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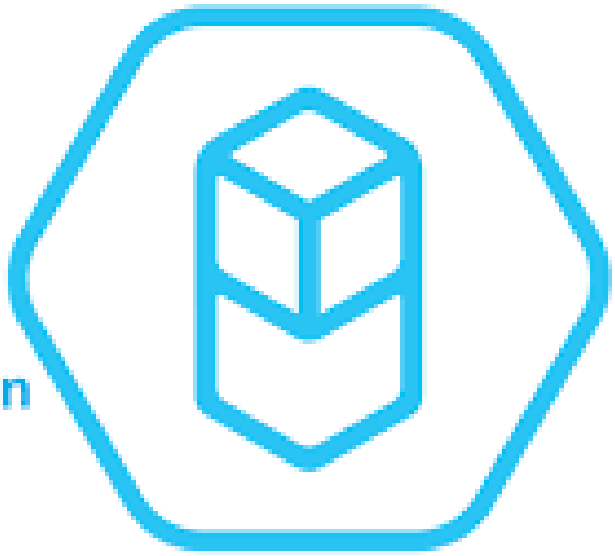

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Dolih Gozalil, Iyan Sopyan, Resmi Mustarichi, Wahyu Priyo Legowo (Author)

IAI CONFERENCE: Activity test of fruit and pomegranate seeds (Punica granatum l) as a hepatoprotector against white male Wistar rats

p. 220-224

PDF

IAI CONFERENCE: The remuneration of the community pharmacist in the developing world: the case in Indonesia

Andi Hermansyah, Anila Impian Sukorini, Abdul Rahem (Author)

p. 36-41

PDF

IAI CONFERENCE: Satisfaction of drug information services implementation in Air Itam, Selindung, Girimaya, Gerunggang and Melintang health centre Pangkalpinang City

Rachmawati Felani Djuria (Author)

p. 246-250

PDF

IAI CONFERENCE: The effect of stress level on the therapeutic outcomes of type 2 diabetes mellitus at the regional public hospital of West Nusa Tenggara province

Baiq Leny Nopitasari, Baiq Nurbaety, Made Krisna Adi Jaya (Author)

p. 67-70

PDF

IAI CONFERENCE: Role of pharmacist in providing drug information and education for patients with chronic diseases during Transition of Care

Umi Athiyah, Abdul Rahem, Catur Dian Setiawan, Andi Hermansyah (Author)

p. 275-280

PDF

IAI CONFERENCE: Formulation and physical evaluation of facial cream preparations from Ceremai fruit juice (Phyllanthus acidus (l.) Skeels)

Danang Indriatmoko, Nani Suryani, Tarso Rudiana, Mila Kurniah (Author)

p. 87-92

PDF

IAI CONFERENCE: Drug interactions in patients with hypertension at Persahabatan hospital in 2015

Zainul Islam, Atika Vitasari, Muhammad Arif Ridwan (Author)

p. 309-314

PDF

IAI CONFERENCE: Formulation and evaluation of Kirinyuh Leaf effervescent granules (Chromolaena Odorata. L) as an antioxidant

Firman Gustaman, Keni Idacahyati, Winda Trisna Wulandari (Author)

p. 123-125

PDF

IAI CONFERENCE: Self-medication and self-treatment with short-term antibiotics in Asian countries: A literature review

p. 152-162

PDF

IAI CONFERENCE: In vivo activity of Phaseolus vulgaris as an anti-hypercholesterolemic

Keni Idacahyati, Yedy Purwandi Sukmawan, Nopi Yanti, Winda Trisna Wulandari, Firman Gustaman, Indra (Author)

p. 184-188

PDF

IAI CONFERENCE: Therapeutic potential of Cymbopogon schoenanthus (L.) developed into nanoparticle technology

Lutfi Chabib, Adnan Muhammad Uno J Hidayat, A.M. Bagas Trianloka, Muhammad Iqbal Pangestu, Arman Suryani, Yulianto (Author)

p. 210-214

PDF

IAI CONFERENCE: In vitro antimalarial activity assay of Ashitaba Leaf ethanolic extract (Angelica keiskei)

