ACTA
PHARMACEUTICA
SCIENCIA

International Journal in Pharmaceutical Sciences, Published Quarterly

61 [1]

MEDIPOL
UNV-YAYIN
MEDİPOL UNIVERSITY PRESS

ACTA PHARMACEUTICA SCIENCIA

Volume: 61, No: 1, 2023

61 [1]

ACTA PHARMACEUTICA SCIENCIA

International Journal in Pharmaceutical Sciences, Published Quarterly

ISSN: 2636-8552
e-ISSN: 1307-2080,
Volume: 61, No: 1, 2023
Formerly: Eczacılık Bülteni
Acta Pharmaceutica Turcica

ACTA PHARMACEUTICA SCIENCIA

International Journal in Pharmaceutical Sciences, Published Quarterly

ISSN: 2636-8552
e-ISSN: 1307-2080,
Volume: 61, No: 1, 2023
Formerly: Eczacılık Bülteni / Acta Pharmaceutica Turcica
Founded in 1953 by Kasım Cemal GÜVEN

ACTA PHARMACEUTICA SCIENCIA

International Journal in Pharmaceutical Sciences
is Published Quarterly
ISSN: 2636-8552
e-ISSN: 1307-2080,
Volume: 61, No: 1, 2023
Formerly: Eczacılık Bülteni/Acta Pharmaceutica Turcica
Founded in 1953 by Kasım Cemal Güven

Address
İstanbul Medipol Üniversitesi
Kavacık Güney Kampüsü
Göztepe Mah. Atatürk Cad.
No: 40 34810 Beykoz/İSTANBUL
Tel: 0216 681 51 00

E-mail
editor@actapharmsci.com
secretary@actapharmsci.com

Web site
http://www.actapharmsci.com

Printing Office
Has Kopyalama Baskı ve Kırtasiye A.Ş
Kavacık Mah. Ekinciler Cad. No:19
Medipol Üniversitesi Kuzey Yerleşkesi
Tel: (0216) 681 53 72

Contents

Aims and Scope of Acta Pharmaceutica Sciencia

Acta Pharmaceutica Sciencia is a continuation of the former "Eczacılık Bülteni" which was first published in 1953 by Prof. Dr. Kasım Cemal GÜVEN's editorship. At that time, "Eczacılık Bülteni" hosted scientific papers from the School of Medicine-Pharmacy at Istanbul University, Turkey.

In 1984, the name of the journal was changed to "Acta Pharmaceutica Turcica" and it became a journal for national and international manuscripts, in all fields of pharmaceutical sciences in both English and Turkish. (1984-1995, edited by Prof. Dr. Kasım Cemal GÜVEN, 1995-2001, edited by Prof. Dr. Erden GÜLER, 2002-2011, edited by Prof. Dr. Kasım Cemal GÜVEN)

Since 2006, the journal has been published only in English with the name, "Acta Pharmaceutica Sciencia" which represents internationally accepted high-level scientific standards. The journal has been published quarterly except for an interval from 2002 to 2009 in which its issues were released at intervals of four months. The publication was also temporarily discontinued at the end of 2011 but since 2016, Acta Pharmaceutica Sciencia has continued publication with the reestablished Editorial Board and also with the support of you as precious scientists.

Yours Faithfully

Prof. Dr. Gülden Zehra OMURTAG
Editor

Acta Pharm. Sci. Vol 61:(1), 2023
DOI: 10.23893/1307-2080.APS6103

Antioxidant and antibacterial activities of ethanolic extract of sintok lancang *(Cinnamomum javanicum* Blume*)* from Central Kalimantan

Susi NOVARYATIIN[1*], Syahrida D ARDHANY[1], Muhammad T RAHMAN[1], Muhammad D MUTTAWALI[1], Hikmah HIKMAH[1], Elin TRI[1], Fahruddin ARFIANTO[2], Nanang HANAFI[3]

[1] Department of Pharmacy, Faculty of Health Sciences, Muhammadiyah University of Palangkaraya, Palangka Raya,Indonesia

