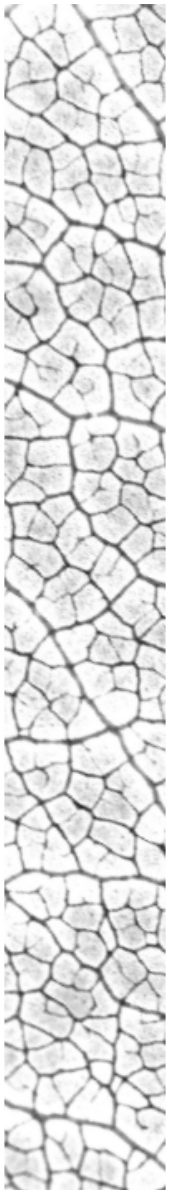

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Melastomataceae leaf extract for masking the bitter flavor of dark chocolate

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Abstract
Bitter is one of the flavors that has low threshold. Eliminating bitter taste is one of the efforts in order to make active compounds in food can be more easily accepted by consumers. This study aims at examining the ability in reducing bitterness of leaves derived from the *Melastomataceae* (DM) against dark chocolate (DC) and its marker compounds, namely theobromine (TB), polyphenols and flavonoids. DM extract was prepared from maceration processby using water (aq), methanol (MeOH), and ethyl acetate (EtOAc). The concentration of polyphenols, flavonoids and percent inhibition of TB was measured by using ultra violet (UV-Vis) spectroscopy. Preliminary confirmation of changes in compound composition was observed by thin layer chromatography (TLC). Changes in the absorbance of covalent bonds were observed by Fourier transformed infra-red (FTIR). Total phenol, flavonoids, and theobromine in DC products mixed with DM extract had decreased, especially in DC combinations with EtOAc or water extract from DM. The decrease in TB concentration ranged from 62.3 to 72.8% compared to pure TB control or between 38.6 and 75.2% in DC products. From the TLC, binding of compounds was obtained at Rf between 0.337 and 0.686. These compounds were detected in Rf between 0.780 to 0.814. From the FTIR interpretation, mixing DM with DC and TB consistently decreased the bonds that were thought to consist of C=CC=O, ON=O, ONOO, RCOO, -OH, $OCOCH_3$, COO-, and R_3NO. In conclusion, DM extract had shown to reduce the bitterness of DC by binding it to TB and changing the composition of compounds in DC, and reducing certain covalent bonds.

Keywords: melastoma leaf, bitter taste reduction, theobromine, polyphenol, TLC, FTIR

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INTRODUCTION

The existence of bitter taste stimulating compounds reduces the effectiveness of functional food consumption. A low threshold causes the bitterness to dominate the overall taste of a functional food, even though the concentrations of the bitter taste compounds exist only small in number (Barborová et al. 2013). DM is a leaf originated from the *Melastomataceae* family, used traditionally to mask the bitter taste for processed vegetable foods such as bitter melon, papaya flowers, and papaya leaves in the Paser Dayak community. Besides being useful for reducing bitterness in vegetables, DM extract is also expected to be able to reduce the bitterness in chocolate, coffee 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: WFP2019089020).

The main source of bitter taste is commonly from alkaloids, terpenoids, and several groups of polyphenols (Gaudette et al. 2013). Efforts to reduce bitter taste have been investigated, for example oleic acid (Homma et al. 2012), sugar or salt (Bakke et al. 2018), cellulose (Arthanarieswaran et al. 2015), amino acids (Gaudette et al. 2013), reverse micelles technology (Ri Huang et al. 2018), or by changing the texture of materials (Gaudette et al. 2013). Furthermore, the bitter taste in processed products can be reduced by using raw materials from other cultivars that has lower bitter compounds (Feng et al. 2013).

Dark Chocolate (DC) is a popular food that usually contains 140 - 600 g/kg of cocoa liquor (Torres-Moreno et al, 2011). The dominant taste of this product is bitter and slightly acidic (Adriaenssens and Callebaut 2010). DC is considered a healthy food because of its antioxidants, the phenolic and alkaloid components, have quenching effects on hydroxyl radicals (Vertuani et al. 2014). The main active ingredient found in DC is theobromine (TB) from the alkaloid group. Other alkaloids contained in DC in fewer concentrations are

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caffeine (Palazzo and Bolini 2013). Alkaloids are often associated as compounds that cause bitter taste (Lenfant et al. 2013). In addition to TB and caffeine, DC also contains high levels of polyphenols and flavonoids (Alañon et al. 2016) which marked by (-)-epicatechin (Hu et al. 2016). The consumer preference of DC based products is highly influenced by bitter taste, acids and the milk content (Harwood et al. 2012).

Qualitative testing of the effectiveness of a compound in inhibiting bitter taste can be done by sensory test methods using both panelists (Owusu et al. 2011) and novel electronic tongues (Newman et al. 2014). Observation of the possibility of binding bitter compounds by coating agents can be approximated by thin layer chromatography (TLC) (Menguy et al. 2009). Quantification of the decrease in bitter causative compounds, in this case DC alkaloids, can be accomplished with TB markers by using the ultra violet-visible (UV-Vis) spectrophotometry method (Xia et al. 2013) which is then verified by high performance liquid chromatography (HPLC) method (Bordiga et al. 2015). Observations on changes in covalent bonds that occur after the addition of bitter flavoring materials can be carried out by using the Fourier transformed infra-red (FTIR) spectrophotometric approach (Alvarez et al. 2012).

