J. Myanmar Acad. Arts Sci. 2019 **Vol. XVII. No.1B**

SMALLANTHUS SONCHIFOLIUS POEPP. (YACON) LEAF ESSENTIAL OIL: ITS PHYSICOCHEMICAL PROPERTIES, CHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY

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Abstract

The present study focused on the extraction of essential oil from yacon leaf and its physicochemical properties as well as antimicrobial activity. Types of organic phytochemical constituents in yacon leaf were determined. The essential oil (0.283 %) was extracted by steam distillation using a Clevenger apparatus. The antimicrobial activity of essential oil was also assessed by agar well diffusion method. The oil was found to show low activities on all tested microorganisms, namely *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*, *Bacillus pumilus, Candida albicans* and *Escherechia coil.* The essential oil of yacon leaf analyzed by gas chromatography-mass spectroscopy (GC-MS) indicated the presence of 3-carene,1-methyl-3-(1-methyl ethyl) benzene, γ-terpinene, D-limonene, caryophyllene,1-methylene-2β-hydroxymethyl-3,3-dimethyl -4β - (3 methyl but -2-enyl) cyclohexaneand 1-formyl-2, 2-dimethyl-3-trans- (3-methylbut-2-enyl)-6-methylene cyclohexane in the essential oil. Some physicochemical properties such as specific gravity (0.936), total acid number (2.805 mg KOH/g) and ester value of essential oil (57 mg KOH/g) were also determined. The present study thus confirmed the presence of essential oil containing mono and sesquiterpenes and the phenolic compounds in yacon leaf, which can be beneficial to human health as a drug or a food.

Keywords: Yacon leaf, essential oil, physicochemical properties, antimicrobial activity, GC-MS phytochemical constituents

Introduction

Yacon (*Smallanthus sonchifolius* Poepp.) is a tuber plant that is native to Andean region. Yacon leaf has been prepared as a traditional medicinal tea that can be useful against chronic diseases, such as diabetes and renal disorder. In Japan, Yacon leaf is used alone or in combination with common yacon leaf to prepare medicinal infusion. In a first attempt to scientifically validate its use (Volpato *et al.*, 1997) suggest that yacon leaf can reduce blood

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glucose levels. A wide range of essential oils of yacon leaf such as β-pinene, caryophylene, y-cadinene, β-phellendrene, β-caryophyllene and β-bourbonene have been reported (Adam *et al.,* 2005). Yacon leaf has been used for centuries by the original inhabitants from the Andes valley as traditional folk medicine to treat chronic diseases. Yacon leaf essential oil contains monoterpene, sesquiterpene and diterpene.

Materials and Methods

The research work was carried out between the months of June and August, 2017 in the Chemistry Laboratory of Chemistry Department, Taunggyi University. A Hewlett Packard GC model HP-6890 equipped with a 30 meter of 5 % phenyl dimethyl siloxan (HP-SMS) capillary column with a Hewlett Packard MS model HP-5973 was used for GC-MS analysis.

Plant Material

The yacon leaf was collected from Hti Wore Village, Taunggyi Township, and Southern Shan State. The plant was verified at the department of Botany, Taunggyi University. The cleaned and air dried sample was powdered by grinder and stored in air tight container to prevent the moisture and other contaminations.

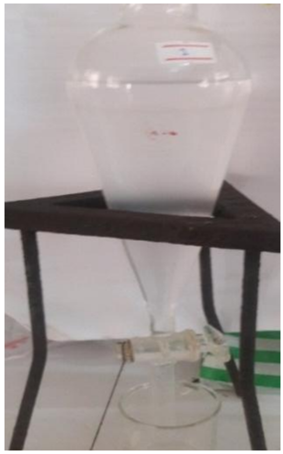
Extraction of the Essential Oil

A modified Clevenger type apparatus was used for the extraction of essential oil from the leaf of yacon through steam distillation (Figure 1). In order to extract the essential oil, 50 g of the dried powder sample was placed in a 500 mL round bottom flask, 250 mL of distilled water added, connected to the Clevenger apparatus and the flask was heated in the heating mantle to boil. The steam carrying the essential oil was condensed and collected in the Clevenger apparatus attached to the condenser. The essential oil was extracted from the water-oil mixture into petroleum ether. The petroleum ether extract was dried over anhydrous sodium sulphate, filtered, and the petroleum ether was removed. The essential oil obtained after evaporation of petroleum ether was kept in a closed bottle in a refrigerator until further analysis.

