DETAIL DOCUMENT
Research

Detail Research

Verified by at 0000-00-00 00:00:00

NIDN Leader
0520077201

Leader Name
SRI HARTATI YULIANI

PDDIKTI Code PT (Leader)
051012

Institution (Leader)
Universitas Sanata Dharma

Title
Formulasi Kapsul Ekstrak Binahong (Anredera cordifolia (Ten) Steenis) Sebagai Penurun Kadar Gula Darah

Skema Abbreviation
PPS-PTM

Skema Name
Penelitian Tesis Magister

The First year of the proposal
Tahun Pertama Usulan
2019

Proposed Year of Activities
Tahun Usulan Kegiatan
2019

The Year of The Activity
Tahun Pelaksanaan Kegiatan
2020

Duration of activity
Lama Kegiatan
1 Year

Proposal Status
didanai

Funds are approved
Rp. 39.250.000,-

SINTA Afiliasi ID
422

Funds Institution
Universitas Sanata Dharma ✔ in sync with Sinta Affiliation

Target TKT
TKT 2

Hibah Program
Penelitian Kompetitif Nasional

Focus Area
Kesehatan

Fund Source Category
Pemerintah

Fund Source

Country Fund Source
ID

Research Member

SRI HARTATI YULIANI
*Registered in Sinta using **SRI HARTATI YULIANI** (Sinta ID : 5977336)*
Status : Leader (Leader) | Universitas Sanata Dharma

UNIVERSITAS
SANATA DHARMA
LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT

SURAT TUGAS PENELITIAN

No. 027/ LPPM USD /III/2020

Ketua Lembaga Penelitian dan Pengabdian Kepada Masyarakat Universitas Sanata Dharma Yogyakarta dengan ini memberikan tugas kepada:

Nama	: Dr. apt. Sri Hartati Yuliani
Pekerjaan	: Dosen
NIP/NIDN	: P.1828 / 0520077201
Jabatan Fungsional	: Lektor Kepala
Program Studi	: S2 Farmasi
Fakultas	: Farmasi
Status	: Ketua Peneliti

Untuk melaksanakan penelitian Tesis Magister yang didanai oleh Kementrian Riset, Teknologi, dan Pendidikan Tinggi Direktorat Jenderal Penguatan Riset dan Pengembangan dengan:

Judul Penelitian	: Formulasi Kapsul Ekstrak Binahong (Anredera cordifolia (Ten) Steenis) Sebagai Penurun Kadar Gula Darah.
Skema Penelitian	: Penelitian Tesis Magister
Waktu Penelitian	: Maret – November 2020

Penerima tugas penelitian wajib mematuhi ketentuan-ketentuan penelitian sebagaimana diatur oleh Kementrian Riset, Teknologi, dan Pendidikan Tinggi Direktorat Jenderal Penguatan Riset dan Pengembangan.

Demikian surat tugas penelitian ini dibuat, mohon dilaksanakan sebagaimana mestinya.

Yogyakarta, 3 Maret 2020
Lembaga Penelitian dan Pengabdian Kepada Masyarakat USD

Dr. rer. nat. Herry Pribawanto Suryawan
Ketua

Tembusan:

1. Yth. Rektor
2. Yth. Wakil Rektor I
3. Yth. Dekan/Direktur Pasca Sarjana
4. Yth. Ketua Program Studi
5. Arsip

Alamat: Jl. Affandi, Mrican, Tromol Pos 29, Yogyakarta 55002 Telp. (0274) 513301 Ext. 1527, Fax (0274) 562383 website: http:/www.usd.ac.id/lembaga/lppm Email : lemlit@usd.ac.id
Acc. No, CIMB NIAGA LEMBAGA PENELITIAN UNIV. SANATA DHARMA 287.01.00277.00.5

HIBAH PENELITIAN TAHUN TUNGGAL
DRPM KEMENRISTEK DIKTI
TAHUN ANGGARAN 2020
UNIVERSITAS SANATA DHARMA-YOGYAKARTA

LAPORAN AKHIR

FORMULASI KAPSUL EKSTRAK BINAHONG (*Anredera cordifolia (Ten) Steenis*) SEBAGAI PENURUN KADAR GULA DARAH

Dr. SRI HARTATI YULIANI, Apt.
OCTAVIANUS BUDI SANTOSA S.Si., Apt

DILAKSANAKAN ATAS BIAYA
Direktorat Riset dan Pengabdian kepada Masyarakat
Deputi Bidang Penguatan Riset dan Pengembangan
Kementrian Riset, Teknologi dan Pendidikan Tinggi sesuai dengan
DIPA No. SPDIPA-042.06-1.401516/2020, tanggal 12 November 2019

UNIVERSITAS SANATA DHARMA

Desember 2020

PROTEKSI ISI LAPORAN AKHIR PENELITIAN
Dilarang menyalin, menyimpan, memperbanyak sebagian atau seluruh isi laporan ini dalam bentuk apapun kecuali oleh peneliti dan pengelola administrasi penelitian

LAPORAN AKHIR PENELITIAN TAHUN TUNGGAL

ID Proposal: 1e6bc7d3-1c71-42af-8861-148b50f1b5b3
Laporan Akhir Penelitian: tahun ke-1 dari 1 tahun

1. IDENTITAS PENELITIAN

A. JUDUL PENELITIAN

Formulasi Kapsul Ekstrak Binahong (Anredera cordifolia (Ten) Steenis) Sebagai Penurun Kadar Gula Darah

B. BIDANG, TEMA, TOPIK, DAN RUMPUN BIDANG ILMU

Bidang Fokus RIRN / Bidang Unggulan Perguruan Tinggi	Tema	Topik (jika ada)	Rumpun Bidang Ilmu
Kesehatan	Teknologi kemandirian bahan baku obat	Pengembangan fitofarmaka berbasis sumber daya lokal	Farmasetika dan Teknologi Farmasi

C. KATEGORI, SKEMA, SBK, TARGET TKT DAN LAMA PENELITIAN

Kategori (Kompetitif Nasional/ Desentralisasi/ Penugasan)	Skema Penelitian	Strata (Dasar/ Terapan/ Pengembangan)	SBK (Dasar, Terapan, Pengembangan)	Target Akhir TKT	Lama Penelitian (Tahun)
Penelitian Kompetitif Nasional	Penelitian Tesis Magister	SBK Riset Dasar	SBK Riset Dasar	2	1

2. IDENTITAS PENGUSUL

Nama, Peran	Perguruan Tinggi/ Institusi	Program Studi/ Bagian	Bidang Tugas	ID Sinta	H-Index
SRI HARTATI YULIANI Ketua Pengusul	Universitas Sanata Dharma	Farmasi		5977336	5
Octavianus Budi Santosa Mahasiswa Bimbingan 1	S2 Farmasi Universitas Sanata Dharma	-	formulasi sediaan kapsul binahong untuk menurunkan kadar gula darah	0	0

3. MITRA KERJASAMA PENELITIAN (JIKA ADA)

Pelaksanaan penelitian dapat melibatkan mitra kerjasama, yaitu mitra kerjasama dalam melaksanakan penelitian, mitra sebagai calon pengguna hasil penelitian, atau mitra investor

Mitra	Nama Mitra

4. LUARAN DAN TARGET CAPAIAN

Luaran Wajib

Tahun Luaran	Jenis Luaran	Status target capaian (*accepted, published, terdaftar atau granted, atau status lainnya*)	Keterangan (*url dan nama jurnal, penerbit, url paten, keterangan sejenis lainnya*)
1	Artikel di Jurnal Nasional terakreditasi peringkat 1-3	Accepted	Jurnal Farmasi Sains dan Komunitas

Luaran Tambahan

Tahun Luaran	Jenis Luaran	Status target capaian (*accepted, published, terdaftar atau granted, atau status lainnya*)	Keterangan (*url dan nama jurnal, penerbit, url paten, keterangan sejenis lainnya*)

5. ANGGARAN

Rencana anggaran biaya penelitian mengacu pada PMK yang berlaku dengan besaran minimum dan maksimum sebagaimana diatur pada buku Panduan Penelitian dan Pengabdian kepada Masyarakat Edisi 12.

