CONSTITUENTS OF ESSENTIAL OIL, TOTAL PHENOLIC, TOTAL FLAVONOID CONTENTS AND ANTIOXIDANT ACTIVITY OF RHIZOME OF *CURCUMA CAESIA* ROXB. (GA MONE TAIN PYAR)

Kay Khine Nyunt[1] , Theingi Wint Thu[2] , Ni Ni Than[3]

Abstract

The preliminary phytochemical screening of *Curcuma caesia* Roxb. revealed the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, starch, tannins, steriods and terpenoids and the absence of cyanogenic glycosides and reducing sugars. The higher total phenol content (μg GAE/mg) was detected in watery extract (234 μg GAE/mg) than ethanol extract (164 μg GAE/mg) of the rhizome of *C. caesia*. The higher total flavonoid content (μg QE/mg) was detected in ethanol extract (58.3 μg QE /mg) than watery extract (136.1 μg QE /mg) of the rhizome of *C. caesia*. The rhizome of *C. caesia* was found to have antioxidant activity. IC_{50} values of ethanol and watery extracts are 10.31 μg/mL and 56.85 μg/mL respectively. Antioxidant potency of ethanol and watery extracts were concluded to be mild if compare with the potency of standard butylated hydroxytoluene (IC_{50} = 6.85 μg/mL). The essential oil from the rhizome of *C. caesia* was extracted by steam distillation method. The components of essential oil were characterized by GC-MS. From resulting data, ten components were observed in the essential oil of *C. caesia*, namely eucalyptol, camphor, *p*-menth-2-en-9-ol, L-α-terpineol, α-bulnesene, iso-caryophllene, γ-muurolene, (5R, 6R)3, 6-dimethyl-5-(prop-1-en-2-yl)-6-vinyl-4,5,6,7-tetrahydrobenzofuran, β-elemene [1-ethenyl-1-methyl-2,4 bis (prop-1-en-2-yl) cyclohexane]and β-elemenone.

Keywords: *Curcuma caesia* Roxb., antioxidant activity, essential oil, total phenol content, total flavonoid content

Introduction

Curcuma caesia Roxb. (Ga Mone Tain Pyar) is a kind of turmeric with bluish-black rhizome belonging to Zingiberaceae (Ginger) famil.*C.caesia* is a perennial herb with bluish-black rhizome native to Northeast, Central India and Myanmar. The plant grows only in the rainy season but it dries in other season. However, it can grow in any type of soil like light, well drained, moist, loamy soil, and light to heavy black soil.

The rhizomes of the plant are aromatic in nature. The inner part of the rhizome is bluish-black in colour and emits a characteristics sweet smell due to presence of essential oil (Pandey, 2003). The plants are rich source of secondary metabolites such as flavonoids, phenolics, carotenoids, coumarins, anthraquinones, tannins, terpenoids, saponins that play a prominent role in inhibiting human carcinogenesis and repair the cell mutations (Ruan, 1989). The part of fresh rhizome is applied in case of snake and scorpion bite. It is recognized as a medicinal herb to possess with various properties such as anti-fungal activity, smooth muscle relaxant and anti-asthmatic activity, bronchodilating activity, antioxidant activity, anxiolytic and CNS depressant activity, locomotor depressant, anti-convulsant, anthelmintic activity, anti-bacterial activity, anti-ulcer activity (Kagyung *et al*., 2010).

[1] Dr, Lecturer, Department of Chemistry, Yangon University
[2] MSc. Candidate, Department of Chemistry, Yangon University
[3] Professor and Head, Department of Chemistry, Yangon University

Botanical Aspects of *Curcuma caesia* Roxb. (Ga Mone Tain Pyar)

Family	:	Zingiberaceae
Genus	:	*Curcuma*
Species	:	*caesia*
Botanical Name	:	*Curcuma caesia* Roxb.
Myanmar Name	:	Ga Mone Tain Pyar
English Name	:	Black Turmeric
Part used	:	Rhizome

Figure 1 Plant and rhizome of *C.caesia*

Medicinal Uses of *C. caesia*

Leaves and rhizomes of *C. caesia* are used in traditional medicine. It contains more amounts of flavonoids and terpenoids which are responsible for the main pharmacological activities like antioxidant, anti-inflammatory, anti-fungal, etc. (Sasikala, 2012).

