ISOLATION OF SOME PHYTOCHEMICAL CONSTITUENTS FROM YACON TUBERS OF TAUNGGYI AREA

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Abstract

The *Smallanthus sonchifolius* (yacon) tubers were dried at the ambient temperature, chopped into bits and percolated with70% ethanol. Qualitative phytochemical screening was determined by the standard procedure suggested the presence of alkaloids, flavonoids, steroids, polyphenols, terpenoids,α-amino acids, glycosides, phenolic compounds, carbohydrate, saponins, tannins, protein, and reducing sugars. Starch and cyanogenic glycosides were not detected in yacon tubers. The profile of the chemical constituents present was established by thin layer chromatography. Six pure organic constituents namely, stigmasteryl acetate (0.003%), β-sitosteryl acetate (0.005%), hexadecanoic acid (0.009%), β-sitosterol (0.02%), chlorogenic acid (0.12%) and β-sitosterol β-D-glucoside (0.8%) based on ethyl acetate extract were isolated from the ethyl acetate soluble fraction of the 70% ethanol extract of the yacon tuber on a silica gel column by gradient elution with petroleum and ethyl acetate (PE-EA, 99:1 to EA only). The isolated compounds were characterized by UV and FTIR.

Keywords : *Smallanthus sonchifolius*, yacon tuber,percolation, phytochemical screening, ethyl acetate extract, UV, FTIR

Introduction

Yacon, *Smallanthus sonchifolius* (Poepp.&Endl.) H. Robinson, is a plant originally cultivated in South America, and the fresh root is eaten like a fruit in this area. Yacon was introduced to Japan in 1985 and has been gradually paid attention to due to its abundant content of fructooligo saccharide, which has some health-promoting effects such as improvement in the intestinal microflora balance, as a storage sugar in place of starch in its root

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(Nakanishi, 1997). Yacon tuber is sometimes used for home cooking but has not been a common foodstuff because of decaying easily or rapid browning of the juice or injured tissues. The browning may be caused by condensation reaction of polyphenols with amino compounds (Yabuta *et al.*, 2001) and enzymatic polymerization of polyphenols (Mayer, 1987). Yacon juice contains 850 ppm polyphenol compounds which generally have anti-oxidative activity and chlorogenic acid was reported as a major antioxidant in yacon as well as tryptophan (Yan *et al.*, 1999). In this study, investigation on ethyl acetate fraction of yacon tubers founds six compounds.

Materials and Methods

Smallanthus sonchifolius (yacon tuber) was collected from Naung Karr Village, Taunggyi Township, Southern Shan State in July 2016 and identified at Department of Botany, Taunggyi University. UV and IR spectra were recorded on Shimadzu 1800 UV-Vis Spectrometer and Shimadzu 8400 Fourier Transform- Infrared Spectrophotometer using KBr pellet. Aluminium backed silica gel GF_{254}precoated plates from Merck were used for TLC analysis.

Isolation of Compounds from YaconTubers

The yacon tuber was dried at ambient temperature and pulverized to coarse grained powder.Dry powder (120 g) was applied to extract with 70% ethanol using percolation method and the resultant crude extract obtained was filtered.The crude extract was concentrated under reduced pressure using a rotary evaporator to obtain a solvent free 70% ethanol extract. This extract (29 g) was stirred with ethyl acetate to obtain ethyl acetate soluble extract (0.5 g), which was separated on silica gel column by gradient elution with PE:EA (99:1, 9:1, 5:1, 3:1, 1:1, 1:2, 1:4 and EA only).Fractions obtained were collected in 10mL flasks. Identical fractions were pooled based on their TLC profiles thus resulting in 6 combined fractions.Further purification using methanol solvent was carried out on fractions that were impure.

Phytochemical Analysis

Qualitative phytochemical screening was carried out to determine the presence or absence of alkaloids, flavonoids, steroids,polyphenols, terpenoids, α-amino acids, glycosides, phenolic compounds, carbohydrate, saponins, tannins, starch, protein, reducing sugars, cyanogenic glycosides and using the standard literature procedure (William and Douglas, 2006).

