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Identification New Derivative Clorogenic Acid From Coffee Pinogu Gorontalo With LCMS Method.

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ABSTRACT

Pinogu coffee (Coffea canephora var. Robusta) is included in the type of Robusta coffee, where Robusta coffee is a type of coffee which contains more chlorogenic acid. Chlorogenic acid is a group of compounds consisting of hydroxycinnamates, such as caffeic acid, ferulic acid, and coumaric acid, associated with quinic acids to form various conjugated structures known as caffeoylquinic acid (CQA), feruloylquinic acid (FQA), and coumaroylquinic all are in several forms of isomers. Chlorogenic acid is a phenylpropanoid compound which has antioxidant activity in reducing uric acid levels. This study aims to isolate and identify the chlorogenic acid contained in the methanol extract of pinogu coffee beans (coffea canephora var. Robusta) using the liquid chromatography-mass spectrometry (LC-MS) method. Isolation is carried out by maceration extraction technique which produces 3 grams of thick extract Methanol thick extract produced phytochemical screening showed positive results containing polyphenol compounds. Methanol thick extract was fractionated using a liquid solid partition method with n-hexane solvent. Where the partitioned methanol extract was concentrated with a rotary evaporator. Thick extract of methanol from the polar fraction partition continued. with the TLC test which aims to obtain the best eluent composition. Then the best composition of eluent is used in the separation by the Preparative Thin Layer Chromatography(PTLC)method using silica gel as a stationary phase and a mixture of eluent ethyl acetate (40:20) n-hexan as the mobile phase, elution process is carried out in the chamber.Type t the single formed on the PTLC plate is then scraped. The results of scrapings are then identified using the LC-MS instrument. The results of the analysis (LC-MS) gave a retention time of 1 minute 34 seconds with injection volume of 0.6 ml / min. Based on the results of the analysis it can be seen that the chlorogenic acid compounds found in pinogu coffee beans (Coffea canephora var. Robusta) have a fragmentation pattern based on the molecular weight of 381 m / z.

Keywords: Pinogu coffee; Robusta coffee; Chlorogenic acid; The liquid chromatography-mass spectrometry.

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INTRODUCTION

Currently coffee is not only consumption, but has become a trend among millennials. One of the best coffee quality indicators is measured by the aroma of coffee. More than 800 volatile Clorogenic acid compounds are one of the most common aromatic compounds contained in coffe, the higher the content of clorogenci acid, the higher the distinctive aroma of a coffee. Clorogenic acd itself has many derivatives, the most common of which are, Caffeoylquinic Acid (CQAs), Dicaffeoylquinic Acid (diCQAs) and feruloyquinic acid (FQAs). Jaiswal et al., (2013) found 70 clorogenic acid derivatives in 7 different types of robusta coffee.

No one has ever identified clogonenic acid derivatives found in Pinogu (Robusta) coffee. The process of isolating the sample using the TLC-preparative method, this makes it possible to obtain isolates that are much easier and faster. then identified using LCMS. Using the Mass Liquid Spectroscopy (Q-Tof Series) detector Chromatography makes it possible to obtain far more accurate data.

MATERIALS AND METHODS

Fraction Extract Procedure

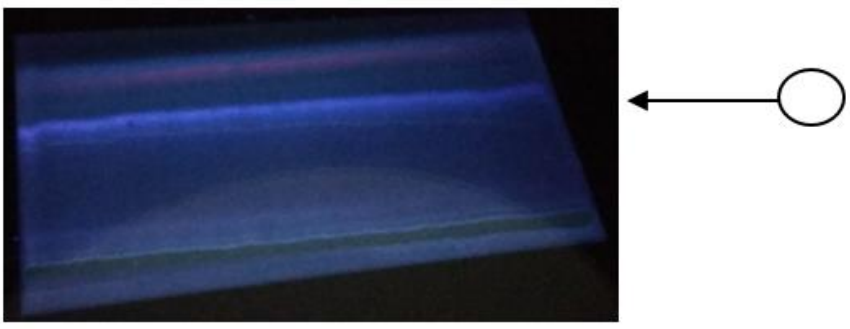
Samples were obtained from the village of Pinogu District, Bone Bolango, Gorontalo. Until the one selected was a young coffee sample. The extraction process uses the maceration method using 80% methanol. The extract was eluted on the TLCP plate using eluent etyl Acetat: n hexan (2: 4). After elution, it is spread under the UV lamp 366, and it appears that the stain of the clorogenci acid will become bluish white in color. After that the stain was separated by centrifuge, then evaporated.

