COMPARISON OF ANTIBACTERIAL ACTIVITY AGAINST *Escherichia coli* AND TOTAL TANNINS CONTENTS BETWEEN DECOCTA AND STEEPINGS OF AGARWOOD (*Aquilariamalaccensis*Lamk.) LEAVES

[1]Endah RismawatiEka Sakti, [2]Elsa Wulandari, [3]Undang Ahmad Dasuki

Program StudiFarmasi,
FMIPA - Universitas Islam Bandung
Jl. Ranggagading no. 8 Bandung
endah.res@gmail.com

ABSTRACT

Background: Agarwood plant (*Aquilariamalaccensis*Lamk.) so far is only used its trunkfor incense material and air freshener. Other parts of this plant, especially leaves are still underutilized, whereas according to the empirical treatment, it can be used as an antidiarrheal drug.
Objective: This study has been carried out antibacterial activity test against *Escherichia coli* and determination of total tanninscontent from steeping and decocta ofagarwood leaves.
Outcome: antibacterial activity and content tannins
Methods: Antibacterial activity test was carried out using agar diffusion method with chloramphenicol as standard comparator. Determination of the total tannins content was done by using spectrophotometer UV-visible, Folin Ciocalteu reagents and tannic acid as standard comparator at 740 nm.
Result: The result showed antibacterial effects of Agarwood leaves in decocta was achieved at concentration 3%-6%, while in leaves steeping was achieved at concentration 4%-6%. Decocta of agarwood leaveshad 1,42% of tannins contents, while leaves steeping had 0,942%.
Conclussion: Decocta ofagarwood leaves had stronger antibacterial activity and content more total tannins than its steeping.

Key words: Agarwood leaves, *Aquilariamalaccensis*Lamk., antibacterial activity, tannin, agar diffusion, FolinCiocalteu.

INTRODUCTION

Traditional therapy by using medicinal plant has been used since long time ago before chemicalmedicine was founded. The development of life style back to nature increases human desire to utilize natural resourcemainly which is derived from plants.

Indonesian traditional medicinal plants are very numerous in nature, one of them is agarwood.Agarwood (*Aquilariamalaccensis*Lamk.) is a member ofgenus Thymelaeaceae, which is found on the island of Sumatra. So far most people only use stem part of agarwood as incense material and air freshener, while the leaves of aloes underutilized. In fact, empirically leaves part of this plant are used to treat variety of diseases, such as rheumatism, cancer, malaria, antimicrobial, abdominal pain, asthma and diarrhea. Utilization of agarwood leaves as anti-diarrhea in community, usually byboiling or steepingwith hot water.

Previous research about *Gyrinopsversteegii* species ofagarwoodleaves showed that active compounds contained in this plant are phenolic compounds, flavonoid and terpenoid compounds (Mulyaningsih and Parman, 2005 in Mega and Swastini, 2010:

188). Another research conducted by Sumama (2002) in Mega and Swastini, 2010: 188) improved that tannin compound had potency as antidiarrhea. Tannin has adstringen property and ability to bind and precipitate the excess protein in the body (Susilawati and Yasmiwar, 2007: 62). Diarrhea can be caused by several factors, one of them is the presence of bacteria *Escherichia coli*. Therefore in the treatment of this disease, antibacterial medicine is also used for several condition. However, the research which investigated correlation between antibacterial activity against *Escherichia coli* with tannin content in agarwood leaves has never been done.

Based on the explanation above, this study investigated antibacterial effect against *Escherichia coli* which could be influenced by total tannin content from agarwood leaves that was prepared by two different wayof processing in community.

METHODS

Plant Material

Agarwood (Aquilariamalaccensis) leaves as material research were obtained from DaikLinga, Riau Islands (Kepulauan Riau). Determination of plant wasconducted in Herbarium BandungenseSITH, Institute Technology Bandung.

Test Microorganism

Pure culture of *Escherichia coli* was derived from Biology Department, Padjadjaran University, Bandung.

Preparation of Plant

Agarwood leaves that had been collected,were cleaned by water, chopped into pieces, then air dried. Dry materialwere stored in an airtight container and protected from light.

