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Submission date: 30-Nov-2021 09:02AM (UTC+0700)
Submission ID: 1715843856
File name: Final_Revision_Paper.doc (137K)
Word count: 1577
Character count: 9404

IN VITRO ANTI-DIABETIC POTENTIAL EXTRACT TEST OF SERI (Muntingia calabura, L.) LEAVES

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ABSTRACT

Objective: The purpose of this study was to determine the potential of the ethanol extract of the of *Muntingia calabura*, L (*M. calabura*) leaves as an anti-diabetic tested by in-vitro using the α-glucosidase enzyme.
Methods: extraction by maceration, phytochemical screening using standard testing and testing for anti-diabetic potential were carried out in vitro with observations of inhibition % of the α-glucosidase enzyme from various concentrations of ethanol extract of *M calabura* (60, 120, 200 and 300 ppm) leaves and acarbose as a standard of comparison.
Results: Phytochemical screening showed the ethanol extract content of *M. calabura* leaves, namely alkaloids, flavonoids, tannins, saponins, triterpenoids and steroids. The ethanol extract of *M. calabura* leaves has potential as an anti-diabetic and the increase in concentration is directly proportional to its potential activity.
Conclusions: The ethanol extract of *M. calabura* leaves has various types of secondary metabolites such as alkaloids, flavonoids, triterpenoids and steroids, saponins and tannins and has potential as anti-diabetic.

Keywords: Seri Leaves, Anti-diabetic, Phytochemical Screening, Acarbose, and α-glucosidase.

INTRODUCTION

The potential of natural resources stores a variety of boon and advantages which have recently been explored for various purposes, for example in the development of herbal-based medicines, functional foods and other[1,2]. One of the series plants or often known as seri is a plant that grows a lot in tropical areas[3], has dense leaves and is used as a shade tree especially in the province of North Sumatera, Indonesia. The seri plant belongs to the Elaeocarpaceae family with the latin name *Muntigia calabura*, L (*M. calabura*)[4]. *M. calabura* is reported to have various activities that show pharmacological effects as an antipoliferative, antioxidant, antinocicetic, cardioprotective, antipyretic[3], anticancer, relieving headache, cold medicine, gastric ulcer, antitumor[4], antimicrobial[5], and anti-diabetic[6].
The plant potential of *M. calabura* is supported by a variety of potential secondary metabolites, including flavonoids, triterpenoids, saponins, steroids[7], alkaloids, and anthraquinones[8]. Based on this information, this study aims to determine the potential activity of the ethanol extract of *M. calabura* as an anti-diabetic which was tested in vitro using inhibition of the enzyme α-gulosidase.

MATERIAL AND METHODS

Preparation sample

M. calabura leaves used in this study were taken from Namorambe Village, Medan Tuntung District, North Sumatera Province, Indonesia. The sample was identified by a botanist at the Herbarium Medanense, University of North Sumatera (No. 5107/MEDA/2020). The leaves of *M. calabura* used were from trees that have been fruitful and were in fresh condition. The samples were cleaned in running water, dried in an open room protected from direct sunlight, then pollinated using a blender and obtained *M.calabura* powder of simplicia.

Preparation and Process of the Extract Plant

500 g of *M. calabura* leaves simplicia powder was extracted with ethanol (p.a) and soaked for 5 days at room temperature and stirred occasionally and then filtered[9]. The filtering process uses Whatman No. 1 paper. Liquid extract obtained by crude ethanol extract in liquid conditions. The ethanol extract was concentrated using a rotary vacuum evavorator at a temperature of 50°C. Phytochemical screening was performed using standard reagents[10,11,12].

Testing Potential Anti-diabetic Activity by Invitro

Testing the anti-diabetic potential of the ethanol extract of *M. calabura* leaves by in-vitro with observing the inhibitory activity of the α-glucosidase enzyme. Tests were carried out by the standard method with slight modifications[13]. In the 96-well micro plate, the mixture was added with 60 µL of phosphate buffer (0.1 M; pH 6.8), 10 µL various concentrations of ethanol extract *M. calabura* leaves (60, 120, 200 and 300 ppm) then incubated at 37°C for 20 minutes. Then proceed with the addition of 20 µL p-nitrohenylglucopyranoseide (p-NPG) (5 mM) as a substrate and re-incubated again for 20 minutes at 37°C. The reaction was stopped by adding 100 µL of Na_2CO_3 (0.2 M). The absorbance of p-NPG release was measured at 425 nm using a multiplate reader. Akarbose used at various concentrations (60-300 ppm) was included as the standard. Control was used without the addition of extracts and without the test material and each test was replicated three times. The results obtained are expressed as the percentage of resistance using the formula[10]:

$$\text{Inhibitory activity } (\%) = \left(1 - \frac{As}{Ac}\right) x\ 100$$

Where: As = Absorbance of sample
Ac = Absorbance of control

RESULT AND DISCUSSION

Preliminary Phytochemical Screening

The weight of the *M. calabura* ethanol extract obtained was 6.34±0.006 g. The results of the screening of the ethanol extract of *M. calabura* leaves are shown in Table 1.