Spectroscopic Analysis

The isolated compounds were characterized by Fourier transform infrared (FTIR) and ultraviolet spectroscopy. FTIR spectra were recorded in which samples were prepared as KBr pellets at the Universities' Research Centre, Yangon University. UV spectra were recorded on methanol solutions at the Chemistry Department, West Yangon University.

Results and Discussion

Phytochemical Analysis

The dried powder sample of yacon tuber showed the presence of various phytochemicals namely, alkaloids, flavonoids, steroids, polyphenols, terpenoids, α-amino acids, glycosides, phenolic compounds, carbohydrate, saponins, tannins, protein, and reducing sugars. Starch and cyanogenic glycosides were not detected in the sample (Table 1).

Table 1 Phytochemicals Present in Tubers of *Smallanthus sonchifolius*(Yacon)

No.	Test	Extract	Reagent Used	Observation	Remarks
1	Alkaloids	1% HCl	Dragendorff's reagent	Orange ppt.	+
2	Flavonoids	95% EtOH	Mg ribbon and conc: H_2SO_4	Pink color	+
3	Steroids	PE	Acetic anhydride and conc: H_2SO_4	Blue color	+
4	Polyphenols	EtOH	1% $FeCl_3$ and 1% $K_3[Fe(CN)_6]$	Greenish-blue color	+
5	Terpenoids	EtOH	Acetic anhydride and conc: H_2SO_4	Red color	+

No.	Test	Extract	Reagent Used	Observation	Remarks
6	α- Amino acids	EtOH	Ninhydrin reagent	Violet color	+
7	Glycosides	H_2O	10% lead acetate	White ppt.	+
8	Phenolic Compounds	H_2O	10% $FeCl_3$	Brown color	+
9	Carbohydrates	H_2O	10 % α-naphthol and conc: H_2SO_4	Red ring	+
10	Saponins	H_2O	Distilled water	Frothing	+
11	Tannins	H_2O	2% NaCl and 1% gelatin	Yellow brown color	+
12	Starch	H_2O	I_2 solution	No blue color	–
13	Protein	Hot H_2O	NaOH and $CuSO_4$	Yellow ppt.	+
14	Reducing Sugars	H_2SO_4 (dil)	NaOH and Benedict's solution	Brick red ppt.	+
15	Cyanogenic Glycosides	H_2O	conc: H_2SO_4, sodium picrate paper	No brick red	–

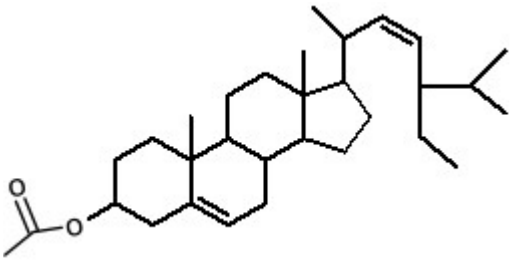
(+) = present, (-) = absent, (ppt.) = precipitate

Identification of the Isolated Compounds

After purification of the fractions from the silica gel column with methanol, six pure compounds were obtained. Thin layer chromatograms of six isolated compounds from EA extract of yacon tuber were identified by UV and IR spectroscopic methods. IR spectral database (AIST) was used for comparison.

Compound M_1

The isolated compound **M_1** could not be observed under short and long wavelength UV lights on TLC plate, but it gave a dark violet spot upon heating with anisaldehyde-sulphuric acid reagent (R_f 0.43, Silica gel GF_{254}, PE:EtOAc, 99:1). This suggests a steroid/terpenoid compound. The IR spectrum (Figure 1) of **M_1** showed absorptions at 1739 and 1261 cm^{-1} corresponding to C=O and C-O stretching vibrations of an acetate ester. Apart from this, the remaining bands are very similar to those of stigmasterol. From this observation, it may be deduced that **M_1** is stigmasteryl acetate. The assignment of IR bands is summarized in Table (2). Therefore **M_1** should be stigmasteryl acetate.