[2] Department of Agrotechnology, Faculty of Agriculture and Forestry, Muhammadiyah University of Palangkaraya, Palangka Raya, Indonesia

[3] Department of Forestry, Faculty of Agriculture and Forestry, Muhammadiyah University of Palangkaraya, Palangka Raya, Indonesia

ABSTRACT

The *C. javanicum* Blume is a typical plant that can be founded in Central Kalimantan. This research aimed to find out the total flavonoids, alkaloids, tannins, and compounds and to evaluate the antioxidant and antibacterial activity of ethanol extract of the stem of *C. javanicum*. The antioxidant activity was tested by two different assays: DPPH and FRAP. Total alkaloids, flavonoids and tannins content were 32.81±0.77 µg caffeine equivalent/mg, 126.96±3.17 µg quercetin equivalent/mg, and 42.89±0.77 µg catechin equivalent/mg, respectively. The IC_{50} value of DPPH was 20.63±0.82 ppm quercetin equivalent while the FRAP method was 968.38±22.25 µmol trolox/g. *C. javanicum* extract had antibacterial potential against the three bacteria tested, with the inhibition zones in the range of 3.17±0.90 - 8.90±1.50 mm. It can be stated that the ethanol extract of the stem of *C. javanicum* has high potential antioxidant activity, however, the antibacterial activity is classified as weak activity.

*Corresponding Author: E-mail: susi_novaryatiin@yahoo.com
ORCIDs:
Susi Novaryatiin: 0000-0003-0696-6546
Syahrida D Ardhany: 0000-0002-8606-8991
Muhammad T Rahman: 0000-0002-0293-0469
Muhammad D Muttawali: 0000-0002-0885-2959
Hikmah Hikmah: 0000-0002-5221-0419
Elin Tri: 0000-0002-1817-2279
Fahruddin Arfianto: 0000-0002-1405-5403
Nanang Hanafi: 0000-0003-2933-8788
(Received 30 May 2022, Accepted 26 Oct 2022)

Keywords: *Cinnamomum javanicum* Blume, DPPH, FRAP, disc-diffusion, medicinal plant

INTRODUCTION

Free radicals are known for their central role in various physiological conditions as well as their involvement in a variety of diseases. The accumulation of free radicals in the body produces oxidative stress, which has been reported to be associated with various diseases such as neurodegenerative disorders, diabetes mellitus, respiratory diseases, cardiovascular diseases, coronary heart diseases, rheumatoid arthritis, the development of cataracts, inflammatory diseases, autoimmune disorders, aging, and various cancers[1-3]. Antioxidants play an important role in neutralizing free radicals[3].

In addition, the resistance of bacterial species to many antibiotics is another major problem in antibiotic therapy that continuously encourages researchers to develop new antibiotics. Recently, most of the newly approved antibacterial agents are derived from natural products or their derivatives. Herbal remedies are one of the most important sources of natural antibacterial agents[1]. The antimicrobial activity was mainly related to the existence of secondary metabolites of phenols, terpenes, and alkaloids present in the plant extracts[2]. Therefore, there is a need to explore new, safe and inexpensive sources of natural antioxidant and antibacterial compounds.

Cinnamomum (Family Lauraceae) is a genus known for its fragrant leaves and bark. This genus has more than 300 species that are widespread in North America, Asia, Australasia, and Southeast Asia. Commercial species sold as spices are *C. verum*, *C. cassia*, *C. burmannii*, *C. zeylanicum*, and *C. loureiroi*[4].

Sintok lancang (*Cinnamomum javanicum* Blume) is a typical plant of Central Kalimantan, in this study found at Mungku Baru Forest (KHDTK), an educational forest managed by the collaboration of Muhammadiyah University of Palangkaraya with the Borneo Nature Foundation (BNF). Empirically, Sintok lancang was used for various diseases such as treating abdominal pain, wounds, and diabetes mellitus.