Total RAB 1 Tahun Rp. 39,250,000

Tahun 1 Total Rp. 39,250,000

Jenis Pembelanjaan	Item	Satuan	Vol.	Biaya Satuan	Total
Bahan	ATK	Paket	1	500,000	500,000
Bahan	Bahan Penelitian (Habis Pakai)	Unit	10	3,875,000	38,750,000

6. HASIL PENELITIAN

A. RINGKASAN: Tuliskan secara ringkas latar belakang penelitian, tujuan dan tahapan metode penelitian, luaran yang ditargetkan, serta uraian TKT penelitian.

Ekstrak daun Binahong (Anredera cordifolia (Ten.) Steenis), salah satu tanaman obat di Indonesia menunjukkan aktivitas menurunkan kadar gula darah. Esktrak ini perlu dibuatkan sediaan agar mudah di distribusi dan dijangkau oleh masyarakat. Bentuk sediaan kapsul dipilih mengingat sifat fisik ekstrak yang cenderung lembab sehingga agak sukar untuk dibuatkan dalam sediaan tablet, memiliki rasa dan bau khas, serta juga kepraktisannya dibandingkan bentuk sediaan serbuk. Tujuan dari penelitian ini adalah menghasilkan kapsul ekstrak daun binahong yang mempunyai aktivitas sebagai penurun gula darah.

Tahapan penelitian yang akan dikerjakan adalah ekstraksi daun binahong, validasi metode analisis viteksin sebagai bahan aktif, optimasi bahan pengering ekstrak binahong, dan optimasi formula sediaan kapsul ekstrak binahong. Hambatan yang dihadapi dalam melaksanakan penelitian ini adalah adanya pandemi covid-19 sehingga selama 3 bulan penelitian terhenti sehingga tahap terakhir yaitu optimasi formula sediaan kapsul. Formulasi sediaan kapsul ekstrak binahong masih dikerjakan dan ditargetkan selesai pada bulan Desember 2020.

Hasil penelitian yang didapat sejauh ini adalah didapatkan metode analisis yang valid untuk digunakan dalam menganalisis viteksin dalam ekstrak etanol binahong. Ekstrak kemudian dikeringkan dengan Coloidal silicone Dioxide (CSC) dan Microcrystalline cellulose (MCC) dengan perbandingan tertentu. Perbandingan yang dipilih adalah CSD:MCC = 0,70:0,30. Pada perbandingan tersebut ekstrak kering memberikan hasil sifat fisis yang paling baik. Formulasi sediaan kapsul sedang dilakukan saat ini dengan melakukan optimasi menggunakan metode desain faktorial.

Luaran yang ditargetkan dalam penelitian ini adalah artikel ilmiah. Artikel ilmiah

berjudul “Chemometric Assisted Quantitative Determination Of Vitexin In Ethanolic Extract Of Binahong (Anredera Cordifolia (Ten.) Steenis) Leaves” merupakan data pada tahap awal penelitian sebelum pandemi terjadi. Artikel tersebut telah publish pada jurnal internasional bereputasi terindeks Scopus Q3 International Journal of Applied Pharmaceutics pada Vol 12, issue 6, November 2020. Artikel tersebut dapat diakses pada web jurnal tersebut dengan link yang langsung menuju naskah sebagai berikut https://innovareacademics.in/journals/index.php/ijap/article/view/39248

B. KATA KUNCI: Tuliskan maksimal 5 kata kunci.

viteksin; binahong; ekstrak bianhong; kapsul ekstrak binahong

Pengisian poin C sampai dengan poin H mengikuti template berikut dan tidak dibatasi jumlah kata atau halaman namun disarankan seringkas mungkin. Dilarang menghapus/memodifikasi template ataupun menghapus penjelasan di setiap poin.

C. HASIL PELAKSANAAN PENELITIAN: Tuliskan secara ringkas hasil pelaksanaan penelitian yang telah dicapai sesuai tahun pelaksanaan penelitian. Penyajian dapat berupa data, hasil analisis, dan capaian luaran (wajib dan atau tambahan). Seluruh hasil atau capaian yang dilaporkan harus berkaitan dengan tahapan pelaksanaan penelitian sebagaimana direncanakan pada proposal. Penyajian data dapat berupa gambar, tabel, grafik, dan sejenisnya, serta analisis didukung dengan sumber pustaka primer yang relevan dan terkini.

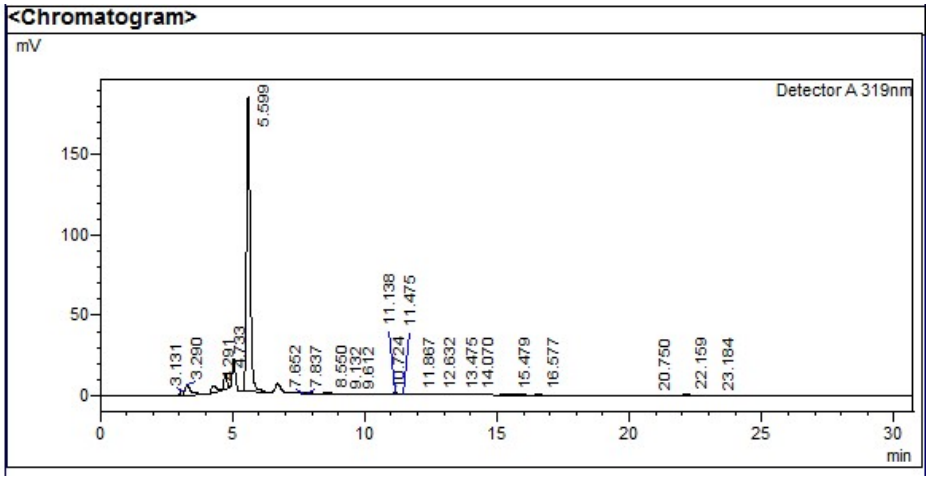
Pengisian poin C sampai dengan poin H mengikuti template berikut dan tidak dibatasi jumlah kata atau halaman namun disarankan seringkas mungkin. Dilarang menghapus/memodifikasi template ataupun menghapus penjelasan di setiap poin.

C. **HASIL PELAKSANAAN PENELITIAN:** Tuliskan secara ringkas hasil pelaksanaan penelitian yang telah dicapai sesuai tahun pelaksanaan penelitian. Penyajian dapat berupa data, hasil analisis, dan capaian luaran (wajib dan atau tambahan). Seluruh hasil atau capaian yang dilaporkan harus berkaitan dengan tahapan pelaksanaan penelitian sebagaimana direncanakan pada proposal. Penyajian data dapat berupa gambar, tabel, grafik, dan sejenisnya, serta analisis didukung dengan sumber pustaka primer yang relevan dan terkini.

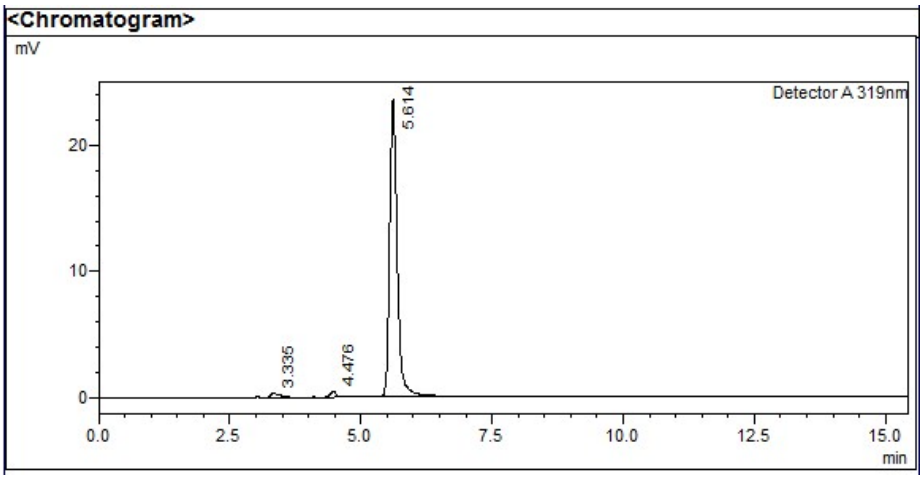
Hasil penelitian ini adalah sebagai berikut:

