International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Special Issue 3, 2022

Original Article

STANDARDIZATION AND A-GLYCOSIDASE INHIBITION OF EXTRACTS AND NANOPARTICLE EXTRACT OF KEMBANG BULAN LEAVES (*TITHONIA DIVERSIVOLIA* (HAMSLEY) A. GRAY)

RATNA DJAMIL[1*], DENI RAHMAT[1], HILMA AZKIA[1], DIAH KARTIKA PRATAMI[1], GREESTY FINOTORY SWANDINY[1]

[1]Faculty of Pharmacy, Universitas Pancasila, South Jakarta, DKI Jakarta, 12640, Indonesia
*Email: ratnadjamilffup@gmail.com

Received: 11 Dec 2021, Revised and Accepted: 21 Mar 2022

ABSTRACT

Objective: The purpose of this study was the determination of the quality parameters of the antidiabetic activity of 70% ethanol extract and Kembang Bulan leaf nanoparticle extract.

Methods: Kembang Bulan powder was macerated kinetic with 70% ethanol, evaporated until a thick extract was obtained using a rotary evaporator, then specific and non-specific parameters were determined, and phytochemical screening was done. The extract was tested for anti-diabetic activity *in vitro* using the α-glucosidase enzyme.

Results: Phytochemical screening results show the presence of flavonoids, saponins, tannins, essential oils, coumarin, steroids, and triterpenoids. The results of quality parameter examination showed that the extract had a thick consistency, blackish-brown color, aromatic odor, 75.20% dissolved compound and 71.97% dissolved compound in ethanol, 9.65% drying shrinkage, 8.73% moisture content, the remaining solvent is 0.43%, the total ash content is 7.12%, the acid insoluble ash content is 0.93%, the Pb level is 0.1167 mg/kg BW, the Cd level is 0.0620 mg/kg BW, the ALT microbial contamination is 0.6827x and Mold and yeast contamination 0.156x. Total flavonoid levels were 1.15%.

Conclusion: The test results can be concluded that the 70% ethanol extract and nanoparticle extracts from Kembang Bulan leaves can inhibit the activity of the αglucosidase enzyme and the inhibitory results obtained by the nanoparticle extract are greater than the 70% ethanol extract.

Keywords: Kembang Bulan leaves, (*Tithonia diversifolia* (Hamsley) A. Gray), Nanoparticles

DOI: https://dx.doi.org/10.22159/ijap.2022.v14s3.10 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Efforts to find alternative treatments by utilizing natural ingredients through the use of medicinal plants have been carried out by many researchers from various research institutes and institutions, which can later be isolated and marketed, such as diabetes mellitus treatment with herbal medicines and one of them by utilizing the anti-hyperglycemic potential of plants, especially which contains polyphenol compounds, including flavonoids. This compound is binding to protein so that it can inhibit carbohydrate decomposing enzymes such as α-glucosidase found in the intestinal wall [1]. Enzymes like α-glucosidase play a role in the hydrolysis of food carbohydrates into glucose and other monosaccharides, in patients with diabetes mellitus, inhibition of this enzyme causes inhibition of glucose absorption, thereby reducing the state of hyperglycemia after eating [2].

One of the natural ingredients that have been researched as a medicinal plant is Kembang Bulan [*Tithonia diversifolia* (Hemsley) A. Gray]. Based on research that has been shown that the Kembang Bulan leaves (KBL) contain flavonoid compounds that can reduce blood glucose levels up to 54.15% [3]. The activity test is based on the reaction mechanism of the α-glucosidase enzyme which can catalyze the reaction of the breakdown of the p-nitrophenol-α-D-glucopyranoside substrate to p-nitrophenol and glucose [4]. For plants that have the ability to inhibit the activity of the enzyme α-glucosidase will cause a decrease in the amount of p-nitrophenol formed. The amount of p-nitrophenol produced was measured at 405 nm by an absorbance microplate reader [5].

One effort to increase the effectiveness of the KBL extract can be increased by forming nanoparticles. Where the solvent used is capmul (glyceryl caprylate), propylene glycol, and glycerin. The component is a solvent that is non-toxic, biodegradable, biocompatible, has a low level of immunogenicity, and can be prepared into nanoparticles, making it very suitable for drug delivery systems. The purpose of this study was the determination of the quality parameters of the antidiabetic activity of 70% ethanol extract and Kembang Bulan leaf nanoparticle extract.

MATERIALS AND METHODS

Material

The material used for this research was the Kembang Bulan (*Tithonia diversifolia* (Hamsley) A. Gray) leaf obtained from the Indonesian Spice and Medicinal Research Institute (BALITRO) Bogor.