The rhizome of the plant is aromatic, contains essential oil and used for a variety of purposes in pharmaceutical and cosmetic industries. The essential oils of *C. caesia* were also reported to have more potent antimicrobial, antioxidant, anticancer, and anti-inflammatory activities than the solvent extract counterparts (Xiang *et al*., 2018).

Materials and Methods

Sample Collection and Preparation

Several samples of *C. caesia* collected from Yangon Region, the soil was removed from the rhizome. The rhizome was carved into very small pieces and allowed to dry well. The dried pieces were made into powder by using grinding machine. The powdered sample was stored in air-tight container to prevent moisture changes and other contaminations.

Preliminary Phytochemical Investigation of *C. caesia*

Phytochemical tests for rhizomeof *C. caesia* was carried out according to the reported methods to investigate the presence and absence of phytochemical constituents (M-Tin Wa, 1972).

Determination of Total Phenol Content

One of the antioxidative factors, total phenolic content was measured spectrophotometrically according to the Folin-Ciocalteu method (Marinova, 2005).

The total phenolic content in each sample was estimated by Folin-Ciocalteu method according to the procedure described by Marinova (2005). First, 0.5 mL of prepared extract solution was mixed with 0.5 mL of methanol. Then, 5 mL of FCR reagent (1:10) was added to the mixture and incubated for 5 min. 4 mL of 1 M sodium carbonate solution was added to each tube and the tubes were kept at room temperature for 120 min and the UV absorbance of reaction mixture was read at λ_{max} 765 nm.The blank solution was prepared as the above procedure by using

distilled water instead of sample solution. Total phenolic content was estimated as milligram gallic acid equivalents per gram of different extract (μg GAE/ mg).

Determination of Total Flavonoid Content

Total flavonoid content was measured spectrophotometrically according to the $AlCl_3$ colorimetric method (Marinova, 2005).

Each extracts solution 0.5 mL was mixed with 1.5 mL of methanol, 0.1 mL of 1 % $AlCl_3$ solution and 2.8 mL of distilled water. The absorbance of reaction mixture was read at λ-max 415 nm. The blank solution was prepared as the above procedure by using distilled water instead of sample solution. Total flavonoid content was estimated as milligram quercetin acid equivalent per gram (μg QE/mg) of extract.

Determination of Antioxidant Activity of *C. caesia*

The antioxidant activity of crude extracts *C. caesia* was measured by using DPPH free radical scavenging assay (Marinova, 2011).

DPPH free radical scavenging activity of ethanol and water extracts of rhizome of *C. caesia* was determined by UV-Visible spectrophotometer (Marinova, 2011). The control solution was prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of ethanol in the brown bottle. The sample solution was also prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of test sample solution. These bottles were incubated at room temperature and were shaken on shaker for 30 min. After 30 min, the absorbance of different concentrations (6.25, 12.5, 25, 50, 100 and 200 μg/mL) of tested sample was measured at 517 nm using UV-7420 spectrophotometer. Absorbance measurements were done in three times for each concentration and the mean value so obtained were used to calculate percentage of radical scavenging activity (% RSA).

Extraction of Essential Oil from the Rhizome of *C. caesia*

The sample of the fresh rhizome of *C. caesia* (100 g) was placed in the glass jacket. The glass jacket is filled with distilled water. The glass jacket was fitted to set which was joined to water condenser. When the glass jacket was heated, the condensed oil and water coming out from condenser will collected in the receiver flask. The oil was extracted with n-hexane in a separating funnel. The n-hexane was evaporated at 60-70 °C to get the essential oil which was then weighed until to be constant weight and kept in air tight bottle (Srivastava, 2003).

