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Two Flavonoids From Stem Bark of *Casimiroa edulis* and Their Antidiabetic and Antioxidant Activities

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Two Flavonoids From Stem Bark of *Casimiroa edulis* and Their Antidiabetic and Antioxidant Activities

K N W Tun[1,2], N S Aminah[3,*], A N Kristanti[3], R Ramadhan[3], Y Takaya[4]
[1] Ph.D. Student of Mathematics and Natural Sciences, Fac. Of Science and Technology, Universitas Airlangga, Komplek Kampus C UNAIR, Jl. Mulyorejo, Surabaya, Indonesia
[2] Dept. of Chemistry, Taunggyi University, Shan State (South), Myanmar
[3] Dept. of Chemistry, Fac. Of Science and Technology, Universitas Airlangga, Komplek Kampus C UNAIR, Jl. Mulyorejo, Surabaya, Indonesia
[4] Fac. Of Pharmacy, Meijo University, 150 Yagotoyama, Tempaku, Nagoya, 468-8503 Japan

* nanik-s-a@fst.unair.ac.id

Abstract: *Casimiroa edulis* Llave et Lex (Rutacae), popularly known as white sapote. The main aim of this study is to isolate and investigate the bioassay of the stem bark of *Casimiroa edulis.* Two flavonoids were isolated from the methanolic fraction of the stem bark of *Casimiroa edulis*. The isolated compounds can be identified as 6,7-dimethoxyflavone (**1**) and 5,6,2′-trimethoxyflavone (**2**) by using advance spectroscopic methods, including FT-IR, UV, 1D NMR, 2D NMR. Compounds 1 and 2 were evaluated for their antidiabetic and antioxidant activities. The result revealed that the two compounds did not have antidiabetic activity and antioxidant activity. This is the first phytochemical study of 6,7-dimethoxyflavone from the genus *Casimiroa*.

Key words: *Casimiroa edulis*, white sapote, Rutaceae, flavonoids

1. Introduction

Natural products are used as medicines for treating and preventing various diseases since prehistoric times. According to the record of fossil, human use of plants as medicines for their diseases may be traced back at least 60,000 years.[**11; 18**]

Casimiroa is a tree belongs to the family of Rutaceae, found in the tropical and subtropical areas of Central America and Mexico, the Caribbean, the Mediterranean region, India, Southeast Asia, South Africa, Australia, and New Zealand. The best-known species is *Casimiroa edulis* **[14; 17]**. It has been widely used as sedative for the treatment of anxiety and dermatological problem. The early pharmacological studies of an aqueous extract and alcohol extracts of the seeds and leaves of *C. edulis* exhibited the cardiovascular, anticonvulsant, sedative, anti-inflammatory, anti-mutagenic, diuretic, hypnotic, anti-hypertension, anti-inflammatory, muscle relaxant and contractile activities [**4; 15].** In Myanmar, local people used this for the treatment of stomach problem.

Many of the phytochemical analysis have been done on the leaves, fruits, seeds and bark of *Casimiroa edulis*. The previous studies indicated that this plant contains flavonoids, coumarin, alkaloids, and limonoids **[1-3, 5-9; 12]**. In this study, two flavonoids namely, 6,7-dimethoxyflavone **(1)** and 5,6,2′-trimethoxyflavone (**2**) have been isolated from the stem bark of *Casimiroa edulis*. Their structures have been elucidated through FT-IR, UV, ^{1}H-NMR, ^{13}C-NMR, and 2D NMR. Furthermore, the antidiabetic and antioxidant activity of isolated compounds were investigated against α-glucosidase inhibition and DPPH assay.

