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1 pesan

Hari Susanti <susantihari@gmail.com> 15 Desember 2023 pukul 09.52
Kepada: aning.rini@staff.uad.ac.id

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---------- Forwarded message ---------
From: **JKKI FKUII** <jkki_fkuii@yahoo.co.id>
Date: Tue, Aug 2, 2016 at 8:51 AM
Subject: Perbaikan naskah
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Kepada

Ibu Hari Susanti
Fakultas Farmasi UAD

Bahwa Naskah ibu **KANDUNGAN TOTAL FENOL DAN AKTIVITAS ANTIOKSIDAN EKSTRAK BINAHONG (*Anredera cordifolia*.)** telah dilakukan telaah ulang oleh editor kami, masukan kami lampirkan dalam bentuk editable text microsoft word.

Masukan dari reviewer juga kami masukkan sebagaimana terlampir dalam email ini. Mohon dilakukan edit ulangterhadap naskah ibu sesuai masukan reviewer.

Kami lampirkan pula template untuk mempermudah ibu dalam melakukan editing. Serta kami mohon untuk mengubah gaya sitasi menjadi Vancouver.

Terimakasih

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KANDUNGAN TOTAL FENOL DAN AKTIVITAS ANTIOKSIDAN EKSTRAK BINAHONG (*Anredera cordifolia*.)

Susanti, H.
Fakutas Farmasi Universitas Ahmad Dahlan Yogyakarta
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ABSTRAK

Latar belakang
Tanaman binahong dilaporkan mengandung senyawa polifenol, flavonoid, dan steroid. Peneliti sebelumnya melaporkan bahwa kandungan polifenol dan flavonoid dalam tanaman sangat berperan pada aktivitas antioksidan.

Tujuan Penelitian
Penelitian ini bertujuan untuk mengetahui seberapa besar kemampuan ekstrak binahong sebagai penangkap radikal bebas DPPH dan kandungan polifenolnya.

Metode
Bagian tanaman yang digunakan adalah seluruh bagian tanaman yang ada diatas tanah. Ekstrak binahong diperoleh dengan cara maserasi bertingkat dengan pelarut berturut-turut adalah heksan, kloroform dan metanol. Uji penangkapan radikal bebas dilakukan secara *in vitro* dengan metode DPPH, dengan asam galat sebagai pembanding. Harga ES_{50} ditentukan dari persamaan regresi linear antara konsentrasi ekstrak versus % penangkapan. Kandungan fenolik total dalam ekstrak ditentukan secara spektrofotmetri dengan pereaksi Folin Ciocalteu.

Hasil
Hasil penelitian menunjukkan bahwa ekstrak heksan, ekstrak kloroform ekstrak metanol binahong mempunyai kemampuan sebagai penangkap radikal bebas. Nilai ES_{50} ekstrak heksan, kloroform, metanol dan asam galat berturut-turut adalah 583,60 µg/ml; 446,22 µg/ml; 237,68 µg/ml; 2,80 µg/ml. Kandungan polifenol total ekstrak heksan, kloroform dan methanol berturut-turut adalah 8,54 GAE mg/g; 17,30 GAE mg/g; 32,5 GAE mg/g .

Kesimpulan
Potensi ketiga ektrak binahong sebagai penangkap radikal bebas DPPH lebih lemah dibanding asam galat.

Kata kunci : binahong, antioksidan, DPPH, fenol total

Commented [lp1]: sebaiknya menekankan bukti potensi binahong

Commented [lp2]: latar belakang dan tujuan belum linear!

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Abstract

Bacground
Binahong was reported to be contained of polyphenol, flavonoids and steroids. Previous study reported that polyphenols,and flavonoidscontent of plant have a big contribute in antioxidant activity. .

Objective
This study aims to determine the ability of binahong extract as a free radicals DPPH scavenger and it's polyphenols content

Method
The aerial part of binahong plant was used in this study. Binahong extract was prepared by maceration using hexane, chloroform and methanol as solvent. The free radicals scavenging activity was done in vitro with DPPH. Gallic acid was used as a positive controler. The ES_{50} value was determined from the linear regression equation between the extract concentration versus % arrest. Total phenolic content in the extract was determined spectrophometrically with the Folin-Ciocalteu reagent.

Result
The results showed that the hexane, chloroform and methanol extract of binahong have the ability as a free radicals scavenger. The ES_{50} value of hexane, chloroform and methanol extract successively were 583.60 µg / ml); 446.22 µg / ml; 237.68 µg / ml; 2.80 ug / ml. Total polyphenol content of hexane , chloroform and methanol extract successively were 8.54 mg GAE/g ; 17.30 mg GAE /g ;32.5 mg GAE /g.

Conclusion
The capability of the three of binahong extract as a free radicals DPPH scavenger were weaker than gallic acid.

Keywords : binahong, antioxidant ,DPPH, TPC

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PENDAHULUAN

Commented [lp8]: penelitian ini terutama mengkaji binahong, melihat kandungan total fenol dan aktivitas antioksidan maka sebaiknya permbahasan mulailah dari ekstrak binahong, bukti2 ilmiah potensinya, baru mulai membahas kandungan binahong serta potensi dari tiap2 kandungan tersebut terutama untuk fenol dan antioksidan! sebaiknya pendahuluan diperbanyak isinya.

Radikal bebas dapat berimplikasi pada penyakit kanker, aterosklerosis, penuaan, inflamasi, diabetes, rambut rontok, dan penyakit degenerative saraf misalnya Alzheimer dan Parkinson (Surveswaran dkk, 2007). Efek radikal bebas dapat diredam jka tubuh memiliki penangkap radikal bebas (antioksidan) yang cukup, dan dengan mengatur pola makan. Karena itu, masyarakat sekarang mulai mengubah pola hidup dengan kembali kepada alam (*back to nature)* (Hermani dan Rahardjo, 2005).

Tubuh manusia sebenarnya memiliki sistem pertahanan endogen terhadap serangan radikal bebas. Jumlah radikal bebas dapat menjadi meningkat karena faktor stes, radiasi, asap rokok dan polusi lingkungan, sehingga sistem pertahanan tubuh menjadi tidak memadai lagi dan tubuh memerlukan tambahan antioksidan dari luar. Dengan adanya antioksidan tambahan tersebut, maka proses oksidasi yang berlebihan dapat dihambat (Halliwell dan Gutteridge, 1999).

Antioksidan adalah senyawa yang mampu menghilangkan, membersihkan (*Scavenging*), menahan pembentukan, ataupun meniadakan efek spesies oksigen reaktif (Lautan, 1997). Beberapa antioksidan sintetik ternyata juga menpenyai sifat toksik dan menunjukkan efisiensi aktivitas yang lebih rendah dibandingkan antioksidan alami (Soong dan Barlaw, 2004). Karenanya, industri makanan dan obat-obatan beralih mengembangkan antioksidan alami.