Organic Compounds in Essential Oil from the Rhizome of *C. caesia*

Organic constituents in essential oil from the rhizome of *C. caesia* were detected by GC-MS Spectroscopic Method at National Analytical Laboratory, Department of Research and Innovation.

Results and Discussion

Phytochemical Constituents of *C. caesia*

The phytochemical tests were done on the rhizome with a view to determine the presence or absence of phytochemical constitutents in *C. caesia*. From the result, alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, starch, tannins, steroids and terpenoids are present but cyanogenic glycosides and reducing sugars are absence.

Total Phenol Content of Crude Extracts of *C. caesia*

The determination of the quantity of phenolic compound was very important in order to determine the antioxidant capacity of sample. Ethanol and watery extracts of *C. caesia* contained large quantities of essential oil contents. The highest content of essential oil can be found on the detection of total phenol content of the sample. The total phenol content of ethanol and watery extracts of rhizome of *C. caesia* are shown in Table 1. According to the results, the total phenolic content of watery extract (234 µg GAE/mg) was higher than ethanol extract (164 µg GAE/mg) of *C. caesia*. Comparison of TPC in ethanol and watery extracts of rhizome of *C. caesia* are represented by a bar graph in Figure 2.

Table 1 Total Phenolic Contents of Ethanol and Watery Extracts of *C. caesia*

Tested samples	Total phenol content (µg GAE/mg of extract)
Ethanol extract	164
Watery extract	234

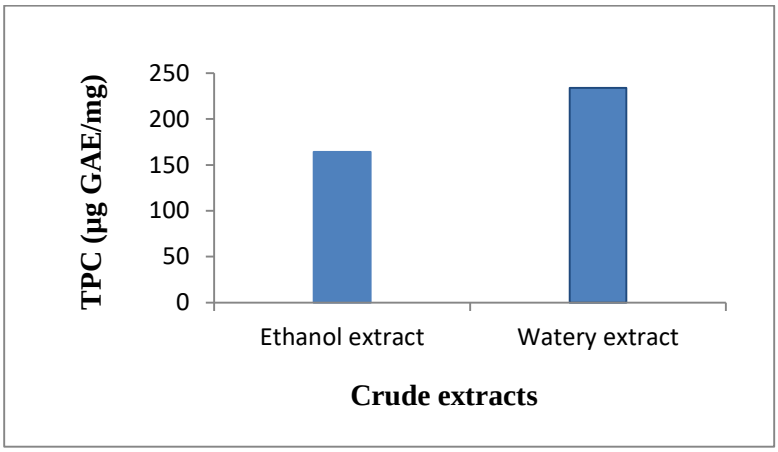


Figure 2 Total phenol contents of the crude extracts of *C. caesia*

Total Flavonoid Contents of Crude Extracts of *C. caesia*

In this study, high flavonoid contents have been found to exert high antioxidant potential. The total flavonoid contents of ethanol and watery extracts of rhizome of *C. caesia* are shown in Table 2. The higher TFC (µg QE/mg) was detected in ethanol (136.1 µg QE/mg) than water (58.3 µg QE/mg) extracts of rhizome of *C. caesia*. According to result, flavonoid compounds were more soluble in ethanol. The different structures and substitutions of flavonoid influence the phenoxyl radical stability, thereby affecting the antioxidant properties of the flavonoids. Comparison of total flavonoid content in ethanol and watery extracts of rhizome of *C. caesia* are represented by a bar graph in Figure 3.