HPLC and mass Spectrometry analysis

LC-MS uses QTOF MS type G2 UPLC waters, Separation using Column C18, with mobile phase, Solvent A: ultra water: 90% acetonitrile, Solvent B: methanol 100% (40:60) Volume Injection 10 ul, 0.6ml Flowrate / min for 4 minutes with the isocratic eluen type. The MS detector uses the ESI method with a mass range of 20 - 1800 m / z.

RESULT AND DISCUSSION

The process of isolating compounds using the Preparative TLC method (TLCP). Methanol extract obtained, previously, was fractionated (centrifuged) with n-hexane, to dispose of artifact compounds and lipid groups. This separation process is based on the solubility affinity of a compound. The residue obtained (the insoluble part n = hexane), then viewed the TLC profile. Mobile Phase used Ethyl acetate (66.6%) - n-hexane (33.3%) Figure 1. From the TLC Profile The results of the TLC chromatogram are fluorescent blue stains under UV lamps suspected of being Clorogenic Acid. The blue fluorosense stain isolation process uses the TLCP with the same mobile phase. After tracing, then the stain is dredged. The process of separating the compound with silica using centrifugation, with the mobile phase solvent used, then evaporated. After evaporating the isolate obtained, prepared for analysis using LCMS.



TLC Preparatif 366 nm. Mobile Phase Etil Acetat (66,6) :n-hexan (33.3)

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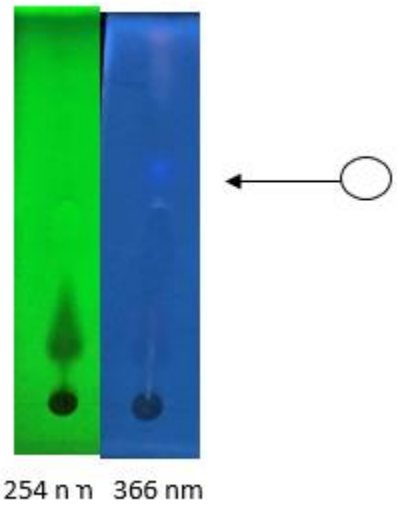

Figure 1: Profil TLC and TLCP From Coffe Pinogu Fraction

The LCMS process, showing the chromatogram obtained only one peak at minute 1.34 (fig 2). This indicates the purity of the isolate, as for noise around the formed peak, indicating an error that might be obtained during the preparation process or from the quality of the reagent used.

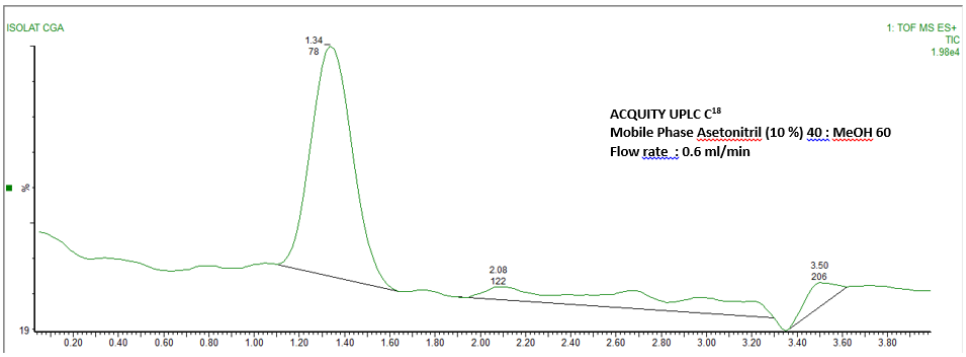

Figure 2: Cromatogram Liquid Cromatography from Isolate Coffe Pinogu

Then the results of Spectrum MS are seen. The isolates obtained indicated Dimethoxy cinnamoylquinic acid (DCA), this was confirmed in the 391 m / z peak (Fig. 3). Dimethoxy cinnamoylquinic acid is formed from two compounds, namely dimethoxy cinnamic acid which binds to Quinic Acid.