This study aims at examining the ability in reduce bitter taste of DC by the addition of aqueous (aq), methanol (MeOH), and ethyl acetate (EtOAc) extracts of DM. The analysis is approached by measuring DC marker compounds namely polyphenols, flavonoids, and TB by UV-Vis spectrophotometer, TLC and FTIR methods.

METHODS

Extraction

Mature DM leaves were separated from the stem and washed in 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, in order to create as much as 2 kg DM powder. 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.

Total Phenol

Measurement of total phenol was carried out based on previous methods (Mu'nisa et al. 2012, Nurhayati et al. 2012). DM powder extract was carefully weighed at 0.3 g, then dissolved to 10 mL in absolute ethanol (SmartLab, Indonesia): aquades (1: 1). The extract solution was taken as much as 0.2 mL. To the solution, 15.8 mL aquadest was added, followed by the addition of 1 mL Folin-Ciocalteu reagent (Sigma-Aldrich, USA) 50% (v/v) in ethanol. The mixture was left to stand for **±**8 minutes, then 3 mL of Na_2CO_3 5% (w/v) (Sigma-Aldrich, USA) was added. The solution was left to stand for **±**2 hours in dark conditions at room temperature (28±2 °C), then the absorbance was measured at 725 nm (Eppendorf BioSpectrometer®, Germany). Quantification of total phenol was carried out using the Gallic acid (Sigma-Aldrich, USA) standard curve which had been prepared in the same way. Total phenol was expressed in mg equivalent Gallic acid per kg dry weight.

Total Flavonoid

The principle in determining the level of flavonoids is the reaction between flavonoids and $AlCl_3$ which produces a yellow complex. Adding NaOH to the complex will form pink complexes in which absorbance can be measured at a wavelength of 510 nm (Zou et al. 2004). About 1 mg of extract was weighed and dissolved to 10 mL in absolute ethanol (Fulltime, China). As much as 0.7 mL of distilled water were added to the dissolved extracts. Then, 0.1 mL of 5% $NaNO_2$ (Sigma-Aldrich, USA) was added to the mixture. After ±5 minutes, 0.1 mL of $AlCl_3$ 10% (Sigma-Aldrich, USA) was added. After ±6 minutes, 0.5 mL of 1 M NaOH (Sigma-Aldrich, USA) was added, then the mixture was incubated for ±10 minutes. Absorbance was measured at 510 nm wavelength (Eppendorf BioSpectrometer®, Germany) with ethanol as a blank. The measurement results were then plotted against the standard curve of catechin (Sigma-Aldrich, USA) which had been prepared in the same way. Total flavonoids were expressed as mg equivalent catechin per kg dry weight.

Total Theobromine

The total measurement of theobromine by UV spectrophotometry was done by modifying the previous method (Li et al. 1990). **Lead solution.** The lead II solution was made by mixing 15 g of Pb (II) acetate (Merck, USA) with 150 ml of water in a 250-ml flask and then the solution was heated while stirring until the color of the solution turned white. **Preparation of controls and mixtures.** The control used was 0.1 g TB (Sigma-Aldrich, USA) or 1 g of DC powder. The mixture used was TB or DC control in which DM extract was with a ratio of 1: 5. The mixture was then transferred into a 150 ml glass and 96 ml of distilled water was added. The solution was heated (70 ± 10 °C) for 5 minutes while stirring it. 4 ml of lead II solution was added and stirred. Distilled water was added until the volume of the solution became 100 mL. To the mixture, 1 g of $NaHCO_3$ was added (Sigma-Aldrich, USA). Then the precipitate was filtered with filter paper for several times until the solution became transparent. 50 mL of filtrate was removed and

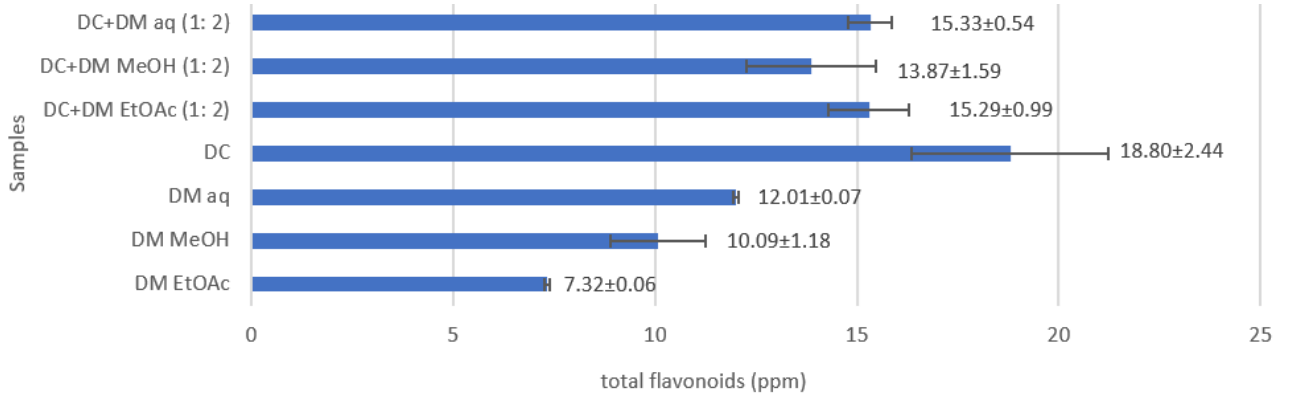


Fig. 1. Total phenol in DC, DM extract, and DC mixture with DM extract
Description: DC = dark chocolate, DM = *Melastomataceae* leaves, DM aq = DM water extract, DM MeOH = methanol extract DM, DM EtOAc = DM ethyl acetate extract

