LETTER TO THE EDITOR

Observation of Bitterness Reducing Ability of *Melastomataceae* Leaf Extracts on Caffeine and Coffee Powder

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Abstract: Aim: The research aims to measure the reduction of bitterness of coffee by *Melastomataceae* leaf (DM) extracts measured by caffeine and polyphenol concentrations and changes in covalent bonds observed with Fourier Transformed Infra-Red (FTIR). **Method:** The ethyl acetate, ethanol absolute, and aqueous extracts of *Melastomataceae* leaves were prepared by overnight maceration followed by dehydration at 50±5 °C. Commercial coffee powder (K) and caffeine standard (CF) were mixed with each extract at ratio of 1:2 and 1:9 (w/w), respectively. Caffeine and polyphenol were spectrophotometrically measured. Dried samples were subjected to FTIR analysis to observe changes in the IR profiles. **Results:** Reductions of caffeine in CF and K were optimum with ethyl acetate extract of DM. The reduction of bitterness of coffee was related to covalent bonds at IR detection range of 1000-1800 and 2500-3700 cm^{-1}. **Conclusion:** *Melastomataceae* leaf can be applied to reduce bitterness on coffee related product. The ethyl acetate extract emerged as the most effective bitterness reducing agent.

Keywords: *Melastomataceae* leaf, polyphenol, caffeine, bitterness reduction, FTIR

1 Introduction

Coffee may stimulate the recording process in long-term memory (De Mejia and Ramirez-Mares 2014), and influence energy intake and appetite control (Schubert et al. 2017). However, coffee is known to taste bitter as a result of caffeine content. Recent research postulates that catechin has a role in the perception of bitter taste (Xu et al. 2018). Together with caffeine, catechin can synergistically increase the bitter taste in coffee (Bedini et al. 2012). The concentrations of these two compounds in coffee end products are influenced by the variety, fruit maturity, and the roasting process (Wei et al. 2014).

Bitter taste can reduce the consumption of functional compounds that are beneficial to the body (Gaudette and Pickering 2013). In the mouth, the perception of bitter taste is caused by receptor i.e. TAS2Rs activation (Lipchock et al. 2017). Various efforts to reduce bitter taste were conducted, for example by designing analogous structures that competitively prevent the activation of bitter taste receptors (Ley et al. 2012; Gaudette and Pickering 2012). Bitter taste reduction in coffee products may be performed by seed fermentation (Drewnowski and Gomez-Carneros 2000), as well as caffeine and catechin polymerization which results in new compounds that are not able to activate bitter taste receptors (Tenney et al, 2017).

Leaf of DM, originated from the *Melastomataceae* family, used traditionally in the Paser Dayak community to mask the bitter taste during the cooking of bitter melon, papaya flowers, and papaya leaves. It is hypothesized that the ability of DM to mask the bitterness of vegetable can be extended further to food industrial and pharmaceutical purposes. The leaf can be extracted and applied to reduce the bitterness of

coffee, tea, dark chocolate, and other food ingredients. The ability and active ingredients of DM extracts as bitter flavor masking have been registered as patents in Indonesia (reg. no: SID201903334). The research aims to measure the reduction of bitterness of coffee by *Melastomataceae* leaf extracts measured by caffeine and polyphenol concentrations and changes in covalent bonds observed with Fourier Transformed Infra-Red (FTIR).

2 Material and Methods

2.1 Extraction

Mature DM leaves were separated from the stem and washed with running water. The leaves were then dried in a dryer at a temperature of 50±5 °C for ±18 hours. Dry leaves were mashed with a grinding machine, so that DM powder was obtained as much as 2 kg. DM powder samples were macerated for 24 hours in water (aq), ethyl acetate (EtOAc) (Fulltime, China), and methanol (MeOH) (Fulltime, China) at room temperature (28±2 °C). During the maceration process, the sample was occasionally shaken. The sample was filtered with a glass funnel with Whatmann filter paper to separate the filtrate from the residue. Macerate was concentrated in the oven at a temperature of 50±5 °C for ±18 hours. The process was repeated several times, so that the aqueous, methanol, ethyl acetate extracts of DM were obtained ±30 g per sample.