Validasi metode analisis viteksin dalam ekstrak binahong dengan kemometrik

- Kromatogram sampel ekstrak daun binahong dan *vitexin* (HPLC)

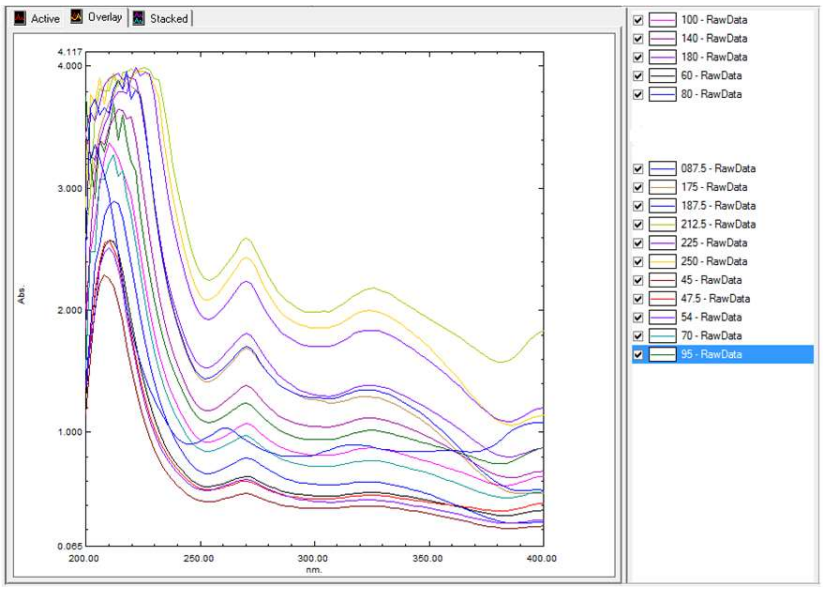


Kromatogram Sampel Binahong

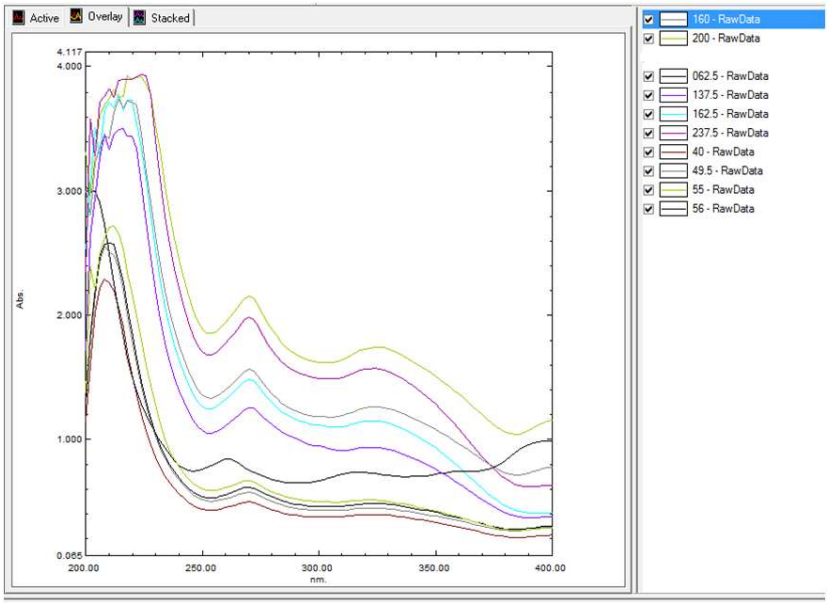


Kromatogram *vitexin* 10 ppm

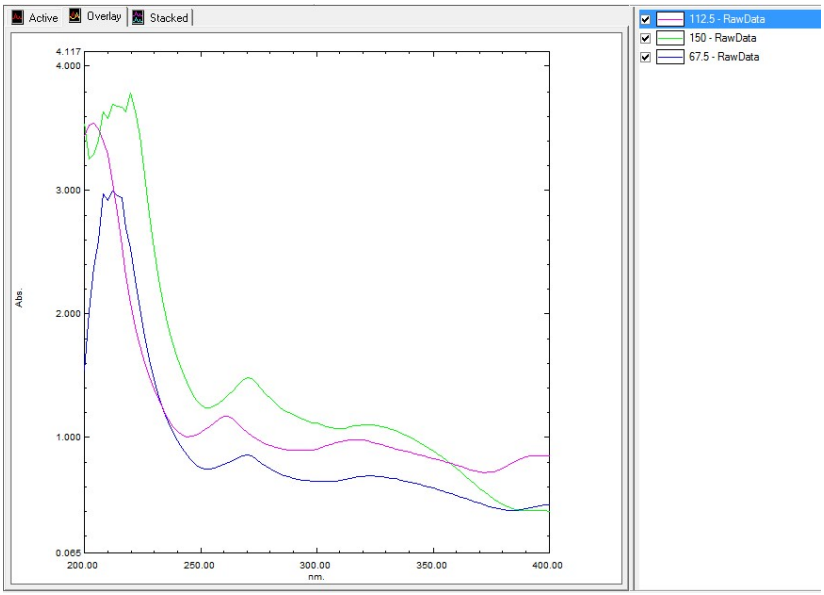
- Spektra data kalibrasi, validasi dan sampel.



Spektra Kalibrasi



Spektra Validasi



Spektra Sampel

- Hasil analisa

Validasi Penetapan Kadar Vitexin (HPLC)

Parameters	Value	Acceptance criteria
System suitability test		
% RSD Tr (min)	0.38	% RSD < 2%
% RSD Area	0.12	
Tailing factor (Tf)	1.247	Tf < 2.0
Number theoretical plate (N)	7488	N > 2000
Selectivity		
Resolution (Rs) sample	Rs = 2.045	Resolution ≥ 1.5
Linearity		
Coefficient correlation (r)	0.9996	0.999
Coefficient determination (R)	0.9992	-
Slope (b)	20349	-
Intercept (a)	-28532	-
Range	2.11 ppm – 31.61 ppm	-
Limit of detection	1.10 ppm	-
Limit of quantitation	3.34 ppm	-
***Accuracy (Recovery)**		
(intra-day)	97.85% – 104.42%	95% – 105%
(inter-day)	101.45% – 103.40%	
***Precision (RSD)**		
(intra-day)	1.63% – 3.04%	< 3.7%
(inter-day)	1.35% – 3.33%	
+Assay content vitexin in ethanolic extract of binahong	0.11 ± 0.01 % w/w with RSD = 1.32%	RSD < 3.7%

Note: * Accuracy and Precision were determined at 3 concentration addition levels of vitexin, 3 replicates and 3 consecutive days for inter-day. +assay content was determined by 3 replicates of ethanolic extract of binahong.

Hasil validasi metode analisis menggunakan HPLC didapatkan kesimpulan bahwa metode yang digunakan dalam memenuhi parameter validitas metode analisis dan selanjutnya dapat digunakan untuk menentukan validitas dari metode kemometrik yang akan digunakan dalam analisis kadar selanjutnya.