2. Experimental Methods

2.1 General

UV spectra were recorded on UV-Vis Shimadzu spectrometer. IR spectra were recorded on FT IR-8400 spectrophotometer. NMR spectra were recorded in $CDCl_3$ by using a JEOL ECA-500 (^{1}H: 500 MHz and ^{13}C: 125MHz). Positive mode HRFABMS was obtained by using a JEOL JMS HX-110 mass spectrometer. Column chromatography was carried out on silica gel (BW-820H). Analytical TLC was performed on silica

on pre-coated Kieselgel silica gel 60 F_{254} aluminium sheets. Melting points were measured by melting point apparatus and are uncorrected.

2.2 Plant material

The stem bark of *casimiroa edulis* Llave et Lex was collected in Namp-see Village, Taunggyi (Shan State), Myanmar during the month of August 2016.

2.3 Extraction and isolation

The air-dried sample of the stem bark of *Casimiroa edulis* (1000 g) was extracted with methanol (3000 mL). Then the methanolic extract was concentrated at room temperature to give MeOH crude extract 250 g. The dried MeOH extracts 250 g were fractionated by partitioning with n-hexane : methanol (v/v) (100 mL × 3). The MeOH extract was evaporated under reduced pressure at 40°C using a rotary evaporator to give the methanolic crude extract 50 g. A methanol extract (50 g) was subjected to VLC separation using 100 g silica gel 60H eluted with a gradient solvent system of *n*-hexane in Et-OAc (100:0, 95:5, 90:10, 80:20, 70:30, 60:40, 0:100) to afford 28 Fractions (1-28). Based on TLC analysis, the fractions can be grouped to be CF-1, CF-2, CF-3, CF-4, CF-5 and CF-6. Fraction CF-6 (6.19 g) was further fractionated by silica gel column chromatography with a gradient solvent system of n-hexane in Et-OAc (100:0, 95:5, 90:10, 80:20, 70:30, 0:100) to afford 270 fractions. Based on TLC analysis, the fractions can be grouped to be SF-1, SF-2, SF-3, SF-4 and SF-5. Fraction SF-2 (120 mg) was further purified by silica gel column chromatography with a gradient of n-hexane in acetone (100:0, 95:5, 90:10, 80:20, 0:100) to give 70 subfractions. Each fraction was checked by TLC and UV lamp. Then, the sub- fractions of the same R_f value were combined and 5 combined fractions (Fra-1 to Fra-4) were obtained. Among them, Fra-2, and Fra-4 gave only one spot on TLC and UV active. The pure compound white crystalline solid form of compound (**1)**, and compound (**2)** were obtained.

2.4 α-Glucosidase inhibition assay and DPPH assay

The α-glucosidase inhibition of two compounds was analyzed according to the method reported by Ramadhan & Phuwapraisirisan [**13**]. Antioxidant activity of two compounds was measured against DPPH radical scavenging activity. The IC_{50} values of the compound were measured by the linear regression.

2.5 Spectra data

6,7-dimethoxyflavone (1)

White crystalline solids ($CHCl_3$) (**1**): UV (MeOH) λ_{max}: 271 nm; IR (ν_{max}, KBr, cm^{-1}): 3070, 2999, 1647, 1571, 1496, 1367, 1288, 1178, 1078, 958, 775; ^{1}HNMR ($CDCl_3$, 500 MHz, δ, ppm, J/Hz) : 7.89 (dd, *J* = 7.7, 1.9 Hz, H-2′ and H-6′), 7.51 (m, H-3′, H-4′ and H-5′′), 7.32 (s, H-5 and H-8), 6.69 (s, H-3), 3.98 (s, OCH_3), 3.94 (s, OCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 178.0 (C-4), 161.6 (C-2), 151.6 (C-9), 150.0 (C-7), 148.0 (C-6), 131.7 (C-1′), 131.4 C-4′, 129.0 (C-3′), 126.1 C-6′), 119.3 (C-10), 119.1 (C-8, 113.4, 108.0 (C-3), 61.9 (7-OCH_3), 57.2 (6-OCH_3).

