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Qualitative Analysis of *Cinnamomum burmannii* Content using GCMS (Gas Chromatography Mass Spectometry) Method

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Abstract

To identify the bioactive compounds in *Cinnamomum burmannii* and their biological activity. Cinnamon bark originating from Batu Malang, East Java, which was located 700-1300 meters above sea level, was processed into dry extract by maceration method with 96% ethanol solvent. Furthermore, cinnamon ethanol extract was analyzed using the GCMS method to look at the content of the bioactive component for further testing biological activity with the server Way2Drug PASS. GCMS results showed 40 active compounds such as trans-cinnamaldehyde, trans-anethole, cinnamyl acetate, calacorene, cadina-1, 4-diene, delta-cadinene. Furthermore, of the 40 compounds, the biological activity potential was tested for 29 bioactive compounds based on PA (probable to be active) values predicted by the Way2Drug PASS server. *Cinnamomum* was tested against the potential as anti-fungi, anti-bacterial, anti-oxidant, anti-inflammatory, anti-diabetic, anti-neoplastic. Trans-cinnamaldehyde showed PA 0,583 as anti inflammatory, L-limonene PA0,818 as anti neoplastic, Tans-anethole PA 0,614 as anti neoplastic, Cinnamyl acetate PA 0,669 as anti inflammatory, calacorene PA 0,698 as anti inflammatory, Delta-cadinene PA 0,651 as anti neoplastic, , Cathechin PA 0,828 as anti oxidant, alpha.-Cubebene PA 0,888 as anti inflammatory and PA 0,837 as anti neoplastic, melilotin PA 0,929 as anti neoplastic, Caryophyllene PA0,915 as anti neoplastic. *Cinnamomum burmannii* had biological potency based on potential activity (PA) 0,432 (+0,117) as antifungi, PA 0,335 (+0,090) as antibacterial, PA 0,304 (+0,199) as antioxidan, PA 0,561 (+0,190) as anti inflammatory, PA 0,373 (+0,170) as antidiabetic, PA 0,584 (+0,234) as antineoplastic.

Keywords: *Cinnamomum burmannii, antifungal, antibacterial, antioxidant, anti-inflammatory, antineoplastic.*

Introduction

The natural wealth of plants in Indonesia includes 30,000 species of plants from a total of 40,000 species of plants in the world, 940 of which are medicinal plants. This amount is 90% of medicinal plants in Asia[1,2,3,4,5,6,7]. One of the medicinal plants that has been used as a traditional treatment product is cinnamon[8].

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Twelve species of cinnamon out of 54 cinnamon species (*Cinnamomum* spp) in the world are found in Indonesia and the most widely planted are *C. burmanii*, *C. zeylanicum* and *C. cassia* in addition to those that grow wild in forests such as *C. massoi* and *C. culilawan* . The five types of cinnamon can produce essential oils, especially from the skin and leaves. Cinnamon plants contain many phytochemical compounds from the phenylproponoids class in the form of cinnamic acid, which function as antioxidants. Cinnamon bark extract contains trans sinamaldehide as an antioxidant compound that can act as radical scavenger and can prevent free radical and be able to repair oxidative damage.[9,10]

Cinnamon is a native plant of South Asia, Southeast Asia and mainland China, Indonesia included. *Cinnamomum burmannii* is a native plant of Indonesia. This plant is generally cultivated by the people and the main producing areas are West Sumatra, Jambi, North Sumatra. Until now, Indonesia is a major producer and exporter of cassia bark which is exported to 44 countries, with the main aim are the United States and a number of European and Asian countries. This is different from Sri Lanka and China which have been able to utilize essential oils from *C. zaelanicum* and *C. cassia* as export commodities.[11]

The leaves containing essential oils, saponins and flavonoids. Besides that, the bark also contains tannin, the leaves contain alkaloids and polyphenols. The chemical content of cinnamon is essential oils, eugenol, safrole, cynnamaldehyde, tannin, calcium oxalate, resin and tanning agent. The part that is used for medicine is bark.[12]