Analysis of the Essential Oil by GC-MS

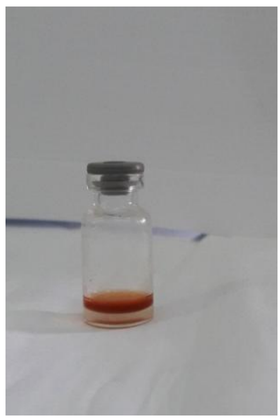
The constituents of the essential oils are volatile and gas chromatography- mass spectrometry (GC-MS) is usually used for their analysis. The mass spectrum of the compound eluted at each retention time from GC was compared to the mass spectrum of the standard by the software to identify the compound.

(a)



(b)

(c)



(d)

Figure 1: Extraction of essential oil from the leaves of *Smallanthus sonchifolius* with Clevenger apparatus, (a) steam distillation, (b) partitioning of the distillate mixture, (c) evaporation of PE in a rotatory evaporator, (d) extracted sample of dry essential oil

Physicochemical Characterization of the Essential Oil of Yacon Leaf

Physicochemical characteristics provide a base line for suitability of oils. Specific gravity, total acid number and ester value were thus determined (Parthiban, *et al.*, 2011).

Determination of percentage oil yield

The percentage of oil yield was calculated by using the following relation (AOAC, 2000).

$$\text{Percentage of oil yield (w/w)} = \frac{\text{Weight of oil}}{\text{Total weight of materials used for oil extraction}} \times 100\,\%$$

Determination of specific gravity

For the determination of specific gravity of oil, a clean 50 mL specific gravity bottle was weighed (W_0). Then the bottle was filled to the brim with water and stopper was inserted. Extra water overflowed out. The water on the stopper and bottle were carefully wiped off and reweighed (W_1). Same process was repeated, but using oil sample instead of water and weighed again (W_2). The specific gravity of the all oil sample was calculated by using the following formula (AOAC, 2000).

$$\text{Specific gravity of test sample} = \frac{W_2 - W_0}{W_1 - W_0}$$

where, W_0 = weight of empty specific gravity bottle

W_1 = weight of water + specific gravity bottle

W_2 = weight of test sample + specific gravity bottle

Total acid number

About 0.2 g of oil was taken in 250 mL conical flask. Then 25 mL of neutral ethyl alcohol was added to it and then boiled on water bath. Phenolphthalein indicator solution (1-2 drop) was added then the mixture while hot was titrated against with standard potassium hydroxide solution with shaking. The end point was noted to the first pink colour which persist for 30 seconds (AOAC, 2000). Acid value was calculated as mg of KOH per gm of oil.

$$\text{Acid value} = \frac{56.1 \times V \times N}{W}$$

where, V = Volume of standard KOH solution in mL

W = Weight of oil sample in grams

N = Normality of standard KOH solution

Ester value

Essential oil (1g) was introduced into a flask and 25 mL of ethanolic solution of KOH (0.5 N) was added. The condenser was connected and the round bottom flask was placed in the heating mantle and heated for one hour. After cooling, 20 mL of distilled water and 5 drops of 0.2 % phenolphthalein as indicator, were added to it. Finally, the excess of KOH solution was titrated with hydrochloric acid (0.5 N), alongside the operation cited, blank was made under the same conditions and with the same reagents (AOAC, 2000). Then the ester value was calculated according to the following equation.

$$\text{Ester Value} = \frac{28.5 \times (B - V)}{W}$$

where, B = volume, in milliliters of 0.5 N hydrochloric acid required for the blank

V = volume, in milliliters of 0.5 N hydrochloric acid required to neutralize the excess of alkali used for the hydrolysis

W = weight, in grams of oil taken

Evaluation of Antimicrobial Activity of the Essential Oil of Yacon Leaf

Antibacterial activity of essential oil of yacon leaf

The antimicrobial activities of essential oil were determined by agar well diffusion method (Agarry *et al*., 2005).

