the world coastline with high diversity of thriving organisms. Mangroves support the conservation of biological diversity for a number of endangered species by providing habitats, nurseries, nutrients, and spawning grounds [1]. Mangroves play also a key role in human sustainability and livelihoods, being heavily used for food, timber, fuel and medicine. They offer protection from catastrophic events, such as tsunami, tropical cyclones and tidal bores and can dampen shoreline erosion [2].

Mangroves contribute to numerous environmental services, including trapping and recycling organic matter, providing shelters and surfaces for terrestrial and aquatic organisms, and contributing to the overall health of coastal environments. The mangrove ecosystem represent an inter phase between terrestrial and marine communities, which receive a daily input of water from the ocean (tides) and freshwater, sediments, nutrients and silt deposits from upland rivers. Mangroves may grow as trees or shrubs according to the climate, salinity of water, topography and edaphic features of the area in which they exist. Mangroves are salt-tolerant evergreen forests found along sheltered coastlines, shallow-water lagoons, estuaries, rivers or deltas in 124 tropical and subtropical countries and areas [3][4].

Mangroves also are well known for their ecological importance and a rich source of several bioactive compounds such as steroids, triterpenes, saponins, flavonoids, alkaloids and tannins having therapeutic significance. These plants have been extensively used in traditional medicine and different studies have

revealed their activity against human, animal and plant pathogens. The leaf and stem extracts of *S. maritima* are rich source of natural antioxidant with moderate antimicrobial activities. It has also been reported that these are an excellent source of antiviral compounds as compared to the seaweeds and sea grasses [5][6]. The ethno-pharmacological consequence pointed out that the mangrove plants are traditionally used for the treatment of rheumatism, painful arthritis, inflammation, asthma, antioxidant, free radical scavenging, anti- inflammatory, antinociceptive, diabetes and hepato-protective actions [7][8]. Apart from medicinal purposes, mangroves are also used as flavoring agents, in textiles, mats, paper, housing, baskets, boats and tapa cloth, tannins etc. [1].

Our previous studies showed that the extract of mangrove leaves tend to be polar. The acetone and methanol extract of the mangrove leaves showed high anti termite and antifungal activity [9].

2. Material and Methods

2.1. Material

Mangrove (*Rhizophora* sp.) fresh and mature leaves was collected from the mangrove forest in Semarang, Central Java Province, Indonesia.

2.2. Extraction and Fractionation

The mangrove leaves were dried and grounded in a hammer mill. The leaves mill (40-60 mesh) then extracted according to the procedure reported previously with slight modification [10]. In this study, leaves mill was used instead of small wood-pieces and the leaves mill was extracted with acetone first and the residue was then extracted again using methanol until the extract solution became colorless. These acetone and methanol extracts were then successively fractionated into n-hexane, ethyl acetate, and water to give their soluble fractions.

2.3. Phytochemical Screenings

The acetone and methanol extracts were tested for the presence of alkaloids, steroids, tannins, saponins and terpenoids. The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

2.3.1. Test for alkaloids. Few mg (about 15 mg) of each extract was separately stirred with 1% HCl (6 mL) on a water bath for 5 min and filtered. These filtrates were divided into three equal parts.

a. Dragendorff's test: to one portion of the filtrate, Dragendorff's reagent (Potassium bismuth iodide solution) (1 mL) was added; an orange red precipitate shows the presence of alkaloids.
b. Mayer's test: to one portion of filtrate, Mayer's reagent (Potassium mercuric iodide solution) (1 mL) was added. Formation of cream colored precipitate gives an indication of the presence of alkaloids.
c. Wagner's test: Potassium iodide (2 g) and iodine (1.27 g) were dissolved in distilled water (5 mL) and the solution was diluted to 100 mL with distilled water. Few drops of this solution were added to the filtrate; a brown colored precipitate indicates the presence of alkaloids [11][12].

