Certificate

This is to certify that

NOVIZAR NAZIR

Has participated in

International Seminar on Food Science and Agricultural Sciences (ISFAS2010)

as Head of Program and Proceeding

Jointly organized by:
Faculty of Agricultural Technology
Andalas University-Indonesia

And

Faculty of Science and Technology
Universiti Kebangsaan Malaysia
Malaysia

Held from February 16-17, 2010
at Hill Hotel and Convention, Bukittinggi Indonesia

Dean of Faculty of Agricultural Technology
Andalas University

Prof. Dr. Isril Berd

Chairman of ISFAS2010

Prof. Dr. Anwar Kasim

ISFAS2010

Universiti Kebangsaan Malaysia
National University of Malaysia

Proceeding

International Seminar on Food & Agricultural Sciences

17 February 2010 Bukittinggi, Indonesia

"Improving the quality of life through food and agricultural sciences"

EDITORS

Novizar Nazir - Universitas Andalas, Indonesia
Aisman - Universitas Andalas, Indonesia
Nazaruddin Ramli - Universiti Kebangsaan Malaysia
Wan Aida Wan Mustapha - Universiti Kebangsaan Malaysia
Ayub Mohd Yatim - Universiti Kebangsaan Malaysia
Mohamad Kassim - Universiti Kebangsaan Malaysia
A. Ploeger - Kassel University Germany

ISFAS 2010

Universiti Kebangsaan Malaysia

ISBN 978-602-96301-0-7

ISBN 978-602-96301-0-7

PROCEEDING

INTERNATIONAL SEMINAR ON FOOD AND AGRICULTURAL SCIENCES-ISFAS2010

16-17 FEBRUARY 2010

HILL HOTEL AND CONVENTION, BUKITTINGGI-INDONESIA

Improving the quality of life through food and agricultural sciences

Jointly organized by:

Faculty of Agricultural Technology,
Universitas Andalas, Padang-Indonesia.

and

School of Chemical Sciences and Food Technology
Faculty of Science and Technology,
Universiti Kebangsaan Malaysia.

PROCEEDING

INTERNATIONAL SEMINAR ON FOOD AND AGRICULTURAL SCIENCES-ISFAS2010

16-17 FEBRUARY 2010

HILL HOTEL AND CONVENTION, BUKITTINGGI-INDONESIA

Improving the quality of life through food and agricultural sciences

Editors:

Novizar Nazir (*Universitas Andalas, Indonesia*)
Aisman (*Universitas Andalas, Indonesia*)
Nazaruddin Ramli (*Universiti Kebangsaan Malaysia*)
Wan Aida Wan Mustapha (Universiti Kebangsaan Malaysia)
Ayub Mohd Yatim (*Universiti Kebangsaan Malaysia*)
Mohamad Kassim (*Universiti Kebangsaan Malaysia*)
A. Ploeger (*Kassel University-Germany*)

First published in 2010 by:

AgriTech Press

Faculty of Agricultural Technology - University of Andalas
Gedung Fateta Level 2- Kampus Unand Limau Manis
Padang, Indonesia 25163- Telp. +62 751 72772. Fax. +62 751 72702
http://www. fateta.unand ac.id

Printed and bound in Malaysia by Pusat Penerbitan dan Percetakan
Universiti Kebangsaan Malaysia, 43000 UKM Bangi-Selangor D.E-Malaysia

Cover design and layout:
Rahmat Hidayat

ISBN 978-602-96301-0-7

Content

Biomass, Bioenergy, Industrial and Process Engineering

ENZYMATIC SYNTHESIS OF FLAVONOID-GLYCOSIDE FROM GAMBIER (*UNCARIA GAMBIER*) BY CGT-ASE FROM *ACTINOMYCETES, BACILLUS LICHENIFORMIS AND ASPERGILUS ORIZAE*

Novizar Nazir[1] Joko Sulistyo[2], Norman Ferdinal[3] and Rini Handayani[2]

[1]Faculty of Agricultural Technology, University of Andalas Padang, Indonesia
[2]Indonesian Institute of Science. Research Centre of Biology. Cibinong, Indonesia
[3]Faculty of Mathematics and Sciences, University of Andalas Padang, Indonesia
e-mail: nazir_novizar@yahoo.com