Alvi Kusuma Wardani, Abdul Rahman Wahid, Miftahul Jannah (Author)

p. 27-30

PDF

IAI CONFERENCE: Formulation and physical properties of lotion Kalakai root ethanol extract (Stenochlaena palustris Bedd)

Rabiatul Adawiyah, Riska Apriliyanti, Agustinawati Umaternate (Author)

p. 235-240

PDF

IAI CONFERENCE: Description of medication adherence in hypertensive respondents at Mandalika Mataram elderly social centre

Anna Pradiningsih, Dzun Haryadi Ittiqo, Neti Puput Arianti (Author)

p. 52-55

PDF

IAI CONFERENCE: Mother's knowledge and practices towards self-medication of fever among children under five years in Muncar Banyuwangi, Indonesia

Sinta Rachmawati, Khusnul Khotimah, Ika Norcahyanti (Author)

p. 264-268

PDF

IAI CONFERENCE: Hypoglycemic activity test on smooth pigweed (Ammaranthus Hybridus L) leaf water extract on male Wistar rats

Asti Yunia Rindarwati (Author)

p. 56-60

PDF

IAI CONFERENCE: Enhancing solubility and antibacterial activity using multi-component crystals of trimethoprim and malic acid

p. 296-304

PDF

IAI CONFERENCE: Correlation between the antioxidant capacity of plasma and blood glucose level

Eva Nurinda, Emelda, Nurul Kusumawardani (Author)

p. 108-115

PDF

IAI CONFERENCE: Acute toxicity study of the ethanolic extract of Eleutherine bulbosa Urb in Wistar rats

Helmina Wati, Rahmi Muthia, Kartini, Finna Setiawan (Author)

p. 143-147

PDF

IAI CONFERENCE: The effect of biofilm formation on the outcome therapy of diabetic foot infections (DFIs) patients in the outpatient clinic and inpatient ward of Dr Sardjito General Hospital Yogyakarta

Ika Puspitasari, Titik Nuryastuti, Rizka Humardewayanti Asdie, Hemi Sinorita, Nusaibah Umaroh4, Wahyu Tri Hapsari (Author)

p. 172-177

PDF

IAI CONFERENCE: Acute toxicity test of 96% ethanol extract of Syzygium myrtifolium leaves in white mice (Mus musculus)

Lusi Indriani, E. Mulyati Effendi, Kevin Christofer Fadillah (Author)

p. 201-204

PDF

IAI CONFERENCE: Adverse drug reactions associated with successful treatment of multidrug-resistant tuberculosis patients in Cempaka Putih Islamic Hospital Central Jakarta

Adin Hakim Kurniawan, Harpolia Cartika, Siti Aisyah (Author)

p. 15-21

PDF

IAI CONFERENCE: Comparing the quality of life of neuropathic patients treated with gabapentin and pregabalin at the neuropathic poly of the NTB provincial hospital in 2019

Nurul Qiyaam, Baiq Leny Nopitasari, Haerul Muhajiji (Author)

p. 225-229

PDF

IAI CONFERENCE: Exploring pharmacist experience and acceptance for debunking health misinformation in the social media

p. 42-47

PDF

IAI CONFERENCE: Medicine management in districts and primary health care centres (PHC) in the national health insurance (JKN) programme

Raharni, Rini Sasanti, Yuyun Yuniar (Author)

p. 251-257

PDF

IAI CONFERENCE: A pharmacoeconomic study: cost-utility analysis of modern wound dressings vs conventional wound dressings in patients with diabetic foot ulcer

Cyntiya Rahmawati, Baiq Leny Nopitasari (Author)

p. 71-76

PDF

IAI CONFERENCE: Proximate analysis on animal feed granules composed of raw material from fish innards wastes

Wahyuning Setyani, Christine Patramurti, Agatha Budi Susiana Lestari, Raysha Mcseer, Day Stella Maris Gewab, Maria Felix Zita Ina Bulu, Maria Regina Lusiana Kya (Author)

p. 281-286

PDF

IAI CONFERENCE: The evaluation of compounding prescription and its availability of a licensed product for children at a private hospital in Yogyakarta, Indonesia

p. 93-97

PDF

IAI CONFERENCE: The potential effect of the green coffee extract on reducing atherogenic index in hyperlipidemic rats