Table 1. Secondary screening metabolites of ethanol Extract *M. calabura* Leaves

Secondary Metabolites	Reagent	Evidence
Alkaloids	Mayer	present
	Dragendroff	present

	Wagner	present
Flavonoids	Shinoda test	present
Triterpenoids and steroids	Libermann Bouchard	present
Saponnis	Forth methods	present
Tannis	$FeCl_3$ 1%	present

The results of phytochemical screening of the ethanol extract of *M. calabura* leaves extracted by maceration method contain various secondary metabolites which include alkaloids, flavonoids, triterpenoids and steroids, saponins and tannins. Glycosides-steroids, saponins, and flavonoids show their potential as blood sugar-lowering[14].

Inhibition of Alfa-glucosidase enzyme

The potential of various natural ingredients with their secondary metabolite content plays an important role for medicinal purposes, one of which is diabetes treatment. In-vitro testing showed that the metabolites contained in the ethanol extract of *M. calabura* leaves showed potential activity as an anti-diabetic. The results of testing data on percent inhibition of alpha-glucosidase enzymes, namely: 32.56; 48.39; 59.89 and 63.21 shown in Figure 1.

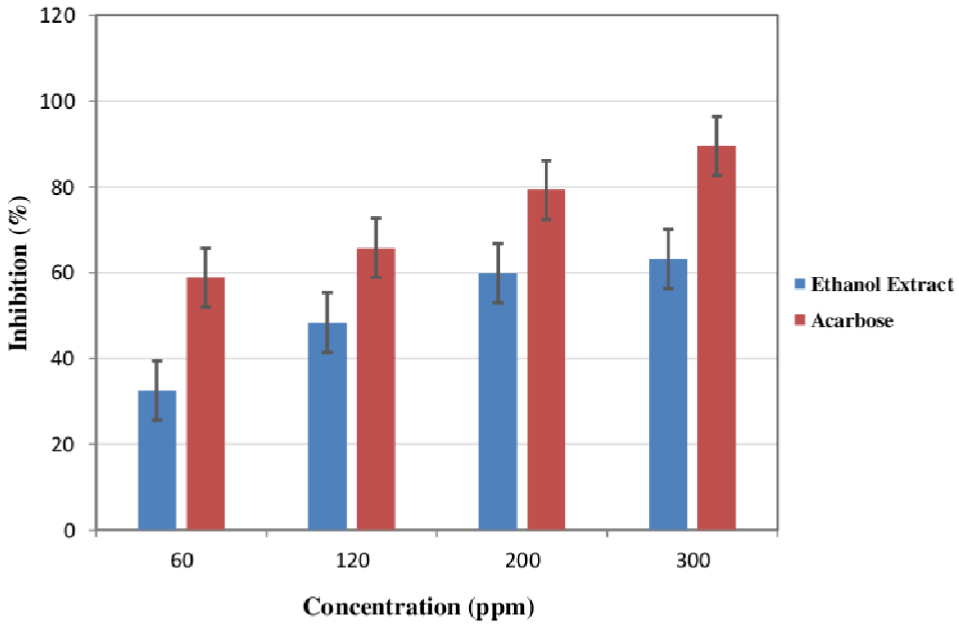

Figure: 1 The potential of *M. calabura* ethanol extract on % inhibition anti-diabetic tested by in-vitro using α-glucosidase enzymes

The results of the ethanol extract of *M. calabura* leaves showed potential activity as an anti-diabetic indicated by its inhibitory ability (%) based on the alpha glucosidase enzyme test. The percentage of α-glucosidase enzyme inhibition was directly proportional to the increase in the concentration of the ethanol extract of *M. calabura* leaves used. The potential for the anti-diabetic activity of *M. calabura* leaves and acarbose as a comparison showed different potentials

where acarbose showed a stronger potential in inhibiting the enzymatic activity of α-glucosidase. The potential of *M. calabura* leaves ethanol extract was supported by the presence of various secondary metabolites contained inside. Previous reports have shown that the compound group of flavonoid has the ability to inhibit activity against the enzyme α-glucosidase[11].

CONCLUSION

The ethanol extract of *M. calabura* leaves has various types of secondary metabolites such as alkaloids, falavonoids, triterpenoids and steroids, saponins and tannins. The presence of a secondary metabolite group of the ethanol extract of *M. calabura* leaves shows potential as an anti-diabetic shown against the inhibition of alpha glucosidase enzymes.

ACKNOWLEDGMENTS

I would like to express my gratitude to the Kementerian Pendidikan dan Kebudayaan, Dikti Indonesia for the opportunity that given to researchers in providing research funds in research schemes for novice lecturers in order to increase the ability of lecturers in research and publication.

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