Abstract

Flavonoid-α- glycoside was synthesized using enzyme of CGT-ase which was isolated from the cultivated Actinomycetes, Bacillus Licheniformis, and Aspergilus orizae. The CGT-ase enzyme has optimum capability at the temperature of 40 ℃, pH 6.5. Yielded 8.9 unit/minute/ml. The pretest of CGT-ase transfer activity was carried out using pyrocatechol and resorcinol as acceptors, and iso-malto and commercial starch solution as glucocyl donors. Subsequently, acceptor was replaced by flavonoid (Etyl acetate and butanol fraction) extracted from four types of Gambier from West Sumatra. The Rf value was compared with the Rf value of arbutin standard. The result showed that there was chemical content variability (qualitatively) and flavonoid fraction among the four types of Gambier: Riau Gadang Type had the highest flavonoid content; and Udang type has higher alkaloid than other types. The CGT-ase Enzyme from Aspergillus oryzae was not potential to be used for flavonoid-α-glicoside synthesis, while CGT-ase Enzyme from Actinomycetes (Strain W 25.3 and W 32) and B. Lichineformis (buffer fosfat) were active as transferase enzymes. Optimal condition for enzyme activity was 40°C at pH = 6.5. TLC analysis showed that all transferred products from Gambier come from organic phase. There were no transferred products identified in water phase. The Rf values of products were about the same with arbutin standard. The sugar total of transferred products was determined by the Dubois method, which was between 672.2 ppm – 2104.8 ppm, depending on types of Gambier and glucocyl donors.

Keywords

Uncaria Gambier, Gambier types, Actinomycetes, Bacillus Licheniformis, Aspergillus oryzae, CGT-ase, flavonoid-α-glicoside

INTRODUCTION

Uncaria Gambir (Hunter) Roxb is one of important commodities of West Sumatra, Indonesia. It has a potential for high economic value and multi-purpose species. Gambier contains flavonoids which can be used as raw material for the manufacture of drugs of anti-hepatitis B, anti-diarrhea [1] inhibiting dental plaque formation (Kozai et al. 1995. *Cit.* [2], antimicrobial, antinematoda [3, 4] and other benefits in support of various pharmaceutical, cosmetic, and agriculture [2]. According to Nazir [2], Indonesia is the only major world exporter of Gambier where nearly 80% of Gambier produced by Indonesia is exported to many countries, especially India. Although Gambier has been traded for a long time, but the processing technology is still simple; Gambier was sold in the form of "raw Gambier". Moreover, bargaining position (bargaining power) of the farmers is still low. This condition can not be allowed; product diversification and utilization, therefore, absolutely must be done. One effort is to make the preparation of Gambier flavonoid compounds which can be used as an antioxidant and antimicrobial. However, flavonoid compounds have a weakness. Flavonoid generally has low solubility and is not stable against the influence of light, oxidation and chemical changes. Therefore, when oxidized, it will change its structure and its function as an active ingredient will decrease and even disappear [5]. One way to improve the solubility and stability of flavonoid compounds is to modify these compounds to form the flavonoid glycosides, glycosides (flavonoids with sugar attached) through transglicocylation, either chemically or enzimatically by the help of enzyme transferase (CGT-ase) [6]. But compared with enzymatic synthesis, chemical synthesis of flavonoid-glycosides compounds is not economical and relatively difficult because it will result in a mixture of products with the configuration of α-and β-glycosides [7,8,9]. Therefore, the synthesis of flavonoids-α - glycosides via enzymatic transfer reaction is a better choice to obtain a relatively stable compound and has high solubility. Sulistyo [8] reported that CGT-ase enzymes can be utilized in reaction using flavonoid transglicocylation compounds as acceptors.

For this research, four types of gambier growing in West Sumatra were used, they are 1) udang type; 2) riau mancik type; 3) riau gadang type and 4) cubadak type [2] which are anatomically [10] and genetically different [11]. Gambier plant extracts are rich in flavonoids that can be used as an antioxidant and antimicrobial [2]. To and develop and increase the potential of these local plants, it is necessary to conduct a research to find the compounds and bioactivity flavonoid-glycosides of the

plants and to test their potentials as antioxidant and antimicrobial. This research is important to answer some questions concerning with : (1) variations in the types of phytochemicals of the four types of gambier ; (2) variations in the flavonoid components of the four types of gambier; (3) the best microbes that produce enzymes CGtase for flavonoid-glycosides synthesis; (d) the best bioprocess conditions for the synthesis of flavonoid-glycosides from gambier

A specific objective of this research was to perform synthesis of flavonoid glycosides of the four types of gambier (*Uncaria gambier Roxb.*) growing in West Sumatra to be used as an antioxidant and antimicrobes. It is expected that the result of study can be applied in the development of traditional medicine, pharmaceutical industries, food and nature-based cosmetic industries, especially of gambier (*Uncaria gambier Roxb.).* Product diversification and utilization are expected to increase the added-values of West Sumatran gambir commodities and reduce the dependence of the farmers from selling the raw gambier so that their their bargaining position could improve.