Kemometrik dengan bantuan software R-studio

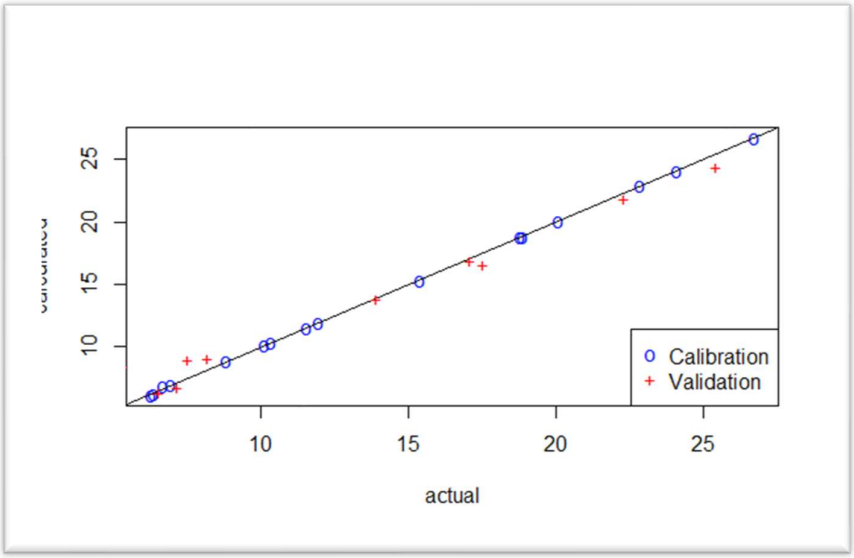
Larutan kalibrasi

Compounds	Multivariate calibrations	Calibration			
		Number of components	R^2	RMSEC	RMSECV
Vitexin	PLS	11	0.9675	0.0738	1.204
Vitexin	PCR	14	0.9648	0.0585	1.253

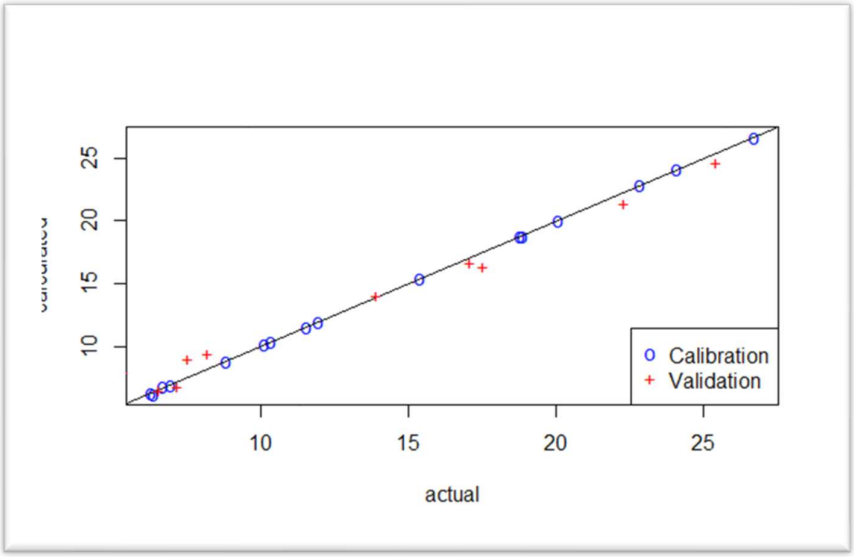
Larutan validasi

Compounds	Multivariate calibrations	Validation		
		# of components	R^2	RMSEP
Vitexin	PLS	11	0.9778	1.2437
Vitexin	PCR	14	0.9820	1.1669

Observation data plot (actual) vs predicted data (calculated) with PLS



Observation data plot (actual) vs predicted data (calculated) with PCR



Hasil penetapakan kadar

Compounds	Method	# of solution test			REP
		1	2	3	
Vitexin	PLS	8.192513	16.950112	12.530707	5.603%
Vitexin	PCR	7.95943	17.01199	12.12951	6.917%
Vitexin	Actual	9.29	16.32	12.57	-

Hasil ini menunjukkan bahwa penetapan kadar vitexin dalam ekstrak etanol daun binahong secara kemometrik bisa dikembangkan sebagai salah satu alternatif yang lebih sederhana dan murah dan untuk selanjutnya maka metode ini akan digunakan untuk penetapan kadar vitexin dalam ekstrak maupun dalam uji keseragaman kadar vitexin dalam kapsul.

Data dan analisis vitexin menggunakan metode kemometri telah dipublikasikan di International Journal of Applied Pharmaceutic dengan judul artikel "Chemometric Assisted Quantitative Determination Of Vitexin In Ethanolic Extract Of Binahong (*Anredera Cordifolia* (Ten.) Steenis) Leaves". Artikel tersebut akan terbit pada Vol 12, issue 6, November 2020.

Pembuatan serbuk ekstrak binahong

Pembuatan serbuk ekstrak binahong dilakukan dengan menambahkan ekstrak cair binahong dengan campuran aerosil dan microcrystaline selulosa (MCC). Terdapat 5 perbandingan sebagai berikut:

P1 = 100% Aerosil

P2 = 75% Aerosil + 25% MCC

P3 = 50% Aerosil + 50% MCC

P4 = 25% Aerosil + 75% MCC

P5 = 100% MCC

Hasil pengeringan pengeringan serbuk ekstrak binahong

Serbuk yang dihasilkan dari pengeringan ekstrak binahong dengan penambahan campuran aerosil dan MCC tersebut kemudian diuji sifat fisisnya meliputi Tap density, LOD dan water uptake. Hasil pengujian tersebut adalah sebagai berikut:

Hasil uji LOD, water uptake, dan Carr's Indeks serbuk kering ekstrak binahong

Formula	LOD (%) ± SD	Water Uptake (%) ± SD	Carr's Index (%) ± SD
P1	3.78 ± 0.205	1.10 ± 0.046	20.00 ± 0.000
P2	4.72 ± 0.161	1.03 ± 0.032	19.33 ± 1.155
P3	1.97 ± 0.085	1.09 ± 0.015	21.33 ± 1.155
P4	2.51 ± 0.146	1.67 ± 0.091	18.67 ± 1.155
P5	3.72 ± 0.200	1.80 ± 0.083	17.33 ± 2.309

Hasil uji LOD, Water uptake dan Carr's Indeks kemudian dianalisis menggunakan software *Design Expert* 12®. Dalam optimasi formula pengering, kriteria penerimaan untuk parameter *Carr's Index*[1,2] yang dimasukkan ke dalam perhitungan adalah kriteria *fair* ke atas (max 20). Kriteria ini dipilih dengan harapan ekstrak kering yang dihasilkan memiliki sifat alir yang baik, sehingga dalam formulasi sediaan kapsul tidak menemui masalah yang bearti. Untuk parameter LOD, batasan penerimaannya adalah 5% (Pustaka). Sedangkan parameter *water uptake*, kriteria yang digunakan dalam perhitungan adalah 0,2 – 2 % (*slighgtly hygroscopic*)[3]. Pemilihan nilai parameter higroskopisitas di sini dengan tujuan menjaga stabilitas sediaan akhir nantinya.

Hasil olah data dengan piranti lunak *Design Expert* 12®.

Number	CSD	MCC	LOD	Water Uptake	Carr's Index	Desirability
1	0.700	0.300	4.106	1.001	19.947	1.000
2	0.250	0.750	2.513	1.667	18.667	1.000
3	1.000	0.000	3.780	1.103	20.000	1.000
4	0.000	1.000	3.720	1.797	17.333	1.000
5	0.750	0.250	4.723	1.033	19.333	1.000
6	0.327	0.673	1.963	1.463	19.890	1.000
7	0.717	0.283	4.318	1.010	19.743	1.000
8	0.961	0.039	4.734	1.140	19.036	1.000
9	0.230	0.770	2.684	1.715	18.344	1.000
10	0.173	0.827	3.197	1.837	17.475	1.000
11	0.042	0.958	3.874	1.889	16.808	1.000
12	0.093	0.907	3.761	1.920	16.739	1.000
13	0.114	0.886	3.646	1.912	16.852	1.000
14	0.289	0.711	2.204	1.564	19.307	1.000
15	0.264	0.736	2.396	1.630	18.900	1.000
16	0.981	0.019	4.299	1.126	19.470	1.000
17	0.731	0.269	4.503	1.020	19.559	1.000
18	0.056	0.944	3.871	1.906	16.731	1.000
19	0.148	0.852	3.402	1.876	17.168	1.000
20	0.072	0.928	3.841	1.917	16.701	1.000
21	0.766	0.234	4.902	1.046	19.144	1.000
22	0.017	0.983	3.816	1.843	17.057	1.000
23	0.193	0.807	3.021	1.800	17.756	1.000

Berdasarkan hasil tersebut, dipilih satu komposisi campuran pengering yang dirasa mempunyai sifat yang memenuhi kriteria. Meskipun semua prediksi komposisi campuran memenuhi kriteria yang ditetapkan, dipilih komposisi campuran yang memiliki respon *water uptake* paling kecil, dalam hal ini terpilih komposisi Coloidal Silicone Dioxide (CSD):Microcrystaline Cellulose (MCC) = 0.700:0.300. Dengan respon *water uptake* yang kecil (higroskopisitas rendah) diharapkan serbuk dapat memiliki stabilitas yang lebih baik. Sebagaimana telah diketahui air adalah media yang baik untuk pertumbuhan mikroorganisme, sehingga dengan higroskopisitas yang rendah, diharapkan dapat menekan pertumbuhannya.