about 5.5 mL of 1 M NaOH (Sigma-Aldrich, USA) was added to produce pH 10.5. Then the filtrate was extracted with chloroform (Fulltime, China) in five repetitions, each of which was added 25-, 20-, 15-, 15- and 10-ml of chloroform. In each repetition, the mixture was shaken for 1 minute and stand for about 5 minutes. Chloroform extract was combined into volumetric flask, and added with chloroform so that it reached a volume of 100 ml. **Measurement.** 10 mL of extract in chloroform added 40 mL of aquadest and 0.55 mL of 10% HCl. Determination of theobromine spectrophotometry was carried out by moving 1 mL of chloroform extract into the cuvette. Absorbance was observed at a wavelength of 302 nm. The results obtained were then plotted in the standard curve of TB (Sigma-Aldrich, USA).

TB Binding Identification with TLC

Identification of TB 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 detected by UV at a wavelength of 254 nm. Calculation of the value of the Retention Factor (Rf) of each compound was determined.

FTIR Analysis

The sample consists of powder and liquid. If the sample was in the thick liquid form, the sample was prepared by coating it with 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).

RESULTS

Total Phenol

The total phenol contained in the DC was 698.36±6.08 ppm. The total phenol in water extracts, MeOH, and EtOAc extracts of DM were 299.97±34.44, 364.69±8.86, and 505.30±14.31 ppm, respectively. After the DC was mixed with DM extract, the total phenol which was measured by the spectrophotometric method decreased, especially in the combination of DC with EtOAc extract from DM (1: 2) and DC combination with water extract from DM (1: 2) (**Fig. 1**).

Total Flavonoids

The concentration of flavonoids from DC was 18.80±2.44 ppm. The concentration of flavonoids in water, MeOH, and EtOAc extracts of DM were obtained at 12.01±0.07, 10.09±1.18, and 7.32±0.06 ppm, respectively. Mixing DC with various extracts of DM also decreased the concentration of flavonoids (**Fig. 2**).

Theobromine

Preliminary confirmation of the binding ability of several DM extracts to TB as a compound that causes bitter taste can be seen in **Fig. 3A**. The ability of binding to TB by several DM extracts also occurred in DC products as shown in **Fig. 3B**. At the same quantity (0.1 g of TB), a decrease in the quantification by spectrophotometry of TB ranged between 62.3 and 72.8% compared to pure TB control. Similar results were obtained for DC products. At the same quantity (1 g of DC), the decrease in TB concentration detected by the UV spectrophotometry method at a wavelength of 302 nm ranged between 38.6 and 75.2%.