5,6,2-trimethoxyflavone(2*)*

White crystalline solids ($CHCl_3$) (**2**) UV (MeOH) λ_{max}: 329, 267, 235 nm; IR (ν_{max}, KBr, cm^{-1}): 3128, 3078, 2972, 2837, 1631, 1612, 1570, 1481, 1357, 1284, 1188, 1083, 964, 744; ^{1}HNMR ($CDCl_3$, 500 MHz, δ, ppm, J/Hz) : 7.85 (1H, dd, *J* = 7.8, 1.7 Hz, H-6′), 7.46 (ddd, *J* = 8.4, 7.4, 1.8 Hz, H-4′), 7.30 (1H, d, *J* = 9.2 Hz, H-7), 7.27 (1H, d, *J* = 9.2 Hz, H-8), 7.09 (1H, td, *J* = 7.7, 1.0 Hz, H-5′), 7.03 (1H, d, *J* = 8.0 Hz, H-3′), 6.98 (1H, s, H-3), 3.98 (3H, s, 2′-OCH_3), 3.93 (6H, s, 5-OCH_3 and 6-OCH_3), NMR ($CDCl_3$, 125 MHz, δ, ppm): 178.4 (C-4), 159.1 (C-2), 158.0 (C-5), 151.9 (C-9), 149.7 (C-6), 147.9 (C-2′), 132.2 (C-4′), 129.1 (C-6′), 120.8 (C-1′), 120.7 (C-5′), 119.2 (C-8), 119.1 (C-10), 113.4 (C-7), 113.1 (C-3), 111.7 (C-3′), 61.9 (2-OCH_3), 57.3 (5-OCH_3), 55.7 (6-OCH_3).

3. Results and discussion

6,7-Dimethoxyflavone (**1**), and 5,6,2′-trimethoxyflavone (**2**) were isolated from the methanolic extract of the stem bark of *C. edulis*. 6,7-dimethoxyflavone was the first phytochemical study of this plant. The isolated compounds identified by interpretation of their ^{1}H NMR and ^{13}C NMR spectral data by comparisons to those available in the literature.

Compound (**1**) was obtained as white crystalline solid with melting point at 236-248°C. IR spectrum of compound (**1)** displayed the absorption band for methoxy (3431 cm^{-1}), sp^2 hydrocarbon (3070 cm^{-1}) sp^3

hydrocarbon (2999-2839 cm^{-1}), carbonyl (1647 cm^{-1}) and aromatic (1639, 1571 cm^{-1}) groups. The UV spectrum showed an absorption band with λ_{max} 271 nm. According to the ^{1}HNMR spectrum, compound (**1)** showed the presence of 14 protons. One singlet sharp peak at δ_H 6.69 indicates the presence of H-3. Furthermore, the H-3 proton showed the correlation with the peak at δ_C 161.6 (C-2), 178.0 (C-4), 131.7 (C-1′) and 119.3 (C-10) in HMBC spectrum. Another two sharp singlets peak at δ_H 3.94 and 3.98 (each, 3H, s) indicate the presence of two methoxy groups on the aromatic ring. Moreover, one singlet sharp peak at δ 7.32 (2H,s) indicates the presence of H-5 and H-6 protons. One doublet-doublet at δ 7.89 ppm (2H, *J* = 7.7, 1.9 Hz) indicate the presence of H-2′ and H-6′ protons. The other remaining one multiplet at δ 7.51 (3H, m) indicates the presence of H-3′, H-4′ and H-5′ protons. The ^{13}CNMR and DEPT spectra of compound (**1)** showed 17 carbon atoms for the comprising of eight sp^2 methine, two oxygenated sp^3 and seven sp^2 quaternary carbons. Therefore, base above information the compound (**1)** was identified as 6,7-dimethoxyflavone [**16**].