2.2 Caffeine, Total Phenol and Total Flavonoid

Samples were collected with a composition of 0.5 g of coffee mixed in EtOAc extract from DM (1: 9), 1 g of ground coffee, or 1 g of ground coffee mixed in EtOAc extract from DM (1: 2). Caffeine quantitative test was carried out using UV-Vis Spectrophotometry at a wavelength of 295 nm (Fitri, 2008). The total phenol determined based on the previous method and expressed in mg equivalent to gallic acid per kg dry weight (Mu'nisa et al., 2012 and Nurhayati et al., 2012). The principle of determining the concentration of flavonoids is the reaction between flavonoids and $AlCl_3$ which produces a yellow complex. The addition of NaOH to the complex will create a pink complex that the absorbance can be read at 510 nm (Zou et al., 2004). The detailed methods were elaborated in the supplemental file.

2.3 Caffeine Binding Identification with TLC

Identification of caffeine (CF) binding with TLC was carried out with respect to the previous method (Oellig et al. 2018). Each fraction of the sample was compared to the standard used in several mobile phases. The mobile phase used was toluene (Merck, USA), acetonitrile (Fulltime, China), and chloroform (Fulltime, China) in a ratio of 4:3:3. The absorption plate used was silica gel 60 (254F) (Merck-Millipore, USA). The plate was dipped in a container containing a saturated mobile phase to reach 1/3 maximum height and left to dry. This procedure was repeated three times, until the mobile phase propagation reached the maximum height. Then, the plate was photographed under UV at a wavelength of 254 nm. Calculation of the value of the Retention Factor (Rf) of each compound was determined.

2.4 FTIR Analysis

The sample consists of powder and liquid. If the sample was in the thick liquid form, the sample was prepared by coating into thin film in the NaCl salt plate (Sigma-Aldrich, USA). All samples then analyzed to obtain infrared (IR) spectrogram with Shimadzu FTIR-8400S (Michelson interferometer, single-ray optical system, globular infrared ceramic source with an S/N ratio of 20000: 1, Happ-Genzel Apodization, and 10x readings at resolution of 4.0).

3 Results

3.1 Caffeine, Total Phenol, and Flavonoids Concentrations

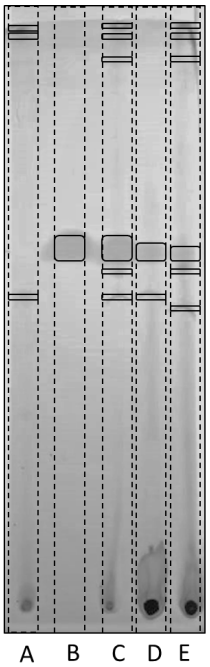
From **Table 1**, the concentration of caffeine in the coffee extract was 10.14±0.01 ppm. Decreased caffeine levels were detected using spectrophotometric methods reaching 54% and 18% in coffee blends

with EtAOc extract or MeOH extract from DM. This showed the binding between caffeine and EtAOc or MeOH extracts from DM. Water extract from DM did not show any binding activity against caffeine in the coffee matrix. However, water extracts from DM showed good binding activity on pure caffeine. From these results it can be concluded that the binding ability of caffeine active compounds depends on the caffeine source to be bound. Simple screening of groups of compounds that might bind caffeine was performed by measuring the total levels of phenolic and flavonoids. From **Table 2**, the binding ability of caffeine in the coffee matrix may be caused by other compounds such as chlorophyll or its derivatives from DM EtAOc or MeOH extracts.

Table 1. The effect of DM extracts on the level of caffeine, phenolic, and flavonoids in coffee sample

Samples	Caffeine (ppm)		Phenolics (ppm)		Flavonoids (ppm)	
Coffee extract + EtAOc extract of DM	4.59±0.07	a	505.30±14.31	a	7.96±0.06	a
Coffee extract + MeOH extract of DM	8.22±0.31	a	364.69±8.86	b	10.60±1.12	a
Coffee extract + Aq extract of DM	10.02±1.54	a	299.97±34.44	c	7.61±5.57	a
Coffee extract	10.14±0.01	a	554.96±6.31	d	45.86±11.16	b
Samples	**Caffeine (%)**		**Phenolics (ppm)**		**Flavonoids (ppm)**	
Caffeine standard + EtAOc extract of DM	52.40±0.09	a	535.71±7.64	a	39.44±1.86	a
Caffeine standard + MeOH extract of DM	81.74±0.09	b	787.08±27.34	b	39.15±6.22	a
Caffeine standard + Aq extract of DM	52.89±0.79	a	548.54±49.44	a	39.93±2.43	a
Caffeine standard	100.00±0.88	c	-		-	