Stigmasteryl acetate (**M_1**)

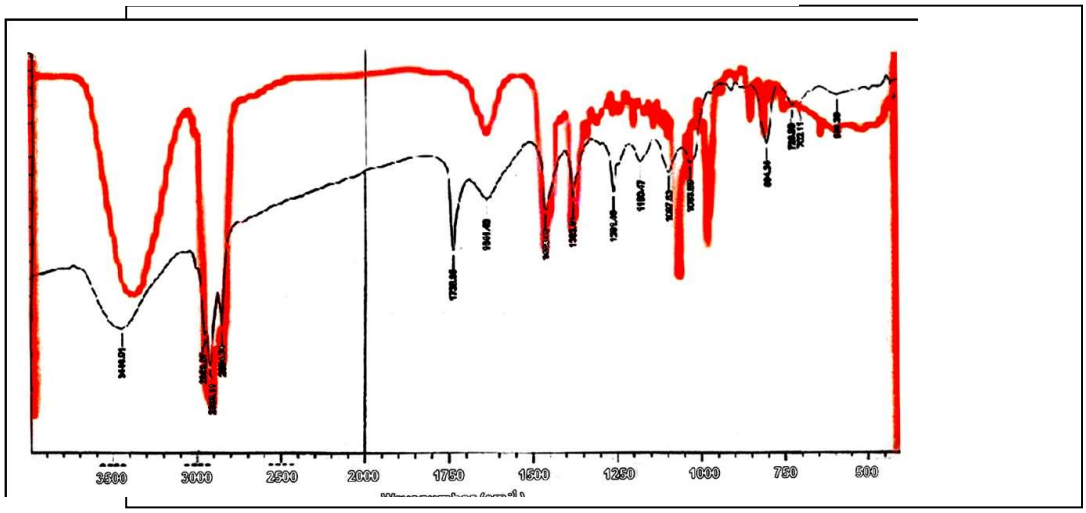


Figure 1 Overlaid IR spectra of M_1(black) and stigmasteryl acetate (red) (AIST)

Compound M_2

The isolated compound **M_2** could not be observed under short and long wavelength UV lights on TLC plate, but it gave a dark violet spot upon heating with anisaldehyde-sulphuric acid reagent (R_f 0.63,Silica gel GF_{254}, PE:EtOAc, 99:1). This suggests a steroid/terpenoid compound. In the IR spectrum (Figure 2) of the compound, the O-H stretching band at 3447 cm^{-1} of β-sitosterol disappeared, while new bands at 1738 cm^{-1} for C = O stretching and 1287 cm^{-1} for C-O stretching showed up, the remaining bands being similar to those of β-sitosterol. This can be seen in the overlaid IR spectra of **M_2** and β-sitosteryl acetate (Figure 2). The assignment of IR bands is summarized in Table (2). Therefore the isolated compound **M_2** should be β-sitosteryl acetate.