Fransiska Maria Christianty, Fifteen Aprila Fajrin, Andrean Roni (Author)

p. 126-131

PDF

IAI CONFERENCE: Antibiotic use on paediatric inpatients in a public hospital in Bangil, Indonesia

Ika Norcahyanti, Malikatur Rosyidah, Abdul Kadir Jaelani, Antonius N.W Pratama (Author)

p. 163-167

PDF

IAI CONFERENCE: Analysis of pharmacists' knowledge and attitude in the pharmaceutical industry of halal certification and their readiness to produce halal medicine

Abdul Rahem, Mustofa Helmi Effendi, Hayyun Durrotul Faridah (Author)

p. 1-7

PDF

IAI CONFERENCE: Microencapsulation of Jeringau Rhizome essential oils (Acorus calamus L.) using β-Cyclodextrin

Ledianasari, Deby Tristiyanti, Elva Maulydha Tanjung, Lovelyta Barani (Author)

p. 189-194

PDF

IAI CONFERENCE: Effectiveness of combination of Moringa Leaf Extract (Moringa oleifera Lamk.) and Papaya Seed Extract (Carica papaya L.) in reducing blood sugar levels of diabetic rats

Novi Ayuwardani, Yetti Hariningsih (Author)

p. 215-219

PDF

IAI CONFERENCE: Identification of herbal products used by families in the campus of Darussalam Gontor University

Amal Fadholah, Solikah Ana Istikomah, Cania Sofyan Islamanda, Evi Rohana Ma'rufi Jannah (Author)

p. 31-35

PDF

IAI CONFERENCE: Effectiveness of public service advertisements on the use of antibiotics in Pangkalpinang

p. 241-245

PDF

IAI CONFERENCE: Correlation between the level of knowledge of drug managers and drug management in several primary health centres in Malang regency

Ayuk Lawuningtyas Hariadini, Nur Ishmah, Hananditia Rachma Pramestutie (Author)

p. 61-66

PDF

IAI CONFERENCE: The comparison of the actual cost to case-mix of type 2 diabetes mellitus inpatient in Pandan Arang Boyolali hospital

Sri Bintang Sahara Mahaputra Kusuma Negara, Devi Ristian Octavia, Primanitha Ria Utami (Author)

p. 269-274

PDF

IAI CONFERENCE: In silico screening of mint leaves compound (Mentha piperita L.) as a potential inhibitor of SARS-CoV-2

p. 81-86

PDF

IAI CONFERENCE: Sambiloto (Andrographis paniculata Nees.) leaf extract activity as an α-Amylase enzyme inhibitor

Yustina Sri Hartini, Dewi Setyaningsih, Maria Josephine Vivian Chang, Maria Cyrilla Iglesia Adi Nugrahanti (Author)

p. 305-308

PDF

IAI CONFERENCE: Fluconazole-tartaric acid co-crystal formation and its mechanical properties

Fikri Alatas, Nia Suwartiningsih, Hestiary Ratih, Titta Hartyana Sutarna (Author)

p. 116-122

PDF

IAI CONFERENCE: Evaluation of antidiarrheal effect of combination of Salam Leaves (Syzygiumpolyanthum) and Jackfruit Leaves (Artocarpus heterophyllus Lam.) infusum in rats induced by castor oil

Husnul Khuluq, Evi Marlina (Author)

p. 148-151

PDF

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RESEARCH ARTICLE

Sambiloto (*Andrographis paniculata* Nees.) leaf extract activity as an α-Amylase enzyme inhibitor

Yustina Sri Hartini, Dewi Setyaningsih, Maria Josephine Vivian Chang, Maria Cyrilla Iglesia Adi Nugrahanti

Faculty of Pharmacy, Sanata Dharma University, Indonesia

Keywords
α-amylase
Aqueous extract
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Sambiloto leaf

Correspondence
Yustina Sri Hartini
Faculty of Pharmacy
Campus III of Sanata Dharma University
Paingan Maguwoharjo Depok Sleman
Yogyakarta
Indonesia
yustinahartini@usd.ac.id