D. **STATUS LUARAN**: Tuliskan jenis, identitas dan status ketercapaian setiap luaran wajib dan luaran tambahan (jika ada) yang dijanjikan pada tahun pelaksanaan penelitian. Jenis luaran dapat berupa publikasi, perolehan kekayaan intelektual, hasil pengujian atau luaran lainnya yang telah dijanjikan pada proposal. Uraian status luaran harus didukung dengan bukti kemajuan ketercapaian luaran sesuai dengan luaran yang dijanjikan. Lengkapi isian jenis luaran yang dijanjikan serta mengunggah bukti dokumen ketercapaian luaran wajib dan luaran tambahan melalui Simlitabmas mengikuti format sebagaimana terlihat pada bagian isian luaran

Jenis luaran adalah publikasi ilmiah. Publikasi ilmiah pada jurnal "International Journal of Applied Pharmaceutics" (Scopus Q3) dengan judul artikel "Chemometric Assisted Quantitative Determination Of Vitexin In Ethanolic Extract Of Binahong (*Anredera Cordifolia* (Ten.) Steenis) Leaves". Status dari luaran ini adalah **publish** pada Vol 12, issue 6, November 2020 (**https://innovareacademics.in/journals/index.php/ijap/article/view/39248**)

E. **PERAN MITRA:** Tuliskan realisasi kerjasama dan kontribusi Mitra baik *in-kind* maupun *in-cash* (jika ada). Bukti pendukung realisasi kerjasama dan realisasi kontribusi mitra dilaporkan sesuai dengan kondisi yang sebenarnya. Bukti dokumen realisasi kerjasama dengan Mitra diunggah melalui Simlitabmas mengikuti format sebagaimana terlihat pada bagian isian mitra

Tidak ada mitra dalam penelitian ini

F. **KENDALA PELAKSANAAN PENELITIAN**: Tuliskan kesulitan atau hambatan yang dihadapi selama melakukan penelitian dan mencapai luaran yang dijanjikan, termasuk penjelasan jika pelaksanaan penelitian dan luaran penelitian tidak sesuai dengan yang direncanakan atau dijanjikan.

Adaya pandemi membuat jalannya penelitian agak terhambat sehingga diputuskan hasil tahapan pertama yaitu validasi metode analisis yang digunakan sebagai data untuk luaran penelitian ini. Hal tersebut cukup membantu karena saat laboratorium harus off waktu masih bisa digunakan untuk menulis luaran.

G. **RENCANA TINDAK LANJUT PENELITIAN:** Tuliskan dan uraikan rencana tindaklanjut penelitian selanjutnya dengan melihat hasil penelitian yang telah diperoleh. Jika ada target yang belum diselesaikan pada akhir tahun pelaksanaan penelitian, pada bagian ini dapat dituliskan rencana penyelesaian target yang belum tercapai tersebut.

Karena alasan adanya pandemi sehingga pengambilan data sempat tertunda sehingga ada data yang belum selesai diambil yaitu formulasi dan uji sifat fisis sediaan kapsul. Kedua hal ini masih dilakukan sampai saat ini dan ditargetkan akan selesai pada akhir Desember 2020. Walaupun ada hambatan tersebut, luaran berupa artikel ilmiah tetap dapat tercapai dengan mengolah data yang pertama.

H. DAFTAR PUSTAKA: Penyusunan Daftar Pustaka berdasarkan sistem nomor sesuai dengan urutan pengutipan. Hanya pustaka yang disitasi pada laporan akhir yang dicantumkan dalam Daftar Pustaka.

1. Earle RR, Ayalasomayajula LU, Naga Raju A, Tanuja Kumari K, Ravi Kumar P. Formulation and evaluation of diclofenac sodium oro dispersible tablets using different superdisintegrants by direct compression technique. Der Pharm Lett. 2016;8(8):227–38.
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Original Article

CHEMOMETRIC ASSISTED QUANTITATIVE DETERMINATION OF VITEXIN IN ETHANOLIC EXTRACT OF BINAHONG (*ANREDERA CORDIFOLIA* (TEN.) STEENIS) LEAVES

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ABSTRACT

Objective: The objective of this study was to develop a UV spectroscopy method in combination with multivariate analysis for determining vitexin in binahong (*Anredera cordifolia* (Ten.) Steenis) leaves extract.

Methods: The partial least square (PLS) regression and the principal component regression (PCR) was performed in this study to evaluate several statistical performances such as coefficient of determination (R^2), root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV), root mean square error of prediction (RMSEP) and relative error of prediction (REP). Cross-validation in this study was performed using leave one out technique.

Results: The R^2 values of calibration data sets resulted from PLS and PCR method were 0.9675 and 0.9648, respectively. The low values of RMSEC and RMSECV both for PLS and PCR method indicated the minimum error of the calibration models. The R^2 values of validation data sets resulted from PLS and PCR method were 0.9778 and 0.9820, respectively. The low values of RMSEP both for PLS and PCR method indicated the minimum error of prediction generated from the calibration data sets. Multivariate calibration techniques were applied to determine the content of vitexin in binahong leaves extract. Predicted values from the multivariate calibration models were compared to the actual values determined from a validated HPLC method. It was found that PLS models resulted in the lowest REP values compared to the PCR models.

Conclusion: The chemometrics technique can be applied as an alternative method for determining vitexin levels in the ethanol solution of binahong leaves extract.

Keywords: Binahong, HPLC, UV Spectroscopy, Vitexin

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INTRODUCTION

Binahong (*Anredera cordifolia* (Ten.) Steenis), one of 6000 types of plants in Indonesia, have been used for several health treatments [1]. Binahong has pharmacological effects such as anti-bacterial [2-5], anti-fungal [6], anti-aging [7], anti-diabetes [8], wound healing [9-11], anti-inflammatory [12], anti-oxidant [13], anti-cancer [14], anti-obesity [15] and anti-hyperuricemia [16]. Due to its pharmacological activities, binahong was potential to be developed as traditional medicine. Hence, it is important to determine the chemicals contained in binahong.

Binahong leaves contain terpenoids, steroids, glycosides, flavonoids, saponins and alkaloids [17]. Several active compounds were reported and obtained from binahong such as ursolic acid, ancordin, apigenin [17] and vitexin [13, 18]. Vitexin was reported due to its pharmacological effects, such as anti-diabetes [19], anti-cancer, anti-oxidant, anti-virus, anti-inflammatory, anti-hypertension, etc [20]. It can be stated that vitexin, one of the active compounds in binahong, can be potentially developed as an anti-diabetic, anti-inflammatory, anti-oxidant and anti-cancer. In this research, vitexin was used as a marker in the standardization of binahong leaf extract.

In previous studies, the determination of vitexin levels has been reported. The most common analytical method used was HPLC [21, 22]. Results of these study indicate that the determination of vitexin levels by the HPLC method has been well validated. However, this kind of method requires considerable expertise, spends a lot of time and requires equipment and costs. Therefore, it is necessary to develop a simple, efficient, and low-cost analytical method to analyze vitexin content in the binahong extract.

The use of spectroscopy, combined with the multivariate technique, was expected to overcome this problem. This study aims to determine the levels of vitexin in binahong (*Anredera cordifolia* (Ten) Steenis) leaf extracts using spectrophotometry combined with multivariate techniques.

MATERIALS AND METHODS

Materials and chemicals

The vitexin (Sigma-Aldrich, Missouri, USA), methanol HPLC grade (Merck Milipore, Darmstadt, Germany) and orthophosphoric acid p. a (Merck Milipore, Darmstadt, Germany), acetonitrile HPLC grade (JT Baker, Phillipsburg, USA), ethanol was purchased from a local store in Yogyakarta, Indonesia. Redistilled water was obtained from the Analytical Instrument Laboratory, Faculty of Pharmacy, Universitas Sanata Dharma, Indonesia. Samples of binahong leaves were obtained from Wirobrajan district in Yogyakarta, Indonesia. The identification of Binahong leaves was confirmed based on the specimen authentication certificate no 14.8.11/UNI/FFA/BF/PT/2019 that was conducted by Biological Pharmacy Departement, Faculty of Pharmacy, Universitas Gadjah Mada.