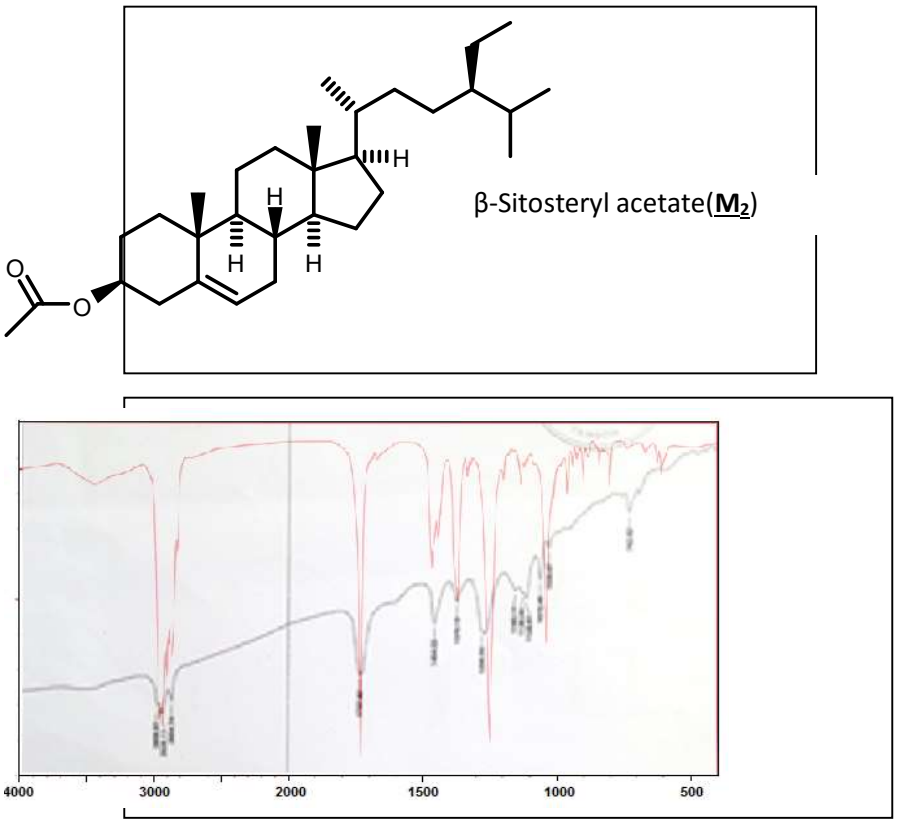


Figure 2 Overlaid IR spectra of M_2 (black) and β-sitosteryl acetate (red) (AIST)

Compound M_3

The isolated compound **M_3** (R_f 0.65, Silica gel GF_{254}, PE:EtOAc, 9:1) was invisible under UV short and long wavelengths on TLC plate, but could be visualized as pink spot upon heating with anisaldehyde-sulphuric acid reagent. The IR spectrum of isolated compound **M_3** suggests a long chain carboxylic acid by the carbonyl stretching band, very broad OH stretching band and an intense CH_2 rocking band, respectively, at 3400-2400, 1697 and 723 cm^{-1} (Figure 3). The assignment of bands is summarized in Table (3). From the close resemblance of the IR spectra (Figure 3) of **M_3** and hexadecanoic acid, **M_3** should be hexadecanoic acid. Hexadecanoic acid is also reported in the plant (Stuardxchange, 2016).

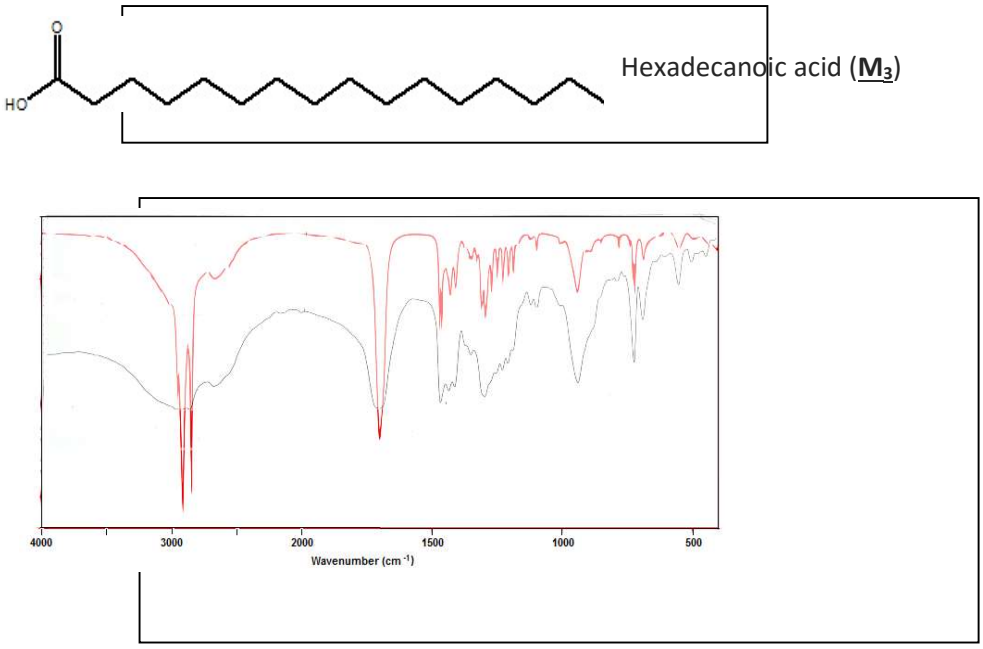
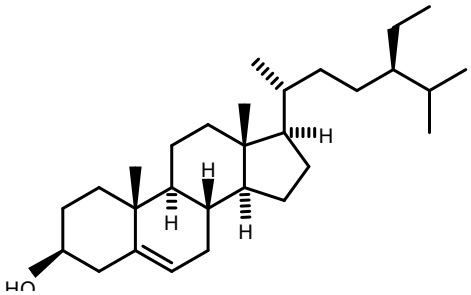