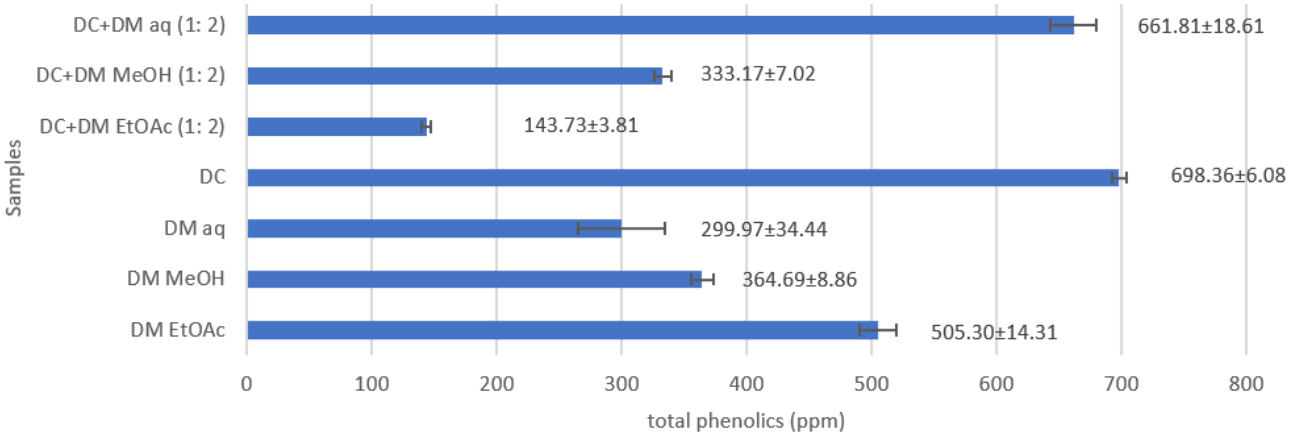
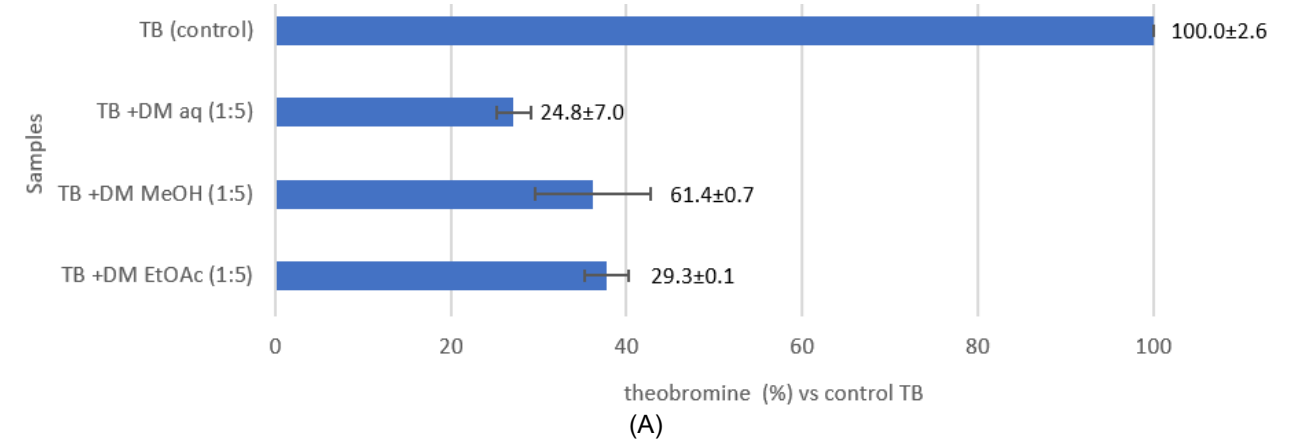
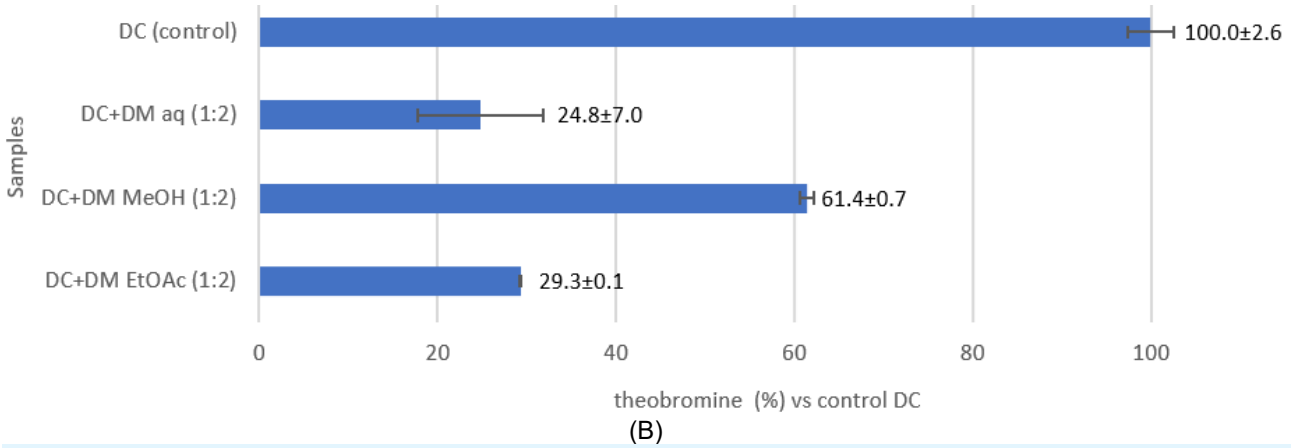


Fig. 2. Total flavonoids in DC, DM extract, and DC mixture with DM extract
Description: DC = dark chocolate, DM = *Melastomataceae* leaves, DM aq = DM water extract, DM MeOH = methanol extract DM, DM EtOAc = DM ethyl acetate extract



(A)



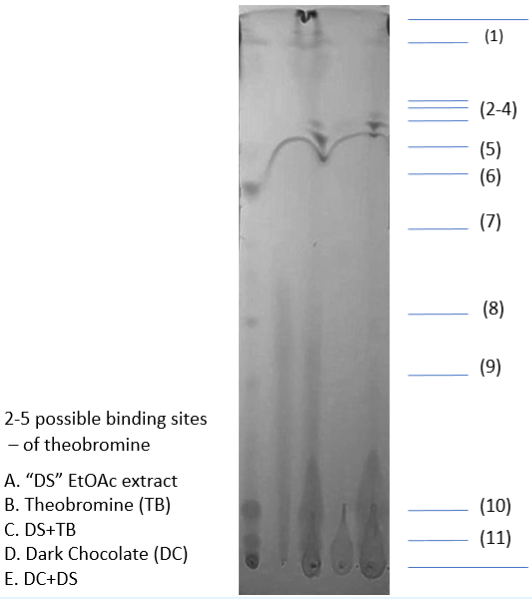
(B)

Fig. 3. Percentage of binding of standard TB and DC in DC by various DM extracts
Description: DC = dark chocolate, DM = *Melastomataceae* leaves, DM aq = DM water extract, DM MeOH = methanol extract DM, DM EtOAc = DM ethyl acetate extract

Binding of Theobromine by EtOAc Extract from DM

In the TLC and subsequent tests, the DM extract used was derived from EtOAc solvent. This decision was based on the production of EtOAc extract of DM which had a higher yield than aqueous and MeOH extracts.