Table 2. Chromatogram and Rf values of EtOAc extract of DM, Caffeine, and a mixture of the DM extract and caffeine



Rf	A	B	C	D	E
	DM EtoAc extract	Caffeine	DM EtoAc extract + Caffeine	Caffeine	DM EtoAc extract + Caffeine
0.991	V		V		V
0.973	V		V		V
0.935			V		V
0.590		V	V	V	V
0.572			V		V
0.529	V		V	V	
0.509					V

Description: A. DM EtOAc extract (DM); B. Caffeine (CF); C. DM+CF; D. Caffeine (CF); E. DM+CF 3.2 CF

Binding Identification with TLC

In addition to phenolic compounds, the chlorophyll component and its derivatives may bind caffeine in the coffee matrix. This phenomenon was shown in the TLC profile in **Table 2**. After caffeine was bound

with DM EtOAc extract, the caffeine spot decreased in intensity and bandwidth (**Table 2**). There was an introduction of new spot at Rf=0.572, or before caffeine identification spots (Rf=0.590). In addition, there was also an introduction of a spot at Rf=0.935. Presumably, it was at these two spots that the interaction between caffeine and DM EtOAc extract occurred.

3.3 FTIR Profile of DM Extracts

Fig. 1(a) shows the FTIR spectrum profile of DM extracts with various solvents. Significant differences were observed in the wavelength range of 2800-3000, 1400-1800, and 1000-1200 cm^{-1}. The difference that occured was expected in the -OH, RRC=O, O=C-NR_2, O=C-NR_2, =C=N- (conjugated, cyclic), =NN=O, CH_3, =CH_2, and CN=O functional groups. **Fig. 1(b)** The FTIR spectrum of DM extracts did not show any similarity in pattern when compared to the FTIR spectrum of the caffeine standard.