Abstract

Introduction: Sambiloto (*Andrographis paniculata*) is an antidiabetic medicinal plant that acts by inhibiting the α-amylase enzyme. Andrographolide, the active compound of sambiloto leaf, is insoluble in water but dissolves in ethanol. **Aim:** This study compared the *in vitro* activity of aqueous extract and ethanolic extract of sambiloto leaf with the α-amylase enzyme. **Methods:** The inhibitory activity test of the α-amylase enzyme was carried out using the ultraviolet-visible spectrophotometric method by measuring the absorbance of the remaining starch, which forms a blue complex with iodine-iodide. **Results:** The inhibitory activity of the α-amylase enzyme of the aqueous extract of sambiloto leaf (with the IC_{50} value of 14.203 ± 0.112 mg/mL) was lower than that of the ethanol extract (with the IC_{50} value of 9.253 ± 0.116 mg/mL). The results of the statistical tests showed significant differences (p <0.05) between the inhibitory activity of the α-amylase enzyme acarbose and the activity of both extracts.

Introduction

The prevalence of diabetes in Indonesia is relatively high and needs comprehensive prevention efforts. Using medicinal plants that have traditionally been employed by people in Indonesia is a means to overcome diabetes. Antidiabetic medicinal plants help maintain normal blood sugar levels. Inhibition of carbohydrate digestion and absorption is one of the strategies for managing blood sugar levels. The α-amylase enzyme plays a role in converting carbohydrates into sugar; thus, inhibiting its activity can suppress the formation of blood sugar (Saad *et al.*, 2017). Sambiloto (*A. paniculata*) has a high level of bitterness; its main constituents include diterpenoids, flavonoids, and polyphenols. Among the single compounds extracted from *A. paniculata*, andrographolide is the major one in terms of bioactive properties and abundance. It is slightly soluble in ethanol and almost insoluble in water. Andrographolide in leaves, stems, or whole plants can be extracted with ethanol (Chao & Lin, 2010). Andrographolide and ethanol extracts of *A. paniculata* leaf showed antidiabetic, hypoglycemic, and antioxidant activity in mice with type 2 diabetes mellitus (DM) (Subramanian *et al.*, 2008). Generally, people use the *A. paniculata* as a medicinal plant by boiling it in water or preparing it according to the Indonesian Traditional Medicinal Herb Formulary (Formularium Ramuan Obat Tradisional Indonesia) (Departemen Kesehatan Republik Indonesia, 2017).

Aqueous extract from some plants showed alpha-amylase inhibiting activity (Bhutkar and Bise, 2012). This study compared the *in vitro* activity of aqueous extract and ethanolic extract of *A. paniculata* leaf with the α-amylase enzyme. Various *in vivo* and *in vitro* methods can be used to examine new antidiabetic drugs. *In vitro* test methods were carried out by testing the inhibitory activity of α-amylase and alpha-glucosidase enzymes (Patil *et al.*, 2012).

Methods

The materials used are sambiloto (*A. paniculata*) leaf

obtained from PT HRL Internasional, East Java in the form of powder, α-amylase enzyme (SIGMA), amylum, andrographolide (SIGMA), 70% technical ethanol, ethanol pro analysis (E. Merck), toluene pro analysis. (E. Merck), chloroform pro analysis (E. Merck), methanol pro analysis (E. Merck), double-distilled water, dimethylsulfoxide pro analysis (E. Merck), acarbose tablets, amylum, sodium phosphate, and sodium chloride. The α-amylase enzyme inhibitory activity test was carried out according to Bhutkar and the authors (2018) with few modifications. The potato starch (1% w/v), 1ml of drug solution (extract, acarbose), 1 ml of α-amylase enzyme (1% w/v) and 2 ml of acetate buffer (0,1M, 7,2 pH) were mixed. The mixture was incubated for one hour, then a 0.1 ml iodine-iodide indicator was added to the mixture. The absorbance measurement used a Shimadzu UV-Vis spectrophotometer using a wavelength of 568.5 nm. The percentage inhibition was calculated as follows:

$$\% \text{ inhibition} = (As-Ac/As) \times 100$$

Ac is the absorbance of the control; As is the absorbance of the sample.