Instrumentation and software

A set of UV-Vis Spectrophotometer (Shimadzu®, Kyoto, Japan) type UV 1800 equipped with quartz cuvette 1 cm (Hellma®, Jena, Germany), ultra-micro analytical balance (RADWAG®, Radom, Poland) series of UYA 2.3Y (max: 2.1 g, min 0.01 mg), a system of (Shimadzu®, Kyoto, Japan) LC-2010HT No. C21255111004 LP with UV/Vis detector, (Retsch®, Haan, Germany) T460 ultrasonicator, membrane filter holder of Whatman® (Maidstone, United Kingdom) (capacity of 300 ml) Cat. No. 1960-004, organic solvent membrane filter of Whatman® (Maidstone, United Kingdom) (0.5 µm pore size, 47 mm diameter); inorganic solvent membrane filter of Whatman® (Maidstone, United Kingdom) (0.45 µm pore size, 47 mm diameter), Millipore® (Darmstadt, Germany) syringe filter (0.20 µm pore size, 25 mm diameter) and a set of Socorex® (Ecublens, Switzerland) micropipettes were used in this study.

Chemometrics method

Extraction of dry leaves of binahong

This procedure was developed from Yuliani *et al.* [23] and conducted as follow: 20 g dry leaves of binahong were weighed,

powdered and macerated using 200 ml ethanol for 90 min at 50 °C and shake at 200 rpm. The macerate was separated and concentrated into 25% of volume. This solution was labelled as the stock solution of ethanolic extract of binahong.

Preparation of calibration set and validation set solutions

Calibration and validation set solution was prepared by diluting a stock solution of ethanolic extract of binahong to achieve 26 compositions of samples as presented in table 1. Each composition was scanned using UV spectrophotometer at the range of 200 nm-400 nm. The absorbance for each composition was recorded with an interval of 2 nm. The obtained absorbance data for each wavelength were used for generating both calibration and validation models. The models were developed by plotting concentration of vitexin in each composition which was determined using HPLC as actual values vs the UV spectrophotometer predicted value.

Table 1: Calibration and validation data sets information for model selection and statistical analysis for determining the content of vitexin in ethanolic extract of binahong

Items	Data sets	
	Calibration	Validation
Number of mixture standards	16	10
Vitexin concentration in ethanolic extract of binahong (µg/ml)		
Mean	14.12	13.11
Range	6.38–26.72	5.36–25.42
Multivariate calibration models	PCR	PCR
	PLS	PLS
Evaluated parameters for model selection	R^2	R^2
	RMSEC	RMSEP
	RMSECV*	

Note: *cross-validation was performed using leave one out (LOO) technique

Spectroscopic analysis and multivariate calibrations

The absorbance values of every single wavelength point achieved from the calibration and validation data sets were statistically analyzed using the R studio software. A statistical package of pls was employed to perform chemometrics data processing. This package has been downloaded and installed by using the function of install. Packages ("pls"). After successfully installed, this package was loaded by using the function of library (pls) before further statistical analysis. A couple of multivariate calibration models, namely PLS and PCR, was generated for ethanolic extract of binahong. The built model multivariate calibration models were statistically evaluated by assessing several performances such as coefficient of determination (R^2), root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV), and root mean square error of prediction (RMSEP). Further, multivariate calibration model for each compound which resulted in a value of R^2 near one and lower RMSEC, RMSECV, and RMSEP were chosen for content determination [24].

Preparations of samples

Samples of diluted ethanolic extract solution of binahong were taken 1 ml. All the diluted and replicated sample solutions were sonicated for 15 min and filtered using Milipore syringe filter before injection. These prepared samples were analyzed both using spectroscopy method for generating the predictive models of multivariate calibration and HPLC method for achieving the actual values for each compound containing in the samples.

HPLC method

Chromatographic conditions

The system of Shimadzu LC-2010 CHT with LabSolution software and UV-Vis detector was developed. This chromatographic system was developed from Mulia *et al.* [25] with the column type, flow rate, pH, and stop time modification. A C18 column of Luna type Phenomenex (250 mm x 4,6 mm, 5 µm) was used. An isocratic elution system was developed. The composition of the mobile phase was methanol-acetonitrile-water (adjusted until pH 3 by orthophosphoric acid) (20:20:60), and mobile phase flow rate was 0.8 ml/min. Chromatographic separation was performed at ambient temperature the injection volume of 10 µl, and stop time setting at 25 min. Quantitation was set with UV detection at 319 nm.

Preparation of vitexin standard solutions

Accurate weight of 0.1 mg of vitexin was transferred into 5 ml volumetric flask. The weighted standard in the volumetric flask was diluted in methanol into the volume. These solutions were labelled as vitexin stock solution.

Standard solutions for UV spectral scanning were prepared from the stock solution. The stock solution was transferred into three separated 5 ml volumetric flasks, respectively, followed by the methanol dilution into the volume.

System suitability parameters

System suitability parameters were analyzed to check the system performance consistency. For system suitability parameters, six times repetition of one concentration of calibration solution vitexin was injected, and column performances like area, retention time, tailing factor, and a number of theoretical plates were observed. Values of the percentage of relative standard deviation (% RSD) for area and retention time parameters were found within the acceptance criteria of system performance. Tailing factor and number of theoretical plates were found for qualitative study based on USP [26].

Analytical method validation

The analytical method for determining the content of vitexin was validated for selectivity, linearity, range, precision, accuracy, detection limit, and quantitation limit, according to the ICH guidelines [27] and AOAC [28].

RESULTS

Validation method and analysis of vitexin in ethanolic extract of binahong using HPLC

The representative chromatogram profile of the sample and vitexin were presented in fig. 1 and 2. The HPLC performance was shown in table 2.

Chemometrics application

The spectra of calibration and validation set solutions can be seen in fig. 3 and 4, while table 3 and 4 presented the performance of PLS and PCR in the calibration and validation solutions. The results of calibration and validation with PLS and PCR can be seen in fig. 5 and 6.

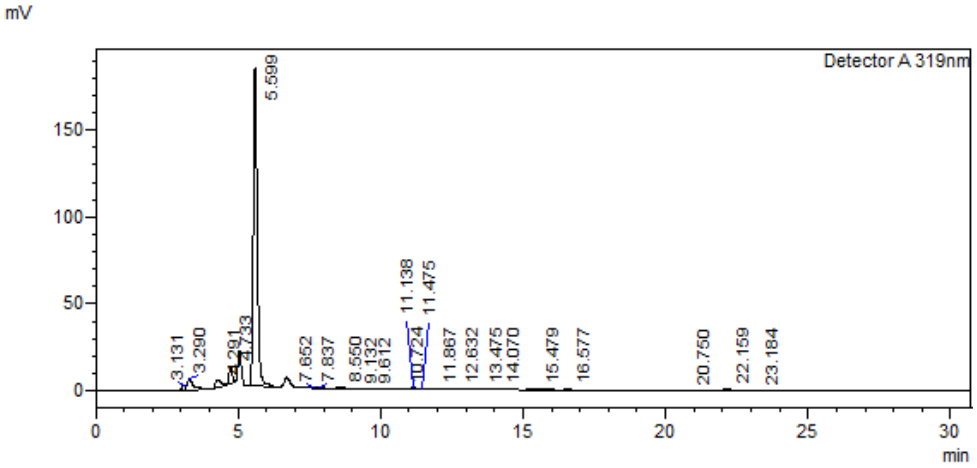

Fig. 1: The representative chromatogram profile of the sample (binahong)