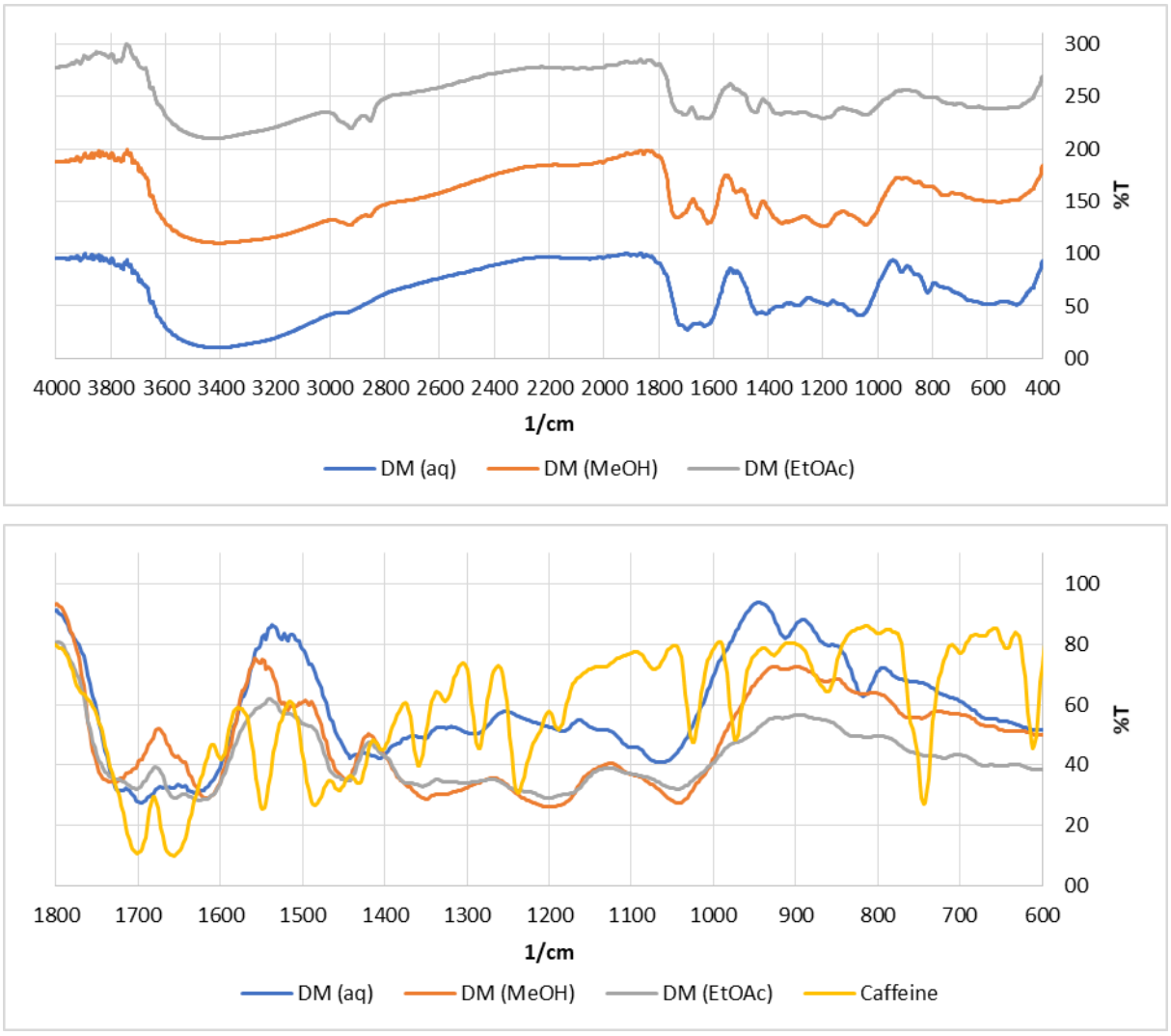


Fig 1. IR spectrum of DM extracts and CF

3.4 FTIR Profile of CF Binding in Pure Form and in Coffee Matrix

Fig. 2(a) shows the FTIR spectrum profile of coffee and coffee mixed with DM EtOAc extract which was then compared to the caffeine standard. A noticeable difference was observed in the wavelength range of 2800-3200, 1000-1800 cm^{-1}. The differences that occur were expected in the functional groups as listed in **Table 3**.