2.3.2. Test for steroids and terpenoids.

a. Salkowski test: The crude extract (about 100 mg) was separately shaken with chloroform (2 mL) followed by the addition of concentrated H_2SO_4 (2 mL) along the side of the test tube, a reddish brown coloration of the interface indicates the presence of terpenoid [13].
b. Liebermann-Burchard test: Each extract (100 mg) was shaken with chloroform in a test tube; few drops of acetic anhydride was added to the test tube and boiled in a water bath and rapidly cooled in iced water. Concentrated H_2SO_4 (2 mL) was added alongside of the test tube. Formation of a brown ring at the junction of two layers and turning the upper layer to green shows the presence of steroids while formation of deep red color indicates the presence of triterpenoids [11].

2.3.3. Test for tannin.

a. Preparation of a Standard Solution of 1000 ppm Tannic Acid
   0.1 gram of tannic acid was dissolved in 100 ml of distilled water. This standard solution should be made new every will running a test [14]. The dilution series preparation were 4, 8, 12, 16, 20 ppm. Each 1 ml was taken from the dilution series and put in a 10 ml pumpkin container containing 35 ml of aquadestilata. In the flask, 0.5 ml of reagent folin type was added, left for 3 minutes and added 1.2 ml of saturated Na_2CO_3 7.5% solution, incubated for 15 minutes. Then the absorption is read at a wavelength of 740 nm.
b. Determination of maximum absorption wavelength
   Taken one of the concentrations of the standard solution, its absorption is measured in the wavelength range of 400-800 nm. The wavelength that shows the highest absorption value is the maximum wavelength. The standard curve is made by connecting the concentration of standard solutions with the absorption results obtained from measurements using a UV-VIS spectrophotometer at a wavelength of 740 nm.
c. Determination of Tannin Levels
   0.5 gram of maserate is weighed and dissolved with aquadest until reach 10 ml volume. If the sample hasn't dissolved completely, it can be apply a device to homogenize the solution. Carefully piped 1.0 ml of sample, put in a 10 ml container containing 7.5 ml of aquadest. 0.5 ml of Folin Denis reagent was added, left to stand for 3 minutes, added 1.0 ml of saturated Na2CO3 solution. Incubated for 15 minutes, then read up at maximum wavelength. Calculated using the standard curve that has been obtained so that the concentration of the measured sample is known.

2.3.4. Test for saponin. Each of extracts (0.5 g) was separately shaken with distilled water (10 mL) in a test tube. The formation of frothing, which persists on warming in a water bath for 5 min, shows the presence of saponins [15].

2.4. Determination of total phenolic contents (TPC) and total flavonoid contents (TFC)

2.4.1. Total Phenolic Content. Total phenolic content was analyzed using the Folin–Ciocalteu colorimetric method with some modifications[16][17]. An aliquot of 0.3 mL of extract was mixed with Folin–Ciocalteu phenol reagent (2.25 mL). After 5 min, 6% sodium carbonate (2.25 mL) was added and the mixture was allowed to stand at room temperature for 90 min. The absorbance of the mixture was measured at 725 nm. Standard calibration curve for gallic acid in the range of 0–200 lg/mL was prepared in the same manner and results were expressed as mg gallic acid equivalent (GAE) per gram of extract.

2.4.2. Total Flavonoid Content. Total flavonoid content was determined using the aluminum colorimetric method [18][19] with some modifications using quercetin as the standard. A calibration curve of quercetin was prepared in the range of 0–200 lg/ mL. Briefly, extract (0.5 mL) and standard (0.5 mL) were placed in different test tubes and to each 10% aluminum chloride (0.1 mL), 1 M potassium acetate (0.1 mL), 80% methanol (1.5 mL) and distilled water (2.8 mL) were added and mixed. A blank was prepared in the same manner where 0.5 mL of distilled water was used instead of the sample or standard, and the amount of aluminum chloride was also replaced by distilled water. All tubes were incubated at room temperature for 30 min. The absorbance was taken at 415 nm. The concentration of flavonoid was expressed as mg quercetin equivalent (QE) per gram of extract.

3. Results and Discussion

3.1. Phytochemical Screening

The results of the phytochemical test carried out on the extracts were recorded as shown in Table 1. Preliminary phytochemical screening revealed the presence of alkaloid, flavonoids, phenolic, and tannin in both of the acetone and methanol extracts. On the contrary, terpenoid, steroid and saponin were absent

in both of the extracts. Alkaloid were much abundant in acetone crude extract according to Dragendorf and Wagner methods, but less abundant according to Mayer method. Alkaloid was found less abundant in methanol crude extract according to Wagner method, but found minute according to Dragendorf and Wagner methods.