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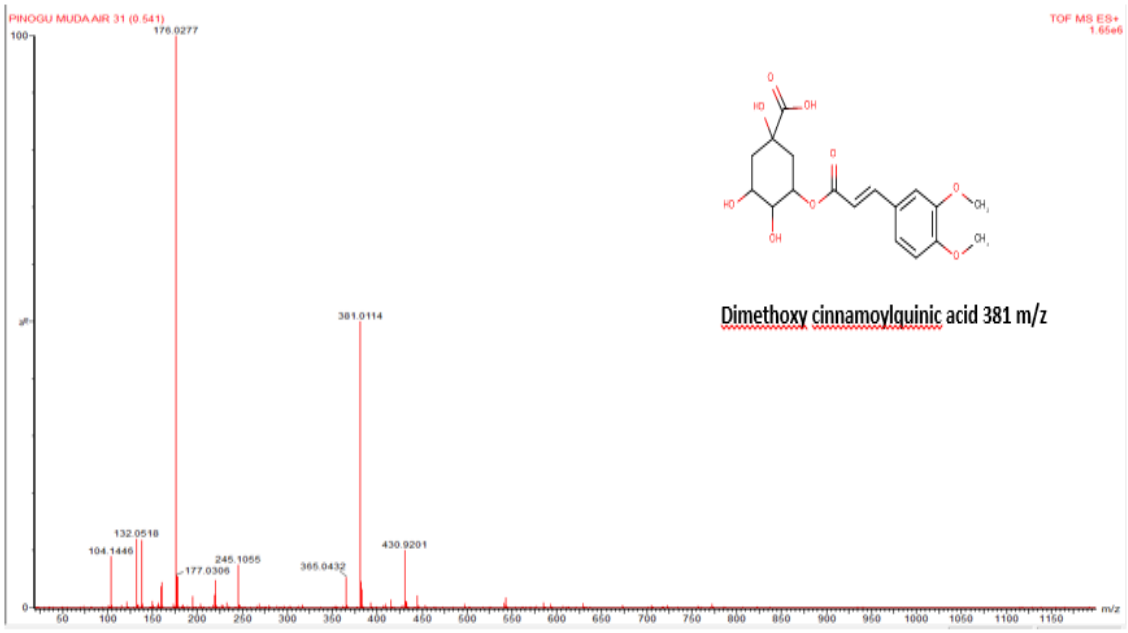

Figure 3: Spekrum MS Isolate Coffe Pinogu

DCA is 1 of 7 clorogenic acid groups (Fig. 4), which characterizes DCA is the presence of two methoxy groups which are bound to the aromatic clorogenic acid. At present there are two types of DCA that have been successfully documented (Fig.5. No 55 & 56).