Table 2 Total Flavonoid Contents of Ethanol and Watery Extracts of *C. caesia*

Tested samples	Total flavonoid content (µg QE/mg of extract)
Ethanol extract	136.1
Watery extract	58.3

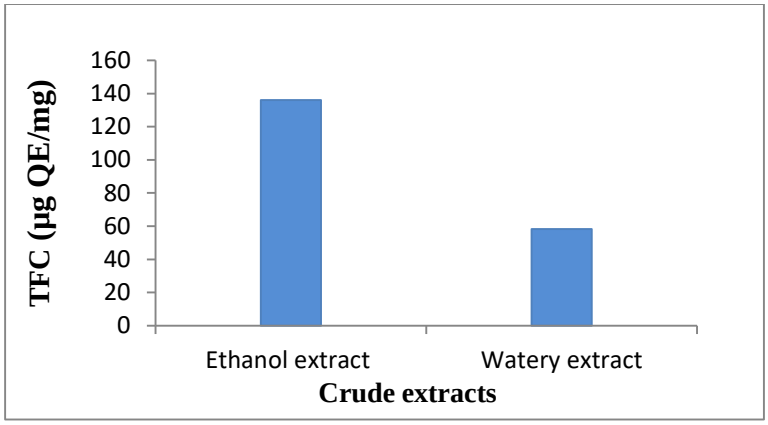


Figure 3 Total flavonoid contents of the crude extracts of *C. caesia*

Antioxidant Activity of *C. caesia*

From the antioxidant activity results, rhizome of *C. caesia* was found to have radical scavenging activity. IC_{50} values of ethanol and watery extracts are 10.31 and 56.85 µg/mL, respectively. So, watery extract has less antioxidant activity than the ethanol extract. All extracts showed lower antioxidant activity when compared to the standard butylated hydroxytoluene (IC_{50} = 6.85 µg/mL). The IC_{50} values of ethanol and watery extracts of rhizome of *C. caesia* and standard butylated hydroxytoluene (BHT) are shown in Table 3 and Figure 4.

Table 3 IC_{50} Values of Crude Extracts from Rhizome of *C. caesia* and Standard BHT

Samples	IC_{50} (µg/mL)
Ethanol	10.31
Watery	56.24
BHT	6.85

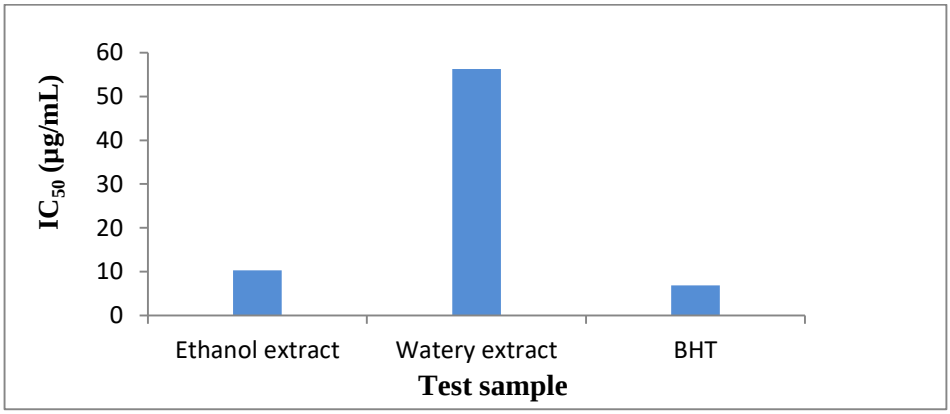


Figure 4 IC_{50} values of the crude extracts of *C. caesia* and standard BHT

Composition of Essential Oil of *C. caesia*

Gas chromatographic-mass spectrometry (GC-MS) is the single most important tool or identification of unknown organic compounds by matching with reference spectra. The GC-MS chromatogram of essential oil from the rhizome of *C. caesia* is showed in Figure 5, Figure 6. Retention indices of the detected compounds were calculated and characterized based on their peaks comparison with reference literature values were shown in Table 1.

