

Artikel

by Madyawati Latief

Submission date: 29-Mar-2021 11:36AM (UTC+0700)

Submission ID: 1545093804

File name: vity_of_isolated_compound_from_perepat_roots_Sonneratiaalba.pdf (1.52M)

Word count: 3263

Character count: 16934

PAPER • OPEN ACCESS

Antioxidant activity of isolated compound from perepat roots (*Sonneratia alba*)

To cite this article: M Latief ⁹ *et al* 2019 *J. Phys.: Conf. Ser.* **1282** 012088

View the [article online](#) for updates and enhancements.



The Electrochemical Society
Advancing solid state & electrochemical science & technology

240th ECS Meeting ORLANDO, FL

Orange County Convention Center Oct 10-14, 2021

Abstract submission due: April 9

SUBMIT NOW

This content was downloaded from IP address 110.137.174.239 on 28/03/2021 at 02:44

Antioxidant activity of isolated compound from perepat roots (*Sonneratia alba*)

M Latief*, A Utami, H Amanda, Muhaimin, and Z Afifah

Chemistry Department, Faculty of Science and Technology, Universitas Jambi, Jalan Jambi-Ma Bulian KM 15, Mendalo Darat Jambi

Email: * madyawatilatief@unja.ac.id

Abstract. *Perepat* (*Sonneratia alba*) is one of the mangrove plants found in Tanjung Jabung Timur, Jambi Province. Research on *perepat* plants has been widely carried out on leaves, fruit and bark, but there is no research on *perepat* roots, so it is necessary to do research on *perepat* roots. This study aims to determine the compounds found in ethyl acetate extract from roots which have antioxidant activity. Extraction and fractionation of *perepat* roots were carried out in multilevel maceration. The isolation technique was carried out using vacuum column chromatography and gravity column chromatography. The isolated compound was identified using UV-Vis spectrophotometer, FT-IR and NMR. Antioxidant activity test of the isolated compound was carried out by DPPH method. Characterization using UV-Vis gives maximum absorption at wavelengths 268 and 313 nm. While the characterization with FT-IR provide information that the isolate containing functional groups of OH, C=O, CH₃ and CH₂. Further identification using NMR suggests that isolate are a mixture of stigmasterol and β -sitosterol compounds, known as phytosterols. Antioxidant activity testing of ethyl acetate extract and isolate gave IC₅₀ values of 223.67 and 439.71 ppm, respectively, indicating that these compounds were classified as less active but still potential as antioxidants.

1. Introduction

Sonneratia alba or commonly known by the community as perepat, which one of the mangroves found on the coasts of countries in Asia, among others in Indonesia, Malaysia, Philippines, India and China. In several regions in Indonesia such as Jawa, Sulawesi and Maluku, the mangrove species *Sonneratia alba* have been used as medicine, drinks and as raw materials for the manufacture of various cakes [1]. The leaves of perepat (*Sonneratia alba*) have antibacterial activity [2]. According to Kusyana [3] *Sonneratia alba* has been widely used by coastal communities traditionally for medicine and food ingredients, and the leaves of *Sonneratia alba* has an activity as antioxidants.

Antioxidants are compounds that can inhibit the oxidation rate of other molecules or neutralize free radicals. Antioxidants are needed to prevent oxidative stress which is an imbalance condition between the available number of free radicals and the amount of the antioxidants in the body [4]. S and Jayaprakasha [5] state that the antioxidant properties of plant extracts are generally caused by phenolic compounds, such as flavonoids, phenolic acids, and tannins.

Based on research conducted by Herawati [6], compounds isolated from the bark of *Sonneratia alba* are phenolic group compounds with lactone rings. This compound is likely to contribute to the antioxidant activity of various plant extracts. The selection of ethyl acetate fraction was carried out based on research conducted by [7]. Ethyl acetate extract had the highest total phenol content of 377.250 mg / g, which was concluded that with high phenol levels the antioxidant activity was also



Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.