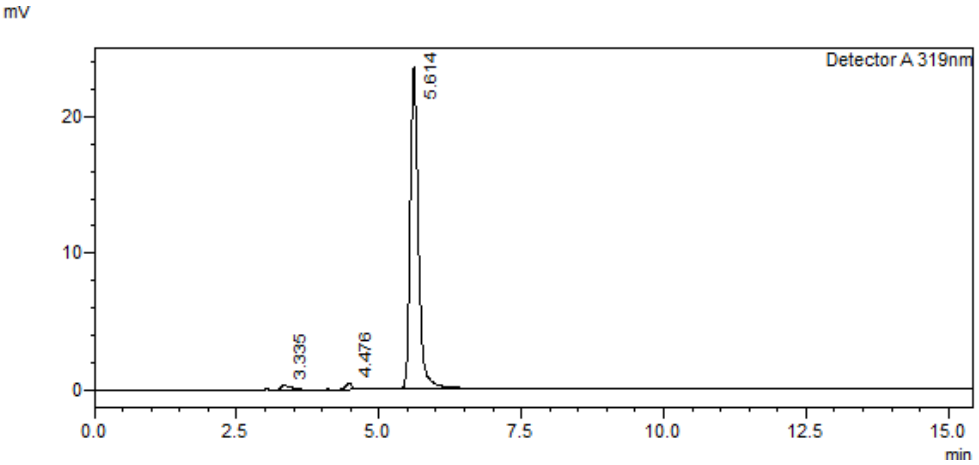

Fig. 2: The representative chromatogram profile of the vitexin (10 ppm)

Table 2: The performance of the validation method and analysis of vitexin in ethanolic extract of binahong using HPLC

Parameters	Value	Acceptance criteria
System suitability test		
% RSD Tr (min)	0.38	% RSD<2%
% RSD Area	0.12	Tf<2.0
Tailing factor (Tf)	1.247	N>2000
Number theoretical plate (N)	7488	
Selectivity		
Resolution (Rs) sample	Rs = 2.045	Resolution>1.5
Linearity		
Coefficient correlation (r)	0.9996	0.999
Coefficient determination (R)	0.9992	-
Slope (b)	20349	-
Intercept (a)	-28532	-
Range	2.11 ppm–31.61 ppm	-
Limit of detection	1.10 ppm	-
Limit of quantitation	3.34 ppm	-
***Accuracy (Recovery)**		
(intra-day)	97.85%–104.42%	95%–105%
(inter-day)	101.45%–103.40%	
***Precision (RSD)**		
(intra-day)	1.63%–3.04%	<3.7%
(inter-day)	1.35%–3.33%	
+Assay content vitexin in ethanolic extract of binahong	0.11±0.01 % w/w with RSD = 1.32%	RSD<3.7%

Note: * Accuracy and Precision were determined at 3 concentration addition levels of vitexin, 3 replicates and 3 consecutive days for inter-day.+assay content was determined by 3 replicates of ethanolic extract of binahong.

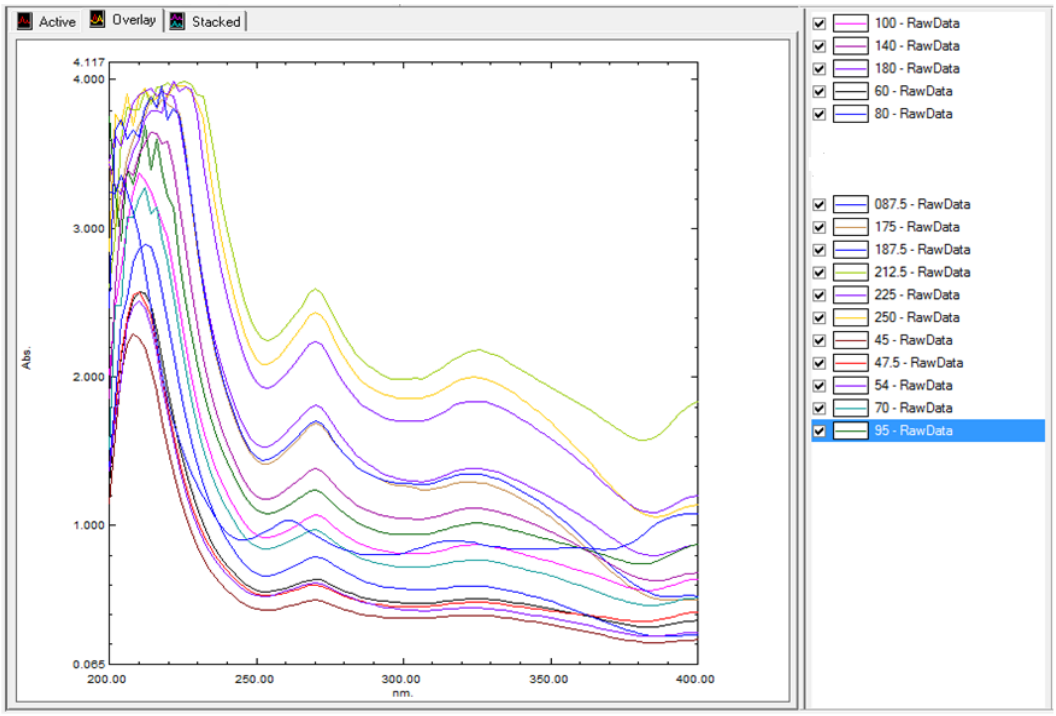

Fig. 3: **The spectra of calibration set solutions**

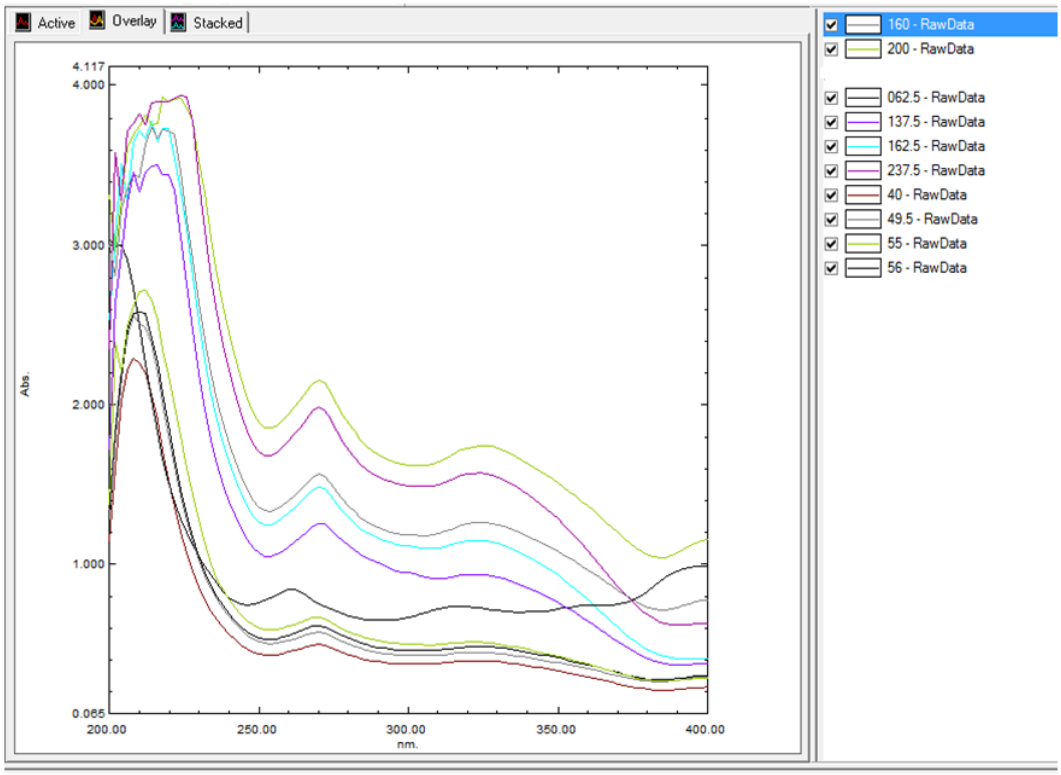

Fig. 4: **The spectra of validation set solutions**

Table 3: The performance of partial least squares (PLS) and principal component regression (PCR) for predicting the content of vitexin in calibration set solutions

Compounds	Multivariate calibrations	Calibration			
		Number of components	R^2	RMSEC	RMSECV
Vitexin	PLS	11	0.9675	0.0738	1.204
Vitexin	PCR	14	0.9648	0.0585	1.253

Table 4: The performance of partial least squares (PLS) and principal component regression (PCR) for predicting the content of vitexin in validation set solutions