Salah satu tanaman yang menarik untuk diteliti adalah *Anredera cordifolia* (Tenore) Steen) yang dikenal dengan nama Binahong yang termasuk dalam familia Anredera. Tumbuhan ini banyak ditemukan di Amerika Selatan. Daun dan rhizoma Binahong bermanfaat sebagai obat penyembuh luka bekas operasi, tipus, radang usus, penurun asam urat, disentri dan ambeien pada umumnya yaitu rhizoma dan daun. Penelitian sebelumnya menunjukkan bahwa daun Binahong terdapat aktivitas antioksidan. Selain mengandung saponin triterpenoid, senyawa flavonoid dan minyak atsiri ditemukan juga pada daun binahong (Rahmawati, 2008). Daun Binahong diketahui mempunyai kandungan asam ursolat (Hammond, 2006).

Efek farmakologis beberapa polifenol termasuk flavonoid sebagai anti-stres, anti-inflamasi, anti-tumorigenesis, hepatoprotektor, diduga erat kaitanya dengan sifat polifenol sebagain antioksidan.

Commented [lp9]: dalam 1 paragraf sebaiknya tidak hanya berisi 1 kalimat saja!

Untuk mengeksplorasi dan mendapatkan senyawa yang berkhasiat sebagai antioksidan dari binahong, maka perlu dilakukan skrining antioksidan dengan beberapa pelarut/penyari dengan berbagai tingkat kepolaran. Sehubungan dengan keterangan di atas maka perlu dilakukan penelitian untuk mengetahui kemampuan ekstrak heksan (non polar), ekstrak kloroform(semipolar) dan ekstrak metanol (polar) dari binahong. Dari hasil penelitian ini diharapkan pemanfaatan binahong dapat lebih optimal terutama dalam bidang kesehatan.

METODE PENELITIAN

Bahan Penelitian meliputi: tanaman binahong yang diperoleh dari daerah Pleret Bantul Yogyakarta ,heksan, kloroform, metanol teknis (Brataco), Metanol pa., pereaksi Folin-Ciocalteu pa. (E.Merck) DPPH, asam galat (Sigma)

Commented [lp10]: apakah diperoleh dari senter tertentu di bantul?

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~~**Jalannya penelitian**~~

Ekstrak binahong dibuat dengan metode maserasi.

Serbuk *aerial part* (bagian tanaman di atas tanah) binahong sebanyak 20 gram dimaserasi secara gradien berturut-turut dengan pelarut heksan, kloroform dan metanol. Masing-masing maserasi dilakukan 2 kali dengan sesekali diaduk. Maserat disaring dengan corong *Buchner* dengan tekanan agar dapat tersaring dengan sempurna. Masing-masing sari ini dikumpulkan kemudian diuapkan dengan *rotary evaporator* hingga terbentuk ekstrak kental.

Commented [lp12]: sebaiknya disebutkan bagian apa saja yang diambil, serta pada jarak berapa sentimeter dari tanah tanaman ini diambil.

Uji aktivitas antioksidan dengan metode DPPH.

Penagkapan radikal bebas DPPH oleh ekstrak binahong diukur dengan mengukur penurunan absorbansi larutan DPPH dalam metanol pada 516,4 nm dengan adanya ekstrak. Absorbansi larutan DPPH 0,15 mM dibaca sebelum ditambah ekstrak dan setelah 30 menit sejak ditambah ekstrak. Sebagai pembanding digunakan asam galat.

Aktivitas antioksidan dihitung dengan menggunakan rumus :

% Penangkapan = $[(A_{kontrol} - A_{uji}/A_{kontrol}) \times 100\%]$ (Khalaf dkk., 2001)

Keterangan : $A_{kontrol}$ = Absorbansi kontrol
A_{uji} = Absorbansi sampel uji

ES_{50} (konsentrasi ekstrak etanol *yang* diperlukan untuk menurunkan absorbansi sebesar 50% dari mula-mula) ditentukan dengan persamaan regresi antara % penangkapan versus konsentrasi. Semakin kecil harga ES_{50} berarti semakin besar daya antioksidan senyawa tersebut. Sebagai pembanding digunakan asam galat. Semua pengujian dilakukan replikasi 3x.

Penentuan total fenolik dalam ekstrak (TPC)

Sebanyak 10,00 mg ekstrak dilarutkan dalam metanol hingga 10,0 ml. Tiga ratus mikroliter larutan ekstrak ditambah 1,5 ml pereaksi Folin-Ciocalteu (yang telah diencerkan 10 kali). Setelah didiamkan 3 menit, ditambah 1,2 ml Na_2CO_3 7,5%, absorbansi dibaca pada 765 nm setelah 1 jam. Sebagai pembanding digunakan asam galat. Kandungan TPC dinyatakan dalam Gallic acid ekivalen (GAE) mg/gram ekstrak.Lakukan replikasi 3x

HASIL DAN PEMBAHASAN

Hasil uji antioksidan dengan metode DPPH

Commented [lp13]: alat yang digunakan apakah dengan spektrofotometer?

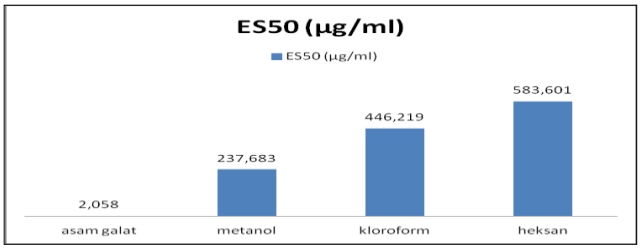
Commented [lp14]: awal paragraph sebaiknya jangan dimulai dengan singkatan ataupun notasi

Commented [lp15]: dipisah antara hasil dan pembahasan. untuk pembahasan mohon dicari apa kroteria suau bahan memiliki aktivitas antioksidan

Uji antioksidan ditentukan dengan metode DPPH. Kemampuan senyawa sebagai penangkap radikal bebas ditunjukkan dengan adanya penurunan absorbansi DPPH pada λ 515-517 nm. Asam galat digunakan sebagai kontrol positif.

Hasil uji aktivitas antioksidan dengan metode DPPH seperti terlihat pada gambar 1 berikut:

Commented [lp16]: G besar→Gambar



Gambar 1. Perbandingan ES_{50} antara asam galat dan ekstrak binahong (rata-rata dari 3 replikasi).