There has not been much research on *C. javanicum* from Indonesia regarding its potential use as an antioxidant and antibacterial agent. One of the studies done on *C. javanicum* from Malaysia showed that the leaf and bark extracts had antioxidant activity[5]. The antibacterial activity of the essential oil of *C. javanicum* found in Borneo was investigated using the broth dilution method, which showed that the inhibition against *S. aureus* with MIC and MBC values

was 250 μg/mL and 500 μg/mL, respectively[4]. Previous studies reported that the ethanolic extract of *C. javanicum* leaves from Central Kalimantan had a high potential for antioxidant activity as tested by DPPH and FRAP methods[6]. However, no studies on the antioxidant activity of these ethanolic extracts of *C. javanicum* stem from Central Kalimantan have been conducted. This study was advanced from the previous study, with the aim being to determine the total number of flavonoid, alkaloid, and tannin compounds and to evaluate the antioxidant and antibacterial activity of the ethanolic extract of stems of *C. javanicum*. This study will help to explore the potential of Indonesian medicinal plants as natural antioxidants and antibacterial agents.

METHODOLOGY

Plant material

C. javanicum was collected from Mungku Baru Forest (KHDTK), an educational forest managed by the cooperation of the Muhammadiyah University of Palangkaraya with the Borneo Nature Foundation (BNF). The collected plant material was identified by Dr. R. Hendrian, MSc (Indonesian Institute of Sciences, Research Center for Plant Conservation and Botanic Gardens, Bogor, Indonesia) with the document number B-833/IPH.3./KS/VII/2020.

Preparation of *Cinnamomum javanicum* extract

The stems of *C. javanicum* were dried in an oven at 45 °C. The dried stems were powdered by the grinder and then percolated using 96% ethanol at room temperature. Percolation is a continuous process in which the saturated solvent is continuously replaced by a new solvent[7]. The solvent is removed from the extract by using a rotary evaporator. Percentage yield (w/w) of the extracts is calculated by formula[8]: Weight of the extract ÷ Weight of the starting plant material) x 100%

Phytochemical qualitative screening

The ethanolic extract of *C. javanicum* was preliminarily screened for phytochemicals such as alkaloids, flavonoids, saponins, tannins, and steroids[9-12].

Total alkaloids content

Ten mg of *C. javanicum* extract were weighed and then dissolved in 10 mL of ethanol. The absorbance of one mL of the extract was measured with a spectrophotometer at 272 nm. The standard used for the calibration curve was caffeine[13]. Total alkaloid content is expressed as μg alkaloids per mg of extract.

Total flavonoids content

Five mg of *C. javanicum* extract were weighed and then dissolved in 10 mL of ethanol. One mL of the extract was placed in the volumetric flask, and one mL of $AlCl_3$ (2% b/v) and 8 mL of acetic acid (5% v/v) were added. After mixing, the solution was incubated for 20 min[14]. The solution absorbance was measured with a spectrophotometer at 412 nm. The standard used for the calibration curve was quercetin[15]. Total flavonoid content is expressed as µg of flavonoids per mg of extract.

Total tannins content

A total of 30 mg of sample was weighed and placed in a 10 mL volumetric flask. Add to the 0.5 mL catechin standard solution, 3.0 mL vanillin 4% and 1.5 mL concentrated HCl. The mixture was incubated for 10 min[15-16]. Absorbance was measured with a UV Vis spectrophotometer at 498 nm. The standard used for the calibration curve was catechin[17]. Total tannin content is expressed as µg tannins per mg extract.

Antioxidant activity by DPPH assay

A 0.4 mM DPPH solution was prepared and the absorbance was measured at 512 nm. The resulting absorbance of the DPPH solution is the absorbance control. The stem extract of *C. javanicum* (sample) was first dissolved in methanol with five variant concentrations of 10, 20, 30, 40, 50 ppm. One mL of 0.4 mM DPPH solution was added in a 5 mL volumetric flask, and then 4 mL of sample solutions of different concentrations were added. The prepared mixture was placed at 25 °C for 30 min, the absorbance was measured at 512 nm[18]. The calculation of the percent inhibition or DPPH scavenging effect was used according to the following formula:

$$DPPH\ Scavenging\ effect\ \% = \frac{(A-B)}{A} X\ 100\%$$

Where A was the absorbance of DPPH solution and B was the absorbance of sample solution[19-20].

