INVESTIGATION OF ESSENTIAL OIL FROM THE LEAF AND ANTIOXIDANT ACTIVITY OF PLANT *APIUM GRAVEOLENS* L. (TAYOKE NAN-NAN)

Soe Soe Tint[12], Saw Hla Myint[3], Ni Ni Than[4]

Abstract

Tayoke Nan-nan is known to be rich in essential oil and phenolic compounds. GC-MS analysis of the essential oil steam distilled from the dry leaf (yield 0.02 %) of *Apium graveolens* L. indicates nine compounds, mostly mono and sesquiterpenes, phthalide derivatives, and a few other compounds. Similar analysis of the essential oil from the fresh leaf (yield 0.07 %) showed fourteen compounds, mostly mono and sesquiterpenes, the phthalide derivatives and a few other compounds. Phthalides are the most abundant constituents in both essential oils. In the determination of antioxidant activity by ferric reducing antioxidant power (FRAP) method, EC_{50} values of the ethanolic extracts of the leaf, stalk and root were 2186, 984 and 2316 μg/mL against 112 μg/mL of ascorbic acid standard. The presence of phthalides and phenolic compounds in the plant, Tayoke nan-nan may be useful as a medicinal drug in certain miner diseases.

Keywords: *Apium graveolens* L., leaf, essential oil, phthalide derivatives, FRAP method

Introduction

All parts of celery (*Apium graveolens* L.) plant yield high proportion of essential oil. This is pale yellow and very fluid, with a strong celery aroma consisted of phthalide derivatives (lactone sedanolide), palmitic acid, hydrocarbons such as limonene (Rożek *et al*., 2016 and Sellami, *et al*.**,** 2012). Traditional processes for extracting essential oils, hydrodistillation and steam distillation, are relatively simple (Sahraoui and Boutekedjiret, 2015 and Božović *et al*., 2017). The chemical composition of the oil was investigated by GC-MS that combines the separation power of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample (Chauhan *et al*., 2014).

The major bioactive compounds in the celery include phenolic compounds, flavonoids and phenols are the most important groups of secondary metabolites and bioactive compounds in plants such as *Apium* plants are good sources of natural antioxidants in human diets (Saxena *et al*., 2012). Spectrophotometric SET-based assays measure the capacity of an antioxidant in the reduction of an oxidant, which changes colour when reduced. Like the antioxidant activity, the reducing power of celery, ethanol extract increases with increasing concentration. In ferric reducing antioxidant power (FRAP) assay, there is an increase in absorbance at a prespecified wavelength as the antioxidant reacts with the chromogenic reagent (Moharram and Youssef, 2014).

The reactions in the FRAP method (Canabady-Rochelle *et al*., 2015) as follow:

$$2\,Fe(CN)_6^{3-} + C_6H_7O_6^- \longrightarrow 2\,Fe(CN)_6^{4-} + C_6H_6O_6 + H^+$$

Ferricyanide (Yellow) — Ascorbate — Ferrocyanide — Dehydro ascorbate

$$3\,Fe(CN)_6^{4-} + 4\,Fe^{3+} \longrightarrow Fe_4[Fe(CN)_6]_3$$

Ferrocyanide — Ferric ion — Ferric-Ferrocyanide (Prussian blue) λ_{max}= 700 nm

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The aim of the present research is to investigate the constituents of essential oils from the dry and fresh leaves of celery and to determine the antioxidant activity by FRAP method of different parts of celery (*A. graveolens*) plant.

Materials and Methods

Plant Material

The plant material (*A. graveolens*, Tayoke Nan-nan) used for this study was collected from Kalaw, Shan state, Myanmar. The leaf samples were dried. Fresh and dry leaf samples were used to extract the essential oil. For the antioxidant activity by FRAP method, dry leaf, stalk and root samples were used.

Experimental Setup

Steam distillation method was used to extract essential oil (Sahraoui and Boutekedjiret, 2015). The schematic diagram of experimental setup is shown in Figure 1. The experiment was conducted in a Clevenger's Apparatus.

1. Boiling flask
2. Biomass flask
3. Connecting distillation adapter
4. Glass stopper
5. Condenser
6. Oil separator apparatus
7. Heating mantle
8. Lab support stand
9. Finger clamp

Figure 1 Essential oil steam distillation kit

Experimental Procedure

Dry leaf sample 25 g or fresh leaf sample 100-150 g were filled in the biomass flask and extracted with steam generated from 350 mL water in the boiling flask until oil distillation ceased after 5-6 h. The essential oil in the distillate was separated by partitioning with *n*-hexane, the extract dried over anhydrous Na_2SO_4 and filtered and kept in the freezer after evaporation of the solvent.