Cinnamomum burmannii is a medicinal plant that is often found in the territory of Indonesia. Cinnamon has been acted as anti-bacterial in *Baccillus aereus*, *Listeria monocytogenes*, *Staphylococcus aereus*, *Helicobacter pylori*, *Salmonella typimurium*, *Salmonella anatum*, and *Eschericia coli*, in addition to acting as an anti-inflammatory, anti-fungal, anti-oxidant, anti-diabetic insecticides and nematicides.[13]

Materials and Methods

Determination test (certification test) of cinnamon

The certification test conducted in Indonesian Institute of Sciences (LIPI) Plant Conservation Center for the Botanical Gardens of Purwodadi, East Java was identified/deteminated based on herbarium and garden collections and scientific references, with the following results:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Laurales

Family: Lauraceae

Genus: *Cinnamomum*

Species: *Cinnamomum burmannii* Ness ex BI

Making cinnamon ethanol extract

The instrument used in making cinnamon ethanol extract is a set of distillation equipment consisting of a distilled kettle, cooler (condenser) and a condensation container and separator funnel. The tools used in making cinnamon ethanol extract are a flouring machine, 30 and 50 mesh sifter, three neck flask, hot plate, turning cooler, rotary evaporator vacuum, pipette, funnel, beaker glass, and filter paper. Cinnamon bark that was tested came from Batu Malang, East Java. Cinnamon sticks are cleaned and scraped off the outer skin and then dried. The dried skin is then mashed up to 1 kg of flour. The 1 kg powder was then soaked with 1.5 liters of 96% ethanol for 24 hours and then filtered and followed by the second and third soaking using 1 lite of 96% ethanol. The yield is collected as much as 300 grams and distilled using a Buchi rotary evaporator to remove residual ethanol. Furthermore, freeze drying is done to make the yield into a dry extract.

The bark of the cinnamon is cleaned and the outer skin is scraped and then dried. The dry skin is crushed into 1 kg of flour. The powder is immersed in 1.5 liters of 96% ethanol for the first 6 hours while stirring occasionally, then left to stand for 18 hours before filtered, filtering is carried out 3 times with the same amount and type of solvent. After the viscous extract was obtained, it was distilled using a Buchi rotary evaporator to remove ethanol residue, then freeze-dried to make a dry extract yield. The collected yield was 300 grams.

GCMS test method

The tools used in this study were 10 mL volumetric flask, micro pipette, volume pipette, 100 mL beaker, Gas Chromatography (HP 6890 GC model number Agilent 19091S-433), column HP-5MS% 30 m long; 250 μm diameter, 0.25 μm film thickness and 1.0 mL/minute flow rate, with Phenyl Methyl Siloxane stationary phase, MSD detector, helium carrier gas (He). 200 mg of cinnamon viscous extract was dissolved with 2 ml ad ethanol and then filtered using 0.45 μm 13mm millipore wathman (Whatman-R nuclepore tract-etched membranes) until a clear solution was obtained, then the solution was pipetted as much as 1.00 μL then injected into a gas chromatography injector. Before measuring the sample, optimization and validation of the gas chromatography conditions were carried out. The analysis conditions used were the injector temperature 3000C, the detector temperature 2300C, with a split ratio of 20.1; 1 The initial temperature of the 1000 C column is held for two minutes at this temperature, gradually increasing by 100C/minute until the temperature reaches a maximum of 1500C with a hold of 21 minutes, a total rate of 28 minutes; The flow rate of the selected column is 1.0 ml/minute. Helium carrier gas with an average velocity of 37 cm/s with a pressure of 10.46 psi. Fraction identification is done by comparing the fragmentation pattern of the mass spectrum with the fragmentation pattern of reference compounds from the NIST02.L and Wiley 275.L databases.[14]

***In silico* test prediction of the bioactive potential of cinnamon**

Based on predictions from PASS Way2Drug, it is proven that cinnamon has a variety of bioactive potential based on the value of potential activity (PA).[15]

Results and Discussion

Based on the average test value of cinnamon bioactive potential is as below:

1. Antineoplastic 0.584285714
2. Anti-inflammatory 0.570818182
3. Antifungal 0.432448276
4. Antidiabetic 0.373333
5. Antibacterial 0,33537931
6. Antioxidant 0.304

Based on the GCMS test results found that in cinnamon obtained the highest content based on the lowest and highest ppm.