According to GC-MS chromatogram, ten compounds were identified from the *C. caesiais*. The compounds identified are eucalyptol, $C_{10}H_{18}O$ (m/z 154), camphor $C_{10}H_{16}O$ (m/z 152), *p*-menth-2-en-9-ol, $C_{10}H_{18}O$ (m/z 154), L-α -terpineol, $C_{10}H_{18}O$ (m/z 154), α-bulnesene, $C_{15}H_{24}$ (m/z 204), *iso*-caryophyllene, $C_{15}H_{24}$ (m/z 204), γ- muurolene, $C_{15}H_{24}$ (m/z 204), (5R,6R)3, 6-dimethyl-5-(prop-1-en-2-yl)-6-vinyl-4,5,6,7-tetrahydrobenzofuran, $C_{15}H_{20}O$ (m/z 216), β-elemene [1-ethenyl-1-methyl-2,4bis(prop-1-en-2-yl) cyclohexane], $C_{15}H_{24,}$ (m/z 204) and β-elemenone, $C_{15}H_{20}O$, (m/z 218).

The following compounds such as eucalyptol, camphor, L-α -terpineol, α -bulnesene, *iso*-caryophyllene,γ-muurolene,(5R,6R)3,6-dimethyl-5-(prop-1-en-2-yl)-6-vinyl-4,5,6,7 tetrahydro -benzofuran, β-elemenone, are in agreement with the reported data whereas, *p*-menth-2-en-9-ol and β-elemene [1-ethenyl-1-methyl-2,4bis(prop-1-en-2-yl) cyclohexane] were absent. This could be due to their geographical variations and the related environmental factors in Myanmar.

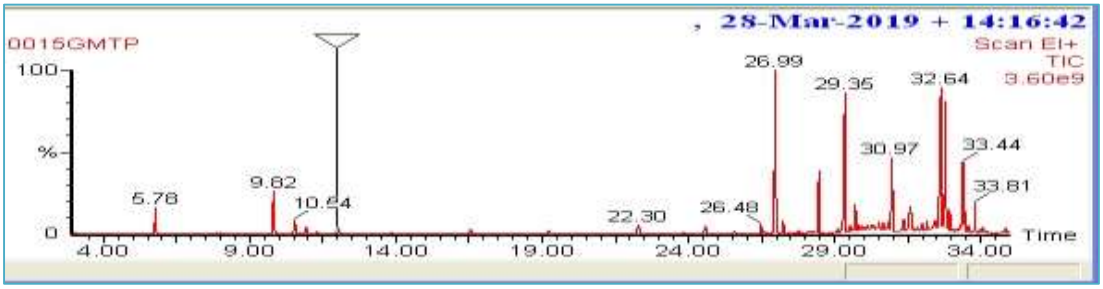
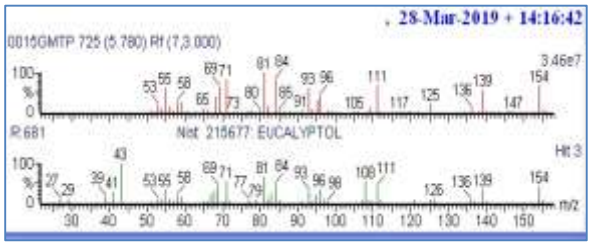
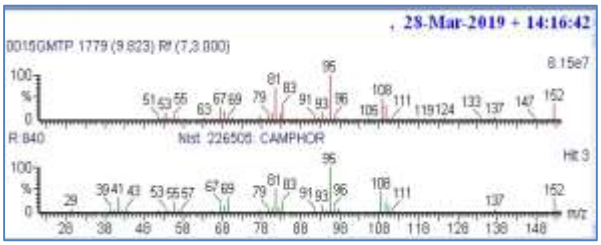


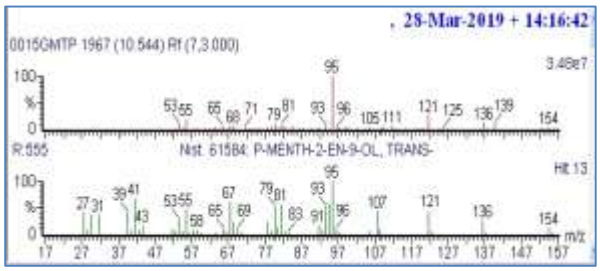
Figure 5 Total ion chromatogram of essential oil from *C. caesia*