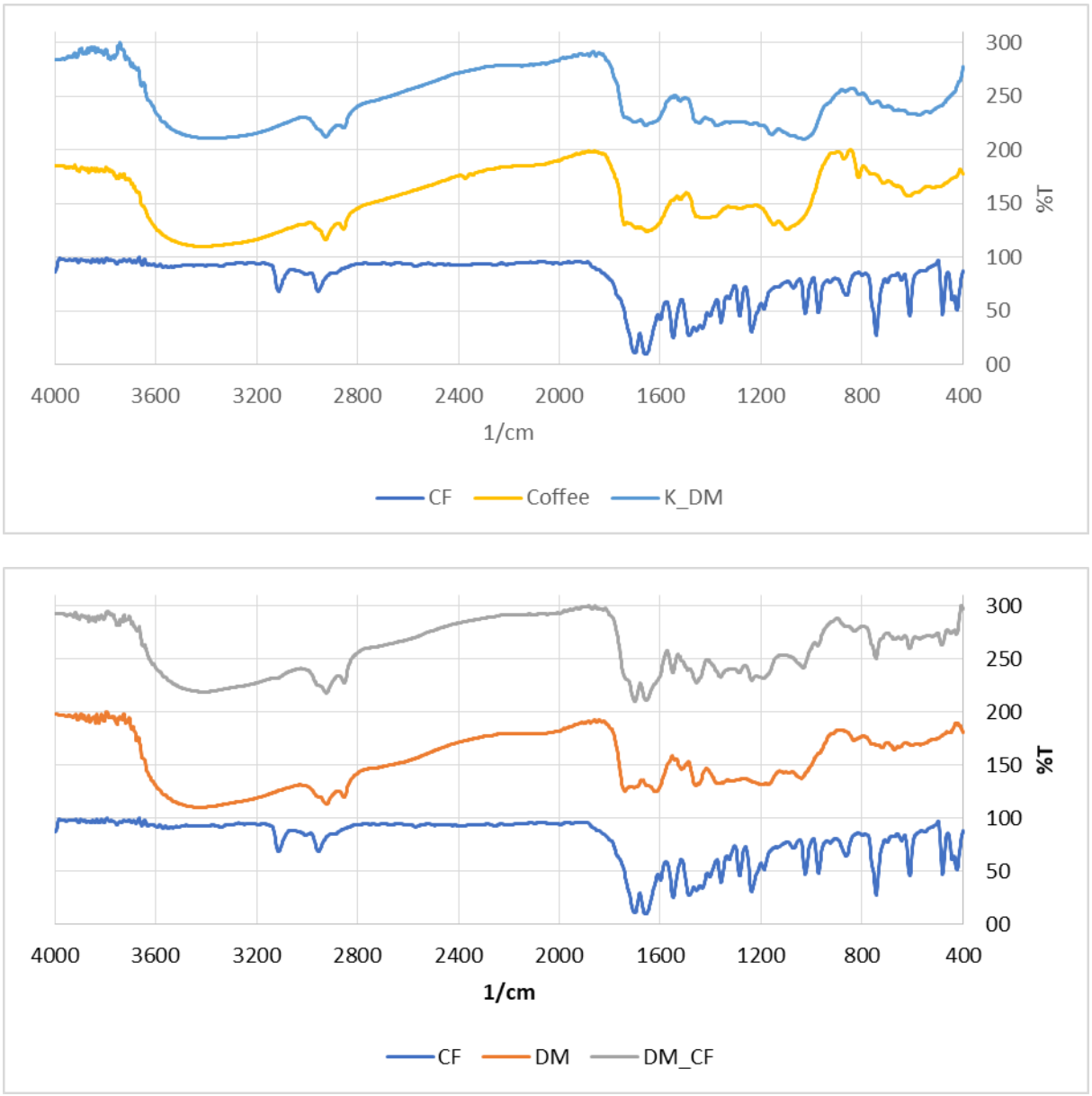


Fig 2. IR spectrum of coffee (K), CF, EtOAc extract of DM mixed with K or CF

The difference in caffeine binding patterns in the coffee matrix compared to pure caffeine was observed from the FTIR profiles. Deducted from the caffeine FTIR spectrum pattern on the coffee matrix, wavelengths of 3111, 2955, 1335, 1294, 1138, and 1070 cm^{-1} were the %T peaks decreased after the binding of coffee with DM EtoAc extract. The -OH, $-CH_3$, $=CH_2$, O=CR-O-CR=O, -COH, O=CR-O-CR=O, and =COC- groups were the interpretations of the related peaks (**Table 3**). For pure caffeine FTIR spectrum patterns, wavelengths of 1485, 1456, 1425, 1412, 1366, 1335, 1294, 1225, 1173, and 1138 cm^{-1} were the peaks of %T that was observed to be different between before and after being bound with DM EtoAc extract. The $-CH_3$, $=CH_2$, C-N=O, RCOO-, -OH, and O=CR-O-CR=O groups were the interpretation of the observed peaks (**Table 3**).

Table 3. Characteristics of covalent bonds in IR Spectrum as a result of DM binding