Published under licence by IOP Publishing Ltd

high. Likewise, the research conducted by Latief *et al.* [8] of ethyl acetate fraction of *Sonneratia alba* leaves and fruit showed antioxidant activity at a concentration of 1000 ppm with inhibition percentages of 79.43% and 69.98%, respectively. Further testing of antioxidant activity with CAP-e method was carried out, which provides information on how antioxidants can scavenge free radicals in red blood cells. This study focuses on knowing the ability of a compound to diminish oxidative stress in red blood cells with thalassemia. The results indicated the activity of scavenging free radicals in normal red blood cells, nature carriers and thalassemia patients [9]. Another study conducted by Papatungan [10], showed that *Sonneratia alba* fruit had antioxidant activity with an IC₅₀ value of 296.54 ppm.

Based on the description above manifested that the leaves, bark and fruit have antioxidant activity. But there is not much information about the chemical content and bioactivity at the root, so it is necessary to do research on *Sonneratia alba* roots. It can be predicted that the root part also has an opportunity as a source of antioxidants.

29

2. Materials and methods

2.1. Plant materials

Sonneratia alba samples in this study were obtained from the river coast in Kampung Laut Village, Kuala Jambi district, Tanjung Jabung Timur Regency, Jambi Province. The sample was then determined in the Laboratory of Biotechnology and Engineering, Faculty of Science and Technology, Jambi University.

2.2. Isolation and purification of compounds

Sonneratia alba roots that have been mashed, then macerated several times using n-hexane solvents until the macerate obtained was no longer colored. Maserate was collected and the solvent was evaporated with a rotary evaporator to obtain a thick n-hexane extract. The residue was dried until the n-hexane solvent evaporated and then macerated with ethyl acetate. Maserate was collected and the solvent was evaporated with a rotary evaporator to obtain thick ethyl acetate extract. Then a phytochemical screening test was carried out on ethyl acetate extract.

Compound isolation was carried out on the most active extract in antioxidant activity, namely ethyl acetate extract. Initial fractionation of ethyl acetate extract was carried out using vacuum column chromatography using silica gel stationary phase. The samples were prepared by pre-absorbed and eluted using n-hexane: ethyl acetate solvent gradient. Eluate was collected in a bottle and each was checked using a Thin Layer Chromatography (TLC) to be grouped. Each fraction was evaporated and tested for antioxidant activity using DPPH stain sprayers. The active stains on the TLC plate were then separated and purified by column chromatography techniques and recrystallized until pure active compounds were obtained.

2.3. Characterization and identification of isolated compounds

Compound characterization was carried out using a UV-Vis spectrophotometer (Biochrom Libra S70) with wavelength of 200-400 nm and FT-IR spectrophotometer (Perkin Elmer) at a wave number of 4000-400 cm⁻¹. Furthermore, isolation compounds were also identified using spectroscopic methods, included ¹H NMR and ¹³C NMR, spectra were recorded using CDCl₃ as solvent.

2.4. Antioxidant activity test of isolated compounds

Testing of antioxidant activity was carried out by DPPH method [11]. For determination of antioxidant activity, samples used ethyl acetate extract and isolated compounds with concentration variations of 0-500 ppm and 0-200 ppm, respectively. A total of 0.2 ml of sample solution was pipette with a micro pipette into vial, then added 3.8 ml of 400 ppm DPPH solution. The mixture was homogenized and incubated for 30 minutes in a dark place. Absorbance value was measured by UV-Vis spectrophotometer at a wavelength of 517 nm. The same procedure was also done for positive control using ascorbic acid.

The antioxidant activity of each sample of ascorbic acid, ethyl acetate extract and isolated compound was expressed by the percentage of free radical inhibition (% inhibition). The concentration values of samples and % inhibition were plotted respectively on the x and y axes in the linear regression equation. The linear regression equation obtained in the form of equation: $y = b(x) + a$, was used to find the IC_{50} of each sample. The IC_{50} value states the sample solution concentration needed to reduce DPPH by 50%.