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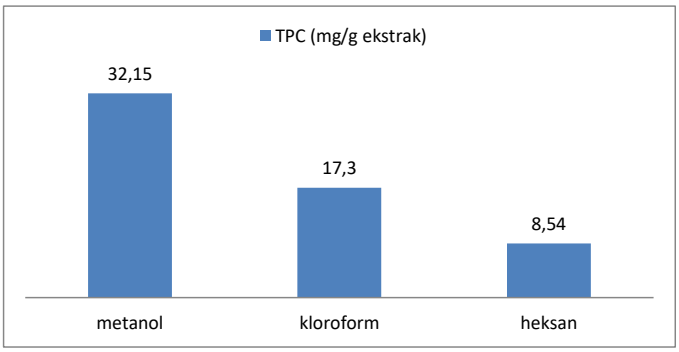
Penentuan *Total Phenoic Content* (TPC) ekstrak binahong

Penentuan TPC dilakukan secara spektrofotometri visibel dengan pereaksi Folin Ciocalteu. Metode ini didasarkan pada terbentuknya komplek warna biru yang dibaca pada panjang gelombang 746 nm. Asam galat digunakan sebagai pembanding. Nilai TPC ketiga ekstrak dinyatakan dalam *Gallic Acid Ekivalen* (GAE) mg/g ekstrak (Gambar 2)



Gambar 2. Profil TPC ketiga ekstrak binahong (rata-rata dari 3 replikasi)

Commented [lp18]: idem komen diatas

Berdasarkan data pada Gambar 1 terlihat bahwa ekstrak metanol memiliki kemampuan sebagai penangkap radikal paling kuat dibandingkan ekstrak kloroform dan ekstrak heksan. Hal ini terlihat dari nilai ES_{50} nya, Semakin kecil nilai ES_{50} suatu zat maka semakin besar kemampuannya sebagai penangkap radikal bebas. Namun kemampuan ekstrak binahong sebagai penangkap radikal bebas masih lebih lemah dibandingkan dengan asam galat. Menurut kriteria/kategori Blois, maka bisa dikatakan ketiga ekstrak masuk dalam kategori tidak aktif karena memilki nilai ES50 lebih dari 200 µg/ml. Hal ini berbeda dengan penelitian yang dilakukan Selawa dkk (2013) tentang kandungan flavonoid dan kapasitas antioksidan total ekstrak etanol daun binahong. Perbedaan terletak pada beberapa aspek, antara lain: bagian tanaman yang digunakan pada penelitian tersebut hanya daun sedangkan penelitian ini menggunakan seluruh bagian tanaman diatas tanah. Metode uji antioksidan pun berbeda, penelitian ini menggunakan metode DPPH sedangkan Selawa dkk menggunakan metode FRAP.

Commented [lp19]: bagaimana hasil penelitian dari peneliti ini?

Commented [lp20]: apa impak dari perbedaan antara penelitian ini dengan penelitian selawa? apakah karena pada penelitian ini menggunakan bagian binahong yang lebih banyak sehingga kadar antioksidannya jadi rendah? apa kaitannya?

Hasil penentuan TPC menunjukkan bahwa ekstrak metanol memiliki kandungan TPC paling tinggi diikuti ekstrak kloroform dan heksan. Hal ini menunjukkan bahwa pelarut yang cenderung polar lebih bisa menyari senyawa polifenol. Berdasarkan data-data tersebut, terlihat adanya korelasi antara kandungan TPC dengan kemampuannya sebagai penangkap radikal bebas. Semakin besar kandungan TPC, maka semakin besar kemampuannya sebagai penangkap radikal bebas (ES_{50} makin kecil). Hal ini sejalan dengan penelitian Susanti (2012) tentang aktivitas antioksidan ekstrak daun dan biji lampes yang menyatakan bahwa

Commented [lp21]: maksud dari mecari senyawa fenol apa?

Kandungan TPC dalam ekstrak daun lampes (41,33 mg/g GAE)> ekstrak biji lampes (26,81 mg/g GAE) dan potensi antioksidan ekstrak daun lampes (ES $_{50}$ = 91,94 µg/ml)> ekstrak biji lampes (ES_{50}=131,81 µg/ml). Hal ini didukung oleh penelitian tentang hubungan antara kandungan total fenol dan antioksidan oleh Buyuktuncel, dkk (2014) yang menyimpulkan bahwa aktivitas antioksidan berkorelasi kuat dengan kandungan total fenol dalam anggur merah.

Commented [lp22]: sebaiknya hindari penggunaan notasi ini

Kesimpulan

1. Ekstrak metanol, ekstrak kloroform dan ekstrak heksan mempunyai aktivitas sebagai antioksidan dengan ES $_{50}$ berturut-turut : 237,68 ; 446,22; 583,60 µg/ml
2. Kadar fenolik total ekstrak metanol, kloroform dan heksan berturut-turut adalah 32,15; 17,30.; 8,54 mg/g GAE

Ucapan Terima Kasih

Kami ucapkan terima kasih kepada Lembaga Penelitian dan Pengembangan Universitas Ahmad Dahlan atas bantuan pendanaan melalui hibah penelitian internal Skim Mandiri

DAFTAR PUSTAKA

Commented [lp23]: mohon ditambahkan lagi daftar pustaka yang digunakan

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“Tuliskan judul naskah disini, awali dengan huruf capital,Cambria,14point”

Pada Bagian ini ditulis tanpa ada identitas apapun demi menjamin system Double Blind review

Abstract (English) – copy paste at this text below

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Keyword (in English) : Proin, ut sodales, metus

Abstrak (Bahasa) – copy paste pada naskah dibawah ini

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Keyword (in Bahasa) : Proin, ut sodales, metus

Gunakan I-M-R-D-C-A-R (Introduction-Methods-Results-Discussion-Conclusion-Acknowledgement-References)

INTRODUCTION

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METHODS

Vivamus suscipit vitae eros sed consequat. In porta ullamcorper metus in finibus. Interdum et malesuada fames ac ante ipsum primis in faucibus. Morbi hendrerit quam a sem pretium, ut cursus lacus hendrerit. Curabitur a libero sit amet sapien elementum cursus. Suspendisse potenti. Praesent pulvinar rutrum enim, vel dignissim lectus ornare vel. Pellentesque ut turpis vel nisl porta tincidunt et quis turpis. Donec finibus at ante quis semper. Quisque dapibus finibus lorem, pretium luctus erat dictum eget. Aenean cursus, tellus sit amet volutpat egestas, nunc sapien tristique dolor, id suscipit neque velit sed lacus.

RESULTS

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DISCUSSION

Integer dolor leo, mattis in cursus egestas, convallis quis dui. Nam quis erat auctor, consequat elit lacinia, porttitor tortor. Suspendisse id faucibus dolor, feugiat finibus libero. Mauris posuere placerat mauris nec vestibulum. Fusce convallis semper erat nec consequat. Quisque maximus vitae mauris sagittis egestas. Donec quam diam, tempor ut mattis quis, congue sit amet tellus. Nullam condimentum vel orci vitae dapibus. Suspendisse libero augue, laoreet id elit a, hendrerit viverra neque. Vestibulum at orci hendrerit, fringilla quam non, tincidunt nibh. Pellentesque egestas consectetur vestibulum. Curabitur ornare ante vulputate, egestas quam vel, tincidunt felis. Aliquam erat volutpat. Sed eleifend scelerisque mauris, sed efficitur libero eleifend ac.