MATERIALS & METHODS

Materials

Cultures used for this research were Actinomycetes (Cr), (G3), *Aspergillus oryzae, and Bacillus licheniformis*, a collection taken from the Laboratorium of Microbiology, Biology, LIPI Research Center, Cibinong, Bogor. Four different species of gambier plants planted in Siguntur area, South Coast Region, West Sumatra, were used for this research. They were Udang type (U), Rriau Gadang type (RG), Riau Mancik type (RM) and Cubadak type (C).

Methods

a. Gambier Extraction : A 100 gram of gambier leaves were boiled for 1 hour (until the leaves were brown), extracted using a blender, filtered, frozen for 12 hours, precipitated and then dried. This is called method A. Method B is that the filtrate of the boiled gambier leaves was not frozen but deposited and dried.
b. Phytochemical screening [13].
c. Extraction of flavonoids [14]
d. Synthesis of Flavonoids-transglikosilasi glycosides via enzymatic reaction using the enzyme CGTase

RESULTS & DISCUSSION

The Extraction of Gambier

Gambier is extracted using two extraction methods. Method A is a modification in which gambier extract was frozen before deposited and dried. Figure 1 shows that Method A is better than Method B for all types of gambier. Research also showed that there are variations in the yield of the 4 types of gambier. It was caused by genetic variations of the gambier used [10, 11]. Other factors that cause the variations in the yield of gambier are genetic factors, means and processing conditions, growing conditions and post-harvest factors [2].

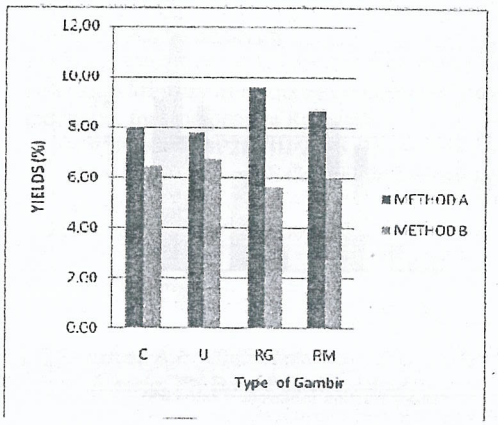

Figure 1. Variation of yield gambier gambier 4 types of West Sumatra

Qualitative phytochemical Identification of Gambier extracts

The results of qualitative analysis showed that gambir contains quinon, terpenoids, alkaloids, tannins, flavonoids and saponin. The biggest amount of alkaloid was found in gambier of Udang type (Table 1).

Table 1. Qualitative phytochemical Identification of Gambier extracts

Group of Chemical compounds	Type of Gambir			
	C	U	RM	RG
Quinon	+	+	+	+
Terpenoid	+	+	+	+
Steroid	-	-	-	-
Alkaloid	+	++	+	+
Tannin	+	+	+	+
Flavonoid	+	+	+	+
Saponin	+	+	+	+

Flavonoids Extraction

There are 3 solvents used in the extraction gambier: chloroform, ethyl acetate and butanol. The results showed that the flavonoid variation of gambier were dependent on the solvent type and by type gambirnya (Fig. 2). The biggest amount of flavonoid was shown by gambier of Riau Gadang type . These variations occurred allegedly because of the genetic variation of gambier [11] and the existence of anatomical variations [10] .

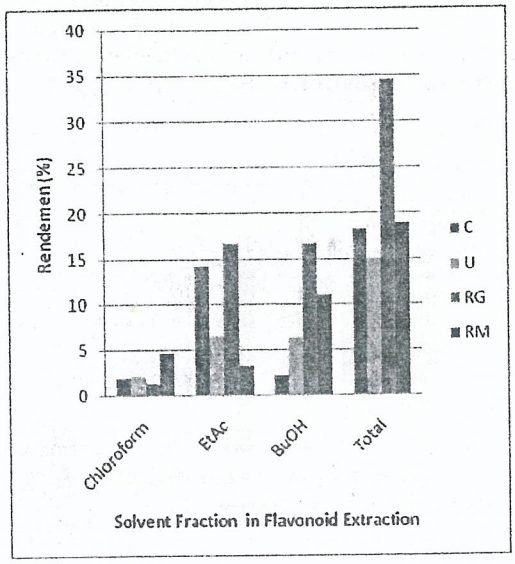

Figure 2. Flavonoid content variability gambier 4 types of West Sumatra by the solvent