Compound (**2**) was obtained as white crystalline solid with melting point at 144-156°C. IR spectrum of compound (**2**) displayed the absorption band for methoxy (3128 cm^{-1}), sp^2 hydrocarbon (3078 and 3003 cm^{-1}), sp^3 hydrocarbon (2972-2837 cm^{-1}), carbonyl (1631 cm^{-1}) and aromatic (1612, 1600 and 1570 cm^{-1}) groups. The UV spectrum showed absorption band with λ_{max} 329, 267 and 235 nm. According to the ^{1}HNMR spectrum, compound (**2)** showed the presence of 16 protons. One singlet sharp peak at δ_H 6.98 (1H, s) indicates the presence of H-3 proton. Furthermore, the H-3 proton showed the correlation with the peak at δ_C 159.1 (C-2), 178.4 (C-4), 119.1 (C-1′) and 120.8 (C-10) in HMBC spectrum. Two doublets at δ_H 7.27 and 7.30 ppm (each, 1H, *J* = 9.2 Hz) indicates the presence of H-7 and H-8. Two singlet sharp peaks at δ_H 3.93 (3H, s) and 3.98 ppm (6H,s) indicate the presence of three methoxy groups on the aromatic ring. One doublet-doublet at δ_H 7.85 (1H, *J* = 7.8, 1.7 Hz) indicates the presence of H-6′ proton. One doublet-doublet-doublet at δ_H 7.46 (1H, *J* = 8.4, 7.4, 1.8 Hz) indicates the presence of H-4′ proton. One triplet-doublet at δ_H 7.09 (1H, 7.7, 1.0 Hz) indicates the presence of H-5′ proton. One doublet at δ_H 7.03 (1H, *J* = 8 Hz) indicates the presence of H-3′ proton. The ^{13}CNMR and DEPT spectra of compound (**2**) showed 18 carbon atoms for the consisting of seven sp^2 methine, three oxygenated sp^3 and eight sp^2 quaternary carbons, respectively. Therefore, base above information the compound (**2**) was identified as 5,6,2′-trimethoxyflavone [**10**].

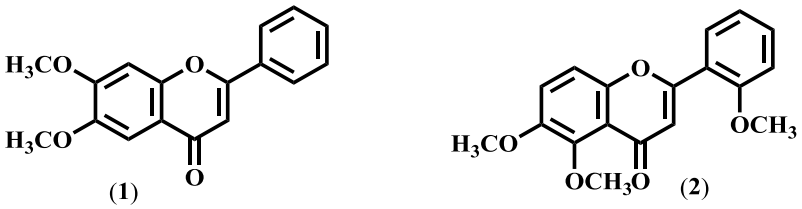

Figure 1. Chemical structure of compound (**1**) and (**2**)

3.1 Anidiabetic and Antioxidant activity

Two compounds were isolated from MeOH fraction of the stem bark of *Casimiroa edulis* were screened for antidiabetic and antioxidant activity against α-glucosidase inhibition and DPPH assay. According to the Table (1), these two compounds did not showed antidiabetic and antioxidant activity.

Table 1. Antioxidant and α-glucosidase inhibition activities of isolated compounds

	IC_{50} mM	
Compound	**Yeast**	**DPPH**
6,7-dimethoxyflavone (**1**)	NI	NI
5,6,2′-trimethoxyflavone (**2**)	NI	NI
Acarbose	0.1030	-

NI = No Inhibition

4. Conclusion

Two compounds were isolated from the stem bark of *Casimiroa edulis*. From their spectroscopic data, these two compounds can be identified as 6,7-dimethoxyflavone (**1**), and 5,6,2′-trimethoxyflavone (**2**). The isolated compounds were evaluated for antidiabetic and antioxidant activities. The result revealed that these two compounds did not have antidiabetic activity and antioxidant activity. Base on our knowledge, 6,7-dimethoxyflavone is isolated for the first time from the genus *Casimiroa*.