Antioxidant activity by FRAP assay

Sample (0.2 g) dissolved with ethanol in a 10 mL volumetric flask. Two mL of sample solution were added to 3 mL of FRAP reagent in a test tube, followed by incubation for 16 minutes. Absorbance was measured with a UV Vis spectrophotometer at 595 nm. Antioxidant activity expressed in µmol trolox/g[19-21].

Antibacterial activity test

The antibacterial activity was determined by disc diffusion method with four variant concentrations 1%, 5%, 10%, and 15% against three bacterial strains: *Cutibacterium acnes/C. acnes* (ATCC 11827), *Staphylococcus epidermidis/S. epidermidis* (ATCC 12228) and *Staphylococcus aureus/S. aureus* (ATCC 25923). Ten mL of McFarland 0.5 standard were prepared in sterilized tubes. The suspension of bacteria was prepared by diluting the colonies of bacteria in a normally sterile saline solution and the turbidity was adjusted to 1-2x10^8 CFU/mL (based on McFarland 0.5 standard). A sterile cotton swab was dipped in a standardized suspension of bacteria and used to inoculate on Mueller-Hinton agar plates[22]. All discs were immersed in the ethanolic extract of *C. javanicum* and then placed on the plates. A disc immersed in 1% clindamycin gel (positive control) was also placed on the plate. These plates were incubated in the aerobic incubator for 24 h at 37 °C. A caliper was used to measure the diameter of the inhibition zone of each extract and positive controls.

RESULTS AND DISCUSSION

Extraction yield

One thousand two hundred grams of *C. javanicum* were extracted into 60.29 g of extract. Based on yield calculation, *C. javanicum* extraction yielded 5.03%. The yield value relates to the number of secondary metabolites that were captured during extraction[8].

Qualitative phytochemical screening

Phytochemical screening of *C. javanicum* stem by using the following standard methods[9-10]. The results of the phytochemical qualitative test of *C. javanicum* stem showed the presence of alkaloids using Mayer's reagents[11], flavonoids using the Shinoda test[12], tannins, saponins, and steroids (Table 1).

Table 1. The qualitative phytochemical of ethanolic extract of *C. javanicum* stem

Phytochemical compound	Result
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Steroids	+

Total alkaloids, flavonoids, and tannins content

C. javanicum stem was extracted by percolation method. Total alkaloids, flavonoids, and tannins content were calculated by standard protocols, with the results of 32.81 ± 0.77 µg caffeine equivalent/mg, 126.96 ± 3.17 µg quercetin equivalent/mg, and 42.89 ± 0.77 µg catechin equivalent/mg, respectively (Table 2). The total flavonoid content is the largest compared to the total alkaloids and tannins content. In addition, the total alkaloids, flavonoids, and tannins of *C. javanicum* stem ethanol extract in this study were greater than the total content of the ethanolic extract of the leaves of *C. javanicum* obtained in the previous studies[6].

Phenolics and flavonoids are commonly found in all parts of the plant. This compound is a group of secondary metabolites made up of a large group of polyphenols that can scavenge free radicals and inhibit lipid oxidation[23]. Most antioxidant activities from plant sources correlate with phenolic and flavonoid contents[24]. Therefore, the measurement of phenol and flavonoid content can be used as a basis for rapid screening of antioxidant activities[1].

Table 2. Total alkaloids, flavonoids, and tannins content of ethanolic extract of *C. javanicum* stem

Assay \ Sample	Ethanolic extract of *C. javanicum*
Total Alkaloid (µg caffeine equivalent/mg)	32.81 ± 0.77
Total Flavonoid (µg quercetin equivalent/mg)	126.96 ± 3.17
Total Tannin (µg catechin equivalent/mg)	42.89 ± 0.77

Antioxidant activity

DPPH and FRAP methods were used to evaluate the antioxidant activity of *C. javanicum* extract. The antioxidant activity results showed that the IC_{50} value of DPPH was 20.63 ± 0.82 ppm quercetin equivalent while the FRAP method was 968.38 ± 22.25 µmol trolox/g (Table 3).