Figure 3 Overlaid IR spectra of M_3 (black) and hexadecanoic acid (red) (AIST)

Compound M4

The isolated compound **M4**(R_f 0.44, Silica gel GF_{254}, PE:EtOAc, 5:1)was invisible under UV short and long wavelengths on TLC plate, but could be visualized as violet spot upon heating with anisaldehyde-sulphuric acid reagent, suggesting a steroid/terpenoid compound. The assignment of absorption bands in IR spectrum (Figure 4) is summarized in Table (2). From the close resemblance between the IR spectra of **M4** and β-sitosterol (Figure 4), **M4** should be β-sitosterol. This is also in accordance with a previous report on the plant (Stuardxchange, 2016).



β-Sitosterol (**M4**)

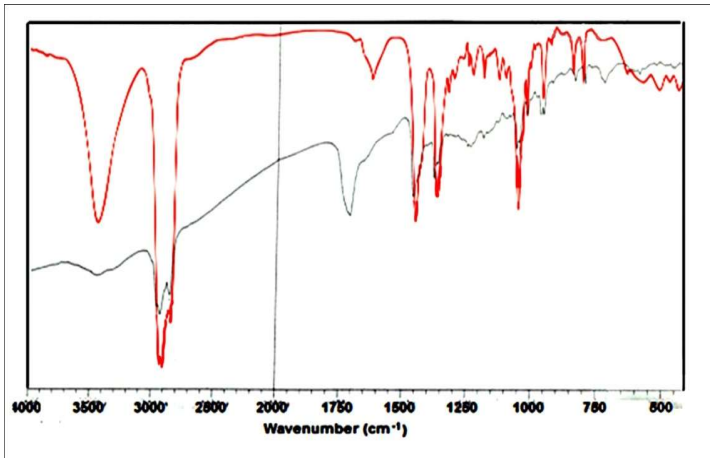
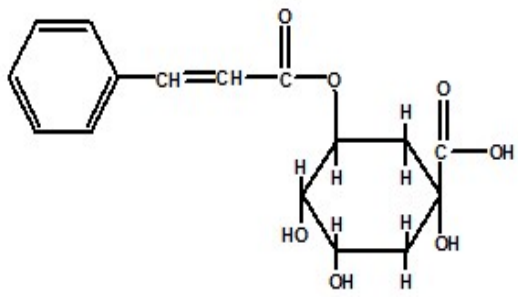


Figure 4 Overlaid IR spectra of M_4 (black) and β-sitosterol (red) (AIST)

Compound M_5

The isolated compound **M_5** (R_f 0.42, Silica gel GF_{254}, PE:EtOAc, 1:4) detectable under UV short wavelength, is a phenolic compound from its positive reaction (an orange spot) on TLC with 10% $FeCl_3$. It also indicated by the alkaline shift from 270 nm (π-π^* transition) to 315 nm (n-π^* transition) of UV absorption bands (Figure 6 and Table 3).The assignment of IR absorption bands (Figure 5) is summarized in Table (2). From the close resemblance between the spectra of **M_5** and chlorogenic acid (Figure 5), **M_5** should be chlorogenic acid. Chlorogenic acid is also previously reported in the plant (Stuardxchange, 2016).