28

3. Results and discussions

3.1. Plants preparation

The sample preparation process is the initial stage of a research series. The samples obtained are washed with clean water and then the samples are cut into small pieces, then dried but still not exposed to direct sunlight, the process of drying the sample aims to reduce the moisture content in the sample. The next sample is grinded, this is done to reduce the sample size. The extraction process is then carried out, using multilevel maceration using n-hexane and ethyl acetate solvents.

3.2. Phytochemical screening test

Phytochemical test was carried out to determine qualitatively the class of compounds contained in the ethyl acetate extract of *Sonneratia alba* roots. According to Harborne (1987) [12], phytochemical screening was carried out to provide an overview of the class of compounds contained in the extracts. The following is a table of phytochemical screening results of ethyl acetate extracts.

Table 1. Phytochemical test results of ethyl acetate extract from *Sonneratia alba* roots.

Compound groups	Result of phytochemical test
Alkaloid	
- Tes Mayer	-
- Tes Dragendorff	-
Flavonoid	-
Tanin	+
Saponin	-
Steroid	+
Terpenoid	+
Fenol	+
Kuinon	-

From table 1, it is known that ethyl acetate extracts of *Sonneratia alba* roots are contains tannin, steroid and phenol compounds.

3.3. Spectroscopic characterization and identification

3.3.1. Uv-Vis characterization. Based on UV-Vis spectrum in Figure 1, there is absorption at maximum wavelengths at 268 nm and 213 nm. Absorption at a wavelength of 268 nm indicates the influence of conjugate bonds, while absorption at a wavelength of 268 nm is an electron transition from $\pi \rightarrow \pi^*$ double bond. For compounds with conjugated double bonds, the transition occurs from $\pi \rightarrow \pi^*$. Where the electron transition from $\pi \rightarrow \pi^*$ indicates the presence of a chromophore which is typical for the C=C double bond system.

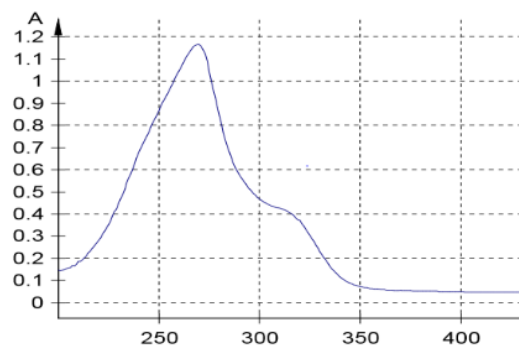


Figure 1. Spectrum absorption of isolated compounds.

3.3.2 *FT-IR Characterization*. Identification using FT-IR spectrophotometer serves to determine the functional group of a compound. Here are the results of measurements using FT-IR spectrophotometer.