Transglikosilasi activity CGT-ase from various sources of culture

Figure 3 showed the activity of transglicocylation of CGT-ase from various source cultures. The activity test on CGT-ase enzyme was done by using starch as a substrate. The results of the test temperature and pH effects on enzyme activity showed that CGT-ase from *Actinomycetes* and *B. lichenifirmis* was optimum at temperature 40 °C (8.87 units / mL) and pH 6.5 (8.89 units / mL). This can be seen on Figure 4 and 5.

The activity of Enzyme CGT-ase was strongly influenced by pH and temperature for optimum activity. This enzyme, however, will lose its activity due to the heat. Based on tests conducted, it was obtained that the activity would decrease with the increasing of temperature and pH, and would stop to increase after the pH is higher than 9 [8, 9, 12].

Synthesis of flavonoids-α-glycosides

Many compounds that have hydroxyl group will be polar, so that its Rf value will be lower. The results of the test transfer activity of flavonoids from gambier Ethyl acetate fraction showed that the Rf value of the product is similar to the transfer of Rf of standard arbutin. TLC results indicated a transfer of products to the organic phase / alcohol (top layer) for all enzymes alpha-amiloglukosidase was 0.005%, while in the water phase of the transfer products are not visible.

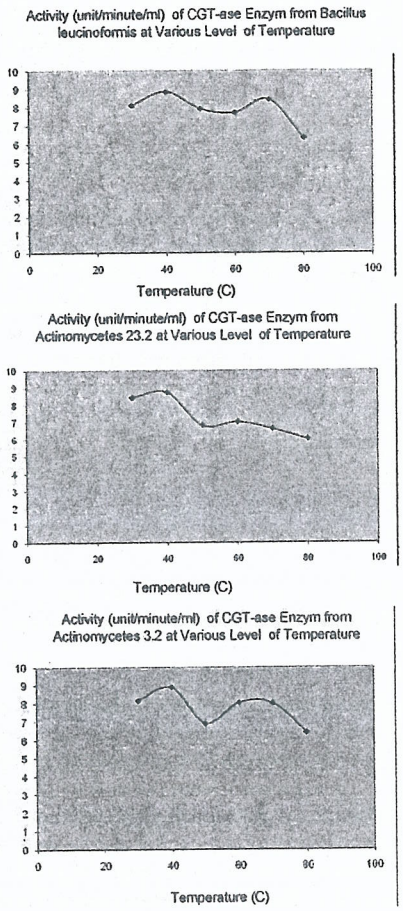

Figure 3. The influence of temperature on the enzyme activity of CGT-ase

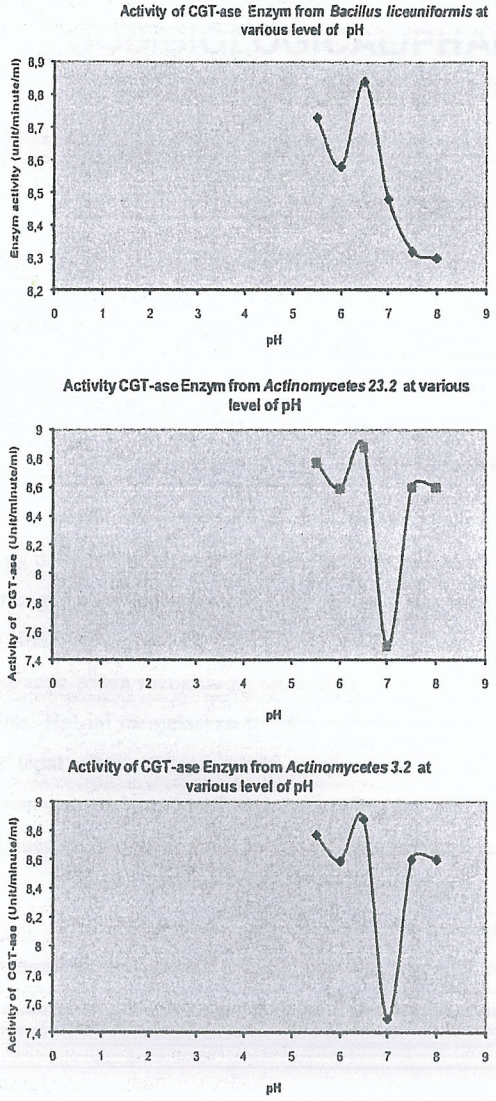

Figure 4. The influence of pH on enzyme activity of CGT-ase

CONCLUSION

The results showed that there is variability in the flavonoid phytochemical properties and among the four types of gambier, where Riau gadang have high flavonoid content, while the highest content of alkaloids found in Gambier Udang type. CGT-ase enzymes from *Aspergillus oryzae* can not be used to synthesize compounds flavonoids-α-glycosides, while the CGT-ase enzymes from *Actinomycetes* (Strain W and 25.3 W 32) and *B. Lichineformis* (phosphate buffer) as a potentially active transferase enzyme.

Sugar content of products is determined by Dubois method ranged from 672.2 ppm - 2104.8 ppm depending on the type of gambier and glucocyl.donor. Flavonoids from all types of gambier (ethyl acetate fraction and butanol) can be used as transfer products, seen from the sugar content. The result showed that glicocyl donor of isomalto looked better than that of the commercial soluble starch.

ACKNOWLEDGMENTS

