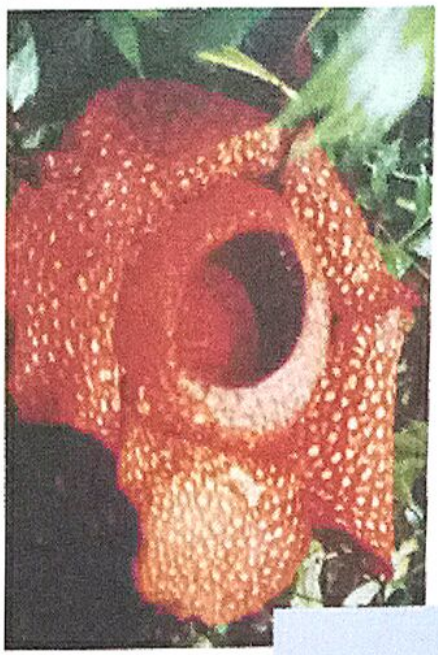
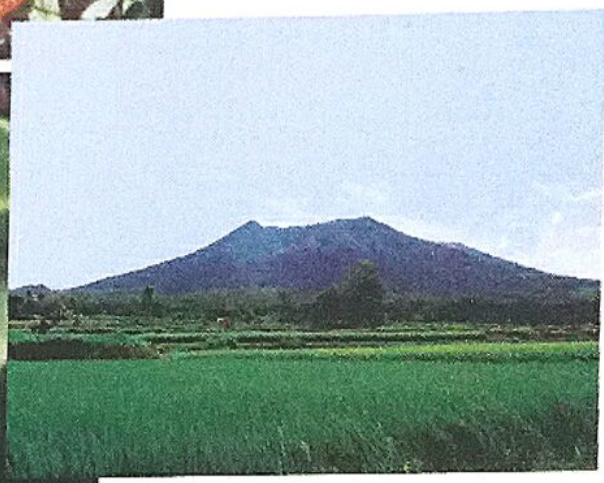
THE TWELFTH ASIAN SYMPOSIUM ON MEDICINAL PLANTS, SPICES AND OTHER NATURAL PRODUCTS (ASOMPS XII)



"Natural Products for Future Healthcare"



13 – 18 November 2006
Bumi Minang Hotel
Padang, West Sumatra
INDONESIA

PROGRAM and ABSTRACTS

Designed by I. Chaniago

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Welcome Message from Chairman of the Organising Committee

First of all, as Chairman of Organizing Committee, it is with great pleasure that I welcome all participants of the 12th Asian Symposium on Medicinal Plants, Spices and other Natural Products (ASOMPS-XII). Here in Padang, the small town you might never heard before, I am particularly happy the ASOMPS-XII has been organized also in line with the Golden Jubile or 50th Anniversary of our University, with the support and collaboration of numbers of governmental and non governmental organizations, research institutions and scientific associations both in Indonesia and overseas.

As you might notice from the scientific program, it is clearly seen that Natural Product Chemistry and Pharmacology like in the previous ASOMPS meting are still dominating, while sessions Botany-Microbiology and Biotechnology-Products Development are less fortunate and not many related participants are coming. To the policymakers this should be noticed. The first group is the mostly basic while the others is application of sciences. I have to mention this because personally it happens to me very often when I talk about these basic sciences only very few would hear. In this respect, as seen from the air before you arrive at the Minangkabau International Airport, you will see a very wide area of this province is till covered by green tropical rain forests. Nobody knows exactly what plants, insects or microbes live in those forests and what marine biota are there along the cost and around the islands off the coast of Sumatra. They all should be researched and developed in the sustainable way for our people in particular and human being in general. It is hoped that after this meeting the new research cooperation can be developed both among the Indonesian scientists and with scientists from overseas.

I also should mention that as the scientists who respects the value of every living creatures in spite of being not fully understand and for what they can be used, we must do all possible efforts to minimize the lost of rainforests where those living creature live, to recognize and protect the cultural heritage, to encourage and recognize biodiversity and research activities as well as to promote national, regional and international cooperation and collaboration related to these rainforest and marine biota. I hope in this Symposium there will be some intensive discussion of the new findings particularly toward "Medicinal, Spices and other Natural Products for Future Healthcare", conservation and supporting the sustainable use of tropical rainforest plants and marine Biota. We hope this meeting will be followed by the better understandings, formal and non-formal cooperation and collaboration that benefit both sides as well as will provide a fair appreciation to the related cultural heritage of tropical rainforest plants marine biota.

I have to mention without neglecting others that the strong support given by the Provincial Government of Sumatra to us for decades especially when we held the similar and related events International Seminars, International Seminar on Tropical Rainforest Plants and Their Utilization for Development (I, Bukittinggi, 1992; II, Padang, 1996; III, Padang; 2001) is highly appreciated. It will too much to mention one by one, we are grateful to the generous donations and support given by our co-sponsors which have enabled us to support the participation of some of our plenary lectures, keynote speakers, contributed lectures and young scientists as well to hold the Welcome Party, Farewell Party and Symposium Dinner.

Last but not least, I am indebted to the members of International Committee for their advice and support especially in the early step of preparation of ASOMPS XII and to all members of Steering and Organizing Committee for without their hard work we might not be here now.

Sincerely,

Prof. Dr. Dayar Arbain, Apt

Welcome message from Rector of The University of Andalas

We would like to express our heartfelt appreciation to the initiative effort and cooperation between the Organizing Committee of the "12th Asian Symposium on Medicinal Plants, Spices and other Natural Products (ASOMPS XII)" for organizing this Symposium in line with the Golden Jubilee commemoration or the 50th Anniversary of the founding of the University of Andalas this year.