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Table 1. Inside of something

An example of a column heading	Column A (t)	Column B (T)
And an entry	1	2
And another entry	3	4
And another entry	5	6

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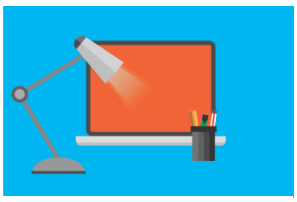

Figure 1. Light on Mac

CONCLUSION

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ACKNOWLEDGMENT (wajib ditulis apabila terdapat dana hibah/grant, serta data pendukungnya)

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Bersama email ini kami lampirkan hasil editing naskah tahap 2 yang berjudul "*KANDUNGAN FENOLIK TOTAL DAN AKTIVITAS ANTIOKSIDAN EKSTRAK BINAHONG (Anredera cordifolia.)*"
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Revisi :

Abstrak

Hasil

Hasil penelitian menunjukkan bahwa Kandungan fenolik total ekstrak heksan, kloroform dan methanol berturut-turut adalah 8,54±0,49 GAE mg/g, 17,30±0,47 GAE mg/g dan 32,5±1,11 GAE mg/g. Ekstrak heksan, ekstrak kloroform ekstrak metanol binahong mempunyai kemampuan sebagai penangkap radikal bebas. Nilai ES_{50} ekstrak heksan, kloroform, metanol dan asam galat berturut-turut adalah 583,601±2,533 µg/ml, 446,219±2,268 µg/ml, 237,683±13,373 µg/ml dan 2,058±0,002 µg/ml.

Abstract

Result

The results showed that the total phenolic content of hexane, chloroform and methanol extract of Binahong was 8.54 ± 0.49 GAE mg/g, 17.30 ± 0.47 GAE mg/g and 32.5 ± 1.11 GAE mg/g. Hexane extract, chloroform extract and methanol extract of Binahong have a free radical scavenger activity. The value of ES50 extracts of hexane, chloroform, methanol, and successive acid errors is 58.601 ± 2.533 µg/ml, 446.219 ± 2.268 µg/ml, 237.683 ± 13.373 µg/ml and 2.058 ± 0.002 µg/ml.

Table 1

Sample	ES_{50} ± SD (µg/ml)
Gallic Acid	2.058 ± 0.002*
Methanol extract	237.68 ± 13.373*
Chloroform extract	446.219 ± 0.2.268*
Hexane extract	583.601 ± 2.533*

Figure 1.

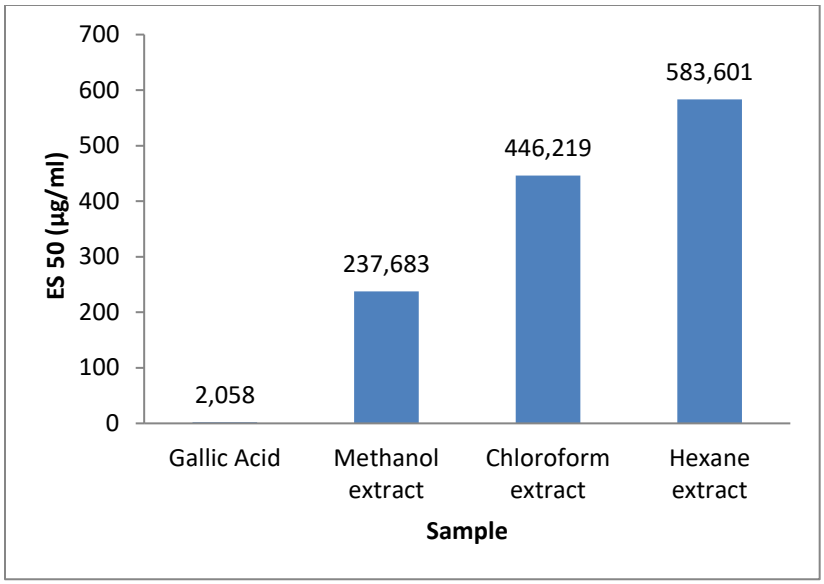

Figure 2.

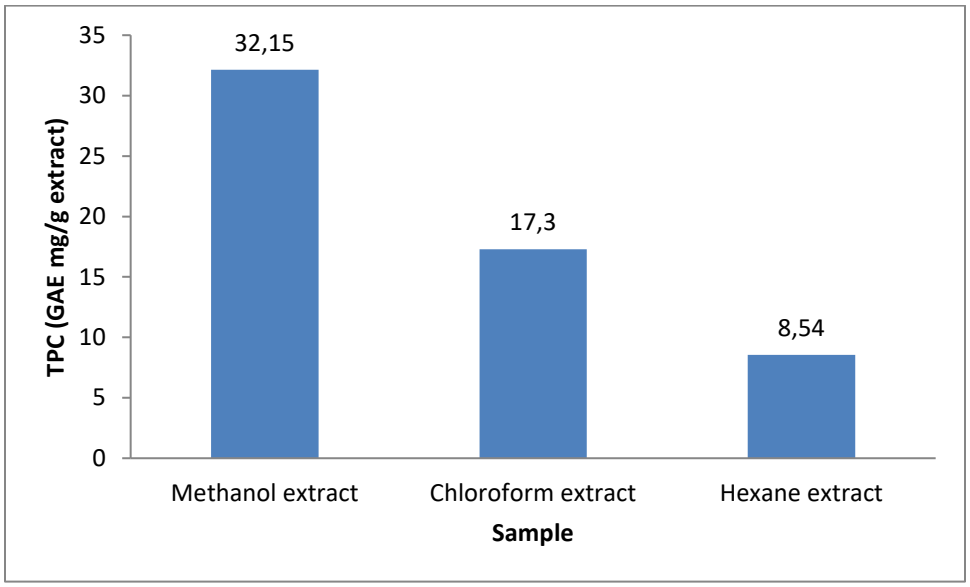

Figure 2. Profile of phenolic total Binahong extract

Total phenolic content and antioxidant activities of binahong (Anredera cordifolia.)

By Hari Susanti

TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITIES OF BINAHONG (*Anredera cordifolia.*)

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Latar belakang
Tanaman binahong dilaporkan mengandung senyawa polifenol, flavonoid, dan steroid. Kandungan polifenol dan flavonoid memiliki peranan penting sebagai antioksidan. Penelitian dengan menggunakan metode FRAP (*Ferric Reducing Ability of Power*) menunjukkan bahwa aktifitas antioksidan dari daun binahong .3,68 mmol/100 g pada simplisia daun kering. Penelitian ini akan mencari sebuah bukti baru aktivitas antioksidan semua bagian binahong yang berada di atas permukaan tanah.