Plant determination

The plants used in this study were determined at the Bogoriense Herbarium, Center for Biological Research, Indonesian Institute of Sciences (LIPI), Cibinong, West Java. Determination is done to ensure the correctness of the plant.

The KBL extraction

The KBL powder was extracted by kinetic maceration using 70% ethanol solvent. The filtrate was filtered with filter paper coated with cotton to separate the pulp and filtrate. Remaceration was then concentrated using a vacuum rotary evaporator to obtain 70% ethanol viscous extract [6].

Phytochemical screening

Preliminary examinations of secondary metabolite compounds on 70% ethanol KBL extracts included identification of the alkaloid, flavonoid, saponin, tannin, quinone, triterpenoid steroid, coumarin, and essential oil groups [7].

Determination of extract quality parameters

Determination of the quality parameters of 70% ethanol KBL extract of spoiled leaves includes specific parameters, namely organoleptic determination and determination of the percentage of dissolved compounds in certain solvents as well as non-specific parameters, namely determination of ash content, determination of drying losses, determination of water content, determination of heavy metal contamination, determination of microbial contamination, determination of residual solvents [8].

Nanoparticles of KBL extract preparation

The nanoemulsion of KBL extract was prepared by the cosolvent method according to the method described by Ratna Djamil *et al.* (2020) [9]. A 100-mg crude KBL extract was dissolved in a cosolvent consisting of 1:2.5:2:10 (v/v) glyceryl caprylate, propylene glycol, glycerine, and water. The mixture was homogenized gently to obtain a nanoemulsion.

Determination of total phenolic levels

As much as 0.4 ml of 70% ethanol extract of spoiled leaves added 0.4 ml of the Folin Ciocalteu reagent (allowed to stand for 3 min in a dark place), 4 ml of 7.5% Na_2CO_3, and aqua dest added to 10.0 ml. The solution is allowed to stand for 70 min at room temperature. The absorption was measured with a UV-Vis spectrophotometer at a wavelength of 759.5 nm. The total phenol content of the 70% ethanol extract of the KBL is expressed in mg Gallic Acid Equivalent/gram extract (mg GAE/g) [10].

Determination of total flavonoid levels

1.0 ml of 70% ethanol extract of KBL was added with 3 ml of methanol, 200 µl of potassium acetate, 200 µl of $AlCl_3$ (aluminum chloride), and aqua dest were added to 10.0 ml. The solution is allowed to stand for 30 min at room temperature. The absorption was measured with a UV-Vis spectrophotometer at a wavelength of 371.0 nm. The total flavonoid *in vitro* antidiabetic activity by using inhibition of alpha-glucosidase enzyme activity was d content of 70% ethanol extract of KBL is expressed in mg Quercetin Equivalent/gram extract (mg QE/g) [11].

Alpha-glucosidase inhibitor assay

This assay was performed according to the method reported by Sahlan *et al.* (2018) with slight modification [2]. The reaction mixture contained 25µl alpha-glucosidase solution (0.2 units/ml), 50 µl of phosphate buffer 0.1 M at pH 7, 25µl 4-nitrophenyl alpha-D-glucopyranoside 20 mmol, 10µl test sample at series concentrations (50-1000 ppm). This reaction mixture was incubated at 37 °C for 30 min and finished by adding 100 µl of sodium carbonate solution 0.1 M. The inhibition activity was monitored at 410 nm using a microplate reader. In this study, we use acarbose as a positive control.

RESULTS AND DISCUSSIONS

Phytochemical screening

Simplisia powder and 70% ethanol extract of leaves of the KBL were analyzed phytochemically to determine the content of the samples used. The result of phytochemical screening is shown in table 1.

Table 1: Phytochemical screening results of kembang bulan leaves

No.	Group identification	Result
1.	Alkaloid	-
2.	Flavonoid	+
3.	Tannin	+
4.	Saponin	+
5.	Quinone	-
6.	Steroid/triterpenoid	+/+
7.	Coumarin	+
8.	Essential oils	-

(+) contain secondary metabolites; (-) does not contain secondary metabolite

Qualitative phytochemistry tests were conducted to analyze secondary metabolites in KBL. It has no alkaloids and volatile compounds. It contained flavonoids, saponins, tannin, saponin, quinone, steroid/triterpenoid, and coumarin. The result was similar to Wahyuningsih *et al.* and Mardihusodo *et al.* [12, 13].