Based on **Fig. 4**, changes in the composition of the composition of EtOAc extract from DM after binding to TB occurred in the Rf range of 0.780 to 0.814. The compounds that were initially detected in Rf between 0.337 and 0.686 became undetectable after the EtOAc extract of DM was mixed with TB and DC. This TLC result showed the potential binding of Rf compounds



Spot	Rf	Spot Visibility				
		A	B	C	D	E
(1)	0.951	✓	-	✓	-	✓
(2)	0.814	-	-	✓	-	✓
(3)	0.802	-	-	✓	-	✓
(4)	0.780	-	✓	✓	✓	✓
(5)	0.734	✓	-	✓	-	-
(6)	0.686	✓	-	-	-	-
(7)	0.591	✓	-	-	-	-
(8)	0.443	✓	-	-	-	-
(9)	0.337	✓	-	-	-	-
(10)	0.101	✓	✓	✓	✓	✓
(11)	0.048	✓	✓		✓	✓

Fig. 4. Chromatogram and Rf values of extracts of DM, TB, DC and a mixture of DM+TB and DC+TB
Description: A. DM EtOAc extract; B. Theobromine (TB); C. DM+TB; D. Dark Chocolate (DC); E. DC+DM

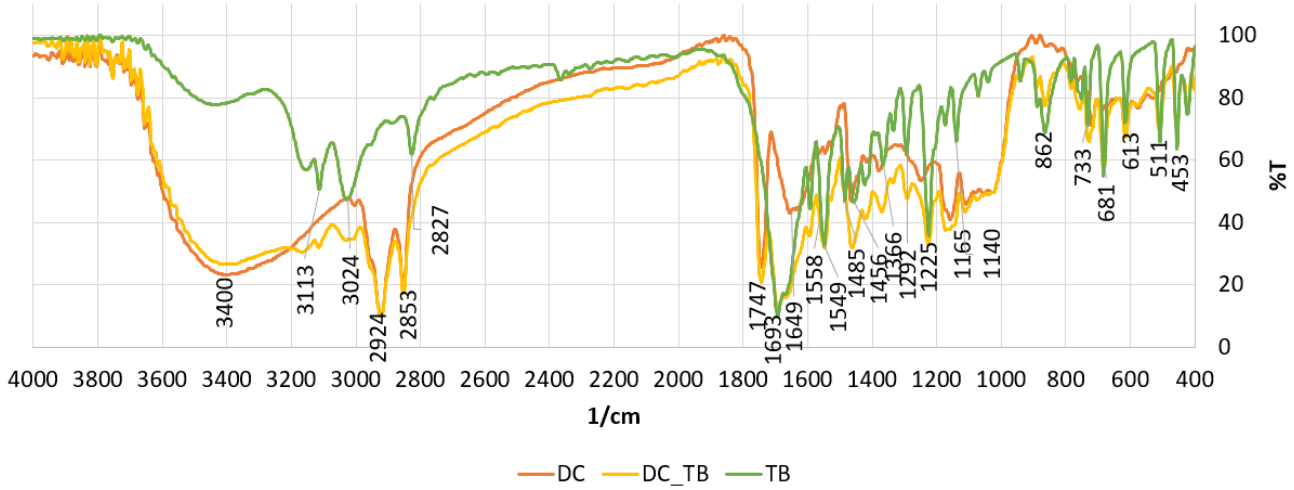


Fig. 5. IR spectrum from DC, TB and DC given additional TB (spiking)

between 0.337 and 0.686 to compounds on Rf between 0.780 and 0.814.

FTIR Analysis

In the first region (ranges from 4000 to 2500 cm^{-1}), TB showed peaks of single bonds, which could be observed i.e. at 3400, 3113, 3024, and 2827 cm^{-1}. Meanwhile, DC contained single bonds with high absorption at wavelengths of 3400, 2924, 2853 cm^{-1}. DC and TB contained less triple bonds. However, DC and TB had a number of double bonds. Absorbance of significant double bonds from DC could be observed at wavelengths of 1747 and 1649 cm^{-1}. Absorbance for double bonds compounds from TB could be observed at wavelengths of 1693, 1558, and 1549 cm^{-1}. DM identification as a bitter taste inhibiting component could be done by referring to FTIR profile. DM contained several single bond functional groups as evidenced by high absorption at wavelength 3400, 2924, 2853 cm^{-1}. However, the single bond functional group of DM had a different absorption compared to the single bond functional group in TB. As with DC, DM and TB contain less triple bonds. DM had a number of double bonds which could be observed at wavelengths of 1745 and 1614 cm^{-1}. Absorbance for double bonds from DM also had no similarity to the absorbance of TB double bonds. DM did not contain TB as evidenced by different peaks in the wavelength ranging from 1500 to 600 cm^{-1}, except at peaks at wavelengths of 733 and 681 cm^{-1}. Peak absorptions as identification for DM were at wavelengths of 1485, 1366, 1165, 1038, 733, and 681 cm^{-1}. The change in %T from the DC combined with EtOAc extract of DM can be observed in **Fig. 7**. There was a change in absorption at wavelength when compared to **Fig. 5** and **Fig. 6**. The DC and DM mixture peak wavelengths were