1. Trans-cinnamaldehyde 2450-54000 (SD 1)
2. cinnamaldehyde 6000-30000 (SD 1)
3. EO 3500-40000 (SD 2.5284410)
4. Eugenol 140-16800 (SD 1.4140787)

Table 1. Bioactive potential based on the value of potential activity (PA) on the highest content of cinnamon.

Compounds	Anti-Fungal	Anti Bacteia	Anti Oxidant	Anti Inflamatory	Anti Diabetic	Anti Neoplastic
Trans-Cinnamaldehyde	0.485	0.287	0.175	0.583	-	0.37
Catechin	0.583	0.35	0.828	0.597	0.396	0.681
Alpha.-Cubebene	0.298	0.278	-	0.888	-	0.837
Trans-Anethole	0.444	0.323	0.323	0.525	-	0.614
Melilotin	0.788	0.553	0.482	0.83	0.531	0.929
Caryophyllene	0.582	0.437	0.174	0.745	-	0.915
Cinnamyl Acetate	0.424	0.345	0.283	0.669	0.193	0.501
Alpha-Caryophyllene	0.339	0.431	-	0.877	-	0.827
Valencene	0.379	0.307	0.142	0.653	-	0.68
Calacorene	0.338	0.209	-	0.698	-	-
Delta-Cadinene	0.482	0.385	0.147	0.492	-	0.651
Stdev	0.11751	0.09033	0.199945	0.190325	0.170136	0.234521

Cinnamon has long been used as a spice, food preservative and food flavoring. Based on the experience of traditional communities cinnamon bark can be efficacious as a medicine for lozenges, mouth ulcers, anti-rheumatism, anti-diarrhea and cough medicines, gout medicines, high blood pressure, stomach ulcers, headaches, flatulence, vomiting, difficulty urinating large, asthma, pain relief and diabetes mellitus.[16]

Kayu manis is a plant native to South Asia, Southeast Asia and mainland China. This plant belongs to the family Lauraceae which has economic value and is an annual plant that requires a long time to be taken. The main products of cinnamon are the bark and branches, while the byproducts are twigs and leaves. This commodity is often used as a spice while its processed products such as essential oils and oleoresin are widely used in the pharmaceutical, cosmetic, food, beverage, cigarette and other industries.[17]

Along with the motto “back to nature” people’s interest in using natural materials is increasing.

This is proven by the existence of small and large industries that use plants as medicinal ingredients. One such medicinal plant is cinnamon plant. Cinnamon processed products can be made in the form of powder, essential oils and oleoresin. Oleorisin and essential oils can be used in the food, beverage, pharmaceutical, flavor (tobacco/cigarette), fragrance, coloring and other industries.[18]

Based on the value of Probability to Be Active, the results of *Cinnamomum burmanii in silico* turned out to have biological potential as anti-neoplastic, anti-inflammatory, anti-oxidant, anti-diabetic, anti-fungal, anti-bacteria predicted PA (probable to be active) value using the Way2Drug PASS server. The Pa (Probability To Be Active) value is a value that describes the potential of a compound being tested. Active compounds that have values that vary between 0.3-0.7, which means low to moderate if tested on a laboratory scale. If the Pa value is more than 0.7, it indicates that the compound is predicted to have high potential in computational and laboratory tests. Meanwhile, if the Pa value is more than 0.3 but less than 0.7, the compound has computationally ability in the activity being tested, but in laboratory tests it has not been proven or its potential is small. If it is less than 0.3, the compound is computationally and laboratory tested has little potential.[15,19,20]

Conclusion

Based on GCMS test the active compounds contained in *Cinnamomum burmannii* which are dominant based on the lowest and highest values are Trans-cinnamaldehyde 2450-54000 (+1), cinnamaldehyde 6000-30000 (+1), EO 3500-40000 (+2.5284410), Eugenol 140-16800 (+1.4140787). So, it can be concluded that based on the results of Way2Drug PASS server *in silico* with probability to be active (PA) values between 0.3-0.7. *Cinnamomum burmannii* has the ability as anti-inflammatory, anti-neoplastic, anti-oxidant, anti-diabetic, anti-bacteria and anti-fungal computationally but in laboratory tests it has not been proven or has little potential.

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