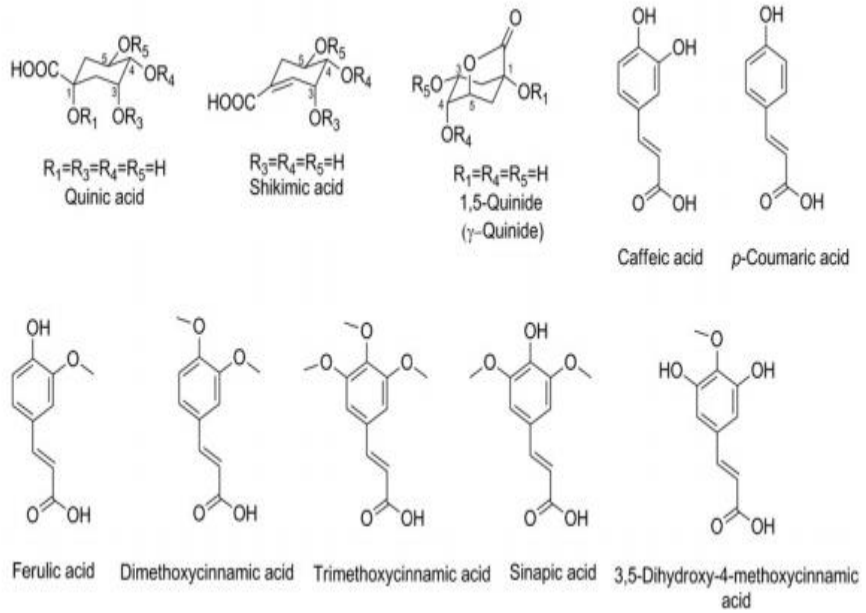

Figure 4: Golongan dan Sistem penamaan Clorogenic Acid

Dimethoxy cinnamoylquinic acid is confirmed through the molecule mass and the fragmentation pattern that is formed. 381 m / z indicates the complete molecular shape of the DCA. The pattern formed by fragmentation formed 366 m / z, 351 m / z and 309 m / z, indicating the loss of two methoxy groups, and the presence of two methoxy groups bound to aromatic rings, and carboxylates in the quinic acid group. 178m / z, 160 m / z and 132 m / z confirm fragments of quinic acid, while 196 m / z 176 m / z and 104 m / z confirm dimethoxy cinnamic acid (Fig. 6).