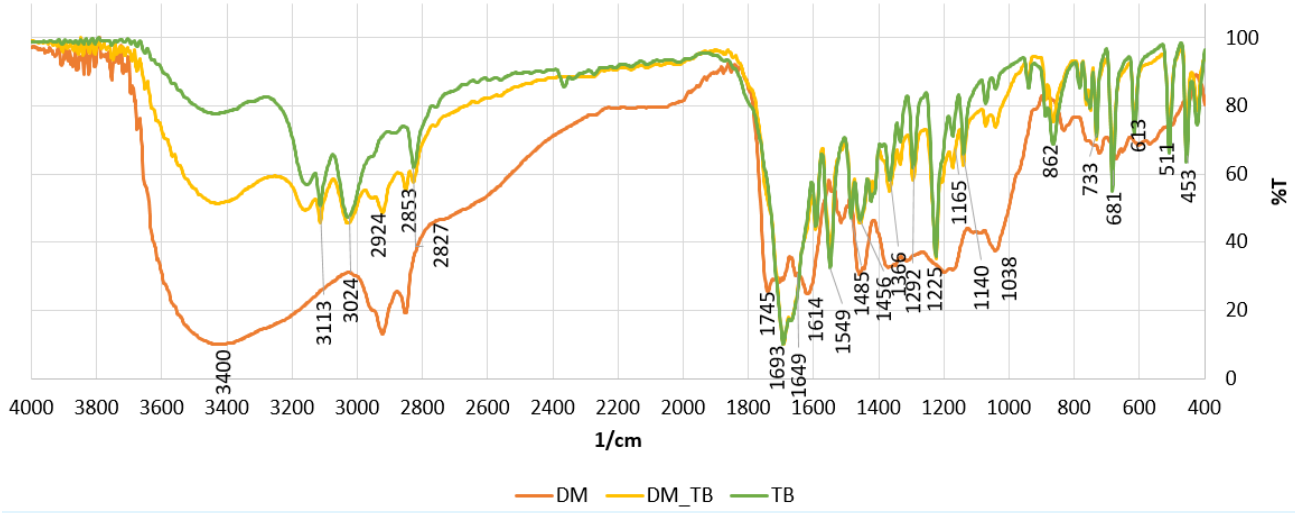


Fig. 6. IR spectrum of DM, TB and TB mixed with DM

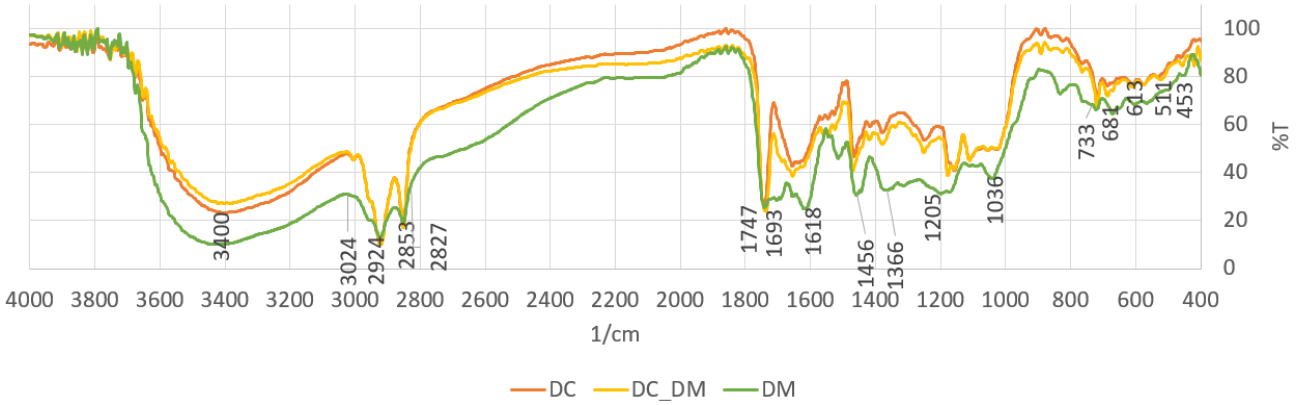


Fig. 7. IR spectrum from DC, DM and DC mixed with DM

not the same with peak wavelengths of DM extract at the range of 3700-2950, 2800-1800, 1690-1180, and 1050-733 cm^{-1}. Furthermore, the %A changes identified can be seen in **Table 1**. The consistency of increase in absorbance as a result of mixing DM was shown in wavelength ranges from 2800 to 2400, from 2300 to 2000, from 1620 to 1600, and from 1500 to 950 except for several peaks between 1500 and 1400, and several peaks between 800 and 600 cm^{-1}.

Characteristics of Covalent Bonds due to DM Binding

Absorbance that tend to decrease in mixing DM with DC and TB could be observed at wavelengths of 1614, 1610, 1367, 1337, 1292, and 1205 cm^{-1}. Mixing DM on DC and TB consistently decreased the bonds that were thought to consist of C=CC=O, ON=O, ONOO, RCOO, -OH, or $OCOCH_3$, COO-, and R_3NO. In addition, modulations of absorbance, increases or decreases, in mixing DM with DC and TB could be observed at wavelengths of 3400, 3113, 1558, 1549, 862, 681 cm^{-1}. DM mixing in DC and TB did not consistently decrease the bonds that were thought to consist of $CONH_2$, NH_2, RCONR, CNO monomers, and CH = CH alkyl.