Determination of specific extract quality parameters

Organoleptic determination aims to determine the identity of the initial introduction to the extract that can be observed visually. The organoleptic result was shown in table 2.

Table 2: The result of organoleptic extract determination

Extract identity	Result
Shape	Thick Extract
Colour	Blackish Green
Smell	Odorless

The dissolve compound in water and the ethanolic solvent as shown in table 3. Determination of dissolved compounds in certain solvents aims to determine the amount of secondary metabolite dissolved in water and ethanol solvents.

Table 3: The results of determining the levels of dissolved compounds in certain solvents

Determination	Result
Water-soluble compounds	75.20±3.21 %
Ethanol soluble compounds	71.85±2.48 %

Data was given in mean±SD, n=3

Based on the results above, the amount of water-soluble compounds is greater than the results of the levels of dissolved compounds in ethanol, this shows that the secondary metabolites of the ethanol extract of KBL leaves are more contained in water than the number of metabolites present in ethanol solvents.

Determination of non-specific quality parameters of extract

Determination of non-specific quality parameters aims to determine the quality of extracts that have standards and safe limits of extracts as a safe and quality natural material product. The results were shown in table 4.

Table 4: Results of non-specific quality parameter estimate

Parameters	Results
Drying losses	9.65%
Water content	8.73%
Total ash content	7.12%
Ash content not acid solubility	0.93%
Residual solvent	0.43%
Lead metal contamination	0.1167 mg/kg
Cadmium total contamination	0.0620 mg/kg
Total plate count	not detected
Yeast and mold	not detected

Determination of non-specific quality parameters aimed to determine the quality of extracts that have standards and safety limits of extracts as a natural material product standard. The result meets the standard of Perka BPOM No 32 Th 2019 [14].

Determination of total phenolic and flavonoid levels

Determination of total phenolic and flavonoid levels was carried out to determine the number of compounds contained in extracts using the colorimetric principle. Determination of total phenol and flavonoid levels in the ethanol extract of 70% lunar leaves using the UV-VIS spectrophotometer method. The result was shown in table 5.

Table 5: Results of determination of total phenol and flavonoid levels

No.	Content determination	Result
1.	Phenolic	8.66±0.23 %
2.	Flavonoid	1.15±0.02 %

Data was given in mean±SD, n=3

Natural alpha-glucosidases inhibitors from plants, and many candidates have transpired to be secondary metabolites including

alkaloids, flavonoids, phenols, and terpenoids [15]. The higher the phenolic and flavonoid content from the extract, the higher its antidiabetic potential activity [1].

Evaluation nanoparticle of KBL extract

Determination Particle Size of KBL extract can be seen in fig. 1.

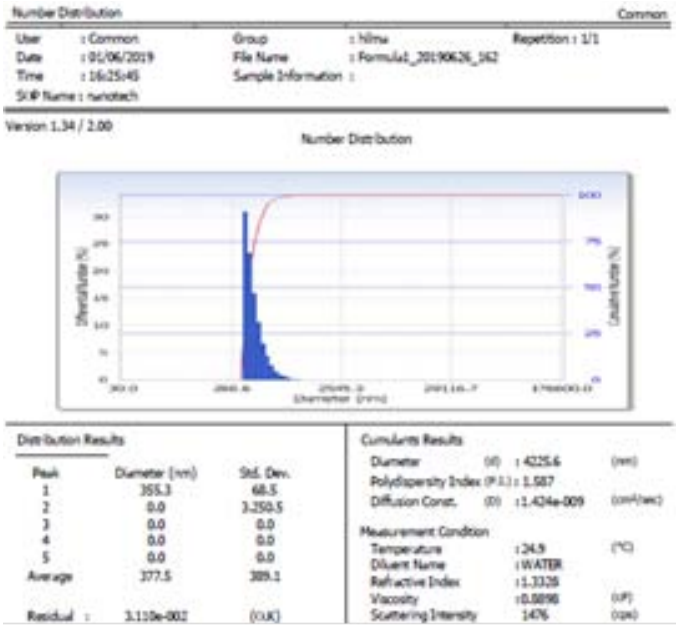

Fig. 1: Results of the determination of the particle size KBE

Potential zeta size

The result of zeta potential is shown in table 6. The adjustment from the zeta test results, obtained a value of-26 mV where the result is less than-30mV, it can be said that the nanoparticle produced is relatively stable.