Table 3. Antioxidant activity of ethanolic extract of *C. javanicum* stem

Assay \ Sample	Ethanolic extract of *C. javanicum*	Quercetin
DPPH (IC_{50} ppm)	20.63 ± 0.82	6.98
FRAP (µmol trolox/g)	968.38 ± 22.25	-

According to some publications, the antioxidant activity with the DPPH method was classified by IC_{50} as very strong (< 50 ppm), strong (50-100 ppm), moderate (101-150 ppm), and low (> 150 ppm)[19,25], while the antioxidant activity with the FRAP method was classified as very low FRAP (< 10 µmol/g), low FRAP (10-50 µmol/g), good FRAP (50-100 µmol/g), high FRAP (100-400 µmol/g) and very high FRAP (> 400 µmol/g)[26]. A low IC50 value (the extract concentration, required to scavenge 50% of DPPH free radicals) means strong antioxidant activity[27]. The ethanolic extract of *C. javanicum* stem is included in the very strong antioxidant activity (20.63 ± 0.82 ppm) and very high FRAP (968.38 ± 22.25 µmol trolox/g). The antioxidant activity in this study was better than in the previous study, where the ethanolic extract of *C. javanicum* leaves included very strong antioxidant activity (26.99 ± 0.27 ppm) and very high FRAP (779.73 ± 19.66 µmol trolox/g)[6]. When compared with the antioxidant activity of *C. javanicum* leaves and bark study conducted in Malaysia with the DPPH method (223.5 ppm and 197.4 ppm)[5], *C. javanicum* in Indonesia, especially Central Kalimantan gives better antioxidant activity.

DPPH is a stable free radical with unpaired electrons distributed throughout the molecule and is widely used to test the free radical scavenging capacity of a variety of samples[28]. The DPPH assay is based on both electron transfer and hydrogen atom transfer reactions. The reduction in absorbance of DPPH caused by antioxidants is due to a reaction between the antioxidant molecules and the radical, which results in radical scavenging by hydrogen donation. This is visualized as purple-to-yellow discoloration. The advantage of the DPPH test is that it is simple, fast, and economical. Although the DPPH test is simple, its sensitivity can be affected by several factors, such as solvent type, reaction time, temperature, and freshness of DPPH reagent[1,29], while the FRAP test is a non-specific, redox-linked, colorimetric assay related to the molar concentration of the antioxidant present. The FRAP assay is a typical method based on electron transfer, which measures the reduction of ferric ion (Fe^{3+})-ligand complex to the ferrous (Fe^{2+}); complexed by antioxidants in acidic media. One limitation of the FRAP assay is its tendency to precipitate, form suspensions, and color

the cuvette. Therefore, the timing of $FeCl_3$ addition is essential to prevent error interpretation. However, the FRAP or DPPH assay is simple, economical, fast, and requires no specialized equipment[29].

Antibacterial activity

In this study, an antibacterial activity test was performed for *C. javanicum* extract and clindamycin gel. Clindamycin is used as a positive control because it is a lincosamide antibiotic used to treat anaerobic, streptococcal, and staphylococcal infections, with *in vitro* bactericidal activity against a wide variety of anaerobic bacteria, including *Bacteroides fragilis* as well as some *Staphylococcus* sp.[30]. It is also known to be one of the antibiotics used to treat acne[31]. The diameters of the zones of inhibition produced by the clindamycin gel against *C.acnes*, *S. epidermidis,* and *S. aureus* were 34.17 ± 2.48 mm, 28.87 ± 0.75 mm, and 29.53 ± 1.06 mm, respectively (Table 4).