We were informed that the number of papers and posters to be presented exceeds 200, covering wide-variety subjects ranging from Botany-Microbiology, Pharmacology, Chemistry and Biotechnology-Product Development. The participants are not limited to scientists from the South-East Asian regions, but other countries from different continents. This event would contribute largely for the promotion of the exchange of scientific information such as to promote awareness among the policy makers of the need for basic research, stimulate studies on medicinal plants, discuss current and new findings, provides opportunity for researchers to update their knowledge and for the establishment of the mutual understanding of culture experiences.

With the new motto "Moment of Change", the interest of the University of Andalas is not only for the well-being of local or Indonesian but reaching further though-out the world. In the century in which information is spreading in a tremendous speed and globalization is a trend, the University of Andalas must prepare for the tough competition that lay ahead. One way to succeed is by initiating and developing collaborative works with many partners from all over the world.

Bearing that in mind I hope that ASOMPS XII is going to be a success, and all participants will benefit from it. Finally, we would like to thank all the organizers of this memorial event for their hard works, and welcome all the participants to the University of Andalas in the City of Padang. We are convinced that this event will contribute to the development of sciences, and promote more understanding and cooperation not only between the researchers of the University of Andalas and other Indonesian counterparts but also with other participants from overseas to create a new culture for human being, which would be beneficial for building a better relationship and bridge among Indonesia and other countries in the world.

Rector,
The University of Andalas

Prof. Dr. Ir. Musliar Kasim, MS

GUBERNUR SUMATERA BARAT

Message from the Governor of West Sumatera

First, I would like to congratulate the University of Andalas for its 50th Anniversary or Golden Jubilee this year. The University of Andalas has played a significant role in the development of this province for decades and I am sure this will continue in the coming years. I am also delighted to welcome you the participants of ASOMPS XII, the respected and prominent scientists who have come from the five Continents to this City of Padang, West Sumatra Province, to continue their long academic and scientific traditions which started at ASOMPS I in Peshawar 1960 in researching medicinal plants and its related disciplines in particular and cooperation in the development of sciences in general.

We have supported similar events in the past when the University of Andalas hosted three other related International Seminars, "*International Seminar on Tropical Rainforest Plants and Their Utilization for Development*" (I, Bukittinggi, 1992; II, Padang, 1996; III, Padang, 2001). Since then, we follow the development of the activities, man power and productivities of the related sciences and technology at the University of Andalas and it seems that it has developed substantially compared to other places in Indonesia. We hope by having this meeting, it will develop faster.

I was informed that in the past with the help of various international organization such as UNESCO, IFS, TWAS, etc. ASOMPS has played an important role in promoting and developing interaction between various scientists and disciplines of Sciences such as Botany, Chemistry, Pharmacology and Biotechnology in Asian countries. It also happened at Andalas University and in Indonesia; I hope this will continue in the future to benefit us all.

West Sumatra is very rich in varieties of ecotype tropical rain forest plants which traditionally have been used for food, spices, medicine, aromatics etc. These plants can be found in a relatively save National Parks and Reserved Forests which cover about 35% of West Sumatran land. We also have unique marine biota in around Mentawai Islands. We do not want to let it just as it is, but it has to be researched, developed and utilized in a sustainable manner for the benefit of us all.

We expect suggestions and proposal for collaborative work among the participants in the country and overseas to research and develop this rich Sumatran biota we have for the benefit of human being "Natural Products for Better Future Healthcare"

Last but not least, we wish all of our visitors and participants coming from outside West Sumatra will enjoy their stay in Padang and increase their understanding of West Sumatra in particular and Indonesia in general.

Padang, 1 November 2006.

Gamawan Fauzi
Governor of West Sumatra

Message from The Mayor of City of Padang

I am deeply honored to welcome you all participants of the 12th Asian Symposium on Medicinal Plants, Spices and other Natural Products (ASOMPS XII) in our City of Padang. I was informed that you come from far away 26 countries spread in the 5 continents.

Beforehand you might not know where Padang is, but I am sure, you have heard the word "Nasi Padang" or "Rendang Padang". Related to the title of this Symposium, the word "medicinal plants" and "spices" are also the common words for us. The use of modern medicine in our society is only for the last few decades, while beforehand our people were very much depended on our traditional plants for their healthcare. I mention this to remind you that our ancestors were expert in using various kinds of our plants, particularly the spices that make the Padang Food well known in Southeast Asia and also can be found in many major cities in the world. As the Mayor of the City of Padang, I am proud that our city has been selected to be the host of this prestigious international event. When you find bad things while you are here please tell us, but if you find good and interesting ones please spread the words to your friends abroad.

I understand that ASOMPS XII 2006 will be a meeting of related top scientists from all over the world. This is an opportunity for Indonesian scientists to discuss their latest research findings and exchange views and ideas. I also hope that Indonesian scientists and academics will benefit from this ASOMPS XII meeting such as to develop new contacts and cooperation for the benefit of both sides.

I also would like to take this opportunity to congratulate the University of Andalas on the celebration of its 50th Anniversary this year. The University of Andalas has come a long way since 1956 and contributed much to the development of our city. Hopefully the scientists and academics from the University of Andalas in particular will take this rare opportunity to learn and to develop our natural resources so that our traditional medicine in the future is not only based on our ancestors experiences but also on scientific proofs. Hopefully this will develop into production of products for national, regional or why not global markets which indirectly also will help our people and our city.

Have a successful Symposium and a nice stay, and please come back some time later.