Yield of Essential Oils

The yield of essential oil of celery leaf was expressed in gram relative to 100 g of raw sample; it was calculated according to following equation:

$$\text{Yield } (\%) = \frac{\text{Amount of extracted oil (g)}}{\text{Amount of raw sample (g)}} \times 100$$

Determination of Composition of Essential Oil by GC-MS

The chemical composition of essential oil was determined by GC-MS analysis on a PerkinElmer system consisting of a Clarus 680 GC model and a Clarus 600 MS model at National Analytical Laboratory, Department of Research and Innovation, Yangon, Myanmar. An Elite5 MS GC column with 5 % diphenyl 95 % dimethyl polysiloxane stationary phase and dimension 30 m (L), 0.25 mm (ID) and 0.25 μm (thickness) was used. The GC settings were as follows: the source temperature was 190 °C and the inlet temperature was 209 °C.

Figure 2 GC-MS instrument

The initial oven temperature was held at 80 °C for 3 min and then heated from 100 to 140 °C at a rate of 2 °C /min, held for 1 min, and then heated to 240 °C at 10 °C /min and held for 3 min. The injector temperature was maintained at 250 °C. The sample in hexane (1 μ L) was injected, with a split ratio of 1: 20. The carrier gas was helium at flow rate of 1.0 mL/min.

Determination of the Functional Groups Present Using FT IR

The FT IR spectrum of the essential oil was measured at National Analytical Laboratory, Department of Research and Innovation, Yangon, Myanmar. The FT IR spectra were reported in % transmittance. The wavenumber region for the analysis was 4000-400 cm^{-1} (in the mid-infrared range).

Determination of Antioxidant Activity by FRAP Method

The 95 % ethanol extracts of dry leaf, stalk and root were used for ferric reducing antioxidant power content (Bhalodia *et al*., 2013).

Preparation of plant extract

The leaf, stalk and root samples (5 g) of celery (*A. graveolens*) were extracted with 95 % ethanol (100 mL).

Preparation of standard solutions

Ascorbic acid (0.01 g) was dissolved in 10 mL distilled water (1000 μg/mL) . This solution was serially diluted with distilled water to give solutions of 5, 10, 25, 50, 75 and 100 μg/mL serially concentrations.

Preparation of test sample solutions

Preparation of test sample stock solutions of samples were prepared by dissolving 0.02 g of each extract in 1 mL of ethanol and diluted with distilled water to make 20 mL solution (2000 μg/mL) . Then sample concentrations of 125, 250, 500, 1000 and 2000 μg/mL were prepared.

Protocol for reducing power

According to this method, 1.0 mL aliquots of each solution in deionized water various concentrations of the standard (5 to 100 μg/mL) and test sample extracts (125 to 2000 μg/mL) were separately mixed with 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of (1 %) potassium ferricyanide. The mixture was incubated at 50 °C in water bath for 20 min after cooling. Trichloroacetic acid (10 %) solution 2.5 mL was added to each mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution 2.5 mL each was mixed with 2.5 mL distilled water and a freshly prepared 0.5 mL of (0.1 %) ferric chloride solution. Increase in absorbance of the reaction mixture indicates increase in reducing power. All experiments were repeated three times.

Results and Discussion

Yield of Essential Oils from Celery Dry and Fresh leaf

The essential oils of dry and fresh leaf of celery (*A. graveolens*) were extracted by steam distillation method. The amount of essential oils obtained were 0.02 % from dry leaf and 0.07 % from fresh sample. Both samples of essential oils have light yellow colour and pleasant aromas. Because of its high volatility, it was stored in an air tight container protected from light in cool place. The essential oil was insoluble in water but miscible in alcohol.

Composition of Essential Oil by GC- MS Analysis

In GC-MS analysis, the obtained essential oil was dissolved in *n*-hexane. Nine volatile compounds in dry leaf and fourteen compounds in fresh leaf essential oils observed are shown in Tables 1 and 2. A high proportion of the essential oils consisted butyl phthalide derivatives (butylphthalide, senkyunolide and sedanolide) and D-limonene and carveol were also contained in the oils of both samples (Figures 3 and 4). Molecular structures of essential oil compounds in dry and fresh leaf are shown in Figure 5. GC-MS analysis of d-limonene and carveol, butylphthalide, senkyunolide and sedanolide of the both essential oils is expressed in Figure 6.

Table 1 Chemical Composition of Essential Oil from Dry leaf sample

Sr. No.	Compound Name		Molecular Formula	Molecular weight	Retention time (min)
1	D- limonene	(**2**)	$C_{10}H_{16}$	136	3.970
2	Carveol	(**6**)	$C_{10}H_{16}O$	152	5.535
3	1-Pentanone 1- phenyl	(**7**)	$C_{11}H_{14}O$	162	8.231
4	Alloaromadendrene	(**9**)	$C_{15}H_{24}$	204	9.980
5	Butylphthalide	(**13**)	$C_{12}H_{14}O_2$	190	11.951
6	Senkyunolide	(**14**)	$C_{12}H_{16}O_2$	192	12.791
7	Sedanolide	(**15**)	$C_{12}H_{18}O_2$	194	12.837
8	Palmitic acid	(**16**)	$C_{10}H_{20}O_2$	256	15.840
9	6-Octa decenoic acid	(**17**)	$C_{18}H_{34}O_2$	282	20.021