Nutrient agar (4.6 g) and agar (1 g) were dissolved in 20 mL of distilled water. The resulting nutrient agar medium was autoclaved at 121 °C for 20 min and cooled in water bath at 60˚C. After cooling, bacterial suspension of each bacterial strain (0.02 mL) was added and poured into petridishes. The seeded plates were allowed to dry in room temperature for 20 min. A standard corn borer of 10 mm diameter was used to cut uniform wells on the surface of the solid medium. Each of the different crude extracts (0.15 mL) were filled into each of the wells and incubated at 37˚C for 24 h. Antibacterial activities in terms of zone diameters of inhibition (mm) were recorded after 24 h of incubation (Lawrence *et al*., 2009).

Antifungal activity of essential oil of yacon leaf

Potatoes (20 g) in 100 mL distilled water were heated on hot plate until boiling and filtered. Potato infusion was obtained. Dextrose (2 g) and agar (1.5 g) were added to potato infusion to obtain the potato dextrose agar medium. The resulting potato dextrose agar medium was autoclaved at 121˚C for 20 min and cooled in water bath at 60˚C. After cooling, fungal suspension of each fungal strain (0.1 mL) was added and poured into petridishes. The seeded plates were allowed to dry in room temperature for 20 min. A standard corn borer of 10 mm diameter was used to cut uniform wells on the surface of the solid medium. Each of the different crude extracts (0.15 mL) were filled into each of the wells and incubated at 37˚C for 72 h. Antifungal activities in terms of zones of inhibition was recorded (Ibrahim *et al*., 2011).

Results and Discussion

Extraction of Essential Oil from Yacon Leaf

Extraction oil of yacon leaf was extracted by using the steam distillation method. The yield percent of essential oil was found to be 0.283 % based on the dry powdered sample.

Chemical Composition by GC-MS Analysis

Gas Chromatography-mass spectrometry (GC-MS) is the single most important method for identification of components of essential oils, by using software to match recorded spectra of compounds with reference spectra in the spectral library and by spectral interpretation also. Traditional “Library search" methods for compound identification find compounds in reference library whose spectra most closely resemble the recorded spectrum. The GC chromatogram of essential oil in yacon leaf is shown in Figure 2. At the retention time 6.70 min, the MS spectrum shows the molecular ion peak at m/z 136 identified as 3-carene by matching with the spectra data base library in Figure 3. Similarly MS spectra of further eluted compounds at retention times 8.86 min, 11.73 min, 11.87 min, 20.03 min, 22.18 min and 22.47 min were also identified as 3-carene, γ-terpinene,1-methyl-3- (1-methylethyl) benzene, D-limonene, caryophyllene,1-methylene-2β-hydroxylmethyl 3,3-dimethyl-4β-(3-methylbut-2-enyl) cyclohexane and 1-formyl-2,2-dimethyl-3 trans- (3-methylbut-2enyl)-6-methylenecyclohexane, respectively, by matching with the spectral data base library in Figures 4, 5, 6, 7, 8, and 9 and Table 1.