Table 4. Antibacterial activity of ethanolic extract of *C. javanicum* stem

Materials	Concentration (%)	Inhibition zone diameter (mm) (mean±SD; n=3)		
		C. acnes	S. epidermidis	S. aureus
Clindamycin gel	1	34.17 ± 2.48	28.87 ± 0.75	29.53 ± 1.06
Ethanolic extract of C. javanicum stem	1	4.20 ± 1.58	7.47 ± 3.87	4.33 ± 0.38
	5	5.10 ± 1.02	7.53 ± 1.80	4.80 ± 0.28
	10	4.00 ± 1.80	7.33 ± 0.38	4.03 ± 1.03
	15	3.17 ± 0.90	8.90 ± 1.50	5.83 ± 0.76

This study showed that *C. javanicum* extract was effective against the three bacteria tested and its inhibition zone was in the range of 3.17 ± 0.90 - 8.90 ± 1.50 mm (Figure 1). The highest antibacterial activity was found for *C. javanicum* extract against *S. epidermidis,* with inhibition zone diameters of 7.47 ± 3.87 mm, 7.53 ± 1.80 mm, 7.33 ± 0.38 mm and 8.90 ± 1.50 mm at concentrations of 1%, 5%, 10% and 15% (Table 4). The antibacterial activity of the extract can be divided into three levels: weak activity (inhibition zone less than 12 mm), moderate activity (inhibition zone 12-20 mm) and strong activity (inhibition zone greater than 20 mm)[32].

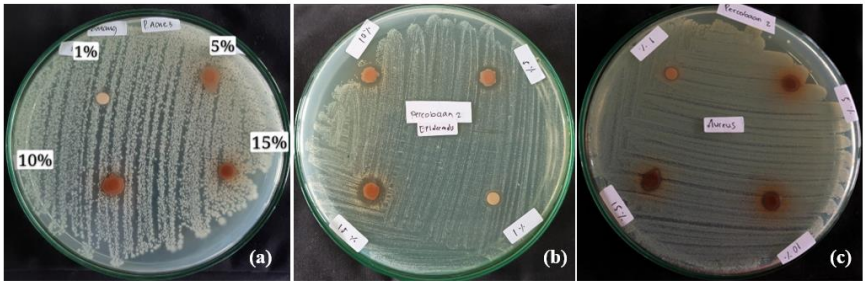

Figure 1. Antibacterial activity of ethanolic extract of *C. javanicum* stem against *C. acnes* (a), *S. epidermidis* (b), and *S. aureus* (c)

It can be concluded that the ethanol extract of the stem of *C. javanicum* has high potential antioxidant activity, but the antibacterial activity is classified as weak activity. The inhibition zones produced in this study fall on the concentration of extract of 10%. Further studies are needed to identify the factor that affects the rise and fall of the inhibition zone. In addition, negative control must be used in further study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING SOURCES

This research was funded by an internal grant from Muhammadiyah University of Palangkaraya and supported by a RisetMu grant batch 5 from Majelis Diktilitbang PP Muhammadiyah for publication.

REFERENCES

1. Safari M, Ahmady-Asbchin S. Evaluation of antioxidant and antibacterial activities of methanolic extract of medlar (*Mespilus germania* L.) leaves. Biotechnol Biotechnol Equip. 2019; 33(1): 372-378. https://doi.org/10.1080/13102818.2019.1577701.

2. Paliwal SK, Sati B, Faudjar S, Sharma S. Antioxidants and antibacterial activities of various extracts of Inula cuspidate C.B. Clarke stem. Beni-Suef Univ J Basic Appl Sci. 2017; 6(20): 97-2015. http://dx.doi.org/10.1016/j.bjbas.2016.10.003.

3. Baba SA, Malik SA. Evaluation of antioxidant and antibacterial activity of methanolic extracts of Gentiana kurroo royle. Saudi J Biol Sci. 2014; 21: 493-498. http://dx.doi.org/10.1016/j.sjbs.2014.06.004.

4. Vairappan CS, Nagappan T, Julip J. The essential oil profiles and antibacterial activity of six wild Cinnamomum species. Nat Prod Commun. 2014; 9(9): 1387-1389. https://doi.org/10.1177/1934578X1400900941.

5. Salleh WMNHW, Ahmad F, Yen KH. Evaluation of antioxidant, anticholinesterase and antityrosinase activities of malaysian cinnamomum species. Dhaka Univ J Pharm Sci. 2015; 14(2): 125-132. https://doi.org/10.3329/dujps.v14i2.28500.