Phytochemical constituents in the various part of the plant vary significantly. Several medicinal plants are used in traditional medicines for curing many diseases. These plants have been extensively used in traditional medicine and different studies have revealed their activity against human, animal and plant pathogens. The leaf and stem extracts of *S. maritima* are rich source of natural antioxidant with moderate antimicrobial activities. It has also been reported that these are an excellent source of antiviral compounds as compared to the seaweeds and sea grasses [5][6]. The study revealed that flavonoids and phenol were found to be present the extract. Several studies reported that, flavonoids show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity. Tannin and phenol are necessary for the animal body for repair and maintenance. Flavonoids are now considered as an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function [20].

Table 1. Phytochemical screening of acetone and methanol extracts of mangrove leaves

Extract	**Alkaloid**			**Terpenoid and Steroid**	**Saponin**
	Mayer	Dragendorf	Wagner		
Acetone extract	++	+++	+++	-	-
Methanol Extract	+	+	++	-	-

Legend: +++ (Much abundant); ++ (Less abundant); + (Minute); - (Absent)

The phytochemical compounds detected are known to have medicinal properties. For example, alkaloids have been reported as powerful poison and many alkaloids derived from medicinal plants show biological activities like, anti-inflammatory [21], antimalarial [22], antimicrobial [23], cytotoxicity, antispasmodic and pharmacological effects [24][25]. Similarly, steroids derived from plants are known to have cardiotonic effect and also possess antibacterial and insecticidal properties [26]. They are very often used in medicines due to their well-known biological activities. Tannins, according to research, are known to have antibacterial [27], antitumor and antiviral activities ([28]. These alkaloids show bioactivity against Gram-positive bacteria and cytotoxicity against leukemia and HeLa cell lines [29]. Alkaloids, flavonoids and xanthones that are potent inhibitors of various oxidative processes in both in vitro and in vivo system. These phytochemical compounds identified in the extracts may be responsible for the biological activities of the mangrove leave extract.

3.2. Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC)

The results as shown in Table 2 revealed that the acetone extract of mangrove leaf has higher values in phenolic, flavonoid and tannin contents compared to the methanolic extract. The total phenolic content was different when extracted with different solvents [30]. Plant polyphenols are the significant group of compounds acting as free radical scavenging or primary antioxidants; therefore, it is justifiable to determine phenolic content in plant extract. Polyphenolic compounds have an aromatic benzene ring with substituted hydroxyl groups, including their functional derivatives. These are able to absorb free radicals and can chelate metal ions that could catalyze formation of ROS which promotes lipid peroxidation. Among polyphenols, flavonoids are of great importance because they help human body to fight against diseases. The ability of flavonoids to act as potent antioxidants depends on their molecular structures, the position of the hydroxyl group and other features in its chemical structure. They are abundantly found in plants as their glycoside [31]. The most abundant flavonol which has a good antioxidant property is quercetin, as it has all the right structural features for free radical scavenging activity [32].

Table 2. Total Phenolic Contents (TPC), Total Flavonoid Contents (TFC) and Total Tannin Contents (TTC) of acetone and methanol extracts of mangrove leaves

Extract	TPC (mg GAE/g)	TFC (mg QE/g)	TTC (%)
Acetone extract	10.024	2.641	6.703
Methanol extract	2.225	1.998	2.736

Tannin and polyphenols extracted from mangal species have also been reported to have cytotoxic, anti-neoplastic, antibacterial and anti-helmintic activities. Saponin and limonoids are reported to possess antimicrobial, inflammatory, cytotoxic, antifeedant and growth regulatory activity against a number of pathogens [1].

Mangroves have been a source of several bioactive compounds. Secondary metabolites like alkaloids, phenolics, saponins, flavonoids, steroids and terpenoids have been characterized from mangroves and have been tested successfully for toxicological, pharmaceutical and ecological importance. Studies on the bioactive compounds of mangrove plants often lead to the discovery of new therapeutic agents. The mangrove plants possess a number of biological activities such as antibacterial, antioxidant, anticancer, cytotoxic, antiproliferative, insecticidal, antimalarial, antifungal, antifeedant, antidiarrheal, central nervous system depressant, antimitotic, antileukemic and anti-plasmodial activities [33][34].