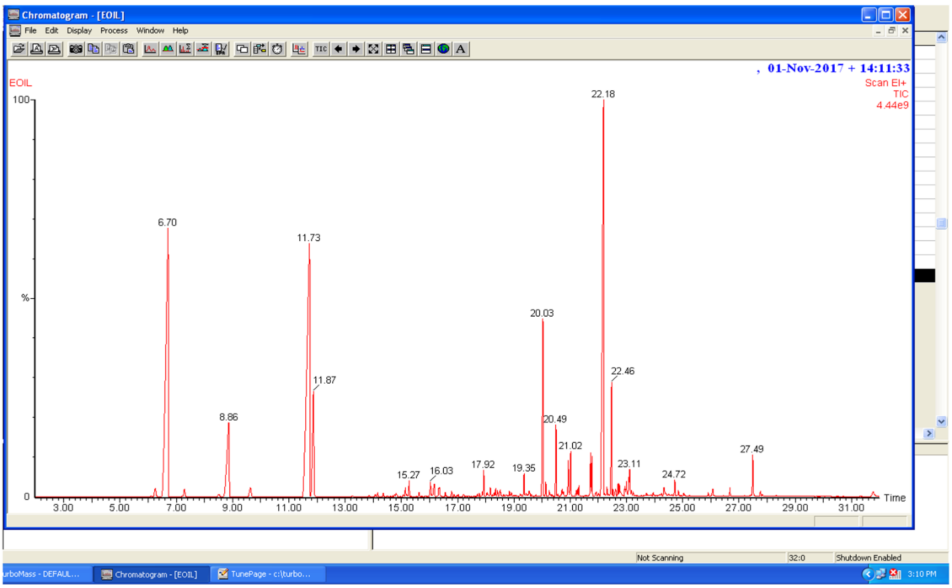


Figure 2 : Gas chromatogram of essential of yacon leaf

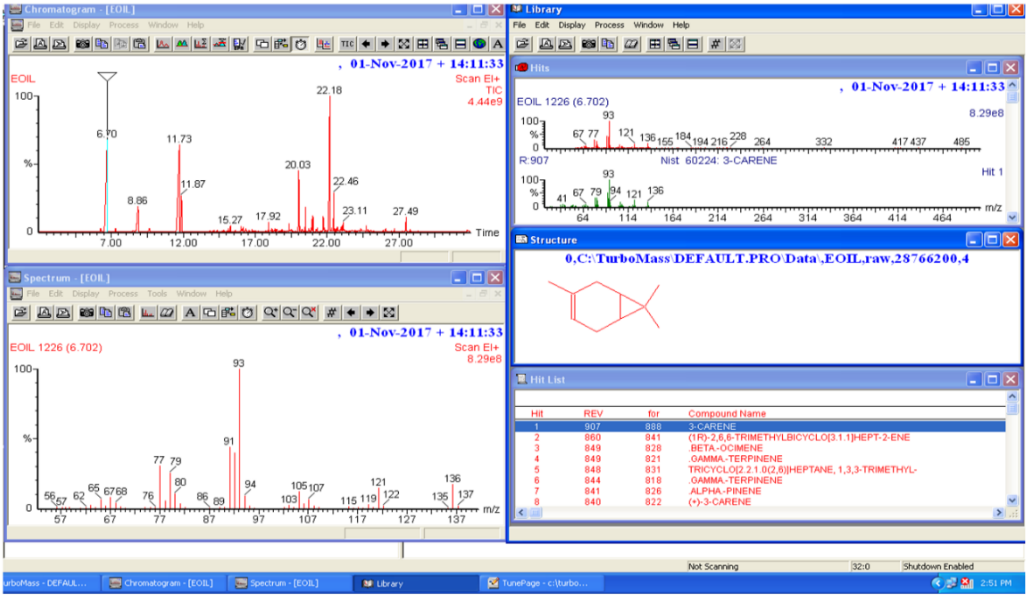


Figure 3: Gas chromatogram and mass Spectrum of the eluted compound at RT 6.70 min compared with the standard spectrum of 3-carene

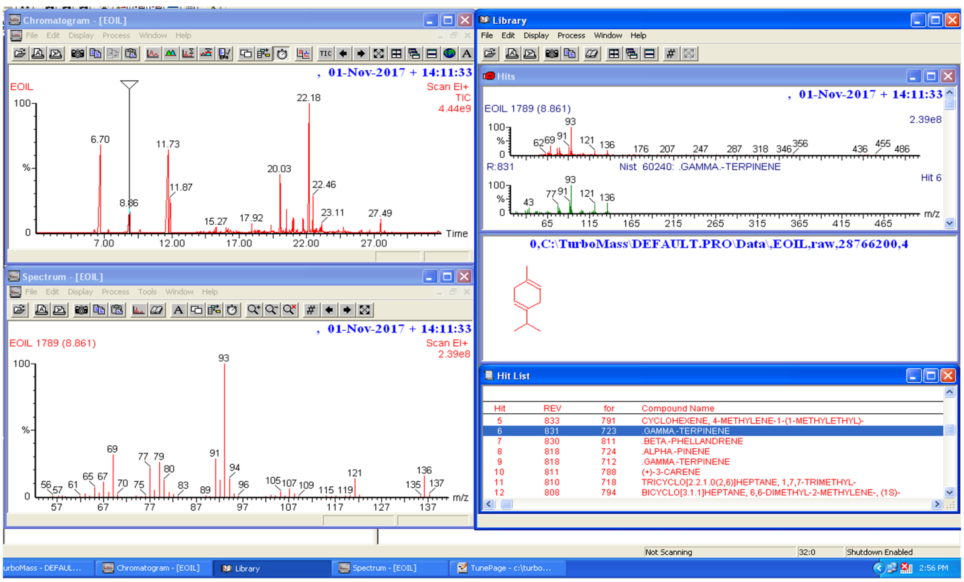


Figure 4: Gas chromatogram and mass Spectrum of the eluted compound at RT 8.86 min compared with the standard spectrum of γ -terpinene