Padang, 2 November 2006

Drs. H. Fauzi Bahar MSi

Acknowledgments

The organizing committee sincerely thank

- The University of Andalas Padang for hosting the symposium website and other supports provided
- All sponsors who has provided excellent supports for the symposium
- Other parties for any support for the success of the symposium

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THE INFLUENCE OF pH AND TEMPERATURE ON THE STABILITY OF CATECHIN ISOLATED FROM GAMBIR (*Uncaria gambir* (Hunter) Roxb)

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Oral Presentation, Chemistry 2 (Organic Chemistry)

The effects of pH and temperature on the chemical stability of catechin isolated from gambir (*Uncaria gambir* (Hunter) Roxb.) have been investigated. Experiments were conducted in buffer solution at 0.1 M and ionic strength of 0.3 at various pHs of 1.5, 7.4, and 8.0, temperatures of 25, 37, and 55^{0}C. The stability of catechin was observed after 10 days of storing. The percentage of catechin remained was determined by reverse phase High Performance Liquid Chromatography (HPLC) using C-18 column and mobile phase consisted of acetonitrile-aquabidestillata-formic acid (18:81:1% v/v/v). The flow rate was adjusted at 1 mL/min. Catechin was detected by UV-Vis detector at maximum absorption wavelength of 279 nm. Results showed that the mean percentage of catechin remained decreased within buffer solution at pH 1.5, 7,4, and 8.0 at various temperatures. The percentage of catechin remained within buffer solution of pH 1.5 at temperature of 25, 37, and 55 were 82.8, 76.1, and 59.0%, respectively. The percentage of catechin remained within buffer solution of pH 7.4 at temperature of 25, 37, and 55 were 50.3, 35.8, and 32.5%, respectively. The percentage of catechin remained within buffer solution of pH 8 at temperature of 25, 37, and 55 were 50.7, 26.2, and 17.0%, respectively. An increase in buffer solution pH resulted in an increase in catechin decomposition. Catechin was more stable in acidic compared to base solution.

ANTIOXIDANT ACTIVITY OF INDUCED RESISTANCE GINGER to WILT DISEASE WITH ANTAGONIST MICROBIAL

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Poster 77, Botany-Microbiology

Ginger (*Zingiber officinale* Rosc.) is one of important traditional medicine plant. Bacterial wilt disease causes by *Ralstonia solanacearum* is a serious problem on Ginger cultivation. This phytophatogen does not just destruct and reduce of ginger rhizome, but also contaminate the soil. Our previous study obtained that antagonist microbial *Pseudomonas fluorescence* and fungi arbuscular mycorryza were effective suppress wilt disease in ginger. The objective of this research was to examine antioxidant activity of two types ginger rhizome (normal and induced rhizome). The result of this study will be presented in this seminar.

The Influence of pH and Temperature on The Stability of Catechin Isolated from Gambir (*Uncaria gambir* (Hunter) Roxb

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ABSTRACT

The pre formulation data is necessary to develop a pharmaceutical dosage form. Information on the degradation processes and/or the stability of drug substance is one of important data needed. So far, data on the stability of catechin are still limited. Aims of the research were to isolate of catechin from gambier (*Uncaria gambir* (Hunter) Roxb and to evaluate the effect of pH and temperature on the chemical stability of catechin. Catechin was isolated by maceration method using ethyl acetate and continuing with hot distilled water. The chemical stability study was performed in buffer solutions at the concentrationof 0.1 M and ionic strength of 0.3 at variables pH of 1.5, 7.4, and 8.0, and at temperatures of 25, 37, and 55 ^{o}C. The stability of catechin was observed after storage time of 10 days. The percentage of catechin remained was determined by reverse phase High Performance Liquid Chromatography (HPLC) using C-18 column and mobile phase consisted of acetonitrile-distilled water-formic acid (18:81:1 v/v/v). The flow rate was adjusted at 1 mL/min. Catechin was detected by UV-Vis detector at maximum absorption wavelength of 279 nm. Results showed that the mean percentage of catechin remained were decreased within buffer solution at pH of 1.5, 7.4, and 8.0 at various temperatures. The percentage of catechin remained within buffer solution of pH 1.5 at temperatures of 25, 37, and 55 ^{o}C were 82.80, 76.08, and 58.96%, respectively. The percentage of catechin remained within buffer solution of pH 7.4 at temperature of 25, 37, and 55 ^{o}C were 50.26, 26.16, and 17.01%, respectively. The higher of the pH of buffer solution, the higher the decomposition of catechin ($p < 0.01$). Catechin was more stable in acidic compared to base solution.

Keywords: stability, catechin, gambier.

INTRODUCTION

*Uncaria gambir*Roxb. or gambier is one of plant in the Rubiaceae family. It contains an officially recognized pharmacological compound (Heitzman *et.al*, 2005). It is an agricultural commodity that has great potential for trading and has been widely used for a long time, both in traditional use and developing with more modern management.

Gambir is a type of compacted sap obtained from processing the leaves and twigs of the gambier plant (Nazir, 2000). Gambier plants (*Uncaria gambir*) are widely available in Southeast Asia, including Indonesia, in the form of shrubs that have twisted hard stems, short-stemmed leaves and light green color. This plant has been widely used by the community in medicine such as diarrhea, gingivitis and throat inflammation, adstringents, burns medicine, mouthwash, mouth ulcers, and fragrances (Bakhtiar, 1991).

This plant contains various components, one of which is catechins. Catechins are a type of tannin in the form of polyphenols which have supplemental properties and are widely

found in plants. Young leaves contain 10-18% more catechins than old leaves. The physiological functions of catechins include: antioxidants, antibacterials, cholesterol control and absorption of UV light (Miller, 1996).

Catechins can form dimers each other called proanthocyanins. This type of tannin is easily oxidized as indicated by a changing in color. Prolonged storage can cause deposits, known as phlobaphenes or phlobatnins (Mills & Bone, 2000). Catechins are included in the phenolic group that has low stability in alkaline solutions and experience faster decomposition in alkaline pH solutions (Gotti *et.al*, 2004).

The content of catechins from Gambir provides good health benefits so that its use as a medicinal ingredient is growing. However, before catechins are formulated into medicinal dosage forms, their preformulation needs to be reviewed so that they can be used to create suitable formulations. One of the preformulation data that can be used in characterizing a drug compound is stability data, both in solution and in a solid state. This stability can be affected by temperature, hydrolysis, oxidation, photolysis, pH etc. (Connors, 1992; Wells, 1998). Therefore, it is necessary to discover the stability profile of catechins in solution form against the influence of pH and temperature during certain storage.