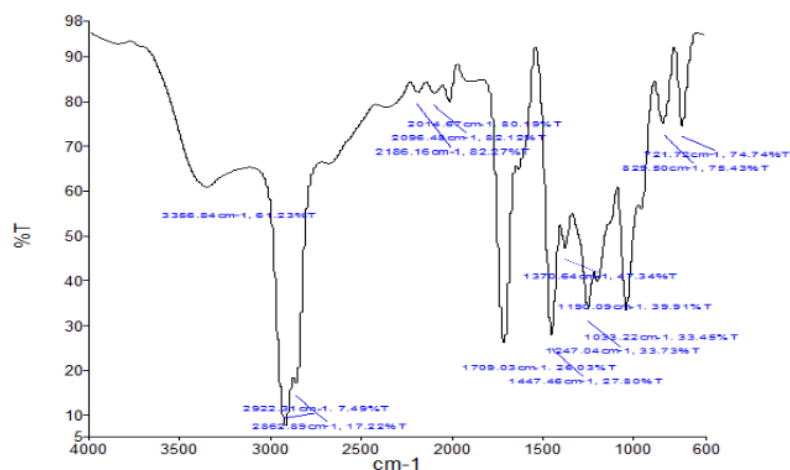


Figure 2. FT-IR spectrum of isolated compounds

From the FT-IR spectrum in Figure 2. shows that the compound obtained gives absorption at wave number 3356.84 cm^{-1} with weak absorption which is assumed to be uptake of OH functional groups from intermolecular hydrogen bonds. The presence of this -OH group is supported by absorption at wave number 1033.22 cm^{-1} from the vibration of C-O stretching on primary alcohol [13]. The presence of sharp bands at wave numbers 2922.31 cm^{-1} and 2862.89 cm^{-1} are the extension of aliphatic C-H groups. According to Socrates (1994), the presence of an aliphatic -CH stretch range absorption indicates the possibility of a methyl group (CH_3) at 2922.31 cm^{-1} and methylene (CH_2) at 2862.89 cm^{-1} . This assumption is reinforced by the appearance of absorption in the wave number regions 1447.46 cm^{-1} and 1370.64 cm^{-1} . Sharp absorption with strong intensity at wave number 1709.03 cm^{-1} which shows strain C=O from carbonyl group. Then the absorption at 1245.04 cm^{-1} appears as an R-O-aromatic stretching vibration.

3.3.3. ^1H and ^{13}C NMR Interpretation. The complete ^1H and ^{13}C NMR spectral assignments of the isolated compounds were made based on HSQC and HMBC spectroscopic data, also by comparing their physical properties reported in the literature [14].

24

Table 2. ^1H and ^{13}C NMR chemical shift values for isolated compound.

Carbon atom number	Chemical Shift Value (ppm)				Nature of carbon
	^{13}C NMR experimental	^{13}C NMR literature	^1H NMR experimental	^1H NMR literature	
1	36.65	36.72			CH_2
2	29.74	29.71			CH_2
3	71.99	71.97	3.54 (m, 1H)	3.53 (m, 1H)	CH
4	42.39	42.35			CH_2
5	140.86	140.94			C=C
6	121.88	121.32	5.35 (s, 1H)	5.38 (s, 1H)	C=CH
7	31.76	31.71			CH_2
8	29.22	29.24			CH
9	50.28	50.03			CH
10	36.30	36.16			C
11	24.45	24.32			CH_2
12	39.93	39.82			CH_2
13	40.64	40.45			C
14	56.92	56.90			CH
15	24.86	24.32			CH_2
16	28.40	28.90			CH_2
17	56.92	56.03			CH
18	12.01	12.06	1.25 (s, 3H)	1.29 (d, 3H)	CH_3
19	19.13	19.06	0.77 (d, 3H)	0.74 (d, 3H)	CH_3
20	39.93	39.82			CH
21	23.22	23.12	1.25 (s, 3H)	1.20 (d, 3H)	CH_3
22	140.86	138.40	5.08 (m, 1H)	5.07 (s, 1H)	C=C
23	121.88	129.34	5.17 (m, 1H)	5.20 (m, 1H)	C=C
24	51.39	51.26			CH
25	34.05	34.01			CH
26	21.23	21.12	0.88 (d, 3H)	0.84 (d, 3H)	CH_3
27	22.84	22.82	0.92 (d, 3H)	0.97 (d, 3H)	CH_3
28	22.55	25.32			CH_2
29	12.13	12.06	0.85 (t, 3H)	1.04 (t, 3H)	CH_3

5

The proton NMR showed the proton of H3 appearing as a multiplet at δ 3.54 ppm and revealed the existence of signals for olefinic protons at 5.08 (m), 5.17 (m), 5.35 (s), and 2.34 (t). The ^{13}C -NMR has shown recognizable signals at 140.86 ppm and 121.88 ppm which are assigned C5 and C6 double bonds respectively. The value at 19.13 ppm corresponds to angular carbon atoms (C19) 39.93 ppm for C-20 and 23.22 ppm for C-21. Spectra shows twenty nine carbon signals including six methyls, nine methylenes, eleven methane and three quaternary carbons.