Characterization of Crude Drug

Agarwood leaves(crude drug) were characterized by phytochemical screening and determination of standard parameters. Phytochemical screening was conducted by using color test specific reagents, to identifypresence of alkaloid, polyphenol, tannin, flavonoid, monoterpenes/sesquiterpenes, steroid/ triterpenoid, quinone and saponin compounds. While standard parameters of crude drug that were determined in this study consisted of organoleptic tests, determination of ash, loss on drying, determination of water and extractable matter.

Preparation of Extract

Two hundred grams of agarwood leaves wereextracted to be decocta and steeping, each of preparation method used 2 liters of water as solvent. Water which is used for decocta was heated at 90°C of temperature, thencrude drug soaked and heated for 30 minutes. While water solvent for steeping, was heated until boiling and then poured into contained that contained crude drug for 5-10 minutes. Both liquid extracts were concentrated by freeze dryer.

Characterization of Plant Extract

Agarwood leaves extractswas characterized by phytochemical screening and determination of standard parameters for extract. Phytochemical screening was done in the same methodwith crude drug, to identify presence of alkaloid, polyphenolic compound, tannin, flavonoid, monoterpenes/ sesquiterpenes, steroids/triterpenoids, quinone and saponin. While standard parameters of extract that were determined in this study consisted of organoleptic tests, determination of density and extractable matter.

Antibacterial Activity Test

Each one gram of extract (decocta and steeping) was added by 10 mL DMSO (dimethyl sulfoxide), then put it into a flask as stock solution (10%). Various concentrations (6%, 5%, 4%, 3%) were made by dilutingstock solution with DMSO.

Suspension of *Escherichia coli*10µL was put into petri dish and then added by 20 mL Nutrient Agar (NA), then shake vigorously until become homogenous media and allowed to solidify. Four wells were made on Agar medium by using perforator. Each of well was filled by samples from the smallest until the largest concentration.Chloramphenicol as comparator antibiotics was used to be a positive control, and DMSO to be a negative control. Furthermore,petri dishes were incubated in incubator at 37°C for 24 hours. The antibacterial activity of extract was indicated by the inhibition zone around the well. Potency of antibacterial activity which was possessed by decocta and steeping of agarwood leaves was determined by comparing the diameter of inhibition zone from each sampel concentration.

Determination of Tannin Content

Determination of total tannin content in the extract was done by using UV-visible spectrophotometer. Five hundreds mg of tannic acid was dissolved with100 ml water in a volumetric flask and made into several concentration of dilution. The solution was pipetted 0.1 mL, put in a flask which already contains 7.5 mL distilled water, added by 0.5 mL Folin-Ciocalteu reagent and 1 mL of 35% Na_2CO_3, then water was added until volume reached 10 mL. The mixture was shake by vortex for 5 minutes, then allowed to stand for 30 minutes and then solution's absorbance was measured by UV-visible spectrophotometer at 400-900 nm. The wavelength which gave the greatest absorbance value was set as maximum wavelength of tannic acid (Tamiselvi et al., 2012: 3261).

Five hundreds mg of each sample was diluted with 100 mL with water, then 0.1 mL of it was put into a flask containing 7.5 mL distilled water, added by 0.5 mL Folin-Ciocalteu reagent and 1 mL of 35% Na_2CO_3, and volume was added until 10 mL. The solution was allowed to stand for 30 minutes, then measured the absorption by UV-visible spectrophotometer at maximum wavelength of tannic acid (Tamiselvi et al., 2012: 3261).

Analysis of Data

Experimental datascollected in this study were analyzed by one-way analysis of variance (one way ANOVA) and the difference between the average of each method was determined by Tukey test using SPSS version 17. AP value less than 0.05 (the value of α) was considered to have statistically significant differences and experimental data were also analyzed by using the average ratio test or independent sample t test (Sugiharto, 2009: 1).

RESULT AND DISCUSSION

Characterization of Crude Drug and Extract

The results of phytochemical screening fromcrude drug and extractof agarwoodleaves can be seen in Table 1.