Table 6: Results of zeta test of potential nanoparticle extract

Result	Peak value
Mobility (cm3/Vs)	-3.307 e-006
Zeta potential (mV	-25.90
Electric field (V/cm)	16.37

According to Trujillo *et al.*, in general particles with zeta potential values exceeding+30mV or less than-30mV, both indicate stability because the electric charge from the droplet is strong enough to repel between dominant droplets in the emulsion system [16]. The zeta potential value has shown that the nanoparticles of KBL are stable because the droplets have a strong enough electric charge to repel the droplets dominant in the nanoemulsion suspension system. High zeta potential is considered the cause of the lower aggregation of particles. The charge on the nanoparticle surface will affect its distribution in the body and increase the uptake of nanoparticles into cells [17].

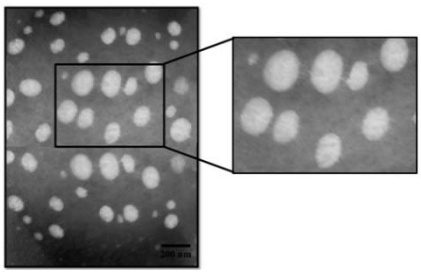

Fig. 2: Determination of nanoparticle morphological result

Determination of morphological of nanoparticle

The morphological shape of nanoemulsion of KBL extract can be seen in fig. 2. From the figure, it can be seen that the nanoparticles can form properly, wherewith a magnification of 60,000x by optical/electron microscopy shows the globules contained in the nanoemulsion of KBL extract has a relatively spherical shape with a size of 200 nm. In addition, from the picture, it can also be seen that the distribution of globule flow is quite evenly distributed**.**

α-glucosidase enzyme activity test

The results of the α-glucosidase enzyme inhibition test in table 7 against diabetes from 70% ethanol extract of KBL obtained an average of 75.25%. While the nanoparticle extract (Formula 1) obtained an average of 71.02%. From these results, there is a difference in activity between the two extracts, namely 50% inhibition concentration (nanoparticle extract is greater than 70% ethanol extract of KBL. However, both extracts have α-glucosidase enzyme inhibitory activity. When compared with previous studies by Sumiyanti (2017) 70% ethanol extract at a concentration of 225 µl/ml has a percentage of inhibition of 76.8%, the result is greater than this study, namely at a concentration of 225 µl/ml has an inhibitory percentage of 75.25% [18]. This can occur due to factors influencing % inhibition such as where to grow, harvest time, and age of the plant.

Table 7: IC_{50} of α-glucosidase assay

Test substance	IC_{50} (ppm)
Acarbose	36.66±2.12
Ethanol extract 70% KBL	75.25±1.02
Nanoparticle extract KBL	71.02±1.18

Data was given in mean±SD, n=3

CONCLUSION

The results of phytochemical screening for simplex powder and KBL leaf thick extract contain flavonoids, saponins, tannins, steroids/triterpenoids, coumarin, and essential oils. Specific quality inspection of KBL extract shows consistency of thick, brownish-green,

slightly bitter taste, and aromatic characteristic, water-soluble extract, and ethanol-soluble extract meet the requirements. Inspection of non-specific quality parameters shows drying losses, moisture content, total ash content, acid-soluble ash content, residual solvents, lead contamination (Pb), cadmium contamination (Cd), Total Plate Count, and Mold and Yeast fig. meet the quality requirements of extracts according to the Indonesian Medicinal Plant Extract Monograph. The result of determining the average total flavonoid content of KBL is 1.15%. Nanoparticles of Thick Extracts of Moon Flower (*Tithonia diversifolia*. (Hamsley) A. Gray) can be made into nanoparticle extracts with a particle size of 377.5 nm and a potential zeta value of-25.98 mV. The results of the α-glucosidase inhibition test against diabetes from ethanol extract of 70% of KBL *in vitro* obtained an average IC_{50} of 75.25 ppm. Whereas on the KBL Nanoparticles obtained an average IC50 of 71.02 ppm. Nanoparticle extract of KBL has a greater inhibition concentration compared to 70% ethanol extract of KBL. So it can be concluded that both extracts can be potential antidiabetic.