Chlorogenic acid (**M_5**)

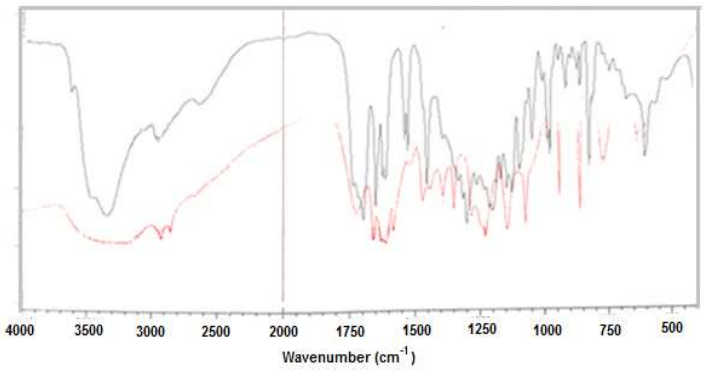


Figure 5 Overlaid IR spectra of M_5 (red) and chlorogenic acid (black) (AIST)

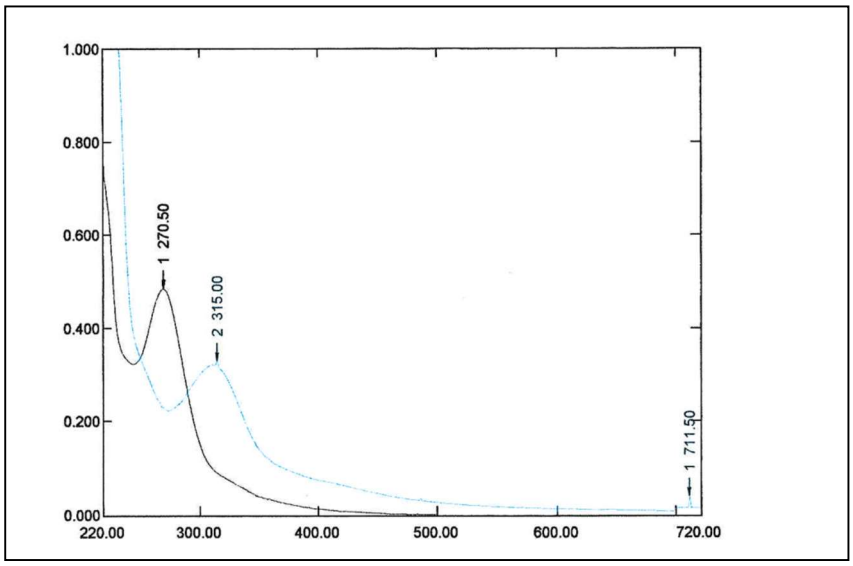


Figure 6 UV spectra of isolated compound M_5 (chlorogenic acid) from yacon tuber

Compound M_6

The isolated compound **M_6** (R_f 0.34, Silica gel GF_{254}, EtOAc) was also invisible under UV short and long wavelengths on TLC plate, but could be visualized as violet spot upon heating with anisaldehyde-sulphuric acid reagent, suggesting a steroid/terpenoid compound. But its higher polarity suggested by its TLC solvent system and intense IR absorption bands for O-H and C-O groups (Figure 7) indicate a glycoside. Apart from these two absorption bands, the remaining bands are very similar to β-sitosterol. Furthermore, the observed bands are very similar to those reported for β-sitosterol β-D-glucoside. Assignment of bands for **M_6** compared with the reported bands (Arunachalam *et al.*, 2009) for β-sitosterol β-D-glucoside is given in Tables 2 and 3.Therefore **M_6** should be β-sitosterol β-D-glucoside.