DISCUSSION

Total Phenol

The total phenol contained in the DC was 698.36±6.08 ppm. The total phenol in water extracts, MeOH, and EtOAc extracts of DM were 299.97±34.44, 364.69±8.86, and 505.30±14.31 ppm, respectively. After the DC was mixed with DM extract, the total phenol which was measured by the spectrophotometric method decreased, especially in the combination of DC with EtOAc extract from DM (1: 2) and DC combination with water extract from DM (1: 2) (**Fig. 1**).

When referring to **Fig. 4**, mixing DC and EtOAc extract from DM changes the composition profile of its constituent compounds. The intended mixing caused changes in the chromophore at a wavelength of 725 nm, so the measurement results for total phenol were lower (Sant'Anna et al. 2013). This chromophore change could be explained as a change in the concentration of covalent bonds detected by using FTIR (**Fig. 7**). This was in line with the previous results on modulation of chromophore concentrations due to mixing phytochemical ingredients (Oliviera et al. 2015, Rolim et al. 2018).

Table 1. Characteristics of covalent bonds in IR Spectrum as a result of DM binding

Wave Length (1/cm)	Possible bond*	delta %A from TB			
		DC	DC_DM	DM	TB_DM
3400	$CONH_2$ or NH_2	55.0	51.0	(68.0)	(41.7)
3113	$CONH_2$ or NH_2	9.5	7.2	(24.9)	(20.2)
3024	NH_3 or CCH-	(0.3)	(1.1)	(16.5)	(14.7)
2924	CH_3, CH_2-, or RCOOH	60.9	60.5	(58.0)	(35.5)
2853	CH_3, CH_2-, or RCOOH	55.9	56.8	(54.7)	(36.5)
1747	C=O, CO-O-, C=C-CO-O-, or esters	32.8	32.9	(32.4)	(27.0)
1745	C=O, CO-O-, C=C-CO-O-, or esters	33.2	33.6	(32.7)	(27.3)
1693	RCOH, aryl, cyclopropyl, or amide	(48.5)	(36.0)	18.9	18.7
1614	C=CC=O, ON=O, or ONOO	4.6	7.3	(26.8)	(22.7)
1610	C=CC=O, ON=O, or ONOO	7.0	9.5	(29.7)	(25.0)
1558	RCONR or monomer CNO	(20.7)	(13.6)	13.1	9.7
1549	RCONR or monomer CNO	(30.5)	(23.8)	24.4	20.5
1456	NN=O or CH_3	(5.0)	(0.1)	(15.4)	(14.5)
1367	RCOO, -OH, or $OCOCH_3$	(1.0)	4.0	(25.4)	(22.1)
1337	OH, RCOO	5.8	11.1	(34.8)	(28.0)
1292	NOO, COO- bond, esters	0.5	4.8	(26.2)	(23.0)
1225	COO- bond	(22.3)	(17.4)	(2.4)	(1.8)
1205	CNO, COO- bond, R_3NO, esters	4.5	9.1	(32.3)	(26.2)
1165	Esters	35.0	35.0	(44.9)	(34.8)
1140	Esters	15.7	15.5	(24.0)	(20.5)
1038	COC	35.4	35.3	(47.9)	(36.8)
1036	COC	36.4	36.3	(48.4)	(37.3)
681	CH=CH alkyl	(20.5)	(16.6)	10.7	6.3

* only strong associated bond listed, from IR Database of St. Thomas "Spectroscopic Tools" (http://www.science-and-fun.de/tools/) based on Socrates (2004) and Hesse et al (2005)

Total Flavonoids

The concentration of flavonoids from DC was 18.80±2.44 ppm. The concentration of flavonoids in water, MeOH, and EtOAc extracts of DM were obtained at 12.01±0.07, 10.09±1.18, and 7.32±0.06 ppm, respectively. Mixing DC with various extracts of DM also decreased the concentration of flavonoids (**Fig. 2**).

The decrease in flavonoids in DC mixtures with various DM extracts was due to changes in the total concentration of flavonoids, where DM extracts had a lower total flavonoid compared to DC. Total flavonoids in DC and DM mixtures were more stable than total phenol testing. Chromophore interference other than flavonoids at wavelength of 510 nm was less than at wavelength of 725 nm (Sengupta et al. 2015).

Theobromine

Preliminary confirmation of the binding ability of several DM extracts to TB as a compound that causes bitter taste can be seen in **Fig. 3A**. The ability of binding to TB by several DM extracts also occurred in DC products as shown in **Fig. 3B**. At the same quantity (0.1 g of TB), a decrease in the quantification by spectrophotometry of TB ranged between 62.3 and 72.8% compared to pure TB control. Similar results were obtained for DC products. At the same quantity (1 g of DC), the decrease in TB concentration detected by the UV spectrophotometry method at a wavelength of 302 nm ranged between 38.6 and 75.2%.

Quantification of TB in a mixture of TB or DC with DM extract showed the ability of DM to bind TB, so that the chromophore was detected less at wavelength of 302 nm (Ionescu et al. 2013). The final concentration of TB as a result of binding TB with DM extracts must be further confirmed by more specific techniques such as HPLC and liquid chromatography mass spectrophotometry (LCMS).