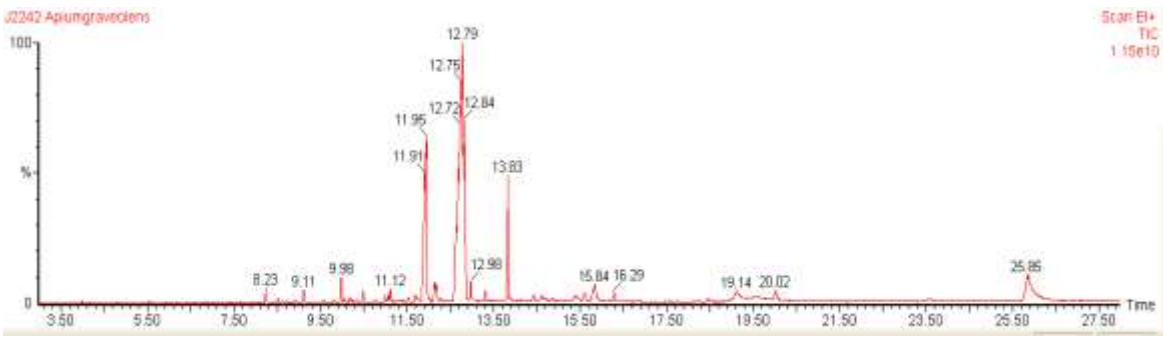

Figure 3 Total ion chromatogram (TIC) of essential oil from dry leaf of *A. graveolens* at retention times 3 -28 min

Table 2 Chemical Composition of Essential Oil from Fresh Leaf Sample

Sr. No.	Compound Name		Molecular Formula	Molecular weight	Retention time (min)
1	α- Pinene	(**1**)	$C_{10}H_{16}$	136	3.690
2	D- limonene	(**2**)	$C_{10}H_{16}$	136	4.219
3	β- Ocimene	(**3**)	$C_{10}H_{16}$	136	4.353
4	3- Carene	(**4**)	$C_{10}H_{16}$	136	4.557
5	Naphthalene	(**5**)	$C_{10}H_{8}$	128	6.298
6	Carveol	(**6**)	$C_{10}H_{16}O$	152	8.208
7	Caryophyllene	(**8**)	$C_{15}H_{24}$	204	9.389
8	Humulene	(**10**)	$C_{15}H_{24}$	204	9.830
9	Naphthalene, decahydro- 4A- methyl- 1-methylene - 7(1-methylethe)	(**11**)	$C_{15}H_{24}$	204	10.245
10	Butylated Hydroxytoluene	(**12**)	$C_{15}H_{24}O$	220	10.298
11	Butylphthalide	(**13**)	$C_{12}H_{14}O_2$	190	12.074
12	Senkyunolide	(**14**)	$C_{12}H_{16}O_2$	192	12.830
13	Sedanolide	(**15**)	$C_{12}H_{18}O_2$	194	12.899
14	Phenol, 2, 2‘- methylenebis [6-(1, 1- dimethylethyl)-4- methyl)	(**18**)	$C_{23}H_{32}O_2$	340	24.048

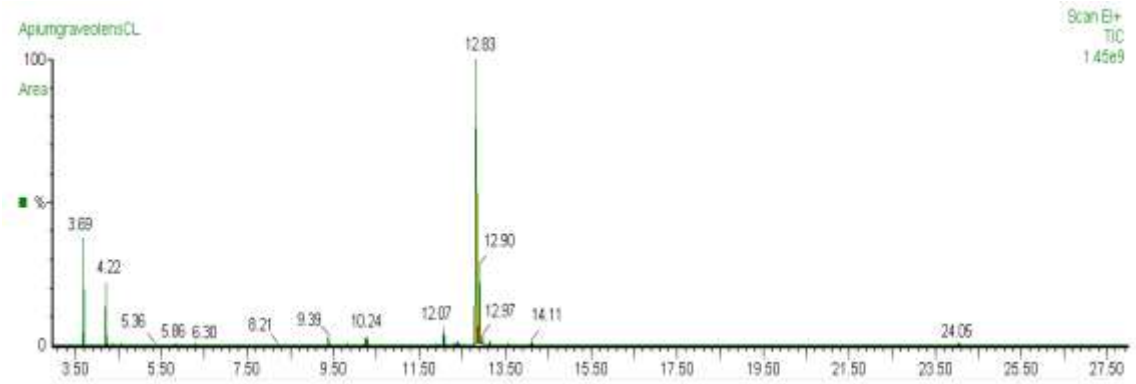

Figure 4 Total ion chromatogram (TIC) of essential oil from fresh leaf of *A. graveolens* at retention times 3 -28 min