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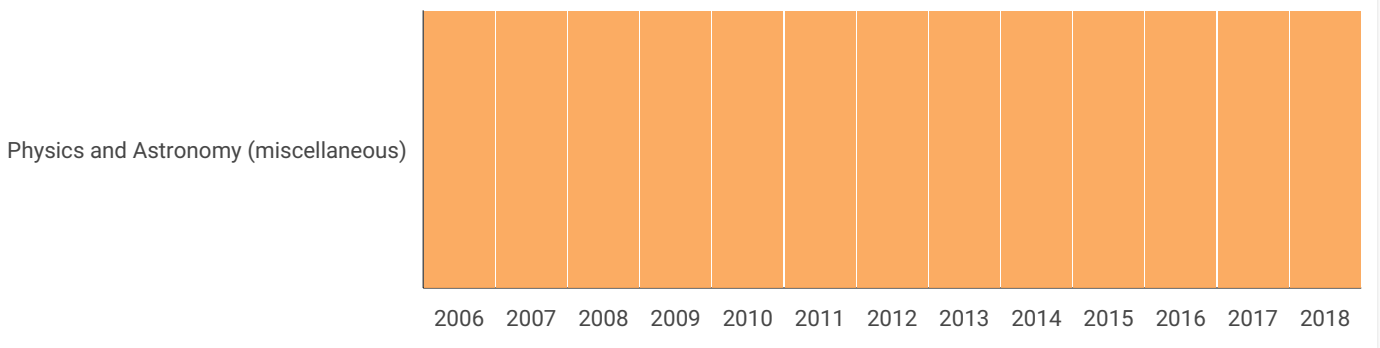

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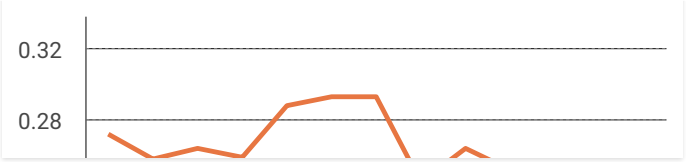