Based on the study of similarity of literature, manifested that the isolated compound is a mixture of stigmasterol and beta-sitosterol compounds, known as phyosterols. According to the literature beta-sitosterol and Stigmasterol are always in a mixture form, it is very difficult to obtain Stigmasterol in pure state. The only difference between the two compounds is the presence of C22=C23 double bond in Stigmasterol and C22-C23 single bond in β -sitosterol, which can see in the figure 3. Furthermore, literatures have shown that sitosterol is difficult to be obtained in pure state. Stigmasterol and beta-sitosterol have the same Rf value 0.55 [15- 17].

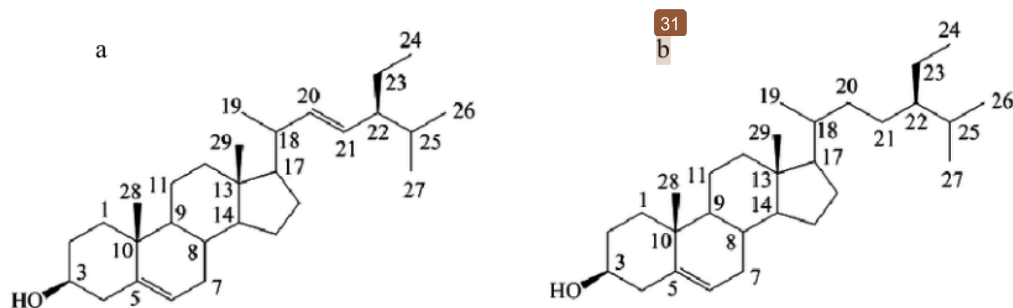


Figure 3. The structure of (a). stigmasterol and (b) beta-sitosterol

3.3.4. Antioxidants Activity from ethyl acetate extracts and isolated compounds. Testing of antioxidant activity was carried out on ethyl acetate extract, isolate compounds (phytosterol) and ascorbic acid (as a positive control), it was done to determine the initial potential of a compound as an antioxidant. Table 3. shows the results of testing antioxidant activity based on the scavenging of a DPPH radical by active compounds.

Table 3. Antioxidat activity of ethyl acetate, isolated compound (Phytosterol) and ascorbic acid.

No	Sample	IC ₅₀ Value	Level of antioxidant
1	Ethyl acetate Extracts	223.67 ppm	Weak
2	Isolated compounds (Phytosterol)	439.71 ppm	Weak
3	Ascorbic acid (positive control)	7.06 ppm	Strong

The IC₅₀ value in the sample obtained from the calculation of the linear regression equation. The coefficient “y” in this equation is as IC₅₀, while the “x” coefficient obtained is the amount of concentration needed to scavage 50% of DPPH radical activity. in the experiment showed an increase in percent inhibition by increasing the concentration of the sample. From the antioxidant activity test, the IC₅₀ values for ethyl acetate extract, isolated compound (Phytosterol) and ascorbic acid were obtained at 223.67 ppm, 439.71 ppm and 7.065 ppm, respectively. From these results it was known that the IC₅₀ value from both ethyl acetate extracts and isolated compound obtained was not close to the IC₅₀ value of the positive control. According to Molyneux [18], that the compound has antioxidant activity if the IC₅₀ value obtained ranges from 200-1000 ppm, where these compounds are classified as weak activity but still have potential as antioxidants.

4. Conclusions

According to the results, isolated compound from the ethyl acetate extract of *Sonneratia alba* roots is a mixture of stigmasterol and beta-sitosterol. Well known as phytosterols. The structure of the isolated compounds were identified on the basis of spectroscopic methods and by comparing their physical properties reported in the literature. Antioxidant activity of ethyl acetate extracts and isolated compounds (Phytosterol) gave IC₅₀ values of 223.67 and 439.71 ppm, respectively. So it can be concluded that the compound was classified as less active (weak) but still has the potential as an antioxidant.

22 knowledgegment

This research is ostensibly supported by DIPA PNBPN, Faculty of Science and Technology.

References

- [1] Bandawayake W M 2002 *Wetlands Ecology ang Managemen.* **10** 421-452.