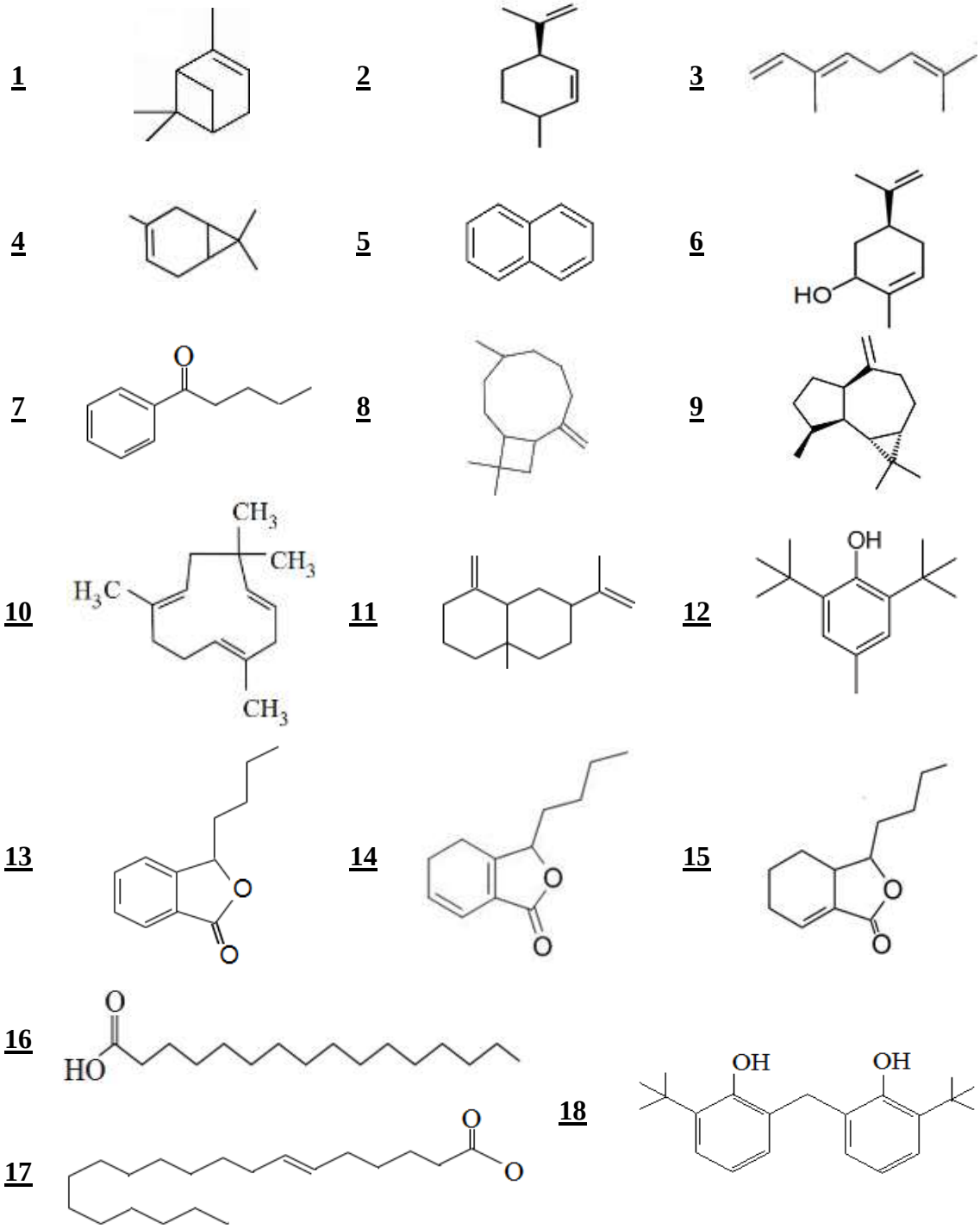

Figure 5 Molecular structures of essential oil compounds from dry and fresh leaves