The IC_{50} calculation was obtained from the linear regression equation after calculating the percentage of inhibition of α-amylase enzyme activity of the test material with a concentration range of 2.5 mg/ml, 5 mg/ml, 7.5 mg/ml, and 10 mg/mL. To compare treatments, analysis of variance (ANOVA) was used, and $p < 0.05$ was considered as statistically significant, alongside the Tukey Post-Hoc Test significance and 95% confidence interval. Linear regression measured the median inhibitory concentration (IC50) to determine the inhibitory activities of α-amylase. IBM SPSS statistics version 22 was used for statistical analysis.

Results

At the same concentration, the ethanolic extract of *A. paniculata* leaf showed a higher percentage of inhibition of the α-amylase enzyme activity than the aqueous extract. The additional concentration of the test materials increased the percentage of inhibition of the α-amylase enzyme activity (Figure 1). Acarbose tablets showed a higher percentage of inhibition than both extracts. The aqueous extract and the ethanolic extract of *A. paniculata* leaf showed an *in vitro* inhibitory activity of the α-amylase enzyme with IC_{50} values of 14.203 ± 0.112 mg/mL and 9.253 ± 0.116 mg/mL, respectively. The thin layer chromatogram of the *A. paniculata* extract showed a spot that is similar to the andrographolide spot (Figure 2).

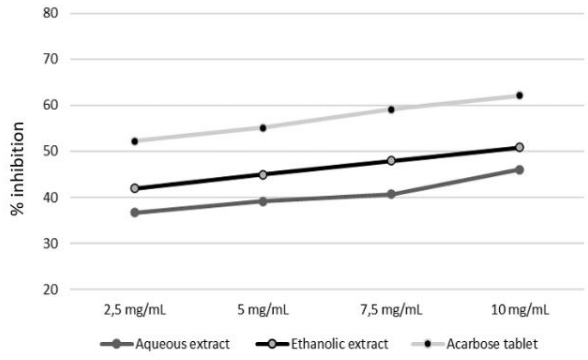
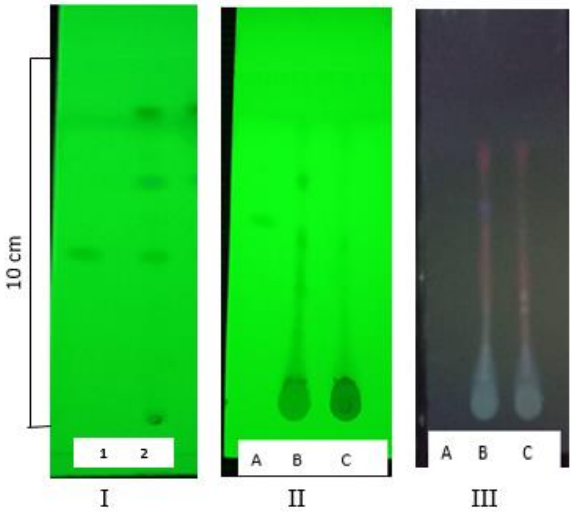


Figure 1: Per cent α-amylase inhibition activity of the aqueous extract, ethanolic extract, and acarbose tablet



(1)Andrographolide; (2)*A. paniculata* leaf powder; (A)Andrographolide; (B)The ethanolic extract of *A. paniculata;* (C)The aqueous extract of *A. paniculata*; (I) and (II) UV_{254} nm detection; (III) UV_{365} nm detection.