Thanks to DP2M of the Directorate General of Higher Education Ministry of National Education of Republic Indonesia, for Competitive Research No.005/SP2H/PP/DP2M/III/2008. Thank you very much also submitted to Research Center for Biology LIPI Cibinong all facilities used in this study. Institute for Research Unand, for their help and cooperation .

REFERENCES

[1] Dharma, A.P. 2005. *Tanaman Obat Tradisional Indonesia.* PN Balai Pustaka. Jakarta.

[2] Nazir N., 2000. *Gambir; Budidaya, Pengolahan dan Prospek Diversifikasinya,* Penerbit Hutanku.

[3] Alen Y., Elvi Rahmayuni dan A. Bakhtiar. 2004. *Isolasi Senyawa Bioaktif Antinematoda Bursaphelenchus xylophilus dari Ekstrak Gambir,* Makalah Poster Seminar Nasional TOI XXVI, 7-8 September 2004.

[4] Bakhtiar, A. 1991. *Manfaat Tanaman Gambir.* Makalah Penataran Petani dan Pedagang Pengumpul Gambir di Kec. Pangkalan 50 Kota. 29-30 Nopember 1991. FMIPA Unand. Padang. 23 hal.

[5] Kitao, S. and H. Sekine. 1994, *α-D-glukosyl transfer to phenolic compounds by sucrose phosphorylase from leuconostoc mesenteroides and production of α-arbutin.* Biosci. Biotech. Biochem. 58(1): 38-42

[6] Kometami, T., Y. Terada, T. Nishimura, T. Nakae, H. Takii and Okada. 1996. Acceptor Specificity of Cyclodextrin Glucanotransferase from an Alkalophilic *Bacillus species* and Synthesis of Glycosyl Rhamnose., *Bioscience, Biotechnology, and Biochemistry* 60 (7): 1176-1178.

[7] Funayama, M. T. Nishino, A. Hirota, S. Murao, S. Takenishi and H. Nakao. 1993- *Enzymatic synthesis of (+) catechin-α-glukosida and its effects on tyronase activity.* Biosci Biotech. Biochem. 57(10): 1666-1669.

[8] Sulistyo, J. Y.S. Soeka. Dan R. Handayani. 2000. *Enzymatic Synthesis of Polyphenol Galactoside and its Antimelanogenesis and Antioxidative Activities.* Makalah pada Kongres Biologi XII

[9] Handayani, R., M. Hawab & J. Sulistyo. 2002. *Antioxidation Activity of Polyphenol Glycosides Synthesized by Enzymatic Transglycosylation Reaction of Bacillus macerans. J. BioSMART.* 4 (2) Hal. 18-22.

li Awal menge-
ir Sumatera Ba-
mian Gambir De-
8 Agustus 2002
netik beberapa Jenis
set S-3 Faperta Unpad)

cyo. 2005. Transesterifica-
Application of Lipase Tech-
as. 6 (5) Hal. 164-167.

[13] Harbo. *. Metode Fitokimia: Penuntun Cara Mo. nganalisis Tumbuhan.* Terbitan ke-2. Terjemahan Kosasih, P dan S. Iwang. Penerbit ITB. Bandung.

. [14] Zaini, R. 2006. Isolasi Komponen Bioaktif Flavonoid dari Tanaman Daun Dewa *Gynura pseudochina (Lour) DC.* Tesis Sekolah Pascasarjana IPB. Bogor