Wave Length (1/cm)	Possible bond	DM	DM_CF	K	DM_K
3111	-OH	-42.49	-5.99	45.08	45.95
2955	-CH3,=CH2	-48.34	-4.48	42.08	49.41
1699	C=C-C=N-, =C=N- (conjugated, cyclic)	18.14	18.78	-16.86	-15.48
1657	RRC=O	20.38	19.81	-14.99	-12.47
1599	O=C-NR2, =C=N- (conjugated, cyclic)	-11.33	-4.08	9.73	10.74
1547	O=C-NR2, =C=N- (conjugated, cyclic)	29.76	16.70	-27.80	-24.32
1485	=C=N- (conjugated, cyclic)	25.08	11.58	-32.70	-18.88
1456	=N-N=O, CH3, =CH2	-0.39	3.82	-6.39	5.24
1425	C-N=O	6.56	-0.38	1.09	8.13
1412	RCOO-, C-N=O	-1.88	-3.18	11.19	18.92
1366	RCOO-, -OH	-15.95	-0.58	8.84	25.69
1335	RCOO-, -OH	-28.54	-3.92	17.45	38.12
1294	=N-N=O, -OH	-24.73	-1.96	14.86	35.13
1225	C-N=O	-9.11	0.27	-5.75	17.78
1173	O=CR-O-CR=O	-33.72	-3.24	27.06	47.98
1138	-COH, O=CR-O-CR=O, =C-O-C-	-29.89	-9.08	40.44	53.83
1070	-COH, O=CR-O-CR=O, =C-O-C-	-28.76	-7.15	42.43	58.53
939	-CH=CH2	-4.77	-7.07	-12.10	36.27
889	-CH=CH2, ethylene oxide	4.56	-3.51	-20.19	23.80
783	3 neighboring aromatic C-H	-8.01	-3.00	1.22	34.98
731	ethylene	8.89	0.94	-15.24	15.53
683	ethylene	-15.40	-3.18	13.17	43.94
615	ethylene	17.90	8.68	-5.09	18.22

Source: IR Database based on Socrates (2004) and Hesse et al (2005)

4 Discussion

4.1 Caffeine, Total Phenolics, and Total Flavonoids Concentrations

The bitter taste in coffee can be caused by caffeine or quinine (alkaloids) by stimulating bitter taste receptor-independent pathways (Poole et al. 2017). Decreased caffeine levels in a mixture of coffee or pure caffeine with DM EtOAC extract indicate a decrease in bitter taste in the final product. However, the bitter taste is not always caused by alkaloids, other groups of phytochemical compounds may play a more important role (Sun-Waterhouse and Wadhawa 2013),

Polyphenolds and flavonoids are thought to play an active role in the perception of bitter taste, including quercetin and catechin (Drewnowski and Gomez-Carneros 2000). Other studies suggested that the role of catechin may dominate or exceed the role of caffeine in activating the bitter taste receptors of TAS2Rs (Yamazaki et al. 2014). To reduce the bitter taste in cocoa beans which also contain high catechins and alkaloids, the polymerization of catechins was carried out by fermentation techniques (Dand 2011). In other products, namely tea, a decrease in catechin occurred as a result of an enzymatic oxidation reaction (Lee et al. 2019). In this study, the total flavonoids as catechins observed in coffee blends and DM EtOAC extracts decreased when compared to total flavonoids in coffee without DM blends. Hence, the bitter taste

that is reduced in the coffee mixture and DM EtOAC extract may be as a result of a decrease in caffeine levels as well as catechin.

4.2 CF Binding Identification with TLC

Identification of candidate groups of compounds that may bind caffeine as well as catechin are chlorophyll or its derivatives. Based on comparative chromatogram data (Buhian et al. 2016), chlorophyll and its derivatives were major components in DM extracts. These components were thought to have the ability to alter the Rf of pure flavonoids and caffeine as observed on the chromatogram (**Table 2**). Furthermore, spectrophotometically, the interaction between chlorophyll-caffeine caused a decrease in absorbance at the observed wavelength (Makarska-Bialokoz 2012).

4.3 FTIR Profile of CF Binding in Pure Form and in Coffee Matrix

Based on **Table 3**, from the FTIR profile, the decrease in bitter taste in caffeine and coffee by DM EtOAC extract may be caused by the binding of the -OH, $-CH_3$, and $=CH_2$ groups. The dominant functional group that binds to the receptor is methyl (CH_3). Some caffeine-like compounds, containing methyl groups and conjugated and cyclic double bonds C=C, are capable of causing positive attenuation of bitter tastes, for example in coffee-like flavor (Yoshida 2019). The hydroxyl group (-OH) also has a role in the binding of flavonoids and alkaloids to bitter receptors as shown in several studies (Hejaz 2012, Ma et al. 2014). This binding may cause polymerization of catechin and caffeine which resulted in Rf changes in TLC analysis (Osowoski et al. 2010), so it may not be read as the same active compound in spectrophotometric based measurements (Makarska-Bialokoz 2012).