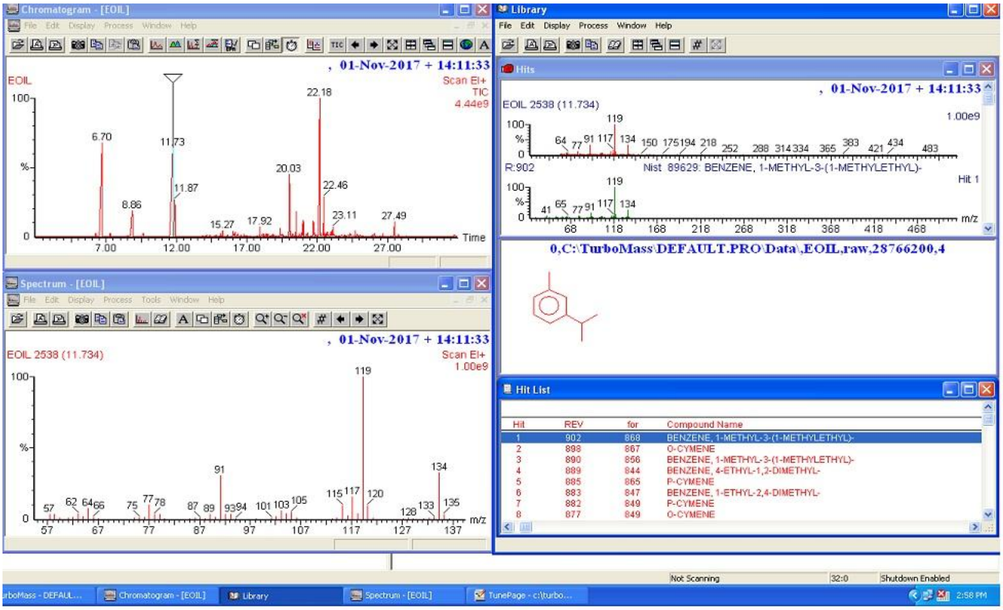


Figure 5: Gas chromatogram and mass Spectrum of the eluted compound at RT 11.73 min compared with the standard spectrum of 1-methyl-3-(1-methyl ethyl) benzene

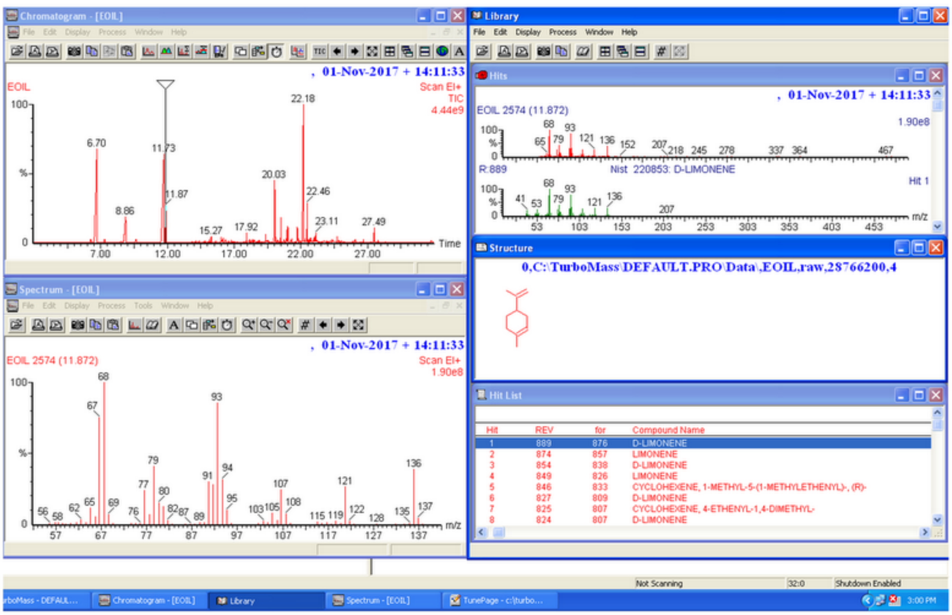


Figure 6: Gas chromatogram and mass Spectrum of the eluted compound at RT 11.87 min compared with the standard spectrum of D-limonene