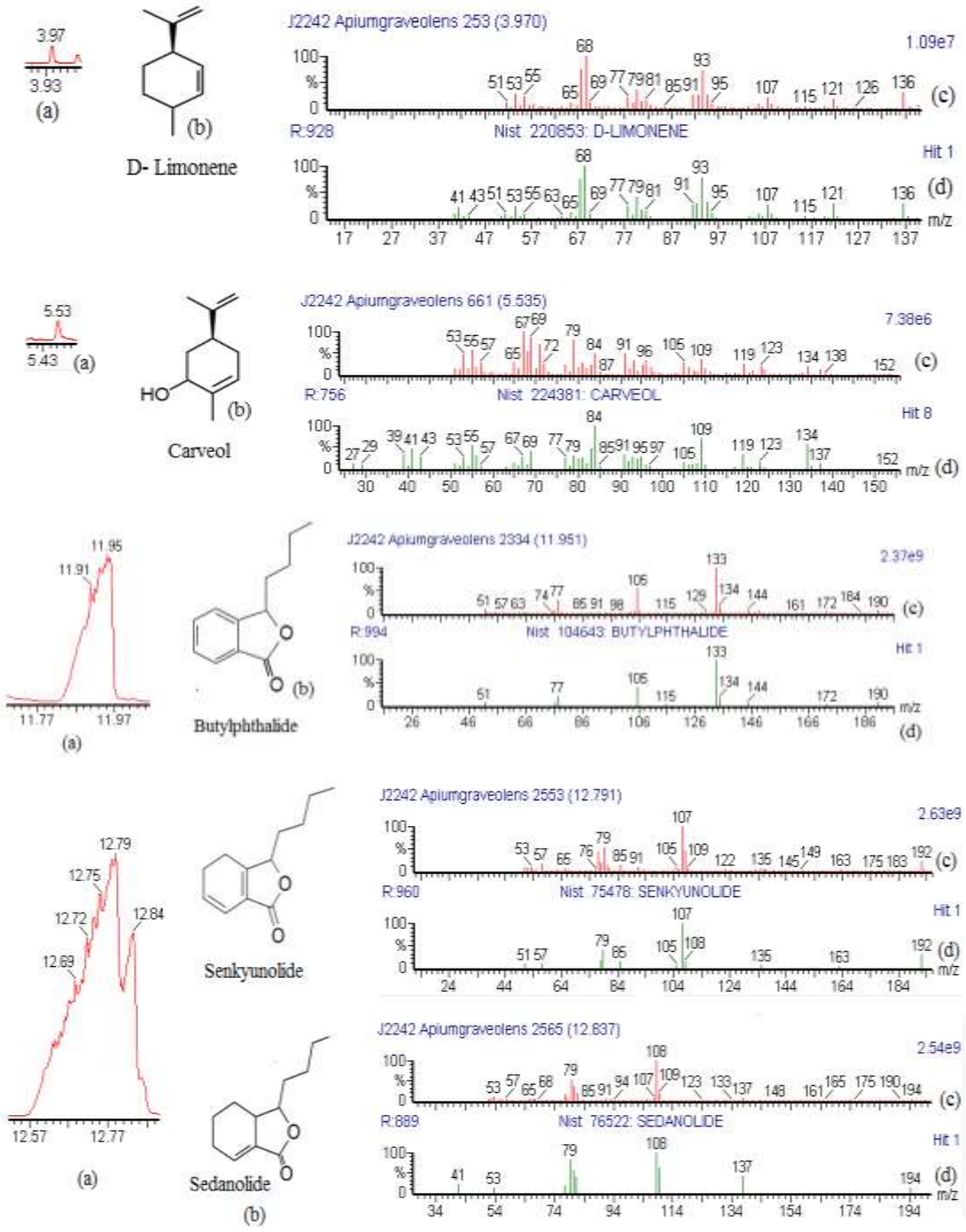

Figure 6 GC-MS analysis of d-limonene, carveol, butylphthalide, senkyunolide and sedanolide present in the essential oil (a) TIC (b) molecular structures (c) mass spectra (recorded) (d) mass spectra (library)

Functional groups present in the essential oil using FT IR

The functional groups present in the essential oil were determined from the wavenumbers of the sample in FT IR the spectrum (Figure 7). The FT IR absorption spectrum of essential oil obtained from dry leaf celery (*A. graveolens*) was measured in the wavelength range 4000- 400 cm^{-1}.

The vibrational band given by C=O bond of ester or five members ring lactone occurs at 1751 cm^{-1}, and the C–O stretching of ester, alcohol, ether leads to bands at 1284 cm^{-1}, 1225 cm^{-1}, 1184 cm^{-1}, 1043 cm^{-1} (Figure 7 and Table 3).