The result of phytochemical screening of agarwoodshowed both crude drug and extract hadsamephytochemical compounds, such as polyphenolic compounds, flavonoids, tannin, quinon. Steroid, monoterpen and sesquiterpen compounds were identified in crude drug, but they were unidentified in extract (both steeping and dekokta).It happened

because the solvent used in the extraction was water. Therefore it had no ability to extract non polar compounds, such as steroid, monoterpen and sesquiterpen

Table 1.Phytochemical Screening of Agarwood Leaves

Classes of Compound	Crude Drug of Agarwood Leaves	Extract of Agarwood Leaves	
		Decocta	Steeping
Alkaloid	(-)	(-)	(-)
Polyfenolic	(+)	(+)	(+)
Flavonoid	(+)	(+)	(+)
Saponin	(-)	(-)	(-)
Tannin	(+)	(+)	(+)
Quinon	(+)	(+)	(+)
Triterpenoid	(-)	(-)	(-)
Steroid	(+)	(-)	(-)
Monoterpen&sesquiterpen	(+)	(-)	(-)

Expl. : (+) identified (-) unidentified

Determination Standard Parameters of Crude Drug and Extract

Standard parameters of crude drug and extract were determined to investigate the quality of crude drug and agarwoodextract used in this study. The results of organoleptic tests of agarwood leaves crude drug which is tested by five respondents showed that it had green color, resembling tea aroma and tastedslightly astringent. Results of standard parameters determination of crude drug andextract of agarwood leaves can be seen in**Tabel 2.**

Tabel 2. Determination of Standard Parameters from Crude Drug and Extract of Agarwood Leaves

Parameter	Crude Drug Agarwood Leaves	Extract of Agarwood Leaves	
		Decocta	Steeping
Extractable matter in water	13.68%	(-)	(-)
Extractable matter in ethanol	21.15%	(-)	(-)
Water content	9.50%	(-)	(-)
Total ash content	6%	(-)	(-)
Insoluble acid Ash Content	2.5%	(-)	(-)
Loss on drying	10%	(-)	(-)
Density	(-)	0.97%	0.94%

The results of extractable matter parameter showed that crude drug of agarwood leaves content polar compounds with less number than nonpolar compounds.It is known by the result of extractable matter in water parameter had less number than extractable matter in ethanol parameter (Depkes RI, 2000: 31).

Determination of water content from agarwood leaves showedthat the water contained in crude drug 9.50%. It was below 10%, so the material still meet the requirements of water content in general. (Depkes RI, 2000: 14). The water content in crude drug should be

limited because water is a source of microbial growth and enzymatic reactions that can damage the quality of crude drug (Arifin et al., 2006: 91).

Determination of ash content aims to measured internal and external mineral content of crude drug from the beginning until the end of crude drug preparation process. (Depkes RI, 2000: 17). Total ash content in agarwood leaveswas 6%, while acid insoluble ash content of agarwood leaves was only 2.5%. It showed that inorganic minerals derived from outside the plant (external) was less in number.

Determination of loss on drying parameter provides maximum limits (range) the amount of compound that is lost in the drying process (Depkes RI, 2000: 13). In this study, loss on drying of agarwoodleaves crude drugwas 10%.

Density parameter of extract was determined from two different extraction methods.The extract obtained by decocta had 0.97 g / mL in density, while the extractobtained by steeping method had 0.94 g / mL in density. The density parameter provides the number of mass per unit volume which indicated the number of compounds which was contained in extract. (Depkes RI, 2000:13).

Agarwood Leaves Extract

The yield of each extract was calculated from the ratio of the extract obtained with the initial crude drug (Depkes RI, 2000: 10). Extract yield fromdekokta(5.19%) was bigger thanstepping (4.44%). It happened because the temperature used during dekokta processwas more constant than steeping. Thiscould make an influence on chemical compounds contained in extract and its pharmacological activity.