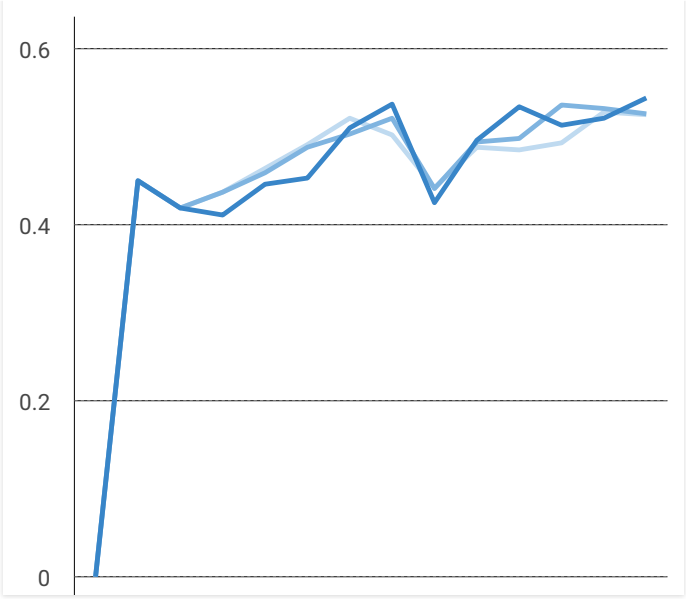

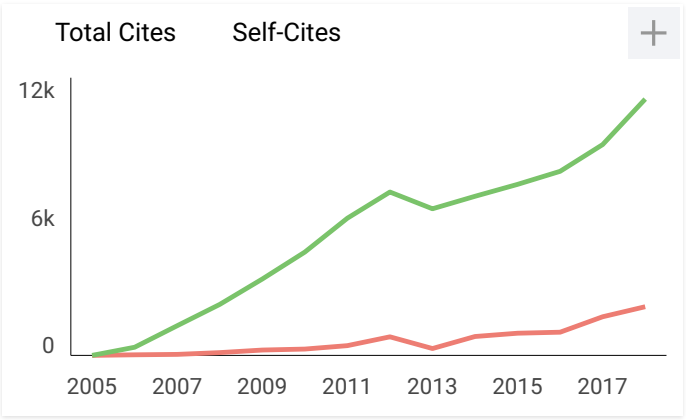

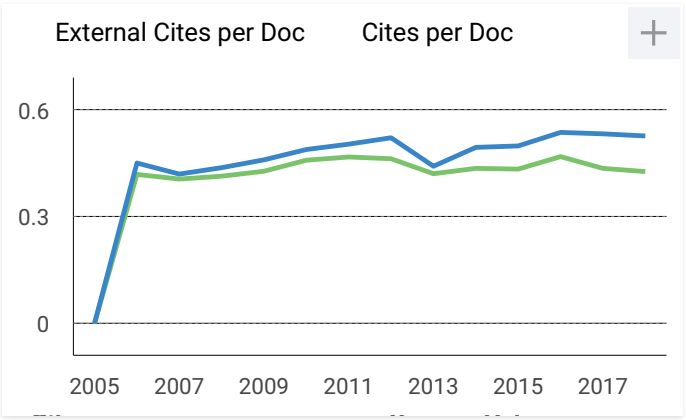

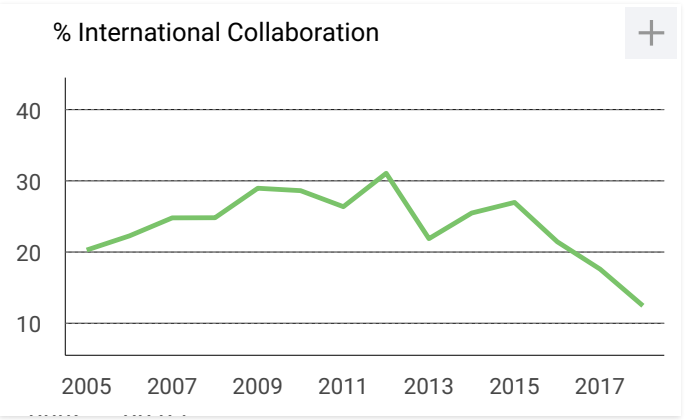

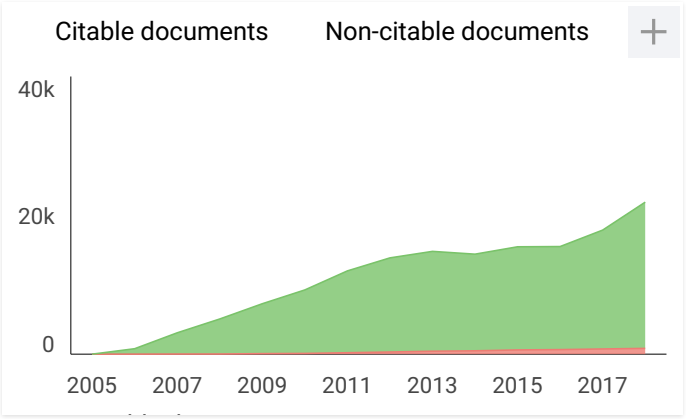

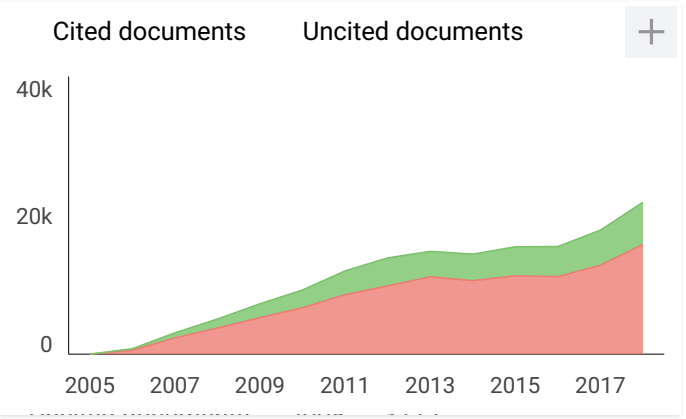

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Preface

Second International Conference "Collaboration Seminar of Chemistry and Industry (CoSCI 2018) in conjunction with 23rd Indonesian Society for Biochemistry and Molecular Biology (ISBMB) seminar was taken place at Universitas Airlangga, Surabaya-Indonesia on October 11-12, 2018. The event presented a theme" Recent Development of Omics Technology For Human Prosperity".