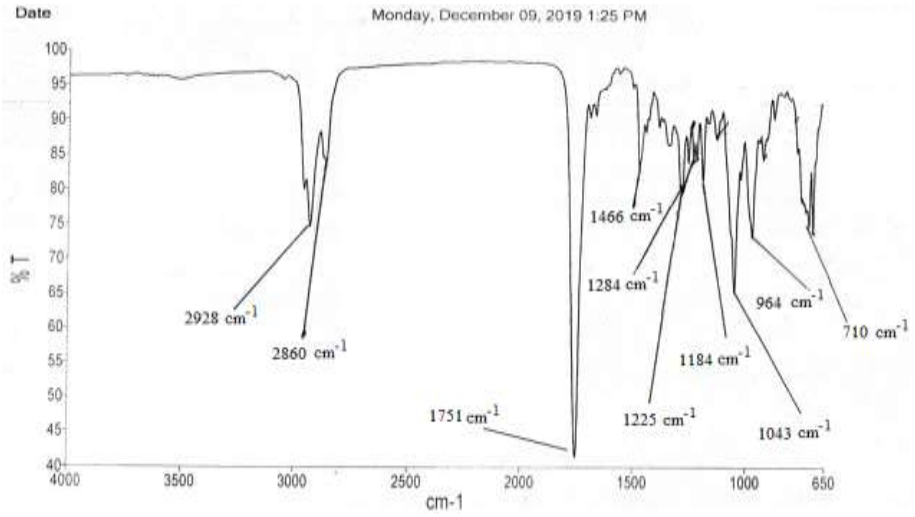

Figure 7 FT IR spectrum of dry leaf essential oil from *A. graveolens*

Table 3 FT IR Spectral Data of Essential Oil from Dry *A. graveolens* Leaf

Wavenumber (cm^{-1})	**Vibrational mode**	**Functional group**
3080	= C-H stretching	Olefin, aromatic compound
2928, 2860	C-H stretching	- CH_3 > CH_2 > CH-
1751	C= O stretching	Ester, 5 members ring lactone
1466	C-H bending	- CH_3 > CH_2 > CH-
1284, 1225, 1184, 1043	C - O stretching	Ester, alcohol, ether
964	= C-H out of plane bending	Trans olefin
710	= C-H out of plane bending	Cis olefin

(silverstein *et al*., 1991)

Activity (Reducing Power Assay)

Celery is valued for the distinctive aroma which it owes to the presence of phthalide- rich essential oil. Moreover, it is known for its antioxidant properties due to the compounds such as phenolic and flavonoid compounds as determined in the previous work (Table 4) (Soe Soe Tint *et al*., 2020).

Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric-ferrous complex that has an absorption maximum at 700 nm. The reducing power of the 95 % ethanol extracts and standard increases with the increase in amount of sample and standard concentrations (Tables 5, 6, 7 and 8). The plot of reducing power shows good linearity in standard (R^2 = 0.9181) and all sample extracts (leaf, stalk and root) are R^2 = 0.983, 0.9553 and 0.9891 respectively for leaf, stalk and root (Figures 12, 13, 14 and 15). EC_{50} values of the ethanolic extracts of leaf, stalk and root were 2186, 984 and 2316 μg/mL against 112 μg/mL of ascobic acid standard (Table 9 and Figure 16).

Table 4 Flavonoid Content, Phenolic Content and Antioxidant Activity of Celery

	Flavonoid content (mg QE/g dry or fresh weight)	Phenolic content (mg GAE/g dry or fresh weight)	Antioxidant activity of ethanol extract IC_{50} value (µg/mL)
Dry leaf	263.453	290.119	99.646
Fresh leaf	29.757	32.769	
Dry stalk	28.743	51.338	45.219
Fresh stalk	3.425	6.117	
Dry root	37.886	53. 987	125.342
Fresh root	4.018	5.726	

(Soe Soe Tint *et al*., 2020)

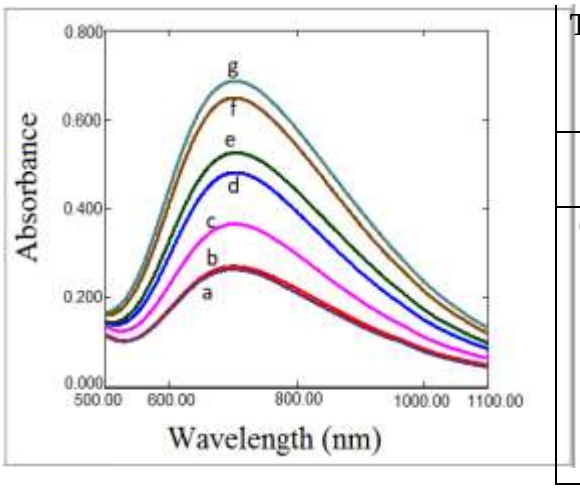

Figure 8 Zero-order overlaid spectra of ferric–ferrocyanide for (a) blank solution (b) 5 µg/mL (c) 10 µg/mL (d) 25 µg/mL (e) 50 µg/mL (f) 75 µg/mL (g) 100 µg/mL standard ascorbic acid solutions

Table 5 Variation of Absorbance with Concentrations of Stand Ascorbic Acid at 700 nm

Conc. (µg/mL)	Abs	Net Abs	EC_{50} (µg/mL)
0, (Blank)	0.264		112
5	0.271	0.007	
10	0.366	0.102	
25	0.481	0.217	
50	0.525	0.261	
75	0.648	0.384	
100	0.686	0.422	

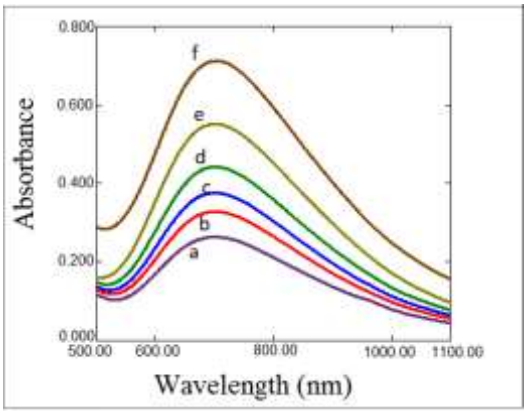

Figure 9 Zero-order overlaid spectra of ferric–ferrocyanide for (a) blank solution (b) 125 µg/mL (c) 250 µg/mL (d) 500 µg/mL (e) 1000 µg/mL (f) 2000 µg/mL celery leaf ethanol extract solutions