METHODS

Equipments and Materials

Shimadzu High Performance Liquid Chromatography (HPLC) consisting of: LC-10AT VP pump, UV-Vis detector (SPD-10A VP Shimadzu), control system (SCL-10A VP Shimadzu), 100 µL syringe (SGE Australia), column C-18 (CLC-ODS M), Millipore filter paper, digital scale, pH meter (Griffin), Ballenkamp incubator, HAAKE F3 Sisons water bath, ultrasonic, oven, refrigerator, Amberlite XAD4 resin column, vacuum filter, amberglass vial and glassware commonly used in the laboratories.

Materials used: Gambir, standard catechins, Amberlite XAD4 resin, Sephadex LH-20, potassium dihydrogen phosphate, potassium hydrogen phosphate, phosphoric acid, distilled water were ordered from commercial source, and organic solvent usual used.

Isolation of Catechin from Gambir

One kilogram of gambier is macerated with ethyl acetate solvent and then it is filtered in vacuo to obtain a thick ethyl acetate extract. Hot water was added to the extract and passed into the Amberlite XAD4 resin column. The filtrate obtained was dried then added with methanol and passed to the Sepahdex LH-20 column, then dried to obtain catechin powder. The catechins were compared with standard catechins using High Performance Liquid Chromatography (HPLC), the C-18 column stationary phase and the mobile phase consisted of acetonityl-water- formic acid (18: 81: 1 v / v / v) with a regulated flow rate of 1 mL/minute.

Evaluation of Catechin Raw Material

Catechin examination includes solubility in water, 6% ethanol, and ethyl acetate; organoleptic examination, and determination of the melting distance (Windholz,1983).

Determination of Catechin Solubility

The solubility of catechins was determined using spectrophotometric method. The maximum absorption wavelength of the catechin standard curve in water, ethanol, and ethyl acetate solvents was determined. 50 mg carefully weighed were dissolved in each solvent (water, 96% ethanol, and ethyl acetate) up to 50 mL to obtain a mainsolution concentration of 1 mg/mL. The standard solutions in each solvent were prepared at concentrations of 20, 30, 40, 50, and 60 µ/mL. The absorption of solution was measured using a UV

spectrophotometer at the maximum absorption wavelength of each solvent. The calibration curve is created based on the absorbance of each standard solution.

One gram of catechins was poured into 10 mL of distilled water. The mixture was stirred using a magnetic stirrer for 3 hours until equilibrium reached, then filtered. The filtrate obtained was diluted with water to obtain a solution absorption ranging from 0.2 to 0.8. Catechin levels were calculated using a standard curve. The determination of the solubility of catechins in ethanol 96% and ethyl acetate was carried out the same as for the determination of the solubility of catechins in distilled water.

Catechin Stability Study

Determination of catechin levels at various pH and temperaturesafter 10 days of storage time

181.25 mg of catechins were weighed and a catechin solution with a concentration of 2.5x 10-3 M was prepared in a buffer solution of pH 1.5 using a 250 mL volumetric flask. 20 mL of the solution is pipetted and placed into the Amberglass vial. For t = 0 days, the catechin solution in the vial was injected directly into the HPLC system. Catechin solutions in other vials were stored at 25, 37, and 55 ^{o}C for 10 days then injected into the HPLC system. The same procedure was performed at pH 7.4 and 8. The flow rate was adjusted to 1 mL/minute.

Catechin solutions were treated at various temperatures and pH within 10 days of storage. Catechin levels in the stability test were measured using High Performance Chromatography (HPLC) using a stationary phase column C-18 with a column length of 25 cm and a mobile phase consisting of acetonitrile-distilled water-formic acid (18:81:1 v/v/v) at a flow rate of 1 mL/min and the pH of 2.5 (31). A correlation curve between catechin concentration and peak height was established.

Preparation of Calibration Curve

145.14 mg of catechins was weighed to make catechin main standard solution at a concentration of 10^{-2} M in the mobile phase in a 50-mL volumetric flask. Then dilution was carried out to obtain 7 standard solutions at levels of 0.5, 1, 1.5, 2, 2.5, 3, and 3.5 x 10^{-3} M. Each standard solution was injected into the reverse phase HPLC system using mobile phase acetonitrile-distilled water-formic acid (18:81:1 v/v/v). The absorption of solution was detected at a maximum absorption wavelength of 279 nm. The flow rate was adjusted at a rate of 1 mL/minute. Calibration curve was created.

Determination of Catechin Levels at Various Temperature and pH

181.25 mg of catechins weighed carefully was dissolved in a buffer solution of pH 1.5 in a 250 mL volumetric flask. 20 mL of the solution was pipetted and put into the amberglass vial. For t = 0 days, the catechin solution in the vial was injected directly into the HPLC system. Catechin solutions in other vials were stored at 25, 37, and 55^{o}C for 10 days then injected into the HPLC system. The same procedure was performed at pH of 4 and 8. The flow rate was adjusted to a rate of 1 mL per minute.

Statistical Analysis

The influence of pH and temperature on the catechins remaining in solutions were analyzed statistically using Two-way ANOVA and Pearson Correlation.

RESULTS AND DISCUSSION

Results of the examination of catechin raw materials including solubility, melting range point, organoleptic, and maximum absorption wavelength can be seen in the following Table 1. The solubility of catechins in water obtained was rather difficult to dissolve, in 96% ethanol it was soluble, and in ethyl acetate it was readily soluble.