Figure 7: Gas chromatogram and mass Spectrum of the eluted compound at RT 20.03 min compared with the standard spectrum of caryophyllene

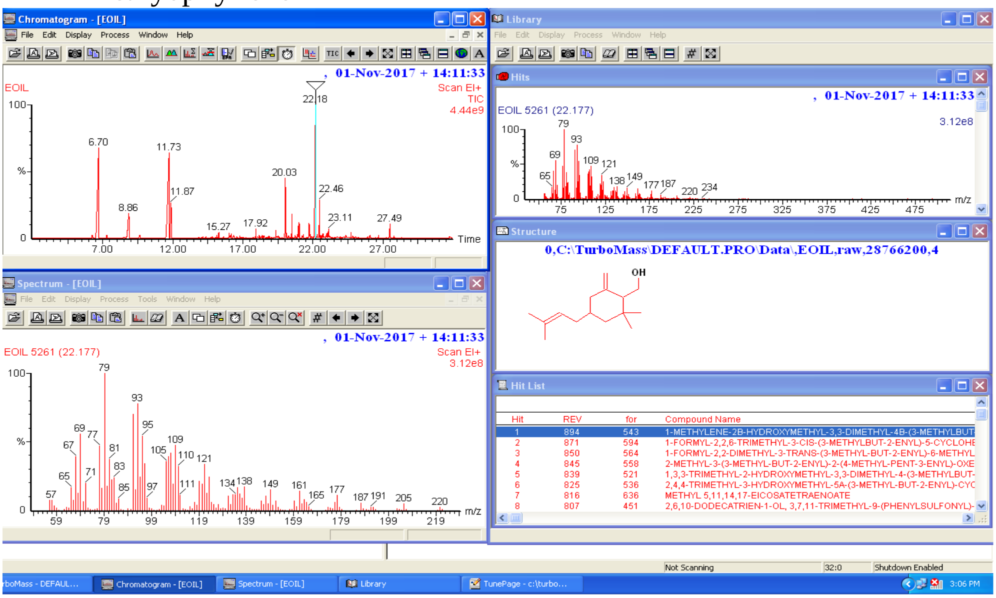


Figure 8: Gas chromatogram and mass Spectrum of the eluted compound at RT 22.18 min compared with the standard spectrum of 1-methylene-2β-hydroxymethyl-3,3-dimethyl-4β-(3methylbut-2-enyl) cyclohexane

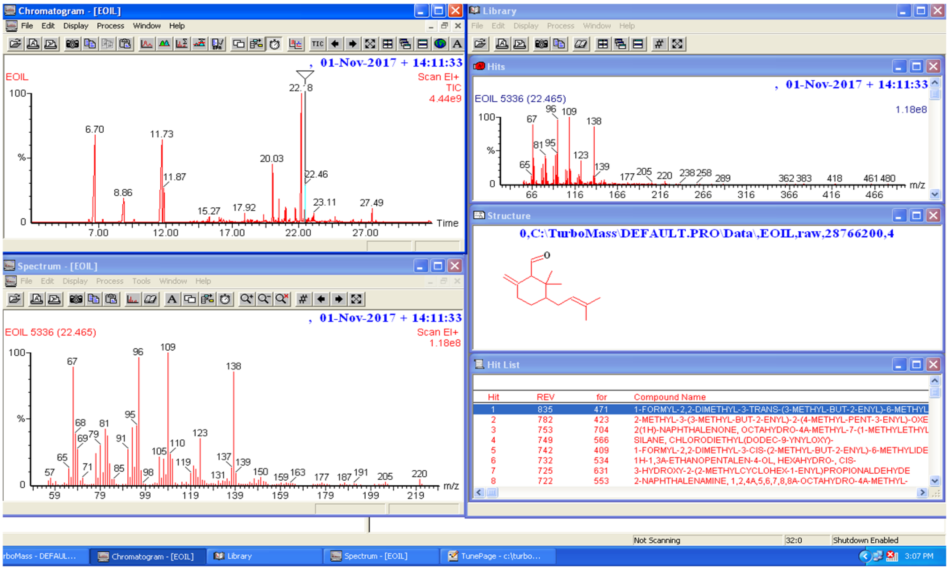


Figure 9: Gas chromatogram and mass Spectrum of the eluted compound at RT 22.47 min compared with the standard spectrum of 1-formyl-2,2-dimethyl-3-trans-(3-methylbut-2-enyl)-6-methylene cyclohexane