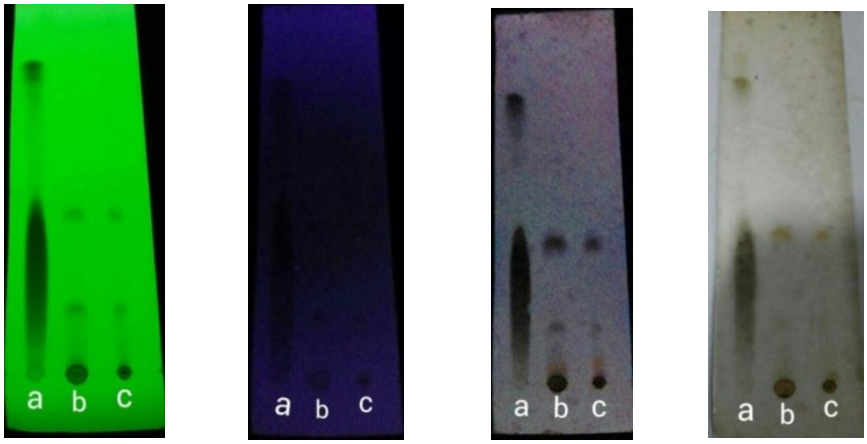
Monitoring Extract by Thin Layer Chromatography

Monitoring TLC profile (**Fig. 1)** of agarwood leaves extract was conducted to analyze qualitatively of tannin content in the extract, by using tannic acid as a comparator. TLC was conducted with mobile phase ethyl acetate: formic acid: and water (9.8: 0.09: 0.09). Value of tannic acid Rf was 0.4375, dekoktahad almost equalRf value (0.41), and so did the steeping (0, 38). This profile showed that tannin was contained in both types of extracts.

Antibacterial Activity Test

Agar diffusion method is antibacterial tests conducted as a preliminary test to detect the presence of antibacterial activity of agar wood leaves extract against bacteria, in this case represented by *Escherichia coli* as negative Gram bacteria that cause diarrhea. Antibacterial activity was investigated by the presence of inhibition zone around the well on solid media. The larger diameter of inhibition zone, the greater antibacterial activity which the extract had. The observation of the antibacterial activity can be seen in **Tabel 3**.

The extract of agarwood leaves decocta and steeping performed antibacterial activity against *Escherichia coli*. This was proved by the presence of inhibition zone of bacterial growtharound the well area.Extract decocta reached its antibacterial activity at 3 mg/mL - 6 mg / mL concentration, whereas extract steeping reached bacterial inhibition at at 3 mg/mL - 6 mg / mL concentration.The inhibition zone results indicated that the extract derived from decocta method had greater inhibition activity against *Escherichia coli* thanthe extract from steeping.



(1) (2) (3) (4)

Figure 1. Thin layer chromatography ofagarwood leaves extract with stationary phase silica gel GF 245 andmobile phase of ethyl acetate: formic acid: and water . (a) tannic acid, (b) agarwood leaves decocta (c) agarwood leaves steeping

Explanation: 1. Chromatogramwas observed withUV 254 nm. 2. Chromatogram was observed with366 nm. 3. Chromatogram was observed with H_2SO_4 0,1%. 4. Chromatogram was observed withF_eCl_3

Tabel 3. Results of Antibacterial Acitivity Test

Concentration	Diameter of Inhibition Zone (mm)		
	Decocta	Steeping	Chloramphenicol
6%	1.63±0.06	1.41±0.07	3.57±0.10
5%	1.22±0.03	1.07±0.06	3.18±0.18
4%	1.13±0.04	0.88±0.10	3.21±0.09
3%	0.96±0.06	0	3.17±0.05

Analysis of Data

The results of the statistical test showed that the inhibition of both extraction methods have significant difference (P-value <a), which means there is a difference antibacterial activity between decocta and steeping. The statistical results in **Table 4** was arranged based on average value of inhibition zone. Decocta extract had greater inhibition activitythan steepingextract . Therefore agarwoodleaves extract prepared by decocta hadstronger antibacterial activity against *Escherichia coli* than steeping.