Table 1. Results of Catechin Raw Material Evaluation

No.	Examination	Requirement (Windholz, 1983)	Observation
1.	Solubility - In distilled water - In ethanol - In ethyl acetate	 Sparingly soluble Soluble Freely soluble	 Sparingly soluble (1:92) Soluble (1:18) Freely soluble (1:4.2)
2.	Melting range	175-177 ^{o}C	169-172 ^{o}C
3.	Organoleptic - Form - Color - Odor	 - - -	 Powder Brownish white Specific
4.	Ultraviolet spectrum in ethyl acetate solution	λ_{max} = 279 nm	λ_{max} = 279 nm

The regression equation of the catechin solution calibration curve in the mobile phase of acetonitrile-distilled water-formic acid (18:81:1 % v/v/v) obtained was Y = (0.27x105) + (1800x105) X and the value of coefficient correlation, r, was 0.994. The precision, standard deviation and coefficient of variation obtained were 1.0065 x 10^{-3} and 0.6%, respectively (n = 6).

Catechins dissolved in buffer solutions at various pH and stored for 10 days at various temperatures were decomposed. It can be observed from the changing in the color of the catechin solution. On day 0, the solution was clear and after being stored for 10 days, the catechin solution becomes brownish and after stored for longer time, the color of the solution will turn dark brown. This is due to the non-enzymatic oxidation of catechins, resulting in color changes in just a few hours (Lopez-Toledano *et.al*, 2002). Catechins are weakly acidic and tend to be stable in acidic solutions (pH <5) (Zhu *et.al*, 2002).

Catechins are flavan-3-ol compounds derived from flavonoids that are easier to decompose in alkaline solutions. The decomposition will be directly proportional to the storage time because the longer the catechins are stored, the more they will decompose. From previous research reports it was known that catechins had an average pKa value of 6.13. Therefore, catechins are more ionized in alkaline solutions so they are easier to decompose (Baweja, 1986).

The instability of catechins in alkaline solutions is catalyzed by hydroxyl ions which dominate at high pH causing the oxidation rate in the solution to be faster. From the catechin structure, it is known that catechins contain many hydroxyl groups and the catechol structure of the B ring is a group that is sensitive to oxidation. This oxidation process causes the catechin structure to be oxidized into quinones so that the solution was brown (Danilewicz, 2003).

The breakdown of catechins also occurs due to epimerization of catechins to form epicatechins which are relatively unstable in alkaline pH solutions but qualitatively, analysis using the HPLC system shows that catechins and epicatechins have the same retention time

so that the change of catechins to epicatechins or vice versa is difficult to distinguish (Zhu *et.al*, 2002: Zhu & Hammerstone, 2003).

Temperature can also increase the rate of the reaction to degrade catechins in the solution. The higher the temperature used, the more catechins will be broken down. In this study, the levels of catechins dissolved in buffer solutions at pH of 1.5, 7.4, and 8 will decompose more rapidly with an increasing of temperature. Increasing the temperature will increase the rate of decomposition of a drug compound. At high temperatures, the number of collisions between reacting molecules per unit time will increase so that the reaction rate increases. The effect of temperature on the rate of decomposition is illustrated by the Arrhenius equation. Temperature also affects the buffer solution. Most alkaline buffers change more easily than acidic buffers due to the influence of temperature (Martin *et.al*, 1983; Wells 1998).

The chromatogram showed the decomposition of catechins, this was indicated by the presence of several new peaks formed after catechin solutions at pH 1.5, 7.4, and 8 stored for 10 days. So far, no research has been found that explains the types of compounds that are formed from the breakdown of catechins. However, when viewed from the catechin structure, it is possible that the compounds formed come from breaking the catechin chain.

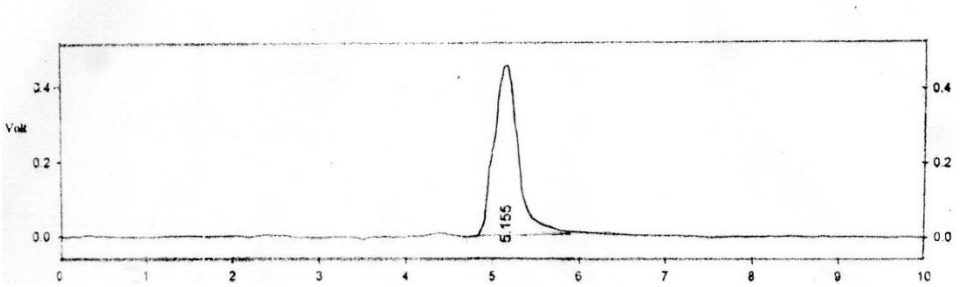


Figure 1a. Standard catechin chromatogram using acetonitrile-water-formic acid mobile phase (18: 81:1 v/v /v), column C-18, at a flow rate of 1 mL/min.

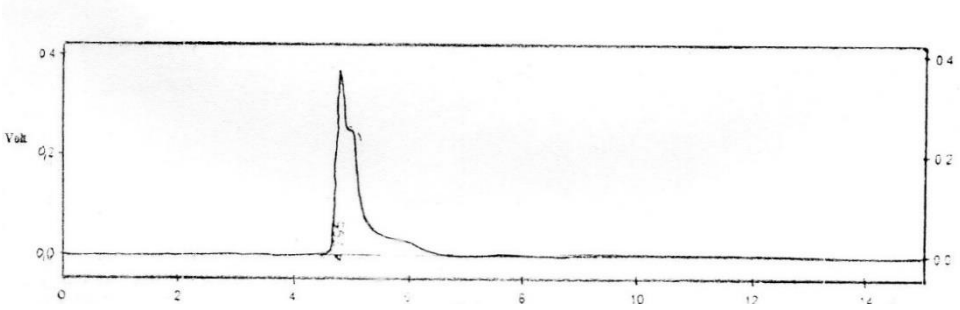
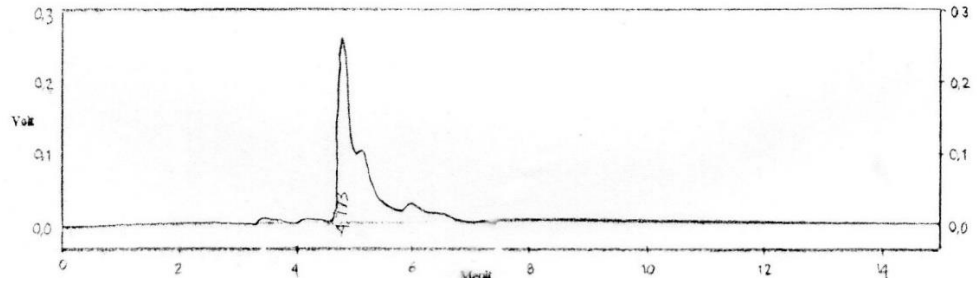
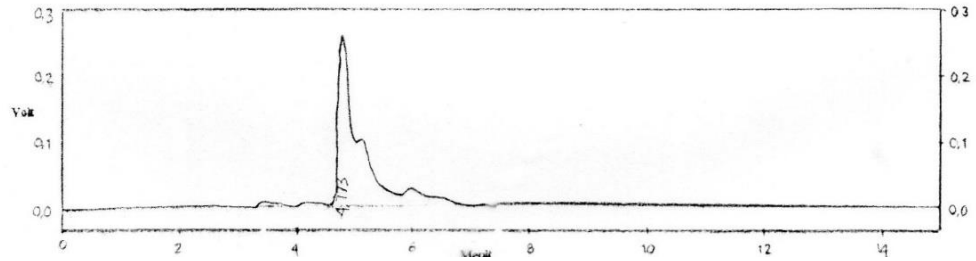