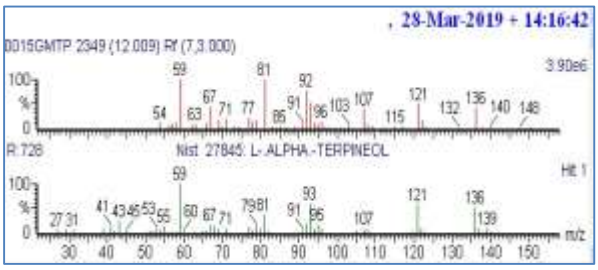
(i) Eucalyptol



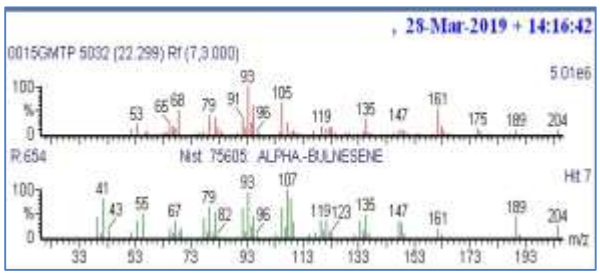
(ii) Camphor



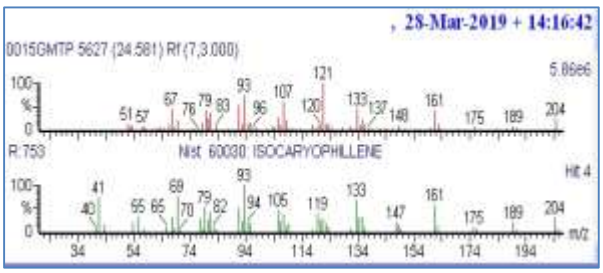
(ii) *p*-Menth-2-en-9-ol



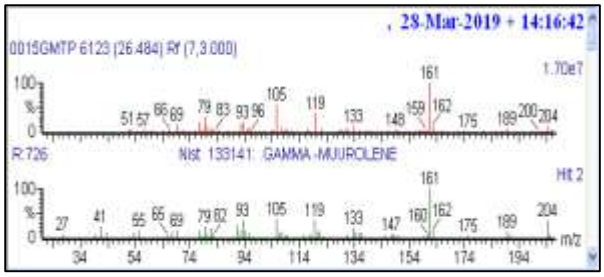
(iv) L-α -terpineol



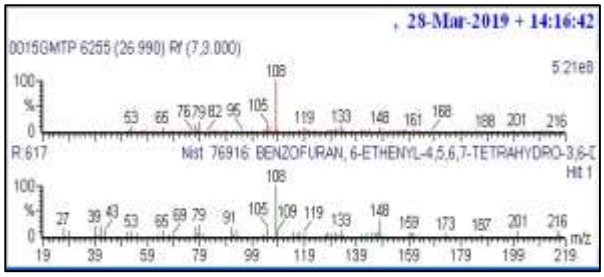
(v) α- bulnesene



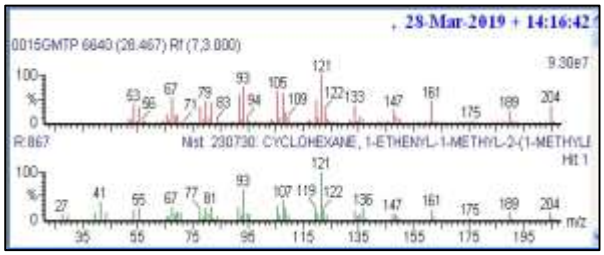
(vi) iso-caryophyllene



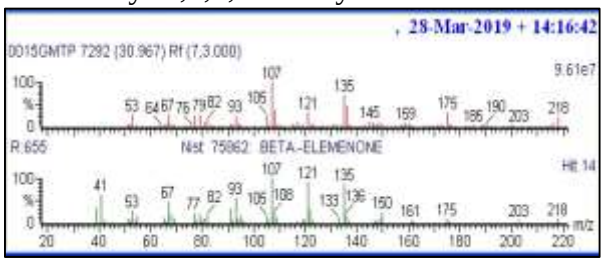
(vii) γ-muurolene



(viii) (5R,6R)3,6-dimethyl-5-(prop-1-en-2-yl) -6-vinyl-4,5,6,7-tetrahydrobenzofuran