Figure 2: Thin layer chromatogram

Discussion

The inhibition of aqueous extract and ethanolic extract of *A. paniculata* leaf against α-amylase enzyme activity was tested *in vitro,* with acarbose being used as a positive control. Acarbose was chosen because of its inhibitory mechanism of action of the carbohydrate hydrolyzing enzyme. The chemical structure of acarbose is similar to that of amylum, which acts as a substrate, where both compounds have a benzene ring and a hydroxyl group that plays a role in binding to the active site of the enzyme; thus, a competitive inhibition mechanism of the enzyme activity can occur (Wright, 2003; Takahama and Hirota, 2017). The decreasing intensity of blue colour in the iodine-amylum complex

is due to the reduced amylum substrate hydrolyzed by the α-amylase enzyme. The additional concentration of the material tests increased the percentage inhibition of α-amylase enzyme activity (Figure 1). At the same concentration, the ethanolic extract of *A. paniculata* leaf showed a higher percentage of inhibition of the α-amylase enzyme activity than the aqueous extract. The ANOVA followed by the Tukey Post-Hoc test (p <0.05) showed a significant difference in the percentages of inhibition of the ethanolic extract of *A. paniculata* leaf, the aqueous extract of *A. paniculata* leaf, and acarbose tablets, on the activity of the α-amylase enzyme.

The level of inhibitory activity against the α-amylase enzyme is expressed as 50% inhibition concentration (IC_{50}), which is the concentration of the test material that can inhibit the enzyme activity by 50% (Subramanian *et al.*, 2008). The value of the percentage inhibition of the test materials is used to calculate IC_{50} by using the linear regression equation formula to determine the equation y = bx + a. The IC_{50} value obtained for the aqueous extract of *A. paniculata* leaf was 14.203 ± 0.112 mg/mL, the IC_{50} value for the ethanolic extract of the *A. paniculata* leaf was 9.253 ± 0.116 mg/mL, while the IC_{50} value of the acarbose tablet was 0.983 ± 0.036 mg/mL. The inhibitory activity of the α-amylase enzyme from the ethanolic extract was greater than that of the aqueous extract. It seems that the active compound that functions as an α-amylase enzyme inhibitor in the leaf of *A. paniculata* is in both extracts, but the amount of the compound in the aqueous extract is lower than that in the ethanolic extract. Qualitative testing used the Thin Layer Chromatography method using Chloroform: methanol (9:1) as mobile phase and silica gel GF 254 nm as stationary phase, and it detected the presence of andrographolide (Figure 2).

The *in vivo* antidiabetic activity of *A. paniculata* aqueous extract had also been reported; a significant reduction in blood glucose level was shown when hyperglycemic rats were treated with 50 mg/kg body weight aqueous extract of *A. paniculata* (Husen *et al.,* 2004). Considering that both extracts showed inhibitory activity of the α-amylase enzyme, andrographolide can be in both ethanolic and aqueous extract, thus suggesting that andrographolide is responsible for the inhibitory activity of the α-amylase enzyme. The overall activity of plant extracts can result from mixtures of compounds with synergistic, additive, or antagonistic activity. It seems that they are more effective than purified compounds due to beneficial "synergistic" interactions (Caesar and Cech, 2019). The inhibitory activity of the α-amylase enzyme from the aqueous extract of *A. paniculata* leaf can occur due to the presence of andrographolide compounds itself, or it can be due to compounds other than andrographolide, present in the extract, that have positive interactions. Further studies are necessary to examine the effect of the combination of compounds in aqueous extract and ethanolic extract of *A. paniculata* leaf as an effort to optimize the safety and benefits of using *A. paniculata* leaf extract. The ethanolic extract of *A. paniculata* leaf had higher α-amylase inhibitory activity than the aqueous extract but lower than acarbose tablets. The aqueous extract and the ethanolic extract of the *A. paniculata* leaf showed *in vitro* inhibitory activity of the α-amylase enzyme with IC_{50} values of 14.203 ± 0.112 mg/mL and 9.253 ± 0.116 mg/mL, respectively. Although having less activity than ethanolic extract, the aqueous extract showed an *in vitro* inhibitory activity of the α-amylase enzyme; it is thus recommended to prepare *A. paniculata* using water.

Conclusion

Both aqueous extract and ethanolic extract of sambiloto (*Andrographis paniculata* Nees.) showed inhibitory activity of the α-amylase enzyme. The inhibitory activity of the α-amylase enzyme of the aqueous extract of *A. paniculata* leaf (with the IC_{50} value of 14.203 ± 0.112 mg/mL) was lower than that of the ethanol extract (with the IC_{50} value of 9.253 ± 0.116 mg/mL).

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