Table 6 Variation of Absorbance with Concentrations of Leaf Extract Solution at 700 nm

Conc. (µg/mL)	Abs	Net Abs	EC_{50} (µg/mL)
0, (Blank)	0.264		2186
125	0.328	0.064	
250	0.375	0.111	
500	0.442	0.178	
1000	0.551	0.287	
2000	0.712	0.448	

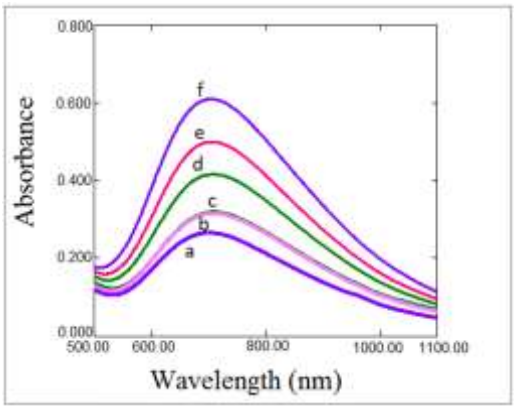

Figure 10 Zero-order overlaid spectra of ferric–ferrocyanide for (a) blank solution (b) 125 µg/mL (c) 250 µg/mL (d) 500 µg/mL (e) 1000 µg/mL (f) 2000 µg/mL celery stalk ethanol extract solutions

Table 7 Variation of Absorbance with Concentrations of Stalk Extract Solution at 700 nm

Conc. (µg/mL)	Abs	Net Abs	EC_{50} (µg/mL)
0, (Blank)	0.264		984
125	0.313	0.049	
250	0.316	0.052	
500	0.408	0.144	
1000	0.498	0.234	
2000	0.609	0.345	

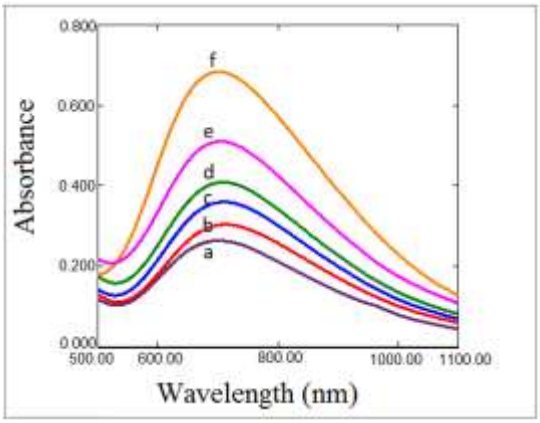

Figure 11 Zero-order overlaid spectra of ferric–ferrocyanide for (a) blank solution (b) 125 µg/mL (c) 250 µg/mL (d) 500 µg/mL (e) 1000 µg/mL (f) 2000 µg/mL celery root ethanol extract solutions

Table 8 Variation of Absorbance with Concentrations of Root Extract Solution at 700 nm

Conc. (µg/mL)	Abs	Net Abs	EC_{50} (µg/mL)
0, (Blank)	0.264		2316
125	0.302	0.038	
250	0.359	0.095	
500	0.408	0.144	
1000	0.51	0.246	
2000	0.683	0.419	