Figure 1b. Chromatogram of catechin after HPLC analysis in buffer solution pH 1.5 at 25 ^{o}C after 10 days of storage time using acetonitrile-distilled water-formic acid mobile phase (18: 81: 1 v/v/v), column C-18, at a flow rate of 1 mL/minute.



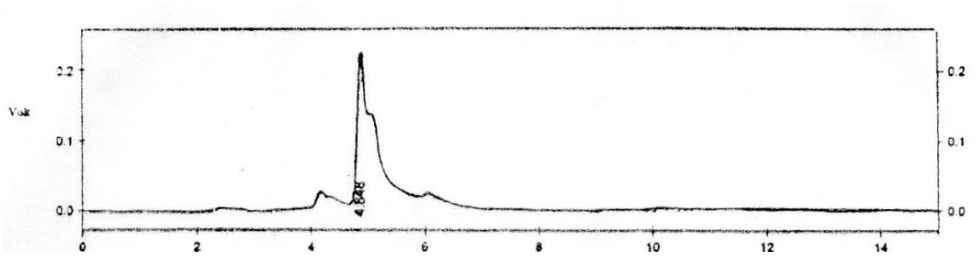
Minutes

Figure 1c. Chromatogram of catechin after HPLC analysis in buffer solution pH 7.4 at 25 ºC after 10 days of storage time using acetonitrile-distilled water-formic acid mobile phase (18: 81: 1 v/v/v), column C-18, at a flow rate of 1 mL minute.



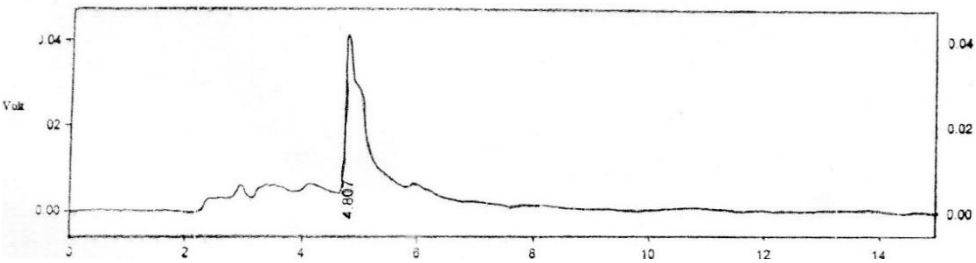
Minutes

Figure 1d. Chromatogram of catechin after HPLC analysis in buffer solution pH 7.4 at 37 ºC after 10 days of storage time using acetonitrile-distilled water-formic acid mobile phase (18: 81:1 v/v/v), column C-18, at a flow rate of 1 mL/minute.



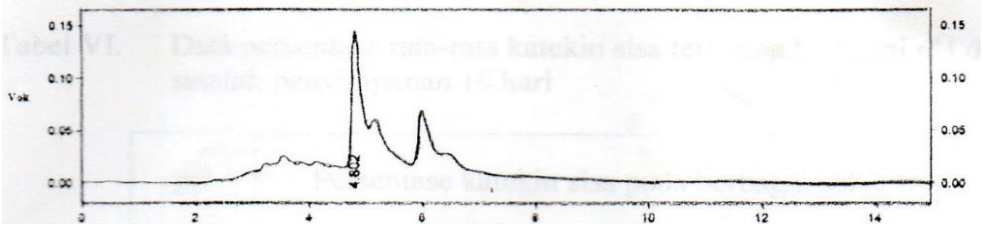
Minutes

Figure 1.e. Chromatogram of catechin after HPLC analysis in buffer solution pH 1.5 at 37 ºC after 10 days of storage time using acetonitrile-water-formic acid mobile phase (18: 81: 1 v / v), column C-18, flow rate 1 mL/minute.



Minutes

Figure 1.f. Chromatogram of catechin after HPLC analysis in buffer solution pH 1.5 at 55 ºC after 10 days of storage using acetonitrile-water-formic acid mobile phase (18: 81:1 v/v/v), column C-18, flow rate at 1 mL/minute.



Minutes

Figure 1g. Chromatogram of catechin after HPLC analysis in buffer solution pH 7.4 at 55 ºC after 10 days of storage using acetonitrile-water-formic acid mobile phase (18: 81:1 v/v/v), column C-18, at a flow rate 1 mL/minute.