Tabel4. Result of Statistical Analysisof Antibacterial Activitywith Independent Sample T test

Extract	Means of Diameter Inhibitio Zone (mm)	Deviation Standard	P-Value
Decocta	1.23	0.26	0.035
Steeping	0.84	0.55	

Chloramphenicol as the positive control showed the greatest inhibition of the growth of *Escherichia coli* when compared with extract samples. Chloramphenicol mechanism inhibits protein synthesis which is required for bacterial cells formation. Therefore chloramphenicol can inhibit the function of RNA from bacteria (Widjajanti, 1991: 78).

Agarwood leaves extract can inhibit the growth of *Escherichia coli* due to the presence of chemical compounds that have antibacterial effects, such as phenolic compounds, included tannin. Tannin can form hydrogen bonds with the protein contained within the bacterial cell. The hydrogen bonds which is formed between tannin with proteins will undergo denaturation of proteins possibility that bacterial metabolism becomes impaired. This reaction becomes the reason why tannin can inhibit the growth of *Escherichia coli* (Makkar, 2003: 41).

Tannin also has ability to react with phospholipids contained in the cell membrane, therefore the cell membrane will be damaged. The damage of cell membrane can make obstruction of food ingredients or nutrients to enter bacteria cell. These materials needed by bacteria to produce energy. Because of it, bacteria will be in growth retardation and even death (Volk and Wheller, 1993: 158). Inhibition of *Escherichia coli*growth from tannin extract contained in agarwood leaves extract supposed also caused by this mechanism

Based on the explanation above, it was proved that differentmethod of making extracts could influence the antibacterial activity results. Usingmore constant temperature during decocta processing than steeping, affected the quantity of compounds that had potential or antibacterial activity, mainly tannin compounds.

Determination of Tannin Content

Tannin total content of both agarwood leaves extract (dekokta and steeping) was determined by absorbance measurement (**Tabel 5)** using spectrophotometer UV-visible with tannic acid as comparator at 740 nm

Tabel 5. Absorbance measurement results tannic acid

Concentration of Tannic Acid (ppm)	Absorbance
20	0.358
40	0.488
60	0.565
80	0.671
100	0.75
120	0.825

Calibration curve of tannic acid can be seen in **Fig. 2,** performed linear regression equation y=0.004x+0.286 with r^2 = 0,992

The highest total tannin content of two different methods of extract preparation was contained in decocta extract for 1.42%, while steeping method had less content 0.942%. Statistical analysis results in **Tabel6.**, showed that total tannin content of both extraction methods have significant difference (P-value <a) which means that there are differences in quantity of total tannin content between two extraction method.

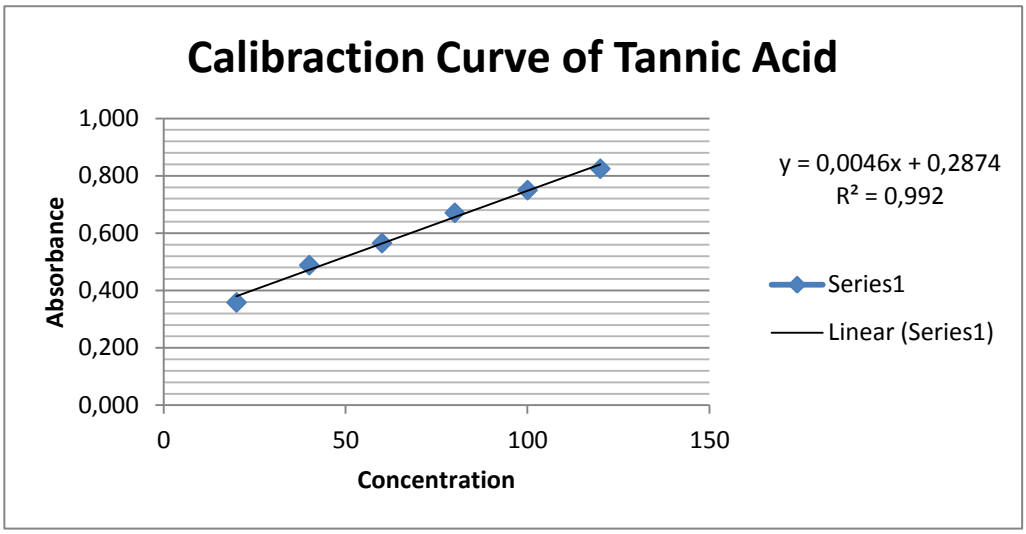


Figure 2. Calibration curve oftannic acid

Tabel 6. Results of Statistical Analysis ofTannin Content with Independent Sample T test