Binding of Theobromine by EtOAc Extract from DM

In the TLC and subsequent tests, the DM extract used was derived from EtOAc solvent. This decision was based on the production of EtOAc extract of DM which had a higher yield than aqueous and MeOH extracts. Preliminary confirmation of changes in composition of compounds and initial detection of compounds could be observed with the TLC method (Marston, 2011). Based on **Fig. 4**, changes in the composition of the composition of EtOAc extract from DM after binding to TB occurred in the Rf range of 0.780 to 0.814. The compounds that were initially detected in Rf between 0.337 and 0.686 became undetectable after the EtOAc extract of DM was mixed with TB and DC. This TLC result showed the potential binding of Rf compounds between 0.337 and 0.686 to compounds on Rf between 0.780 and 0.814.

FTIR Analysis

In general, the interpretation of IR based on wavelength is divided into four categories, namely (1) 4000 to 2500 cm^{-1}: single bond functional groups which generally consist of N-H, C-H, and C-O; (2) 2500-2000 cm^{-1}: consist of functional groups with triple bonds; (3) 2000-1500 cm^{-1}: consist of functional groups with double bonds, and (4) 1500-600 cm^{-1} functional groups with single bonds which are used as identification of identical compounds when comparing measurement results from two different samples (Altemimi et al. 2017). In each set of tests (**Figs. 5-7**), spiking was carried out as an effort to ensure that there was no significant shift in reading between the samples used (Koch et al. 2013). Percent absorbance (% A) from FTIR measurement results was

the opposite of percent transmittance (% T), meaning that If %T was low then %A was high.

In the first region (ranges from 4000 to 2500 cm^{-1}), TB showed peaks of single bonds, which could be observed i.e. at 3400, 3113, 3024, and 2827 cm^{-1}. Meanwhile, DC contained single bonds with high absorption at wavelengths of 3400, 2924, 2853 cm^{-1}. DC and TB contained less triple bonds. However, DC and TB had a number of double bonds. Absorbance of significant double bonds from DC could be observed at wavelengths of 1747 and 1649 cm^{-1}. Absorbance for double bonds compounds from TB could be observed at wavelengths of 1693, 1558, and 1549 cm^{-1}.

As one of the compounds that cause bitter taste, theobromine or 3,7-dihydro-3,7-dimethyl-1H-purine-2,6-dione ($C_7H_8O_4N_2$) has the highest peak absorbance of IR at wavelengths around 1695, 1660, 1590, 1550, 1490, 1450, 1370, 1220, 890, 860, 750, 720, 690 and 610 cm^{-1} (SpectraBase™, Biorad, 2018). There was a slight difference in absorption between the standard used in this study and that used by Biorad. The standard used in this study came from Sigma-Aldrich (USA) with a purity of >97%, while Biorad used a standard originating from Eastman Organic Chemicals (USA) with unspecified purity. The functional groups of one pure compound that are identical in the mixture can be detected in the fourth region, namely at a wavelength of 1500 dd. 600 cm^{-1} (Batista et al. 2016). The FTIR profile of the bitter causative compounds of TB also appeared in DC extracts at the similar wavelengths of 1456, 1366, 862, 733, 681, and 613 cm^{-1}.

DM identification as a bitter taste inhibiting component could be done by referring it to FTIR profile. DM contained several single bond functional groups as evidenced by high absorption at the wavelength of 3400, 2924, 2853 cm^{-1}. However, the single bond functional group of DM had a different absorption compared to the single bond functional group in TB. As with DC, DM and TB contain less triple bonds. DM had a number of double bonds which could be observed at wavelengths of 1745 and 1614 cm^{-1}Absorbance for double bonds from DM also had no similarity to the absorbance of TB double bonds. DM did not contain TB as evidenced by different peaks in the wavelength ranging from 1500 to 600 cm^{-1}, except at peaks at wavelengths of 733 and 681 cm^{-1}. Peak absorptions as identification for DM were at wavelengths of 1485, 1366, 1165, 1038, 733, and 681 cm^{-1}.

The change in %T from the DC combined with EtOAc extract of DM can be observed in **Fig. 7**. There was a change in absorption at wavelength when compared to **Fig. 5** and **Fig. 6**. There were DC and DM mixture peak wavelengths that were not the same with peak wavelengths of DM extract at the range of 3700-2950, 2800-1800, 1690-1180, and 1050-733 cm^{-1}. Furthermore, the %A changes identified can be seen in **Table 1**. The consistency of increase in absorbance as a result of mixing DM was shown in wavelength ranges from 2800 to 2400, from 2300 to 2000, from 1620 to 1600, and from 1500 to 950 except for several peaks between 1500 and 1400, and several peaks between 800 and 600 cm^{-1}.

CONCLUSIONS

DM extracts were proven to reduce the bitter taste of DC. Total phenol, flavonoids and theobromine in DC products mixed with DM extract decreased, especially in DC combinations with EtOAc or water extracts of DM. The decrease in TB concentration ranged from 62.3 to 72.8% compared to pure TB control or between 38.6 and 75.2% in DC products. Changes in the composition of compounds in EtOAc extract from DM after binding TB or DC were the disappearance of the compounds in Rf between 0.337 and 0.686 and the appearance of compounds in the Rf range of 0.780 to 0.814. Based on the results of the FTIR analysis, there were changes in the wavelengths of 1614, 1610, 1367, 1337, 1292, and 1205 cm^{-1} as a result of mixing DM in DC and TB interpreted as covalent bonds of C=CC=O, ON=O, ONOO; RCOO, -OH, $OCOCH_3$; COO-, and R_3NO.

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