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No.	Name	Abbreviation	R^1	R^3	R^4	R^5
1	3-*O*-caffeoylshikimic acid	3-CSA	-	C	H	H
2	4-*O*-caffeoylshikimic acid	4-CSA	-	H	C	H
3	5-*O*-caffeoylshikimic acid	5-CSA	-	H	H	C
4	1-*O*-caffeoyl-1,5-quinide lactone	1-CQL	C	H	H	-
5	3-*O*-caffeoyl-1,5-quinide lactone	3-CQL	H	C	H	-
6	4-*O*-caffeoyl-1,5-quinide lactone	4-CQL	H	H	C	-
7	3-*O*-feruloylshikimic acid	3-FSA	-	F	H	H
8	4-*O*-feruloylshikimic acid	4-FSA	-	H	F	H
9	5-*O*-feruloylshikimic acid	5-FSA	-	H	H	F
10	1-*O*-feruloyl-1,5-quinide lactone	1-FQL	F	H	H	-
11	3-*O*-feruloyl-1,5-quinide lactone	3-FQL	H	F	H	-
12	4-*O*-feruloyl-1,5-quinide lactone	4-FQL	H	H	F	-
13	3-*O*-*p*-coumaroyloylshikimic acid	3-*p*-CoSA	-	*p*Co	H	H
14	4-*O*-*p*-coumaroyloylshikimic acid	4-*p*-CoSA	-	H	*p*Co	H
15	5-*O*-*p*-coumaroyloylshikimic acid	5-*p*-CoSA	-	H	H	*p*Co
16	1-*O*-*p*-coumaroyl-1,5-quinide lactone	1-*p*-CoQL	*p*Co	H	H	-
17	3-*O*-*p*-coumaroyl-1,5-quinide lactone	3-*p*-CoQL	H	*p*Co	H	-
18	4-*O*-*p*-coumaroyl-1,5-quinide lactone	4-*p*-CoQL	H	H	*p*Co	-
19	1-*O*-caffeoylquinic acid	1-CQA	C	H	H	H
20	3-*O*-caffeoylquinic acid	3-CQA	H	C	H	H
21	4-*O*-caffeoylquinic acid	4-CQA	H	H	C	H
22	5-*O*-caffeoylquinic acid	5-CQA	H	H	H	C
23	1-*O*-feruloylquinic acid	1-FQA	F	H	H	H
24	3-*O*-feruloylquinic acid	3-FQA	H	F	H	H
25	4-*O*-feruloylquinic acid	4-FQA	H	H	F	H
26	5-*O*-feruloylquinic acid	5-FQA	H	H	H	F
27	1-*O*-*p*-coumaroylquinic acid	1-*p*-CoQA	*p*Co	H	H	H
28	3-*O*-*p*-coumaroylquinic acid	3-*p*-CoQA	H	*p*Co	H	H
29	4-*O*-*p*-coumaroylquinic acid	4-*p*-CoQA	H	H	*p*Co	H
30	5-*O*-*p*-coumaroylquinic acid	5-*p*-CoQA	H	H	H	*p*Co
31	1-*O*-dimethoxycinnamoylquinic acid	1-DQA	D	H	H	H
32	3-*O*-dimethoxycinnamoylquinic acid	3-DQA	H	D	H	H
33	4-*O*-dimethoxycinnamoylquinic acid	4-DQA	H	H	D	H
34	5-*O*-dimethoxycinnamoylquinic acid	5-DQA	H	H	H	D
35	1,3-di-*O*-caffeoylquinic acid	1,3-diCQA	C	C	H	H
36	1,4-di-*O*-caffeoylquinic acid	1,4-diCQA	C	H	C	H
37	1,5-di-*O*-caffeoylquinic acid	1,5-diCQA	C	H	H	C
38	3,4-di-*O*-caffeoylquinic acid	3,4-diCQA	H	C	C	H
39	3,5-di-*O*-caffeoylquinic acid	3,5-diCQA	H	C	H	C
40	4,5-di-*O*-caffeoylquinic acid	4,5-diCQA	H	H	C	C
41	1-*O*-caffeoyl-3-*O*-feruloylquinic acid	1C-3FQA	C	F	H	H
42	3-*O*-caffeoyl-4-*O*-feruloylquinic acid	3C-4FQA	H	C	F	H
43	3-*O*-feruloyl-4-*O*-caffeoylquinic acid	3F-4CQA	H	F	C	H
44	3-*O*-caffeoyl-5-*O*-feruloylquinic acid	3C-5FQA	H	C	H	F
45	4-*O*-feruloyl-5-*O*-caffeoylquinic acid	4F-5CQA	H	H	F	C
46	3,4-di-*O*-feruloylquinic acid	3,4-diFQA	H	F	F	H
47	3,5-di-*O*-feruloylquinic acid	3,5-diFQA	H	F	H	F
48	4,5-di-*O*-feruloylquinic acid	4,5-diFQA	H	H	F	F
49	3-*O*-*p*-coumaroyl-4-*O*-caffeoylquinic acid	3*p*Co-4CQA	H	*p*Co	C	H
50	3-*O*-caffeoyl-4-*O*-*p*-coumaroylquinic acid	3C-4*p*CoQA	H	C	*p*Co	H
51	3-*O*-*p*-coumaroyl-5-*O*-caffeoylquinic acid	3*p*Co-5CQA	H	*p*Co	H	C
52	4-*O*-*p*-coumaroyl-5-*O*-caffeoylquinic acid	4*p*Co-5CQA	H	H	*p*Co	C
53	3-*O*-*p*-coumaroyl-4-*O*-feruloylquinic acid	3*p*Co-4FQA	H	*p*Co	F	H
54	4-*O*-*p*-coumaroyl-5-*O*-feruloylquinic acid	4*p*Co-FCQA	H	H	*p*Co	F
55	3-*O*-dimethoxycinnamoyl-4-*O*-caffeoylquinic acid	3D-4CQA	H	D	C	H
56	3-*O*-dimethoxycinnamoyl-5-*O*-caffeoylquinic acid	3D-5CQA	H	D	H	C
57	4-*O*-caffeoyl-5-*O*-dimethoxycinnamoylquinic acid	4C-5DQA	H	H	C	D
58	4-*O*-dimethoxycinnamoyl-5-*O*-caffeoylquinic acid	4D-5CQA	H	H	D	C
59	4-*O*-dimethoxycinnamoyl-5-*O*-feruloylquinic acid	4D-5FQA	H	H	D	F
60	3-*O*-caffeoyl-4-*O*-sinapoylquinic acid	3C-4SiQA	H	C	Si	H
61	3-*O*-sinapoyl-4-*O*-caffeoylquinic acid	3Si-4CQA	H	Si	C	H
62	3-*O*-feruloyl-4-*O*-sinapoylquinic acid	3F-4SiQA	H	F	Si	H
63	3-*O*-trimethoxycinnamoyl-5-*O*-feruloylquinic acid	3T-5FQA	H	T	H	F
64	1-*O*-caffeoyl-4-*O*-feruloylquinic acid	1C-4FQA	C	H	F	H
65	1-*O*-caffeoyl-4-*O*-dimethoxycinnamoylquinic acid	1C-4DQA	C	H	D	H
66	4-*O*-feruloyl-5-*O*-dimethoxycinnamoylquinic acid	4F-5DQA	H	H	F	D
67	1-*O*-caffeoyl-3-*O*-sinapoylquinic acid	1C-3SiQA	C	Si	H	H
68	1-*O*-feruloyl-4-*O*-sinapoylquinic acid	1F-4SiQA	F	H	Si	H
69	1-*O*-feruloyl-3-*O*-sinapoylquinic acid	1F-3SiQA	F	Si	H	H
70	1-*O*-caffeoyl-3-*O*-trimethoxycinnamoylquinic acid	1C-3TQA	C	T	H	H