- [2] Putri R R and K R H 2016 Antibacterial Activity Test and Fltochemical Test of Sonneratia alba Mangrove Leaf Extract *Journal of Aquaculture Science and Technology* 43-50.
- [3] Kusyana D Y 2014 *Exploration of the Potential of Active Ingredients Efficacious Antioxidants Mangrove Leaves and Fruits Types of Sonneratia alba (JE Smith, 1816)* (Bogor: Department of Marine Science and Technology Faculty of Fisheries and Marine Sciences Bogor Agricultural University).
- [4] Werdl¹¹ A 2014 *Jurnal Biotek Medisiana Indonesia* **3** 59-68.
- [5] Sighn R P, Murthy K N C and Jayaprakasha G K 2002 *J Agric Food Chem* **2** **50** 81-6.
- [6] Herawati N 2011 *Chemica* **12** 9-13.
- [7] Septiana A T and Ari A 2012 **6** 22-28.
- [8] Latief M, Nazarudin and Nelson 2015 Antioxidant Activity of Perepat Leaf and Fruit (*Sonneratia alba*) Origin of Tanjung Jabung Timur Jambi Province *Proceedings of SEMIRATA 2015 in the field of MIPA West BKS-PTN* (Pontianak: Universitas Tanjungpura) pp 112-117.
- [9] Latief M, Utami A, Fadhilah N, Bemis R, Amanda H, Heriyanti, Rahayu M A, Yusnaidar, Syahri W and Muhaimin 2018 *Pharm. Sci. & Res* **10** 2160-2162.
- [10] Paputungan, Zulkifli, Djuhria W, and Bartie E K 2017 *Journal of Fisheries Product Technology Media* **5** 3.
- [11] Selvia T, Joseph G S, and Jayaprakarsa G K 2003 *J. Food Microbiology* **20** 455-460.
- [12] Harborne J B 1987 *Phytochemical Method: A Guide to Modern Ways to Analyze Plants* Translator: Kosasih P and Soediro I. (Bandung: Institut Teknologi Bandung).
- [13] Socrates G 1994 *Infrared Characteristic Group Fre-quencies, Tabel and Charts* 2nd Edition ⁷ (London: John Wiley and Sons London)
- [14] Pierre L L and Moses M N 2015 Isolation and Characterisation of Stigmasterol and Beta-Sitos¹³ from *Odontonema Strictum* (Acanthaceae) *JIPBS* **2** 88-95.
- [15] Pollock J R A and Stevem R S 1965 *Dictionary of organic compounds 4th edition* vol 5 (London: Eyre and spottis²⁵ode (Publishers) Ltd).
- [16] Anjoo K and Ajay K S 2011 *International Journal of Pharmacy and Pharmaceutical Sciences* **3** ¹⁰ 94-96.
- [17] Pateh U U, Haruna A K, Garba M, Iliya I, Sule I M, Abubakar M S and Ambi AA 2008 *Nigerian Journal of Pharmaceutical Sciences* **7** 19 – 25.
- [18] Molyneux P 2004 *Journal Science of Technology* **26** 211-219.

ORIGINALITY REPORT

18%

SIMILARITY INDEX

11%

INTERNET SOURCES

14%

PUBLICATIONS

10%

STUDENT PAPERS

PRIMARY SOURCES

1

vital.lib.tsu.ru

Internet Source

1%

2

Submitted to Mansoura University

Student Paper

1%

3

Submitted to University of Ghana

Student Paper

1%

4

Venkata Sai Prakash Chaturvedula, Indra Prakash. "Isolation of Stigmasterol and ?-Sitosterol from the dichloromethane extract of Rubus suavissimus", International Current Pharmaceutical Journal, 2012

Publication

1%

5

Submitted to Jawaharlal Nehru Technological University

Student Paper

1%

6

Submitted to University of Wales, Bangor

Student Paper

1%

7

Fatma Tuğçe Gurağaç Dereli, Mert Ilhan, Esra Küpeli Akkol. "Identification of the main active

1%

antidepressant constituents in a traditional
Turkish medicinal plant, *Centaurea kurdica*
Reichardt", Journal of Ethnopharmacology,
2020