6. Ardhany SD, Novaryatiin S, Hanafi N. The phytochemical screening and antioxidant potential of *Cinnamomum javanicum* Blume leaves from Central Kalimantan. Bull Pharm Sci. 2021; 44(1): 81-88. https://doi.org/10.21608/BFSA.2021.174133.

7. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: a comprehensive review. Chin Med. 2018; 13(20): 1-26. https://doi.org/10.1186/s13020-018-0177-x.

8. Kusuma SAF, Mita SR, Firdayani I, Mustarichie R. Study on the antibacterial activity of fruit extracts of klutuk banana (*Musa balbisiana* Colla) against *Shigella dysenteriae* ATCC 13313. Asian J Pharm Clin Res. 2017; 10(7): 220-223. https://doi.org/10.22159/ajpcr.2017.v10i7.18561.

9. Vijayan K, Gopinathan M, Ambikapathy V. Phytochemical screening and antioxidant activity of *Diospyros ebenum* J. koening ex retz, leaves extract. Int J Pharm Sci Res. 2020; 11(10): 5163-5169. https://doi.org/10.13040/IJPSR.0975-8232.11(10).5163-69.

10. Ahmed Z, Aziz S, Hanif M, Mohiuddin SG, Khan SHA, Ahmed R, et al. Phytochemical screening and enzymatic and antioxidant activities of *Erythrina suberosa* (Roxb) Bark. J Pharm Bioallied Sci. 2020; 12(2): 192-200. https://doi.org/10.4103/jpbs.JPBS_222_19.

11. Senhaji S, Lamchouri F, Toufik H. Phytochemical content, antibacterial and antioxidant potential of endemic plant *Anabasis aretioides* Coss. & Moq. (Chenopodiaceae). Biomed Res Int. 2020; 2020(6): 1-16. https://doi.org/10.1155/2020/6152932.

12. Mondal S, Rahaman ST. Flavonoids: a vital resource in healthcare and medicine. Pharm Pharmacol Int J. 2020; 8(2): 91-104. https://doi.org/10.15406/ppij.2020.08.00285.

13. Sathishkumar T, Baskar R. Screening and quantification of phytochemicals in the leaves and flowers of *Tabernaemontana heyneana* Wall. A near threatened medicinal plant. Indian J Nat Prod Res. 2014; 5(3): 237-243.

14. Hasan AEZ, Nashrianto H, Juhaeni RN, Artika IM. Optimization of conditions for flavonoids extraction from mangosteen (*Garcinia mangostana* L.). Der Pharm Lett. 2016; 8(18): 114-120.

15. Beyene BB, Alem FA, Ayana MT. Determination of antioxidant and antibacterial activities of leaf extracts of *Plumbago zeylanica* (amira). Cogent Chem. 2020; 6(1831715): 1-16. https://doi.org/10.1080/23312009.2020.1831715.

16. Hayat J, Akodad M, Moumen A, Baghour M, Skalli A, Ezrari S, et al. Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of *Marrubium vulgare* L. from 2 different localities of Northeast of Morocco. Heliyon. 2020; 6(11): e05609. https://doi.org/10.1016/j.heliyon.2020.e05609.

17. Formagio ASN, Volobuff CRF, Santiago M, Cardoso CAL, Vieira MDC, Pereira ZV. Evaluation of antioxidant activity, total flavonoids, tanins and phenolic compounds in Psychotria leaf extracts. Antioxidants. 2014; 3: 745-757. https://doi.org/10.3390/antiox3040745.

18. Manssouri M, Znini M, Majidi L. Studies on the antioxidant activity of essential oil and various extracts of *Ammodaucus leucotrichus* Coss. & Dur. fruits from Morocco. J Taibah Univ Sci. 2020; 14(1): 124-130. https://doi.org/10.1080/16583655.2019.1710394.

19. Sukweenadhi J, Yunita O, Setiawan F, Kartini, Siagian MT, Danduru AP, et al. Antioxidant activity screening of seven Indonesian herbal extract. Biodiversitas. 2020; 21(5): 2062-2067. https://doi.org/10.13057/biodiv/d210532.