Compounds	Multivariate calibrations	Validation		
		# of components	R^2	RMSEP
Vitexin	PLS	11	0.9778	1.2437
Vitexin	PCR	14	0.9820	1.1669

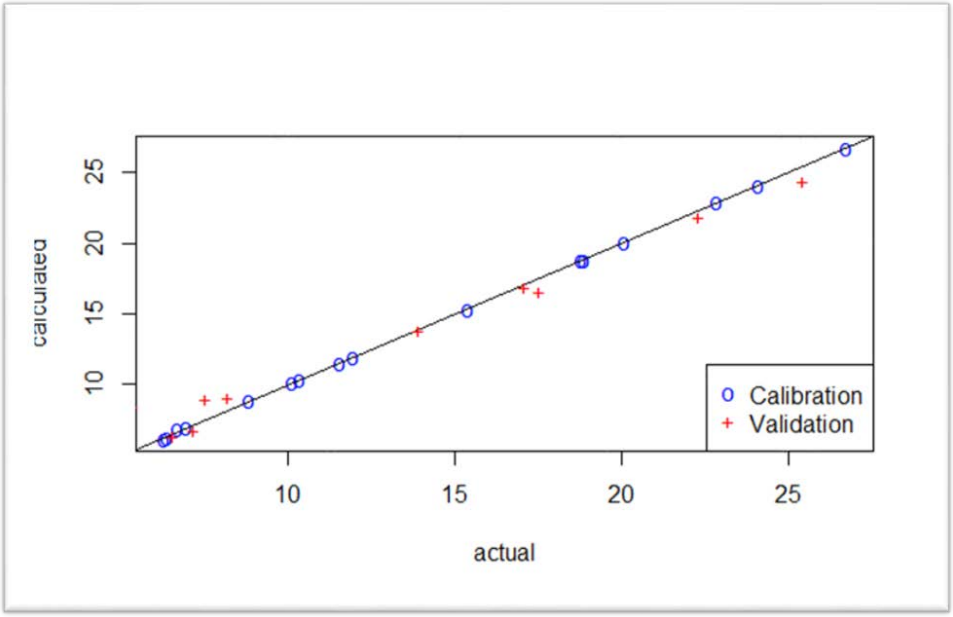

Fig. 5: Observation data plot (actual) vs predicted data (calculated) with PLS

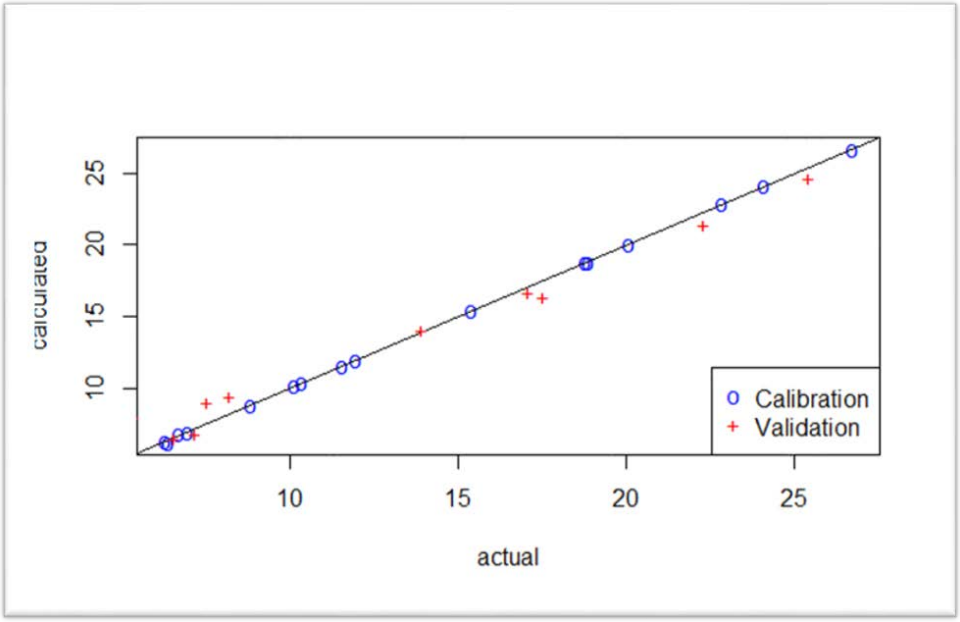

Fig. 6: Observation data plot (actual) vs predicted data (calculated) with PCR

Table 5: The assay of vitexin at the ethanolic extract of binahong leaves result

Compounds	Method	# of solution test			REP
		1	2	3	
Vitexin	PLS	8.192513	16.950112	12.530707	5.603%
Vitexin	PCR	7.95943	17.01199	12.12951	6.917%
Vitexin	Actual	9.29	16.32	12.57	-

The assay of vitexin

Result of the assay of vitexin at the ethanolic extract of binahong leaves can be seen in table 5.

DISCUSSION

The HPLC method was chosen as a reference method, according to the previous study [25]. This reference method was modified and validated due to the different parameters of the instrument compared to the previous study. The representative chromatogram profile of the sample was presented in fig. 1. This study was validated according to ICH (selectivity, linearity, range, the limit of detection, the limit of quantitation, accuracy and precision) [27]. Parameters of system suitability test and validation study were presented in table 2.

The actual data of vitexin levels in binahong leaves extract should be determined to conduct a multivariate analysis. The actual data was obtained from the HPLC analysis. The results of the validation method for determining levels of vitexin by HPLC are presented in table 2. The system suitability test and all validation parameters have been met with the requirement with high sensitivity, since the LoD and LoQ were 1.10 ppm and 3.34 ppm, respectively. This method gave valid, simple and fast method to determine vitexin in binahong if it was compared to Dwitiyanti *et al.* [29] they reported using gradient eluation to determine vitexin compound in binahong, but in this study, isocratic elution was developed to determine vitexin compound. Mulia *et al.* [25] also reported HPLC method to determine vitexin in binahong extract, but they use no ethanol as a solvent to extract the binahong powder. Mulia *et al.* [25] and Dwitiyanti *et al.* [29] also reported no validation data in their research, so this method can be made an alternative method to standardize binahong extract.

The output of UV spectral data was exported to Excel (Microsoft) and converted into. csv formatted files for further chemometrics data processing. Statistical analysis and multivariate calibrations were performed using R Studio software version 1.1.456 with pls packages. This software can be freely downloaded from https://rstudio.com/products/rstudio/download/.

The results of R^2 both for PLS and PCR were 0.9675 and 0.9648, respectively. These coefficient correlations indicated that the predicted value (model) and the observed value (HPLC) resulted in a very good correlation. RMSEC values resulted from the PLS and PCR models were 0.0738 and 0.0585, respectively, while RMSECV obtained from LOO cross-validation for PLS and PCR models were 1.204 and 1.253, respectively. Less values both for RMSEC and RMSECV indicated that minimum errors in calibration models.

The results of calibration and validation with PLS and PCR can be seen in fig. 5 and 6. Table 4 presented the performance of PLS and PCR in the validation solution that resulted in R^2 of 0.9778 and 0.9820, respectively. These results indicated that the predicted value and observed values showed a good correlation. RMSEP values given by the PLS and PCR models were 1.02437 and 1.1669, respectively. Hence, it can be assumed that the generated model resulted in minimum errors of prediction.

Spectral data from the measurement results of the samples were collected and processed statistically to achieve the prediction results of vitexin levels. The prediction results were then calculated by the REP value against the results of the determination of levels by HPLC, as shown in table 5.

Based on the results of the determination of the levels of vitexin in the ethanol extract solution of binahong leaves (see table 5), by experimenting with 3 sets of test solutions, the results using the PLS model showed values of 8.192513, 16.950112 and 12.530707, respectively. When compared with the actual value (HPLC), an error value (REP) of 5.603% is obtained, which means very good. Likewise, the results using PCR showed successive values of 7,95943, 17,01199 and 12.12951. When compared with the actual value (HPLC), an error value (REP) of 6.917% is slightly greater, but it can still be said to be very good.

CONCLUSION

The UV spectroscopy combined with chemometrics technique can be developed as an alternative method for determination of vitexin levels in the ethanol solution of binahong leaves extract.

ACKNOWLEDGMENT

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The authors declare that they have no conflicts of interest.

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