Figure 5: Derivat Clorogenic Acid (C =caffeoyl; D =dimethoxycinnamoyl; F =feruloyl; pCo =p-coumaroyl; H = hydrogen; T = trimethoxycinnamoyl)

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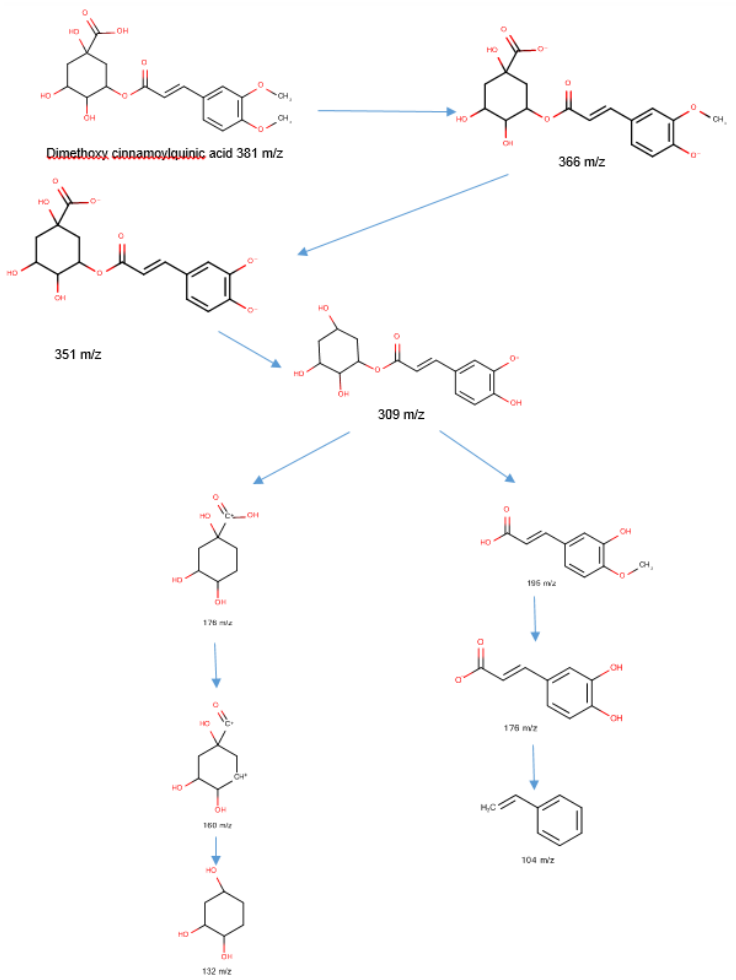

Figure 6: Fragmentasi Isolate Coofe Pinogu

CONCLUSION

Based on this study, it was found that Clorogenic acid contained in Coffe pinogu is a Dimethoxy cinnamoylquinic acid derivative. This DCA is a metabolite product formed from the biosynthetic pathway of quiniq acid and cinnamic acid. Coffe types used are coffe that are still young, and this has implications for the quantity of aromatic compounds. The more mature the coffe, the more aromatic products formed will be.

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