20. Andres AI, Petron MJ, Lopez AM, Timon ML. Optimization of extraction conditions to improve phenolic content and in vitro antioxidant activity in craft brewers' spent grain using response surface methodology (RSM). Foods. 2020; 9(1398): 1-13. https://doi.org/10.3390/foods9101398

21. Mitrevska K, Grigorakis S, Loupassaki S, Calokerinos AC. Antioxidant activity and polyphenolic content of North Macedonian wines. Appl Sci. 2020; 10(6): 1-10. https://doi.org/10.3390/app10062010.

22. Novaryatiin S, Ardhany SD. The antibacterial activity of bawang Dayak (*Eleutherine bulbosa* (Mill.) Urb.) from Central Kalimantan against acne-causing bacteria. Int J Appl Pharm. 2019; 11(Special issue 5): 22-25. https://doi.org/10.22159/ijap.2019.v11s5.T0032.

23. Kamtekar S, Keer V, Patil V. Estimation of phenolic content, flavonoid content, antioxidant and alpha-amylase inhibitory activity of marketed polyherbal formulation. J App Pharm Sci. 2014; 4 (9): 61-65. https://doi.org/10.7324/JAPS.2014.40911.

24. Azam M, Eden WT, Fibriana AI, Rahayu SR. Antioxidant properties of capsule dosage form from mixed extracts of *Garcinia mangostana* Rind and *Solanum lycopersicum* fruit. Acta Pharm Sci. 2021; 59(1): 69-80. https://doi.org/10.23893/1307-2080.APS.05904.

25. Najafabadi SF, Safaeian L, Zolfaghari B. In vitro antioxidant effects of different extracts obtained from the leaves and seeds of *Allium ampeloprasum subsp. Persicum*. J HerbMed Pharmacol. 2019; 8(3): 256-260. https://doi.org/10.15171/jhp.2019.37.

26. Fernandes RPP, Trindade MA, Tonin FG, Lima CG, Pugine SMP, Munekata PES, et al. Evaluation of antioxidant capacity of 13 plant extracts by three different methods: cluster analyses applied for selection of the natural extracts with higher antioxidant capacity to replace synthetic antioxidant in lamb burgers. J Food Sci Technol. 2016; 53(1): 451-460. https://doi.org/10.1007/s13197-015-1994-x.

27. Iduğ T, Hızlı H, Şen A, Koç F. In vitro antimicrobial and antioxidant activity of some berry species. Acta Pharm Sci. 2018; 56(3): 51-59. https://doi.org/10.23893/1307-2080.APS.05617.

28. Kusuma IW, Arung ET, Kim YU. Antimicrobial and antioxidant properties of medicinal plants used by the Bentian tribe from Indonesia. Food Sci Hum Wellness. 2014; 3(3-4): 191–196.

29. Shahidi F, Zhong Y. Measurement of antioxidant activity. J Func Foods. 2015; 18(Part B): 757-781. https://doi.org/10.1016/j.jff.2015.01.047.

30. Markey L, Pugliese A, Tian T, Roy F, Lee K, Kumamoto CA. Decreased ecological resistance of the gut microbiota in response to clindamycin challenge in mice colonized with the fungus *Candida albicans*. mSphere. 2021; 6(1): 1-16. https://doi.org/10.1128/mSphere.00982-20.

31. Novaryatiin S, Ardhany SD. Potential anti-acne: bawang Dayak (*Eleutherine bulbosa* (Mill.) Urb.) from Central Kalimantan-Indonesia. Pharmacog J. 2020; 12(1): 52-57. https://doi.org/10.5530/pj.2020.12.9.

32. Shahbazi Y. Antibacterial and antioxidant properties of methanolic extracts of apple (*Malus pumila*), grape (*Vitis vinifera*), pomegranate (*Punica granatum* L.), and common fig (*Ficus carica* L.) fruits. Pharm Sci. 2017; 23: 308-315. https://doi.org/10.15171/PS.2017.45.