Table 1: GC-MS Analyzed Chemical Compounds in Leaf Oil of Yacon

Sr.no	Compounds Present	Retention Time (min)
1.	3-carene	6.70
2.	γ-terpinene	8.86
3.	1-methyl-3- (1-methyl ethyl)benzene	11.73
4.	D-limonene	11.87
5.	caryophyllene	20.03
6.	1-methylene-2β-hydroxylmethyl 3,3-dimethyl-4β-(3-methylbut-2-enyl)cyclohexane	22.18
7.	1-formyl-2, 2-dimethyl-3 trans- (3-methylbut-2-enyl)-6-methylene cyclohexane	22.47

Physicochemical Investigation of Essential Oil in Yacon Leaf

In the present study, essential oil obtained from leaf of *S. sonchifolius* was evaluated for physicochemical characteristics (Table 2). Specific gravity is the ratio of the density of a respective substance to the density of water at 4° C. Specific gravity value of oil 0.936 are less than 1 for most of the oils except for those containing oxygenated aromatic compounds. In the present study the essential oil from leaf of yacon has low specific gravity value of 0.936 (AOAC, 2000).

The acid number measures the amount of acids present in oil. This is expressed as the number of milligrams of KOH, which are required to neutralize the free fatty acids present in one gram of oil. Acid value is an indirect method for determination of free fatty acid of amount in oil samples and its edibility. Oil with low free fatty acids has more significant usage. The total acid number value recorded in the present study is 2.805 mg KOH/g. This value is within the permissible limits i.e. 10 mg KOH/g of oil and therefore found to be suitable for dietary purposes, as they contain lower fatty acid content.

Ester value determined was found to be 57 mg KOH/g for essential oil of yacon leaf. This value is expressed as the number of milligrams of KOH required by combining with fatty acids present in the glyceride form in 1 g of oils of fats.

Table 2: Physicochemical Properties of Essential Oil

Properties	Values
Specific Gravity	0.936
Acid Value	2.805 mg KOH/g
Ester Value	57 mg KOH/g

Antimicrobial Activity of Essential Oil

In vitro study showed the effectiveness of essential oil of yacon leaf by inhibiting six tested microganisms by agar well diffusion method. The well diameter was 10 mm in this experiment. The larger the zone diameter, the more active against the tested microorganisms.

The antimicrobial activity of essential oil in yacon leaf was low activity on all tested microorganisms, namely, *Bacillus subtillis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia colli*. Inhibition zones of essential oil are shown in Figure 10 and Table 3.

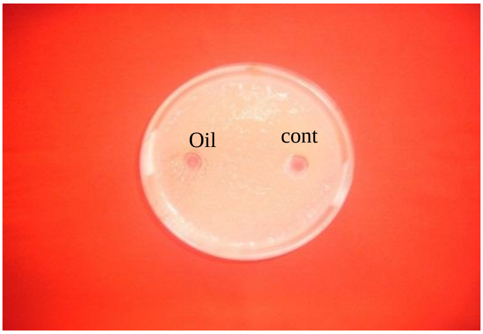
Table 3: Antimicrobial Activity of Essential Oil of Yacon Leaves

		Zone Diameters of inhibition of Microorganisms (mm)					
Samples	**Solvent**	***B.subtilis***	***S.aureus***	***P.aeruginosa***	***B.pumilus***	***C.albicans***	***E-coli***
Control	pet-ether	-	-	-	-	-	-
Essential oil	Pet-ether	14 (+)	13 (+)	13 (+)	14 (+)	14 (+)	14 (+)

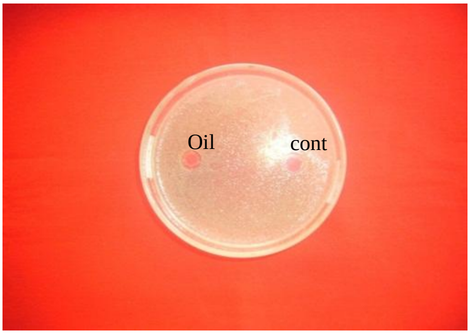
Organisms

Agar Well – 10 mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)