References

[1] Gouda S, G. Das, J K Patra 2015 *Mangroves: A Rich Source of Natural Bioactive Compounds*. *Recent Advances in Natural Products* (USA: Studium Press LLC)

[2] Carugati L, B Gatto, E Rastelli, M L Martire, C Coral, S Greco, R Danovaro 2018 *Impact of mangrove forests degradation on biodiversity and ecosystem functioning*. Scientific Reports (2018) 8:13298. www.nature.com/scientificreports/

[3] Hakim, L, D Siswanto, N Nakagoshi 2017 Mangrove Conservation in East Java: The Ecotourism Development Perspectives *The J of Tropical Life Sci.* **7**(3) pp 277–285

[4] Blankespoor B, S Dasgupta, G M Lange 2016 Mangrove as Protection from Storm Surges in a Changing Climate Policy Research Working Paper 7596 World Bank Group Development Data Group Surveys and Methods Team & Development Research Group Environment and Energy Team, March 2016.

[5] Patra J K, N K Dhal, H N Thatoi 2011 In vitro biaoctivity and phytochemical screening of Suaeda maritima (Dumort): A mangrove associate from Bhitarkanika, India *Asian Pacific Journal of Tropical Medicine* (2011) 727–734

[6] Sahoo, G, N S S Mulla, Z A Ansari, C Mohandass 2012 Antibacterial Activity of Mangrove Leaf Extracts against Human Pathogens. *Indian J. of Pharm. Sci.* **74** (4) pp 348–351

[7] Roome T, Dar A, and Naqvi A 2011 Evaluation of antinociceptive effect of Aegiceras corniculatum stems extracts and its possible mechanism of action in rodents *Journal of Ethnopharmacology* **135** pp 351–358

[8] Prabhakaran J and D Kavitha 2012 Ethnomedicinal importance of mangrove species of Pitchavaram. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, **3**(2) pp 611–614

[9] Syahidah and N Subekti 2018 *Biological Activity of Mangrove Leaves Extract (Rhizophora sp.).* (Makassar, Indonesia: Proceeding of The 1st Biennial Conference of Tropical)

[10] Wang S Y, P F Chen, S T Chang 2005 Antifungal activities of essential oils and their constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) leaves against wood decay fungi. *Bioresour. Technol.* **96** pp 813–818

[11] Joshi A, Bhobe M, Saatarkar 2013 A Phytochemical investigation of the roots of Grewia microcos Linn. *J. Chem. Pharm. Res.* **5** pp 80– 87

[12] Abdullahi M N, Ilyas N, Ibrahim H 2013 Evaluation of phyto- chemical screening and analgesic activity of aqueous extract of the leaves of Microtrichia perotitii dc (Asteraceae) in mice using hotplate method. *Med. Plant Res*. **3** pp 37–43