Tujuan Penelitian
untuk mengetahui seberapa besar kandungan fenolik total dari ekstrak binahong serta mengetahui kemampuan ekstrak binahong sebagai penangkap radikal bebas dengan menggunakan metode DPPH

Metode
Bagian tanaman binahong yang digunakan pada penelitian ini adalah seluruh bagian tanaman yang ada diatas tanah. Ekstrak binahong diperoleh dengan cara maserasi bertingkat dengan menggunakan pelarutheksan, kloroform dan metanol. Kandungan fenolik total dalam ekstrak ditentukan secara spektrofotmetri dengan pereaksi Folin Ciocalteu. Uji penangkapan radikal bebas dilakukan secara *in vitro* dengan metode DPPH, dengan asam galat sebagai pembanding. Harga ES_{50} ditentukan dari persamaan regresi linear antara konsentrasi ekstrak dan % penangkapan.

Hasil
Hasil penelitian menunjukkan bahwa Kandungan fenolik total ekstrak heksan, kloroform dan methanol berturut-turut adalah 8,54±0.49 GAE mg/g, 17,30±0.47 GAE mg/g dan 32,5±1.11 GAE mg/g. Ekstrak heksan, ekstrak kloroform ekstrak metanol binahong mempunyai kemampuan sebagai penangkap radikal bebas. Nilai ES_{50} ekstrak heksan, kloroform, metanol dan asam galat berturut-turut adalah 583,60±0.43 µg/ml, 446,22±0.51 µg/ml, 237,68±5.63 µg/ml dan 2,80±0.1 µg/ml.

Kesimpulan
Potensi ketiga ektrak binahong sebagai penangkap radikal bebas DPPH lebih lemah dibanding asam galat.

Kata kunci : binahong, antioksidan, DPPH, fenolik total

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Abstract

Background

Binahong plants are reportedly containing polyphenols, flavonoids, and steroid compounds. The content of polyphenols and flavonoids plays an important role as antioxidants. Research using the method of FRAP (Ferric Reducing Ability of Power) indicates that the antioxidant content of the binahongleaves extract is 3.68 mmol/100 g in dried leaves. This study offers new proof of antioxidant activity of all the aerial part of binahong.

Objective

This research aims to determine the total phenolic content from binahong extract and its ability as free radical scavenger using DPPH method.

Method

The plant used in this research is every part of plants on the ground. The binahong extract is obtained by a maceration method with various solvents (hexane, chloroform, and methanol). Total phenolic content in the extract is spectrophotometrically determined with the Folin Ciocalteu reagent. Antioxidant activity assay is performed in vitro by the method of DPPH, with Gallic acid as a comparator. ES50 parameter is determined from the linear regression equation between the concentration of extracts and % scavenging.

Result

The results showed that the total phenolic content of Hexan extract, chloroform, and successive methanol was 8,54 ± 0.49 GAE mg/g, 17,30 ± 0.47 GAE mg/g and 32,5 ± 1.11 GAE mg/g respectively. Hexan extract, chloroform extract, and methanol extract of binahong show an ability as a free radical scavenger. The value of ES50 extracts of hexane, chloroform, methanol extract , and gallic acid is 583,60 ± 0.43 µg/ml, 446,22 ± 0.51 µg/ml, 237,68 ± 5.63 µg/ml and 2.80 ± 0.1 µg/ml.

Conclusion

The capability of the three of binahong extract as a free radicals DPPH scavenger were weaker than gallic acid.

Keywords: Binahong, antioxidant, DPPH, TPC

INTRODUCTION

Free radicals may play a role in various degenerative diseases such as cancer, atherosclerosis, ageing, inflammation, diabetes, hair loss, and Parkinson's (1) (2). The effects of free radicals can be mitigated if the body has enough antioxidants and by regulating diet, such as by consuming foods and drinks that contain a high level of antioxidants. Therefore, people are starting to change their lifestyles by using nature-sourced materials which are believed to be healthier and safer for the body (3).

The human body is capable of producing limited amounts of endogenous antioxidants. However, the limited amount of antioxidants is not able to fight the increasing free radicals inside the body. Therefore, exogenous antioxidants are needed to fight the increase in free radicals (4).

Antioxidants can eliminate, clean (scavenging), resist formation, or negate the effects of free radicals (4, 5). There are 2 types of antioxidants; synthetic and natural antioxidants. Natural antioxidants have higher effectiveness but lower toxic properties compared to synthetic antioxidants (6). Therefore, the food and medicine industry has shifted to develop natural antioxidants.

Binahong (Anredera cordifolia) or Tenore Steen is found in South America and one of the interesting plants to study. Growing evidence suggests that the binahong plant has clinical potentials. Binahong leaves and rhizoma are known to be useful as a healing agent for scars, typhus, inflammation of the intestine, lowering uric acid, dysentery, and haemorrhoid. Binahong leaves contain triterpenoid saponins, flavonoid compounds, and ursolic acid (7) (8).

Binahong plants need to be explored to obtain its active compounds of the antioxidants property. It is necessary to screen the antioxidant potential with some extraction methods and compound with various levels of polarity. Previous studies have shown that the ethanol extract of binahong leaves has antioxidant activity. (9) (10) Accordingly, this study using all parts of plants above ground with hexane (non-polar), chloroform (semipolar), and methanol (polar) extracts to obtain extracts tested for free radical scavenging activity. The results of this study suggest that the use of binahong can be optimized, especially in the health sector.

RESEARCH METHODS

Binahong plants were obtained from the Bantul Pleret area of Yogyakarta. Determination of plants was carried out in the Laboratory of Pharmaceutical Biology, Gadjah Mada University.

Extraction procedures

The process of extraction in this study was done by using a gradual maceration method. 20 g of dried powder from all parts of the Binahong plant above ground level was extracted with gradually organic solvents from non polar to polar organic solvents (hexane, chloroform and methanol). After maceration periods, the soaked powder-solvent mixtures were filtered by using Buchner funnel. The residue left in the funnel was re-extracted twice follow the same procedure and filtered. Each extract was concentrated and dried by by using rotary evaporator to obtain a thick extract.**Antioxidant activity**

The free radical scavenging activity of hexane, chloroform, and methanol extracts of Binahong was measured using DPPH assay. The extracts were added into tubes containing 0,15 mM methanolic DPPH solution. The reduction of absorbance at 516 nm was measured twice, before and 30 minutes after extracts were added into tubes.

Antioxidant activity is calculated by the following equation:

$$\% \text{ Capture} = [(A_{control} - A_{test}/A_{control}) \text{ X } 100\%] \qquad (11)$$

Note : $A_{control}$ = Control absorbance
A_{test} = Control absorbance test sample

The concentration of extract needed to reduce absorbance by 50% from the initial state (ES50) was determined by the regression equation between the percentage of capture and concentration. The smaller the value of ES50 means the greater the antioxidant power of the compound. As a comparison for this method used gallic acid, and all tests were replicated 3 times.