Extraction Method	Means	Deviation Standard	P-value
Dekokta	0.5700	0.0104	0.000
Steeping	0.473	0.0072	

From the data obtained, it was proven that constant heating in extraction method effected on the quantity of tannin content which can be extracted from crude drug. Total tannin content of the decocta extract and steeping have differences, because although both extraction method using heating, but the heating time of decocta was longer than steeping. Besides it, the stability of temperature used during decocta processing was relatively more stable at 90°C, while the temperature using on steeping method decrease progressively.

CONCLUSION

Crude drug, decocta and steeping extract of agarwood(*Aquilariamalaccensis*Lamk.) leaves contained tannin positively.The highest total tannin compoundswas containedin decocta extraxt of 1.42%, whereas in steeping method was 0.942%. Antibacterial activity against *Escherichia coli* which gives stronger inhibition is decocta of agarwood leaves at concentration of 3% to 6%, while inhibition of steeping was performed the concentration of 4% to 6%.Therefore it can be concluded that there was correlation between tannin total content with antibacterial activity. Higher content of tannin in decocta extract, gave stronger inhibition as antibacteria against *Escherichia coli* than steeping extract.

DISCLOSSURE:

We declare that we have no conflict of interest

ACKNOWLEDGEMENT

The author would like to acknowledge to Principal ofUniversitas Islam Bandung, Principal of Mathematics and Natural Science of Universitas Islam Bandung, Principal of Pharmacy Department Universitas Islam Bandung for providing some facilities to conduct this study. We are also thankful to Pharmacy Department ITB and Herbarium Bandungense SITH ITB for support this research

REFERENCES

Arifin, H., Anggraini, N., Handayani, D., Rasyid, R.,(2006). *Standarisasi Ekstrak Etanol Daun* Eugenia cumini*Merr.,*[Abstract], Jurusan Farmasi Fakultas Matematika dan Ilmu Pengetahuan Alam, UniversitasAndalas, Padang.

Departemen Kesehatan Republik Indonesia.(2000). *Parameter Standar Umum Ekstrak Tumbuhan Obat*, Depkes RI, Jakarta.

Makkar H.P.S. (2003). *Effect and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feedingtanninn-rich feeds,* Animal Production and Health Section, Joint FAO/IAEA Division. International Atomic Energy Agency, Vienna, Austria.

Mega, M. dan Swastini, D.A. (2010). Screening Fitokimia dan Aktivitas Antiradikal Bebas Ekstrak Metanol Daun Gaharu (*Gyrinops versteegii), Jurnal KimiaUniverstitasUdayana*. 4(2) :187-188.

Sugiharto, T. (2009). *Bahan kuliah statistika 2,* Fakultas Ekonomi, Universitas Gunadarma, Depok.

Sumama, Y. (2002). *Budidaya Gaharu.*Seri agribisnis, PenerbitSwadaya, Jakarta.

Susilawati dan Yasmiwar.(2007). *Flavonoid Tanin-Polifenol,* Fakultas Farmasi, UniversitasPadjadjaran, Bandung.

Tamiselvi, N., Krishnamoorthy, P., Dhamotharan, R., Arumugam, P., Sagadevan, E.. Analysis of Total Phenols, Total Tannins and Screening of Phytocomponents in *Indigofera aspalathoides* (Shivanar Vembu) Vahl ex DC. Coden (USA).*Journal of Chemical and Pharmaceutical Research.*Vol 4.No.6.

Volk. W. A and Wheeler, M.F (1993). *Mikrobiologi Dasar,* alih bahasa : Markham, PT. GeloraAksaraPratama,Jakarta.

Widjajanti, V.N. (1991). *Obat-obatan.*Penerbit Kanisius, Yogyakarta.