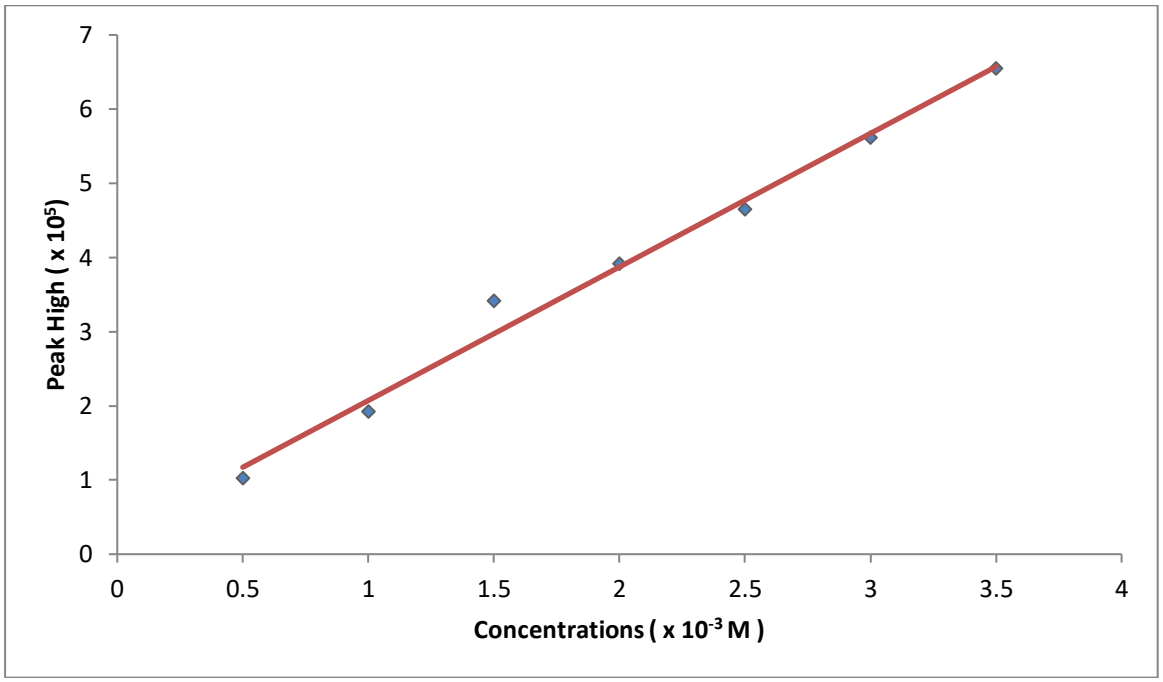


Figure 2. Curve of standard catechins in mobile phase acetonitrile-distilled water-formic acid (18: 81:1 v/v/v), column C-18, at a of flow rate 1 mL/min.

Percentage of catechins remaining in buffer solutions at pH of 1.5 after stored for 10 days at 25, 37, and 55 ºC, were 82.80 ± 0.15, 76.08 ± 2.39, and 58.96 ± 1.82%, respectively. The percentage of catechins remaining in buffer solutions pH 7.4 stored for 10 days at 25, 37, and 55 ºC were 50.26 ± 0.52, 35.78 ± 2.34, and 32.48 ± 0.15%, respectively. The percentage of catechins remaining in buffer solutions of pH 8.0 which were stored for 10 days at 25, 37, and 55 ºC were 50.69 ± 0.46, 26.16 ± 2.11, and 17.01 ± 0.47%, respectively.

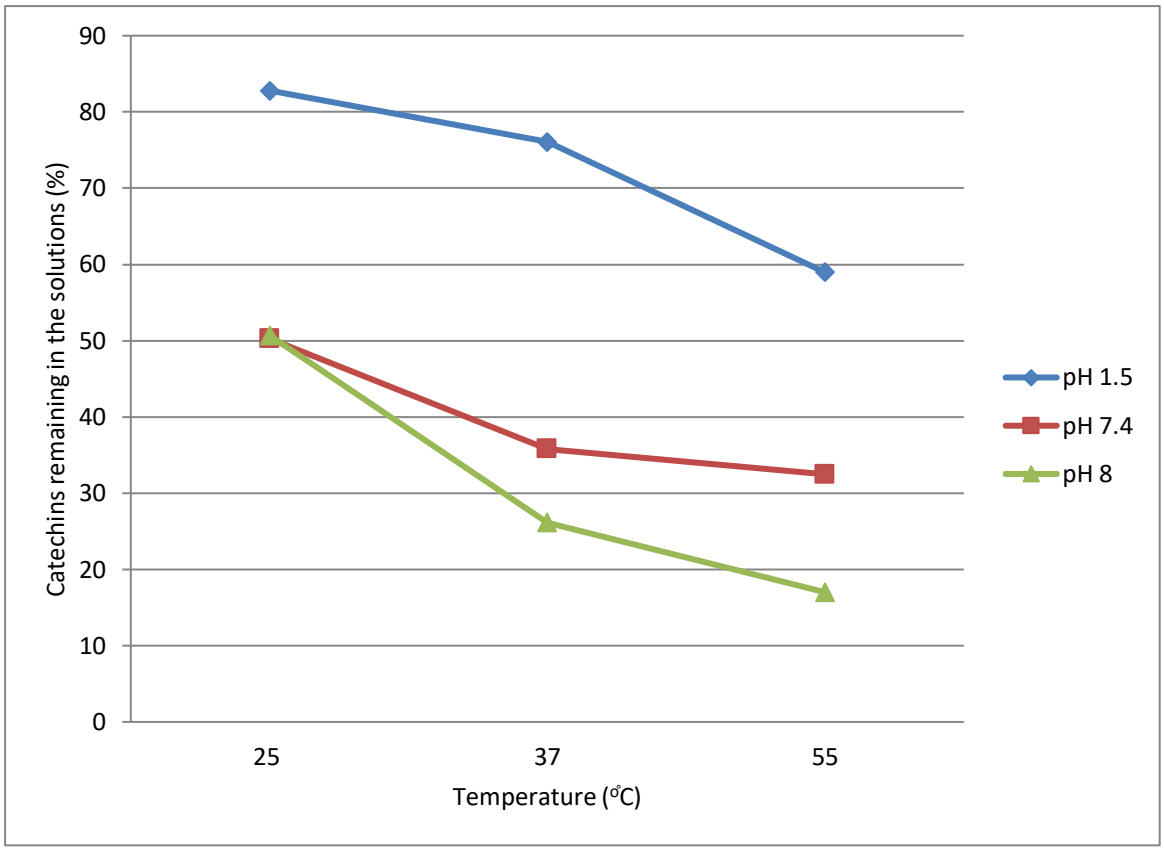


Fig. 1. The profile of catechins remaining in the solutions at various pH after 10 days of storage time at temperatures of 25, 37, and 55