(1) *Bacillus subtilis* (N.C.T.C – 8236)
(2) *Staphylococcus aureus* (N.C.P.C – 6371)
(3) *Pseudomonas aeruginosa* (6749)
(4) *Bacillus pumilus* (N.C.I.B – 8982)
(5) *Candida albicans*
(6) *E – oil* (N.C.I.B – 8134)



Bacillus subtilis



Staphylococcus aureus

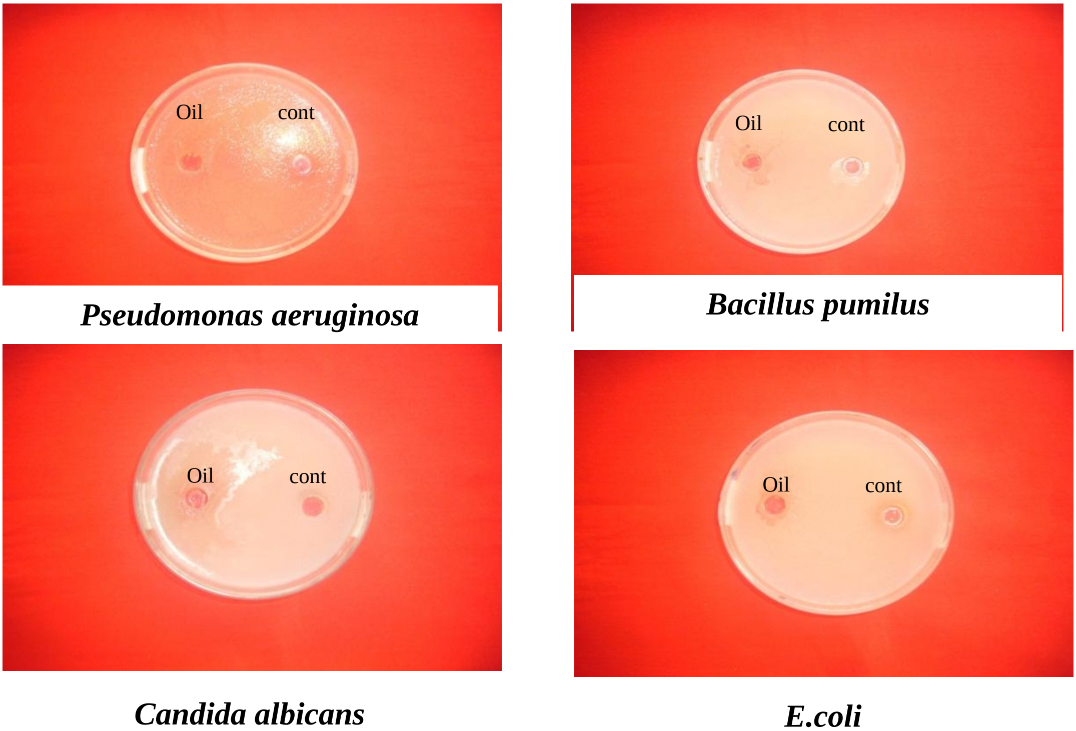


Figure 10: Antimicrobial activities of essential oil of *Smallanthus sonchifolius* (yacon) leaf

Conclusion

The essential oil (yield 0.283 %, based on the dry powdered sample) was extracted from yacon leaves by steam distillation method using Clevenger apparatus. GC-MS analysis showed that 3-carene,γ -terpinene, 1-methyl-3-(1-methyl ethyl)bezene, D-limonene, caryopohyllene, 1-methylene-2β-hydroxymethyl-3, 3-dimethyl- 4β- (3-methylbut-2enyl) cyclohexane and 1-formyl-2, 2-dimethyl -3-trans- (3-methylbut-2-enyl) -6-methylene cyclohexane were present in the essential oil. Additionally, physicochemical properties data are very important to control the quality of essential oil. The specific gravity value recorded in the present study was 0.936. Total acid number was 2.805 mg KOH/g. Ester value determined was 57 mg KOH/g. The essential oil of yacon leaf exhibited antimicrobial activities against all tested microorganisms. Therefore yacon leaf has a good potential of giving valuable health benefits for human beings.

Acknowledgements

The authors would like to thank the Myanmar Academy of Arts and Science for allowing to present this paper and Professor and Head Dr Ah Mar Yi, Department of Chemistry, Taunggyi University for her kind encouragement.

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