Publication

8	ijpsr.com Internet Source	1 %
9	Submitted to City University Student Paper	1 %
10	Submitted to Higher Education Commission Pakistan Student Paper	1 %
11	www.kau.edu.sa Internet Source	1 %
12	Submitted to University of Embu Student Paper	1 %
13	S. B. Solabannavar, V. B. Helavi, U. V. Desai, R. B. Mane. "Application of Amberlite IRA-400 (Basic) as a Base in Heck Reaction", Synthetic Communications, 2003 Publication	1 %
14	manualzz.com Internet Source	1 %
15	Widayat, B Cahyono, H Satriadi, S Munfarida. " Antioxidant activity and total phenolic content in	<1 %

16

Chun Liu, Fangyan Chen, Yu-bin Tang, Peng-wei Huo. "An Environmentally Friendly Nanocomposite Polypyrrole@Silver/Reduced Graphene Oxide With High Catalytic Activity for Bacteria and Antibiotics", Research Square, 2021

Publication

<1 %

17

Submitted to Cita Hati Christian High School

Student Paper

<1 %

18

www.bioflux.com.ro

Internet Source

<1 %

19

garuda.ristekdikti.go.id

Internet Source

<1 %

20

Malgorzata Zimniewska, Mariola Pawlaczyk, Izabella Krucinska, Iwona Frydrych et al. "The influence of natural functional clothing on some biophysical parameters of the skin", Textile Research Journal, 2018

Publication

<1 %

21

ejournal.upi.edu

Internet Source

<1 %

22

www.jpsr.pharmainfo.in

Internet Source

<1 %

- | | | |
|----|---|------|
| 23 | Supanee Tipnee, Aranya Jutiviboonsuk, Paveena Wongtrakul. "The Bioactivity Study of Active Compounds in Wolffia globosa Extract for an Alternative Source of Bioactive Substances", Cosmetics, 2017
Publication | <1 % |
| 24 | patents.google.com
Internet Source | <1 % |
| 25 | www.esciencecentral.org
Internet Source | <1 % |
| 26 | Josiane Alhage, Hoda Elbitar, Samir Taha, Jean-Paul Guegan, Zeina Dassouki, Thomas Vives, Thierry Benvegna. "Isolation of Bioactive Compounds from Calicotome villosa Stems", Molecules, 2018
Publication | <1 % |
| 27 | Feni Iranawati, Rizqi Narulitai, Citra Satrya Utami Dewi, Sunanto Arifin. " Web evaluation of maceration length period on antioxidant potency of leaf ", E3S Web of Conferences, 2020
Publication | <1 % |
| 28 | L Lizawati, A Riduan, N Neliyati, Y Alia, D Antony. " Genetic diversity of cinnamon plants (BL.) at various altitude based on morphological character ", IOP Conference Series: Materials Science and Engineering, 2018
Publication | <1 % |

- | | | |
|----|--|------|
| 29 | Sultana, B.. "Antioxidant potential of corncob extracts for stabilization of corn oil subjected to microwave heating", Food Chemistry, 2007
Publication | <1 % |
| 30 | Yamazaki, E.. "Antioxidant activity of Japanese pepper (<i>Zanthoxylum piperitum</i> DC.) fruit", Food Chemistry, 2007
Publication | <1 % |
| 31 | siloblog
Internet Source | <1 % |
| 32 | Grue-Sorensen, G.. "New 1 α ,25-dihydroxy vitamin D ³ analogues with side chains attached to C-18: synthesis and biological activity", Bioorganic & Medicinal Chemistry, 199811
Publication | <1 % |
| 33 | Kumaran, A.. "In vitro antioxidant activities of methanol extracts of five <i>Phyllanthus</i> species from India", LWT - Food Science and Technology, 200703
Publication | <1 % |
| 34 | Shin, Y.. "Synthesis and biological evaluation of (-)-dictyostatin and stereoisomers", Tetrahedron, 20070827
Publication | <1 % |

Exclude quotes Off

Exclude bibliography Off

Exclude matches Off