"Omics is a general term for a broad discipline of science and engineering for analyzing the interactions of biological information objects in organism. The main focus is on: 1) mapping information objects such as genes, proteins, and ligands; 2) finding interaction relationships among the objects; 3) engineering the networks and objects to understand and manipulate the regulatory mechanisms; and 4) integrating various omes and omics subfields.

The event was held to facilitate for the scientists, scholars, engineers and students from universities, research institutes and industries to present ongoing research activities, especially in Biochemistry, Biology, Chemistry, Medicine and other in related fileds. It was also to encourage future collaboration among all participants.

The conference of CoSCI and ISBMB created proceedings from the papers that were submitted by participants, after they were reviewed by committee members and international reviewers. This volume intends to provide readers with the recent advances in the Omics Technology such as Chemistry, Biochemistry and Molecular Biology, and Medicine field.

We would like to thank to all authors who contributed to the proceedings and also to the organizing committee, reviewers, speakers, sponshor, and all the conference participants for their supporting in the conference of CoSCI 2018 and 23rd Seminar of ISBMB.

Dr. Purkan, M.Si
Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia, Nov. 10, 2018

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Conference Photographs

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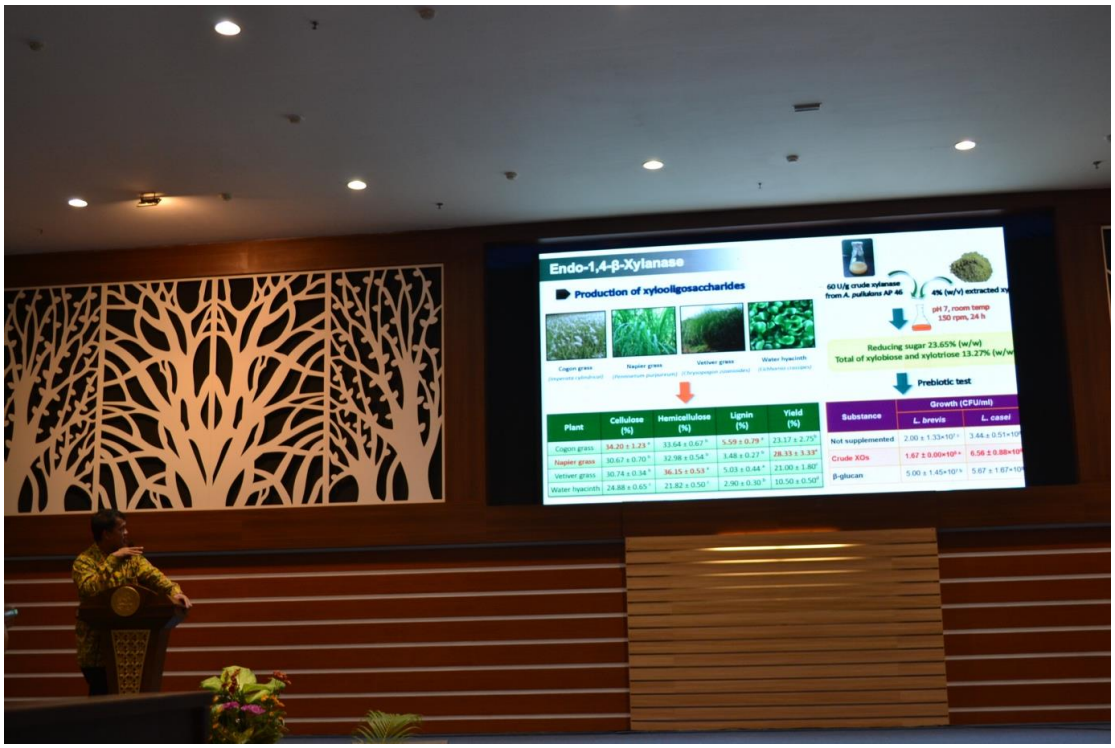
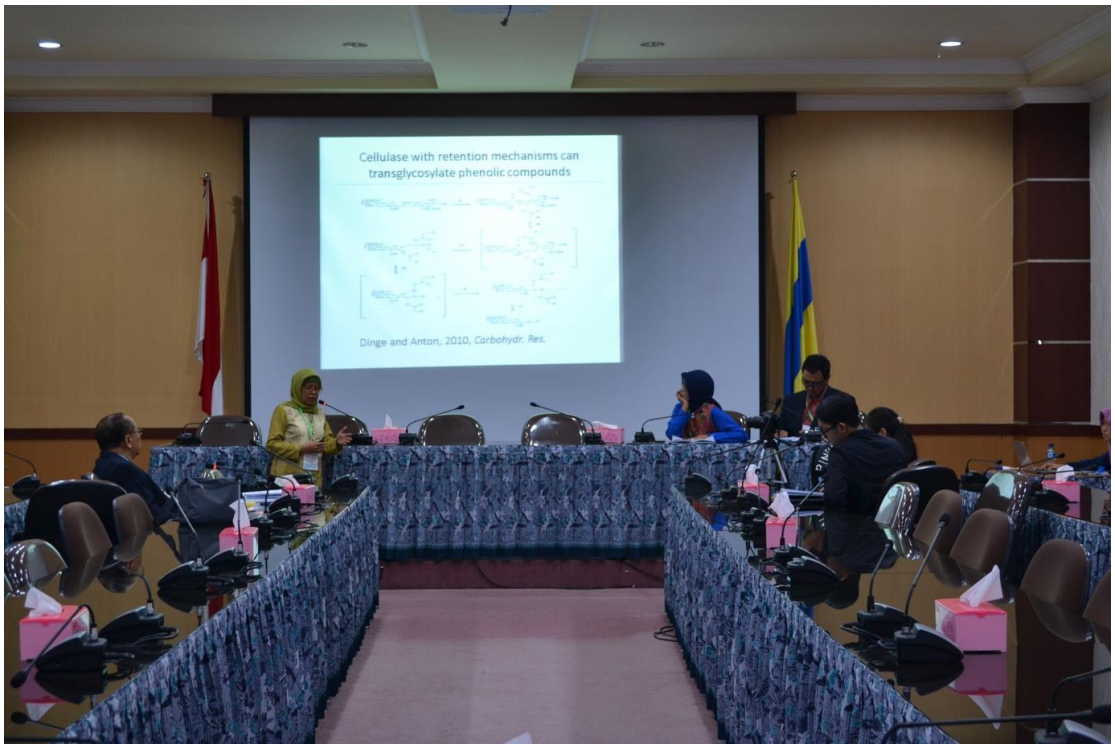
2nd International Conference "Collaboration Seminar of Chemistry and Industry (CoSCI) in conjunction with 23rd Indonesian Society for Biochemistry and Molecular Biology (ISBMB) seminar

October 11-12, 2018, Universitas Airlangga, Surabaya


Endo-1,4-β-Xylanase
Production of xylooligosaccharides
Cogon grass
Napier grass
Vetiver grass
Water hyacinth
Plant
Cellulose (%)
Hemicellulose (%)
Lignin (%)
Yield (%)
Cogon grass
Napier grass
Vetiver grass
Water hyacinth
Prebiotic test
Substance
Growth (CFU/ml)
L. brevis
L. casei
Not supplemented
Crude XOs
β-glucan


Cellulase with retention mechanisms can transglycosylate phenolic compounds
Dinge and Anton, 2010, Carbohydr. Res.

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