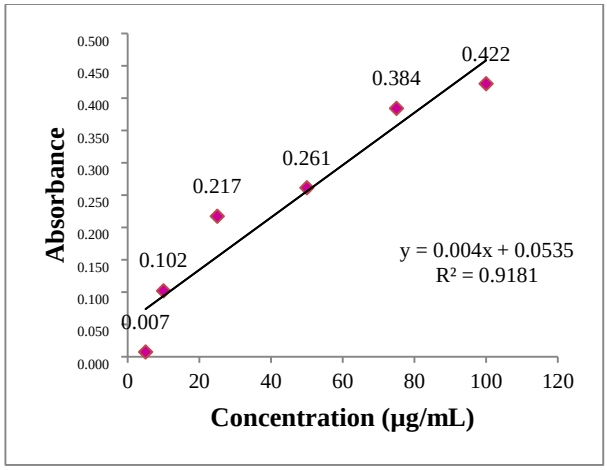

Figure 12 Ferric reducing power determination of standard ascorbic acid

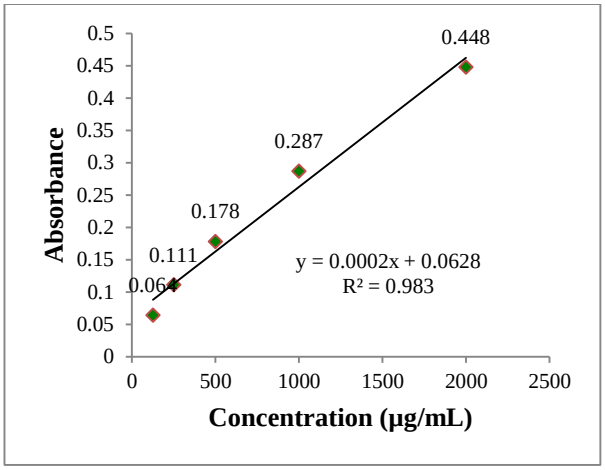

Figure 13 Ferric reducing power determination of celery leaf extract solutions

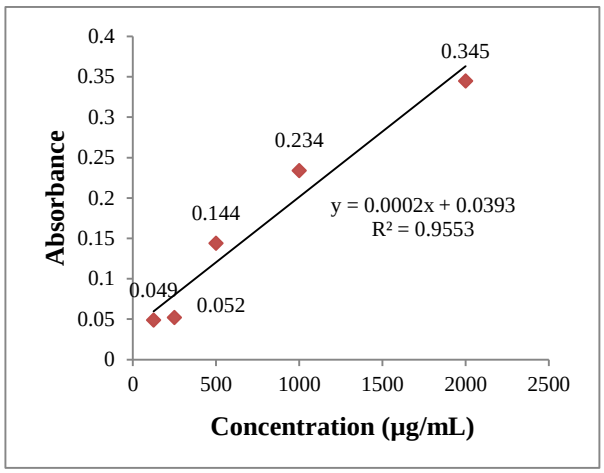

Figure 14 Ferric reducing power determination of celery stalk extract solutions

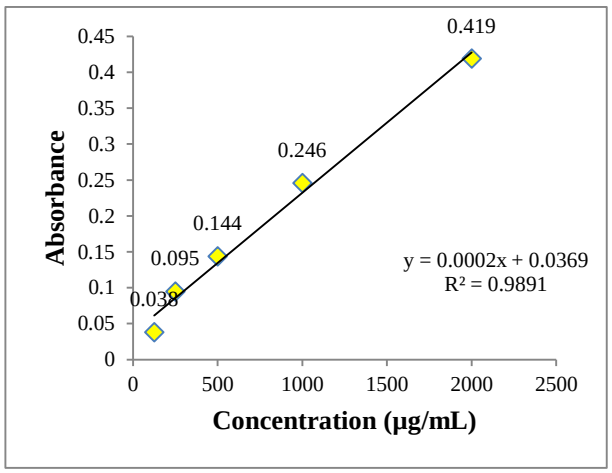

Figure 15 Ferric reducing power determination of celery root extract solutions

Table 9 EC_{50} Values of Ferric Reducing Power for Ascorbic acid and Ethanol Extract of Celery Leaf, Stalk and Root

EC_{50} (µg/ mL)			
Ascorbic Acid	**Celery Leaf**	**Celery Stalk**	**Celery Root**
112	2186	984	2316

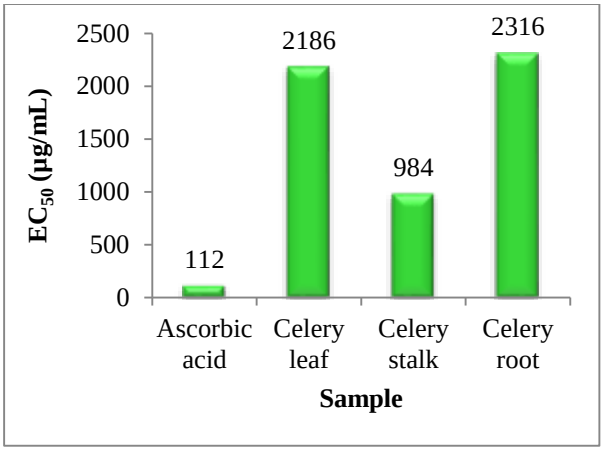

Figure 16 A bar graph of EC_{50} values of ascorbic acid and ethanol crude extracts of leaf, stalk and root of celery from Kalaw, Shan State, Myanmar

Conclusion

The GC-MS analysis of essential oil of dry leaf of *Apium graveolens* L. (Celery, Tayoke Nan-nan) provides nine volatile compounds and fourteen compounds in fresh leaf. Butyl phthalide derivative compounds are most abundant in dry and fresh leaf essential oils. Senkyunolide as the main compound among them.

FT IR spectroscopy is an extremely effective method for determination of presence or absence of a wide variety of functional groups in a molecule. In this study, the absorption frequencies (cm^{-1}) express a variety of functional groups in the essential oil. The prominent peak at 1751 cm^{-1} for C = O stretching of five member ring ester or lactone agrees with the major component phthalides in the oil.

The leaf, stalk and root ethanol extracts of *A. graveolens* (Celery, Tayoke Nan-nan) show antioxidant activity by ferric reducing power (FRAP) in the decreasing order, stalk, leaf, root, which is the same order given by DPPH method. The reducing power shows good linear relation in standard (R^2 = 0.9181) and all sample extracts (leaf, stalk and root).

The Presence of phthalides and phenolic compounds in the plant, Tayoke nan-nan may be useful as a medicinal drug in certain miner diseases.

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