Determination of total phenolic binahong extract

Determination of Total Phenolic Content (TPC) was performed by visible spectrophotometry with Folin Ciocalteu reagent. This method is based on the formation of a blue complex that is read at a wavelength of 746 nm. Gallic acid was used as a comparison. The third TPC value of the extract was expressed in Gallic Acid Equivalent (GAE) mg / g extract. A total of 10.00 mg of each type of extract was dissolved in methanol to amount to 10.0 ml. Three hundred microliters of the extract solution were added to 1.5 ml of the Folin-Ciocalteu reagent (which had been diluted 10 times). After settling for 3 minutes, the solution is added with 1.2 ml of 7.5% Na2CO3. The absorbance will be read at 765 nm after 1 hour. As a comparison used Galat acid (12) All tests were replicated 3 times.

ES50 data and TPC content were statistically analyzed, namely, Anova continued with LSD with a 95% confidence level using the SPSS program.

RESULTS

The extract yield obtained from each solvent was 1.2% Hexane; chloroform 4.2%; and 6.3% methanol respectively. The ability of compounds as free radical scavengers is shown by a decrease in absorbance of DPPH at 1516.4 nm. The results of antioxidant activity tests by DPPH method as shown in Table 1 and Figure 1 below:

Sample	ES_{50} ± SD (µg/ml)
Gallic Acid	2,058 ± 0,002*
Methanol extract	237,683 ± 13,373*
Chloroform extract	446,219 ± 2,268*

Hexane extract	583,601 ± 2,533*

n=3 * $p<0.05$

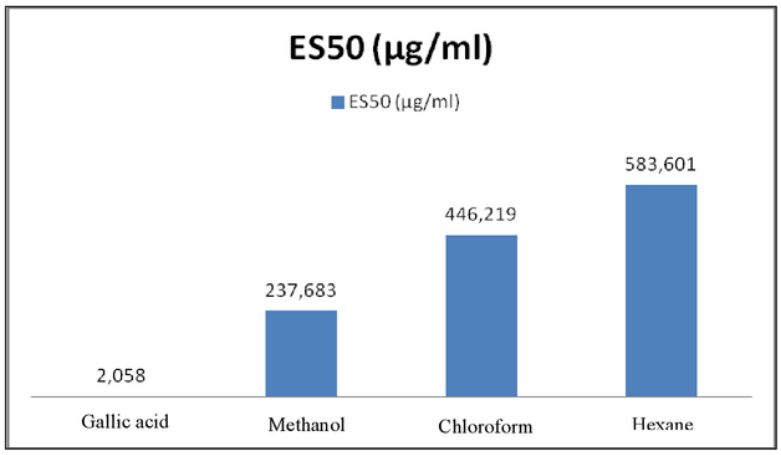

Figure 1. Comparison of ES50 between Gallic acid and binahong extract.

The results of determining the extract TPC content are presented in (Figure 2)

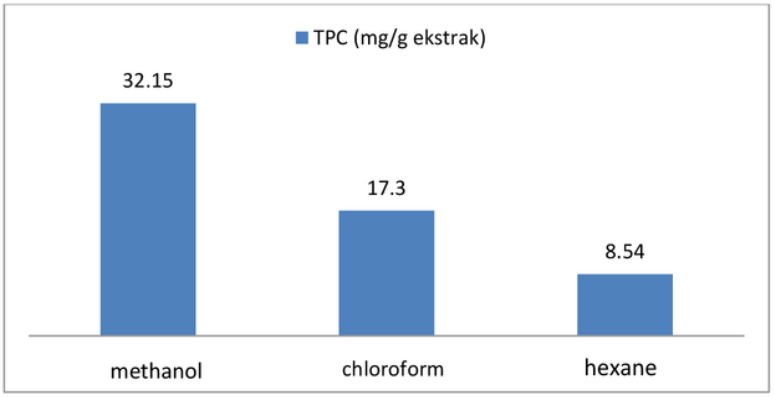

Figure 2. Profile of the third TPC of binahong extract

Discussion

Figure 1 shows that methanol extract has the most powerful ability as the free radical scavenger compared to chloroform extract and hexane extract. Based on the ES50 value of methanol extract, the smaller the ES50 value of a substance, the greater its ability as a free radical scavenger. The results of this study are in line with another research (13) which states that the methanol extract of binahong leaves has higher free radical scavenging activity than hexane extract. However, the ability of binahong extract as a free radical scavenger is still weaker than gallic acid. According to Blois criteria (14), those three extracts are in the inactive category because they have an ES50 value of more than 200 µg / ml. Different from the previous research, this study uses not only the leaves but also all parts of the plant above ground level (both stems, young leaves, and old leaves). Moreover, we studied the content of flavonoids and the total antioxidant capacity of the ethanol extract of binahong leaves. Antioxidant test methods used were also different, in which this study uses the DPPH method while Selawa et al. using the FRAP method.

The results of TPC determination showed that methanol extract has the highest TPC content followed by chloroform extract and hexane extract. This shows that more polar solvents tend to find polyphenol compounds. Based on these data, it is seen that there is a correlation between TPC content and its ability as a free radical scavenger with a correlation coefficient (R) value of 0.994. Accordingly, recent research states that the TPC content, as well as its antioxidant potential of lampes leaf extract, is greater than the seed extract (16). This is supported by Büyüktuncel et al which suggests that antioxidant activity is strongly correlated with total phenolic content in red wine (17).

Conclusion

The antioxidant activity of methanol extract was higher compared to chloroform extract and hexane extract. The total phenolic level oftotal methanol extracts was higher compared to chloroform extract and hexane extract.

Acknowledgement

We thank Ahmad Dahlan University Research and Development Institute for funding assistance through the Independent Scheme Independent research grant.

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UJI AKTIVITAS ANTIOKSIDAN EKSTRAK DAUN BINAHONG (Anredera Cordifolia (Tenore) Steenis) DENGAN 1,1-DIFENIL-2-PIKRILHIDRAZIL (DPPH) MENGGUNAKAN SPEKTROFOTOMETER UV-VIS

Antioxidant Activity of Binahong (Anredera Cordifolia (Tenore) Steenis) Leafs Extracts With 1,1-diphenyl-2-picrylhydrazyl (DPPH) Using UV-Vis Spectrophotometer

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Abstract

Testing of antioxidant activity of Binahong (Anredera Cordifolia (Tenore) Steenis) leafs extracts has been done with 1,1-diphenyl-2-picrylhydrazyl (DPPH) using UV-Vis Spectrophotometer. The aim of this research was to determine the antioxidant activity of Binahong leafs extracts. Concentration of 1,1-diphenyl-2-picrylhydrazyl (DPPH) after addition of Binahong leafs extracts was determined using UV-Vis Spectrophotometer. Various concentrations of Binahong leafs extracts were 20 ppm, 40 ppm, 60 ppm and 80 ppm. Vitamin C was the positive control used at similar variation concentrations, whereas DPPH solution dissolved in absolute ethanol was as the negative control. The results showed that the IC50 values obtained for Binahong leafs extracts and vitamin C were 40.27 ppm and 49.20 ppm. Based on the IC50 data, it can be seen that Binahong leafs extracts are stronger antioxidant than vitamin C.