ACKNOWLEDGMENT

Support from Universitas Pancasila has gratefully acknowledged

FUNDING

The research has no specific funding to declare.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The author has no conflicts of interest to declare

REFERENCES

1. Djamil R, Winarti W, Zaidan S, Abdillah S. Antidiabetic activity of flavonoid from binahong leaves (Anredera cordifolia) extract in alloxan induced mice. J Pharmacogn Nat Prod. 2017;3(2):139-42. doi: 10.4172/2472-0992.1000139.
2. Rahmawati O, Pratami DK, Raffiudin R, Sahlan M. Alpha-glucosidase inhibitory activity of stingless bee honey from Tetragonula biroi and Tetragonula laeviceps. AIP Conf Proc. 2019:30001.
3. Sari AR, Saraswati TR, Yuniwarti EYW. Antihyperglycemic activity of aqueous extract of insulin leaves (Tithonia diversifolia) on hyperglycemic rats (Rattus norvegicus). J Bio Bio Edu. 2018;10(3):636-41. doi: 10.15294/biosaintifika.v10i3.15845.
4. Carrasco H, Raimondi M, Svetaz L, Liberto Di M, Rodriguez MV, Espinoza L. Antifungal activity of eugenol analogues. Influence of different substituents and studies on mechanism of action. Molecules. 2012;17(1):1002-24. doi: 10.3390/ molecules17011002, PMID 22262200.
5. Scarano S, Palladino P, Pascale E, Brittoli A, Minunni M. Colorimetric determination of p-nitrophenol by using ELISA microwells modified with an adhesive polydopamine nanofilm containing catalytically active gold nanoparticles. Mikrochim Acta. 2019;186(3):146. doi: 10.1007/s00604-019-3259-2, PMID 30707372.
6. Djamil R, Zaidan S, Rahmat D, Pratami DK, Hakim F. Nanoemulsion of okra fruit extract as antidiabetic treatment. Int J App Pharm. 2020;12:138-42. doi: 10.22159/ijap.2020v12i5.37805.
7. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. 3rd ed. Springer Netherlands; 1998.
8. Djamil R, Rahmat D, Zaidan S, Latifah MN. Anticholesterol activity of okra fruit extract (Abelmoschus esculentus (L) Moench) and its nanoemulsion *in vivo*. Pharmacogn J. 2020;12(2):316-20. doi: 10.5530/pj.2020.12.50.
9. Desmiaty Y, Rahmat D, Maulidina NS. Preparation of standardized aqueous extract of Annona muricata Linn. Leaf and its potency as antioxidant. Pokjanas; 2016. p. 30.
10. Saikia S, Mahnot NK, Mahanta CL. Optimisation of phenolic extraction from Averrhoa carambola pomace by response surface methodology and its microencapsulation by spray and freeze drying. Food Chem. 2015;171:144-52. doi: 10.1016/j.foodchem.2014.08.064, PMID 25308654.
11. Sari AC, Elya B, KK. Antioxidant activity and lipoxygenase enzyme inhibition assay with total flavonoid assay of garcinia porrecta laness. Stem Bark Extracts. Pharmacogn J. 2017;9(2):257-66. doi: 10.5530/pj.2017.2.44.
12. Wahyuningsih MSH, Wijayanti MA, Budiyanto A. Hanafi M. Isolation and identification of potential cytotoxic compound from kembang bulan [Tithonia diversifolia (Hemsley) A. Gray] leaves. Int J Pharm Pharm Sci. 2015;7:298-301.
13. Mardihusodo HR, Wahyuningsih MSH, Astuti I. The effect of active compound isolated from the leaves of kembang bulan [Tithonia diversifolia (Hemsley) A. Gray] on cell cycle and angiogenesis of WiDr cell line. J Med Sci. 2013;45:101-11.
14. BPOM RI, Nomor PB. Tahun 2019 persyaratan keamanan dan mutu obat tradisional. Badan Pengawas Obat Makanan. 2019;32:1-37.
15. Assefa ST, Yang EY, Chae SY, Song M, Lee J, Cho MC. Alpha glucosidase inhibitory activities of plants with focus on common vegetables. Plants (Basel). 2019;9(1):2. doi: 10.3390/plants9010002, PMID 31861279.
16. Aghababaei F, Cano-Sarabia M, Trujillo AJ, Quevedo JM, Ferragut V. Buttermilk as encapsulating agent: effect of ultra-high-pressure homogenization on chia oil-in-water liquid emulsion formulations for spray drying. Foods. 2021;10(5):1059. doi: 10.3390/foods10051059, PMID 34064917.
17. Gupta PK, Pandit JK, Kumar A, Swaroop P, Gupta S. Pharmaceutical nanotechnology novel nanoemulsion-high energy emulsification preparation, evaluation and application. Pharm Res. 2010;3:117-38.
18. Laksmitawati DR, Fatimah S, Jannah RF, Sumiyati Y, Marwati U. Activity of porang flour and Moringa extract to blood glucose and lipid levels in alloxan induced diabetic mice. J Pharm Sci Res. 2018;10:231-9.