In the analysis of the Two-way ANOVA test data at the 95% confidence level, it was seen that there was a significant effect of the pH and temperature variables on the percentage of residual catechin which were significant with F counts 67.7 and 21.5, respectively. The interaction of pH and temperature variables on the percentage of residual catechins gave an F value of 2.1.

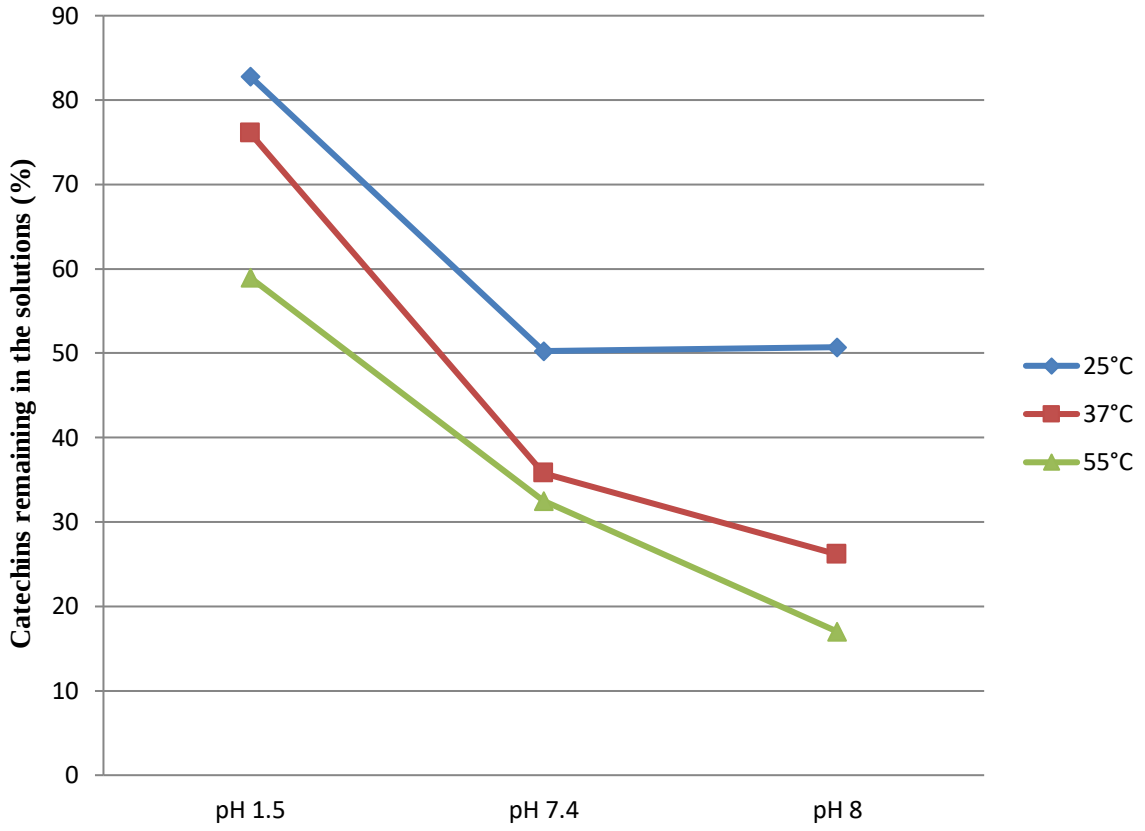


Fig. 1. The profile of catechins remaining in the solutions at various temperatures after 10 days of storage time at pH of 1.5, 7.4, and 8.

After the bivariate correlation analysis using the Pearson Correlation test, it was known that the correlation coefficient value is -0.767 between the pH variable and the variable percentage of residual catechins. While the Pearson correlation coefficient, the relationship between temperature and the percentage of catechins left is -0.415. The probability value less than 0.05 indicates a significant effect of pH and temperature variables on the percentage of residual catechins. The negative sign indicates the opposite relationship. The results of this analysis indicate an inversely proportional relationship between pH and temperature on the stability of catechins. The higher the pH and temperature of the catechin solution, the less catechin stability and residual catechin levels will be.

CONCLUSION

The percentage of catechins remaining in buffer solutions at pH of 1.5 which was stored for 10 days at 25, 37, and 55 ^{o}C were 82.80 ± 0.15, 76.08 ± 2.39, and 58.96 ± 1.82%, respectively. Meanwhile, the percentage of catechins remaining in the buffer solution pH 7.4 stored for 10 days and the same temperature were 50.26 ± 0.52, 35.78 ± 2.34, and 32.48 ± 0.15%, respectively. Whereas at pH 8.0 which was stored at the same time and temperature intervals, the percentage of catechins remaining in the buffer solution were 50.69 ± 0.46, 26.16 ± 2.11, and 17.01 ± 0.47%, respectively.

Catechin solutions in weak acid solutions were more stable than weak alkaline solutions. There was a decrease in catechin levels in pH 1.5, 7.4, and 8 solutions when stored at 25, 37, and 55 ^{o}C for 10 days. Increasing pH and temperature causes a decreasing in catechin levels.

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