Keywords: Antioxidant, Binahong, 1,1-diphenyl-2-picrylhydrazyl (DPPH), UV-Vis Spectrophotometer

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Review

Free radicals and antioxidants in normal physiological functions and human disease

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Total phenolic content and antioxidant activities of binahong (Anredera cordifolia.)

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ABSTRACT

Background: Binahong plants are reportedly containing polyphenols, flavonoids, and steroid compounds. The content of polyphenols and flavonoids plays an important role as antioxidants. Research using the method of FRAP (Ferric Reducing Ability of Power) indicates that the antioxidant content of the binahongleaves extract is 3.68 mmol/100 g in dried leaves. This study offers new proof of antioxidant activity of all the aerial part of binahong.

Objective: This research aims to determine the total phenolic content from binahong extract and its ability as free radical scavenger using DPPH method.

Methods: The plant used in this research is every part of plants on the ground. The binahong extract is obtained by a maceration method with various solvents (hexane, chloroform, and methanol). Total phenolic content in the extract is spectrophotometrically determined with the Folin Ciocalteu reagent. Antioxidant activity assay is performed in vitro by the method of DPPH, with Gallic acid as a comparator. ES parameter is determined from the linear regression equation between the concentration of extracts and % scavenging.

Results: The results showed that the total phenolic content of Hexan extract, chloroform, and successive methanol was 8,54 ± 0.49 GAE mg/g, 17,30 ± 0.47 GAE mg/g and 32,5 ± 1.11 GAE mg/g respectively.

Hexan extract, chloroform extract, and methanol extract of binahong show an ability as a free radical scavenger. The value of ES50 extracts of hexane, chloroform, methanol extract , and gallic acid is 583,60 ± 0.43 µg/ml, 446,22 ± 0.51 µg/ml, 237,68 ± 5.63 µg/ml and 2.80 ± 0.1 µg/ml.

Conclusion: The capability of the three of binahong extract as a free radicals DPPH scavenger were weaker than gallic acid.

Latar Belakang: *Tanaman binahong dilaporkan mengandung senyawa polifenol, flavonoid, dan steroid. Kandungan polifenol dan flavonoid memiliki peranan penting sebagai antioksidan. Penelitian dengan menggunakan metode FRAP (Ferric Reducing Ability of Power) menunjukkan bahwa aktifitas antioksidan dari daun binahong .3,68 mmol/100 g pada simplisia daun kering. Penelitian ini akan mencari sebuah bukti baru aktivitas antioksidan semua bagian binahong yang berada di atas permukaan tanah.*

Tujuan: *Penelitian ini bertujuan untuk mengetahui seberapa besar kandungan fenolik total dari ekstrak binahong serta mengetahui kemampuan ekstrak binahong sebagai penangkap radikal bebas dengan menggunakan metode DPPH.*

Metode: *Bagian tanaman binahong yang digunakan pada penelitian ini adalah seluruh bagian tanaman yang ada diatas tanah. Ekstrak binahong diperoleh dengan cara maserasi bertingkat dengan menggunakan pelarutheksan, kloroform dan metanol. Kandungan fenolik total dalam ekstrak ditentukan secara*

spektrofotmetri dengan pereaksi Folin Ciocalteu. Uji penangkapan radikal bebas dilakukan secara in vitro dengan metode DPPH, dengan asam galat sebagai pembanding. Harga ES_{50} ditentukan dari persamaan regresi linear antara konsentrasi ekstrak dan % penangkapan.

Hasil: *Hasil penelitian menunjukkan bahwa Kandungan fenolik total ekstrak heksan, kloroform dan methanol berturut-turut adalah 8,54±0.49 GAE mg/g, 17,30±0.47 GAE mg/g dan 32,5±1.11 GAE mg/g. Ekstrak heksan, ekstrak kloroform ekstrak metanol binahong mempunyai kemampuan sebagai penangkap radikal bebas. Nilai ES_{50} ekstrak heksan, kloroform, metanol dan asam galat berturut-turut adalah 583,60±0.43 µg/ml, 446,22±0.51 µg/ml, 237,68±5.63 µg/ml dan 2,80±0.1 µg/ml.*

Kesimpulan: *Potensi ketiga ektrak binahong sebagai penangkap radikal bebas DPPH lebih lemah dibanding asam galat.*

INTRODUCTION

Free radicals may play a role in various degenerative diseases such as cancer, atherosclerosis, ageing, inflammation, diabetes, hair loss, and Parkinson's.[1,2] The effects of free radicals can be mitigated if the body has enough antioxidants and by regulating diet, such as by consuming foods and drinks that contain a high level of antioxidants. Therefore, people are starting to change their lifestyles by using nature-sourced materials which are believed to be healthier and safer for the body.[3]

The human body is capable of producing limited amounts of endogenous antioxidants. However, the limited amount of antioxidants is not able to fight the increasing free radicals inside the body. Therefore, exogenous antioxidants are needed to fight the increase in free radicals.[4]

Antioxidants can eliminate, clean (scavenging), resist formation, or negate the effects of free radicals.[4,5] There are 2 types of antioxidants; synthetic and natural antioxidants. Natural antioxidants have higher effectiveness but lower toxic properties compared to synthetic antioxidants.[6] Therefore, the food and medicine industry has shifted to develop natural antioxidants.

Binahong (*Anredera cordifolia*) or Tenore Steen is found in South America and one of the interesting plants to study. Growing evidence suggests that the binahong plant has clinical potentials. Binahong leaves and rhizoma are known to be useful as a healing agent for scars, typhus, inflammation of the intestine, lowering uric acid, dysentery, and haemorrhoid. Binahong leaves contain triterpenoid saponins, flavonoid compounds, and ursolic acid.[7,8]

Binahong plants need to be explored to obtain its active compounds of the antioxidants property. It is necessary to screen the antioxidant potential with some extraction methods and compound with various levels of polarity. Previous studies have shown that the ethanol extract of binahong leaves has antioxidant activity.[9,10] Accordingly, this study using all parts of plants above ground with hexane (non-polar), chloroform (semipolar), and methanol (polar) extracts to obtain extracts tested for free radical scavenging activity. The results of this study suggest that the use of binahong can be optimized, especially in the health sector.

METHODS

Binahong plants were obtained from the Bantul Pleret area of Yogyakarta. Determination of plants was carried out in the Laboratory of Pharmaceutical Biology, Gadjah Mada University.