[13] Ayoola G A, Coker H B, Adesegun S A, Adepoju-Bello A A, Obaweya K, Ezennia E C, Atangbayila T O 2008 Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria. *Trop. J. Pharm. Res*. **7** pp 1019–1024
[14] Cunnif P 1996 *Official Method Of Analysis Of AOAC International sixteeth edition Vol II*, (USA: AOAC international Suite 500, 481 North freederick Avenue Gaithersburg: Maryland 20877-2417)
[15] Banso A, Adeyemo S, 2006 Phytochemical screening and anti- malarial assessment of Abutilon mauritianum, Bacopa monnifera and Datura stramonium. *Biokemistri* **18** pp 39–44
[16] Cai Y Z, Luo Q, Sun M, Corke H, 2004 Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci*. **74** pp 2157–2184
[17] Chlopicka J, Pasko P, Gorinstein S, Jedryas A, Zagrodzki P 2012 Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads. LWT – *Food Sci. Tech*. **46** pp 548–555
[18] Chang C, Yang M, Wen H, Chern J 2002 Estimation of total flavonoid content in propolis by two complementary colorimetric methods *J. Food Drug Anal*. **10** pp 178–182
[19] Stankovic M S 2011 Total phenolic content, flavonoid concentra- tion and antioxidant activity of *Marrubium peregrinum* L. extracts. *Kragujevac J. Sci*. **33** pp 63–72
[20] A N Pache, A D Diwan, S R Chandra 2016 Flavonoids: An overview. *J Nutr Sci* **2016** (5) pp 1–15
[21] Augusto L S, Josean F T, Marcelo S, Margareth F M, Petronio F A, Jose M B 2011 Anti-inflammatory activity of alkaloids: an update from 2000 to 2010 *Molecules* **16** pp 8515–8534
[22] Dua V K, Gaurav V, Bikram S, Aswathy R, Upma B, Dau D A, Gupta N C, Sandeep K, Ayushi R 2013 Anti-malarial property of steroidal alkaloid conessine isolated from the bark of Holarrhena antidysenterica. *Malaria J*. **12** pp 1–6
[23] Benbott, A., Yahyia, A., Belaı¨di, A., 2012. Assessment of the antibacterial activity of crude alkaloids extracted from seeds and roots of the plant Peganum harmala L. J. Nat. Prod. Plant Resour. 2, 568–573.
[24] Ameyaw, Y., Duker-Eshun, G., 2009. The alkaloid contents of the ethno-plant organs of three antimalarial medicinal plant species in the eastern region of Ghana. Int. J. Chem. Sci. 7, 48–58.
[25] Thite S V, Chavan Y R, Aparadh V T, Kore B A 2013 Preliminary phytochemical screening of some medicinal plants. *Int. J. Pharm. Chem. Biol. Sci*. **3** pp 87–90
[26] Alexei Y B, Joseph I S, Olga V F 2009 Endogenous cardiotonic steroids: physiology, pharmacology and novel therapeutic targets. *Pharmacol. Rev*. **61** pp 9–38
[27] Hisanori A, Kazuyasu F, Osamu Y, Takashi O, Keiji I 2001 Antibacterial action of several tannins against Staphylococcus aureus. *J. Antimicrob. Chemother* **48** pp 487–491
[28] Kumari M, Jain S 2012 Review paper, Tannins: an antinutrient with positive effect to manage diabetes. Res. *J. Recent Sci*. **1** pp 70–73
[29] Omar S, Chee C L, Fasihuddin A, Ni J X, Jaber H, Huang J, Nakatsu T 1992 Phenanthrene lactams from Goniothalamus velutinus. *Phytochem* **31** 4395–4397
[30] Abdelwahab S I, Abdul A B, Elhassan M M, Mohan S, Al- Zubairi A S, Mariod A A 2009 Antimicrobial and free radical scavenging activities of dichloromethane extract of Goniothalamus umbrosus. *Int. J. Trop. Med*. **4** 32–36
[31] Rajanandh M G, Kavitha J 2010 Quantitative estimation of b- sitosterol, total phenolic and flavonoid compounds in the leaves of Moringa oleifera. *Int. J. Pharm Tech Res*. **2** pp 1409–1414
[32] Kalita P, Barman T K, Pal T K, Kalita R, 2013. Estimation of total flavonoids content (TFC) and antioxidant activities of methanolic whole plant extract of Biophytum sensitivum linn. *J. Drug Delivery Ther*. **3** pp 33–37
[33] Patra J K and Thatoi H N 2011 Metabolic diversity and bioactivity screening of mangrove plants: A review. *Acta Physiologiae Plantarum* **33** pp 1051–1061
[34] Thatoi H N, Patra J K and Das S K 2011 Free radical scavenging and antioxidant potential of

mangrove plants: a review. *Acta Physiologiae Plantarum* **36**(3) pp 561– 579

Phytochemical Analysis of Mangrove Leaves (Rhizophora sp.)

ORIGINALITY REPORT

23%	20%	16%	13%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

1%

★ Feras Q. Alali, Khaled Tawaha, Tamam El-Elimat, Maha Syouf et al. "Antioxidant activity and total phenolic content of aqueous and methanolic extracts of Jordanian plants: an ICBG project", Natural Product Research, 2007

Publication

Exclude quotes	On	Exclude matches	< 15 words
Exclude bibliography	Off		