5 Summary

Melastomataceae leaf can be applied to reduce bitterness on coffee related product. The ethyl acetate extract emerged as the most effective bitterness reducing agent. The bitter taste that is reduced in the coffee mixture and DM EtOAC extract is as a result of a decrease in caffeine levels as well as flavonoids (catechin equivalents). The decrease in bitter taste in caffeine and coffee by DM EtOAC extract is caused by the binding of the -OH, $-CH_3$, and = CH_2 groups. This binding may cause polymerization of catechin and caffeine, therefore altering Rf in TLC analysis. Therefore, it cannot be read as the same active compound in spectrophotometric based measurements. Based on a comparative chromatogram, the binding ability of caffeine and catechin to the coffee matrix may be sourced from other compounds such as chlorophyll or its derivatives from DM extracts.

Acknowledgement

The authors would like to thank the University of Mulawarman IsDB Grant Project for funding this research in 2019.

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Supplemental File

Caffeine Determination

About 0.5 g of caffeine mixed with EtOAc extract from DM (1: 9), 1 g of coffee powder, or 1 g of coffee powder mixed with EtOAc extract of DM (1: 2) are added to the beaker then 150 mL of hot distilled water is added into it while stirring. The coffee solution was filtered through a funnel with filter paper into Erlenmeyer, then 1.5 g of calcium carbonate (CaCO3) and the caffeine solution were put into a separating funnel and extracted 4 times, each with the addition of 25 mL chloroform. The lower layer is taken, then the extract (the chloroform phase) is evaporated with an evaporator (temperature 50 ± 5 ° C) until the chloroform completely evaporates. Solvent-free caffeine extract was put into a 100 mL measuring flask, diluted with distilled water to the mark line and homogenized, then determined by UV-Vis spectrophotometry at a wavelength of 295 nm. The absorbance values are plotted into the caffeine standard curves made at concentrations of 3, 6, 9, 12, and 15 ppm.

Total Phenol Determination

Samples were collected with a composition of 0.5 g of mixed coffee in EtOAc extract from DM (1: 9), 1 g of ground coffee, or 1 g of ground coffee mixed in EtOAc extract from DM (1: 2). The samples were then carefully weighed at 0.3 g, then dissolved into 10 mL in absolute ethanol (SmartLab, Indonesia): aquades (1: 1). Extract solution was taken as much as 0.2 mL. For benefits, 15.8 mL is added, followed by adding 1 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, USA) 50% (v / v) in ethanol. The mixture was left for ± 8 minutes, then 3 mL 5% Na_2CO_3 (w / v) (Sigma-Aldrich, USA) was added. The solution was left for ± 2 hours in dark conditions at room temperature (28 ± 2 ° C), then the absorbance started at 725 nm (Eppendorf BioSpectrometer®, Germany). Quantification of total phenols was carried out using standard Gallic acid curves (Sigma-Aldrich, USA) which had been prepared in the same manner.

Total Flavonoid Determination

Samples were collected with a composition of 0.5 g of mixed coffee in EtOAc extract from DM (1: 9), 1 g of ground coffee, or 1 g of ground coffee mixed in EtOAc extract from DM (1: 2). The sample was weighed at 0.1 mg and dissolved to 10 mL in absolute ethanol (Full Time, China). A total of 0.7 mL of distilled air was added to the dissolved extract. Then, 0.1 mL of 5% $NaNO_2$ (Sigma-Aldrich, USA) was added to the mixture. After ±5 minutes, 0.1 ml of AlCl3 10% was added (Sigma-Aldrich, USA). After ±6 minutes, 0.5 mL 1 M NaOH (Sigma-Aldrich, USA) was added, then the mixture was incubated for ± 10 minutes. Absorbance at a wavelength of 510 nm (Eppendorf BioSpectrometer®, Germany) with ethanol as a blank. The measurement results are then plotted against the catechin standard curve (Sigma-Aldrich, USA) which has been prepared in the same way. Total flavonoids are determined in mg equivalent of catechins per kg dry weight.