Extraction procedures

The process of extraction in this study was done by using a gradual maceration method. 20 g of dried powder from all parts of the Binahong plant above ground level was extracted with gradually organic solvents from non polar to polar organic solvents (hexane, chloroform and methanol). After maceration periods, the soaked powder-solvent mixtures were filtered by using Buchner funnel. The residue left in the funnel was re-extracted twice follow the same procedure and filtered. Each extract was concentrated and dried by by using rotary evaporator to obtain a thick extract.

Antioxidant activity

The free radical scavenging activity of hexane, chloroform, and methanol extracts of Binahong

was measured using DPPH assay. The extracts were added into tubes containing 0,15 mM methanolic DPPH solution. The reduction of absorbance at 516 nm was measured twice, before and 30 minutes after extracts were added into tubes.

Antioxidant activity is calculated by the following equation[11]:

$$\% \text{ Capture} = [(A_{control} - A_{test}/A_{control}) \text{ X } 100\%]$$

Note: $A_{control}$ = Control absorbance

A_{test} = Control absorbance test sample

The concentration of extract needed to reduce absorbance by 50% from the initial state (ES_{50}) was determined by the regression equation between the percentage of capture and concentration. The smaller the value of ES50 means the greater the antioxidant power of the compound. As a comparison for this method used gallic acid, and all tests were replicated 3 times.

Determination of total phenolic binahong extract

Determination of Total Phenolic Content (TPC) was performed by visible spectrophotometry with Folin Ciocalteu reagent. This method is based on the formation of a blue complex that is read at a wavelength of 746 nm. Gallic acid was used as a comparison. The third TPC value of the extract was expressed in Gallic Acid Equivalent (GAE) mg / g extract. A total of 10.00 mg of each type of extract was dissolved in methanol to amount to 10.0 ml. Three hundred microliters of the extract solution were added to 1.5 ml of the Folin-Ciocalteu reagent (which had been diluted 10 times). After settling for 3 minutes, the solution is added with 1.2 ml of 7.5% Na_2CO_3. The absorbance will be read at 765 nm after 1 hour. As a comparison used Galat acid (12) All tests were replicated 3 times. ES_{50} data and TPC content were statistically analyzed, namely, Anova continued with LSD with a 95% confidence level using the SPSS program.

RESULTS

The extract yield obtained from each solvent was 1.2% Hexane; chloroform 4.2%; and 6.3% methanol respectively. The ability of compounds as free radical scavengers is shown by a decrease in absorbance of DPPH at λ 516.4 nm. The results of antioxidant activity tests by DPPH method as shown in Table 1 and Figure 1 below:

Table 1. The results of antioxidant activity tests by DPPH

Sample	ES_{50} ± SD (µg/ml)
Gallic Acid	2,058 ± 0,002*
Methanol extract	237,683 ± 13,373*
Chloroform extract	446,219 ± 2,268*
Hexane extract	583,601 ± 2,533*

Note: n=3 * p<0.05

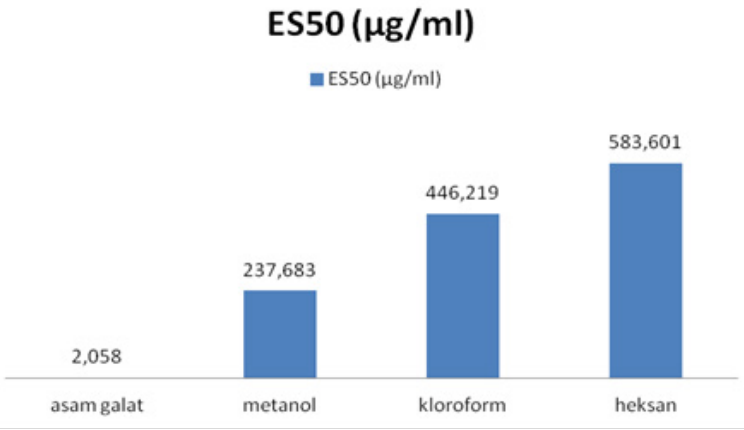

Figure 1. Comparison of ES_{50} between Gallic acid and binahong extract

The results of determining the extract TPC content are presented in Figure 2.

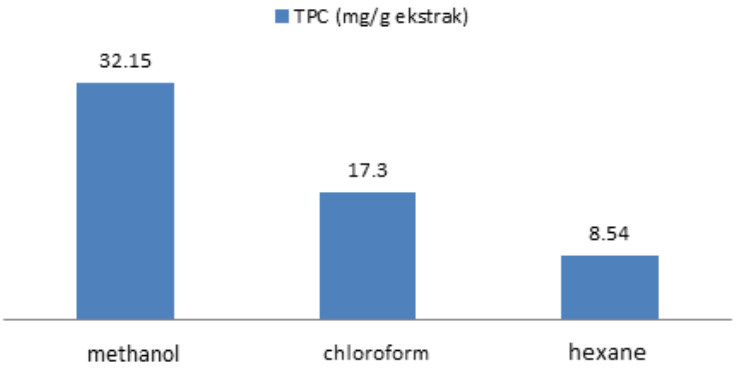

Figure 2. Profile of the third TPC of binahong extract

DISCUSSION

Figure 1 shows that methanol extract has the most powerful ability as the free radical scavenger compared to chloroform extract and hexane extract. Based on the ES_{50} value of methanol extract, the smaller the ES_{50} value of a substance, the greater its ability as a free radical scavenger. The results of this study are in line with another research which states that the methanol extract of binahong leaves has higher free radical scavenging activity than hexane extract.[13] However, the ability of binahong extract as a free radical scavenger is still weaker than gallic acid. According to Blois criteria, those three extracts are in the inactive category because they have an ES_{50} value of more than 200 µg / ml.[14] Different from the previous research, this study uses not only the leaves but also all parts of the plant above ground level (both stems, young leaves, and old leaves).[15] Moreover, we studied the content of flavonoids and the total antioxidant capacity of the ethanol extract of binahong leaves. Antioxidant test methods used were also different, in which this study uses the DPPH method while Selawa et al. using the FRAP method.

The results of TPC determination showed that methanol extract has the highest TPC content followed by chloroform extract and hexane extract. This shows that more polar solvents tend to find polyphenol compounds. Based on these data, it is seen that there is a correlation between TPC content and its ability as a free radical scavenger with a correlation coefficient (R) value of 0.994. Accordingly, recent research states that the TPC content, as well as its antioxidant potential of lampes leaf extract, is greater than the seed extract.[16] This is supported by Büyüktuncel et al which suggests that antioxidant activity is strongly correlated with total phenolic content in red wine.[17]

CONCLUSION

The antioxidant activity of methanol extract was higher compared to chloroform extract and hexane extract. The total phenolic level oftotal methanol extracts was higher compared to chloroform extract and hexane extract.

CONFLICT OF INTEREST

None declare.

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