(ix) β-elemene



(x) β-elemenone

Figure 6 Comparison of the EI mass spectra of compounds and databank from essential oil of *C. caesia*

Table 4 Chemical Compositions of Essential Oil from the Rhizome of *C. caesia*

No.	Compounds from *C.caesia*	Compounds from *Curcuma* Species *	Molecular Weight	Retention Time (min)
1	Eucalyptol	Eucalyptol	154	5.78
2	Camphor	Camphor	152	9.82
3	*p*-Menth-2-en-9-ol		154	10.54
4	L-α -terpineol	L- α -terpineol	154	12
5	α- bulnesene	α -bulnesene	204	22.29
6	iso-caryophyllene	iso-caryophyllene	204	24.58
7	γ -muurolene	γ -muurolene	204	26.48
8	(5R,6R)3,6-dimethyl-5-(prop-1-en-2-yl)-6-vinyl-4,5,6,7-tetrahydrobenzofuran,	(5R,6R)3,6-dimethyl-5-(prop-1-en-2-yl)-6-vinyl-4,5,6,7-tetrahydrobenzofuran,	216	26.99
9	β-elemene [1-ethenyl-1-methyl-2,4 bis (prop-1-en-2-yl) cyclohexane]		204	28.46
10	β-elemenone	β-elemenone	218	30.97

MW=Molecular Weight* Dosoky, 2018

Conclusion

In this study, investigation of phytochemical constituents, total phenolic content, total flavonoid content and organic compounds from essential oil of the rhizome of *C. caesia* were reported.

The result of preliminary phytochemical screening of different crude extracts of *C. caesia*

revealed the presence of alkaloids, amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, saponin, starch, tannins, steroids and terpenoids and the absence of cyanogenic glycosides and reducing sugars.

The higher TPC was detected in watery extract (234 µg GAE/mg) than ethanol extract (164 µg GAE/mg) of the rhizome of *C. caesia*. From the result data, phenolic compounds of *C. caesia* were more soluble in water.

The higher TFC was detected in ethanol extract (136.1 µg QE/mg) than watery extract (58.3 µg QE/mg) of the rhizome of *C. caesia*. From the experimental data, flavonoid compounds were more soluble in ethanol.

The rhizome of *C.caesia*. is found to have antioxidant activity. IC_{50} values of ethanol and watery extracts are 10.31 µg/mL and 56.24 µg/mL, respectively. Antioxidant potency of ethanol extract significantly higher than watery extract of *C.caesia*. Radical scavenging activity both extracts were concluded to be mild if compare with the potency of standard butylated hydroxytoluene (IC_{50} = 6.85 µg/mL).

The components of essential oil from *C.caesia*. are analyzed by GC-MS. From resulted data, ten components namely eucalyptol, camphor, *p*-menth-2-en-9-ol, L-α-terpineol, α-bulnesene, iso-caryophyllene, γ- muurolene, (5R,6R)3,6-dimethyl-5-(prop-1-en-2-yl)-6-vinyl-4,5,6,7-tetrahydrobenzofuran, β-elemene [1-ethenyl-1-methyl-2,4bis(prop-1-en-2-yl) cyclohexane] and β-elemenone were identified from essential oil of *C. caesia*.

From the research data, rhizome of *C. caesia*. possess antioxidant activity and may probably derived from compounds such as flavonoids and phenols. Organic compounds from essential oil should be useful in the formulation of traditional medicine. This primary information will help in conducting further studies for identification of chemical constituent, medicinal uses of essential oil components and their effectiveness in medicine.

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