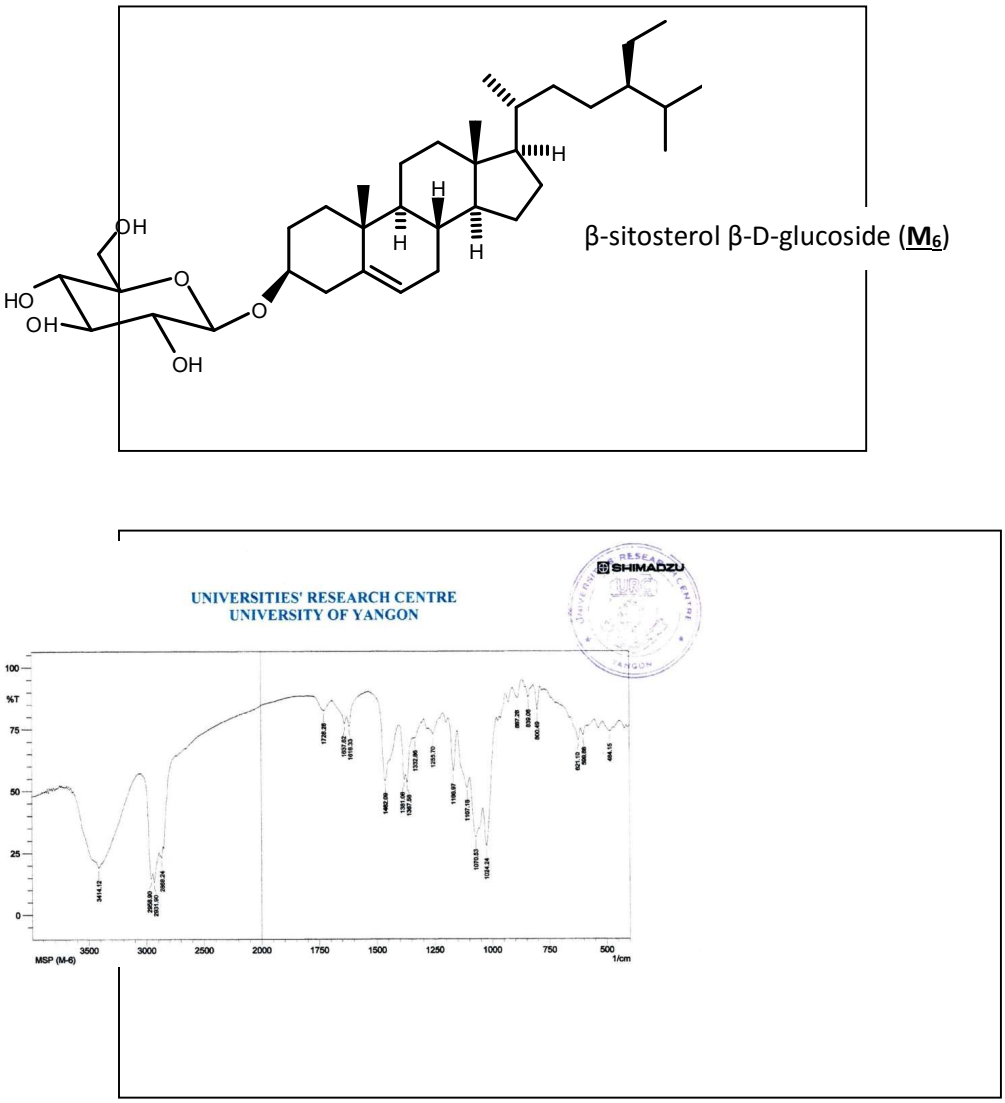


Figure 7 FTIR spectrum of isolated compound M_6 (β-sitosterol β-D-glucoside) from yacon tubers

Table 2 FTIR Spectral Data of The Isolated Compounds from Ethyl Acetate Extraction of Yacon Tuber

Sample code	Wavenumber (cm^{-1})									
	v O-H	v=C-H	v C-H	First overtone of δ CH_2	v C=O	v C=C	δas C-H	δs C-H	v C-O	δoop=C-H
M_1	-	-	2957, 2926, 2855	-	1739	-	1464	1383	1261	804, 723, 702
M_2	-	-	2960, 2926, 2855	-	1738	-	1464	1379	1287	800
M_3	3550-2500	-	2970, 2840	2675	1697	-	1466, 1412	1350	1296, 1229, 1207	939, 723
M_4	3447	-	2980, 2850	-	-	-	1464	1381, 1370	1064, 1074	839, 800
M_5	3550-2500	3100	2970, 2850	-	1726, 1659	1613, 1580, 1520, 1470	1470	-	1144, 1074	943, 864
M_6	3414	-	2960, 2850	-	-	-	1462	1381,1368	1167, 1107 1070, 1024	839, 800

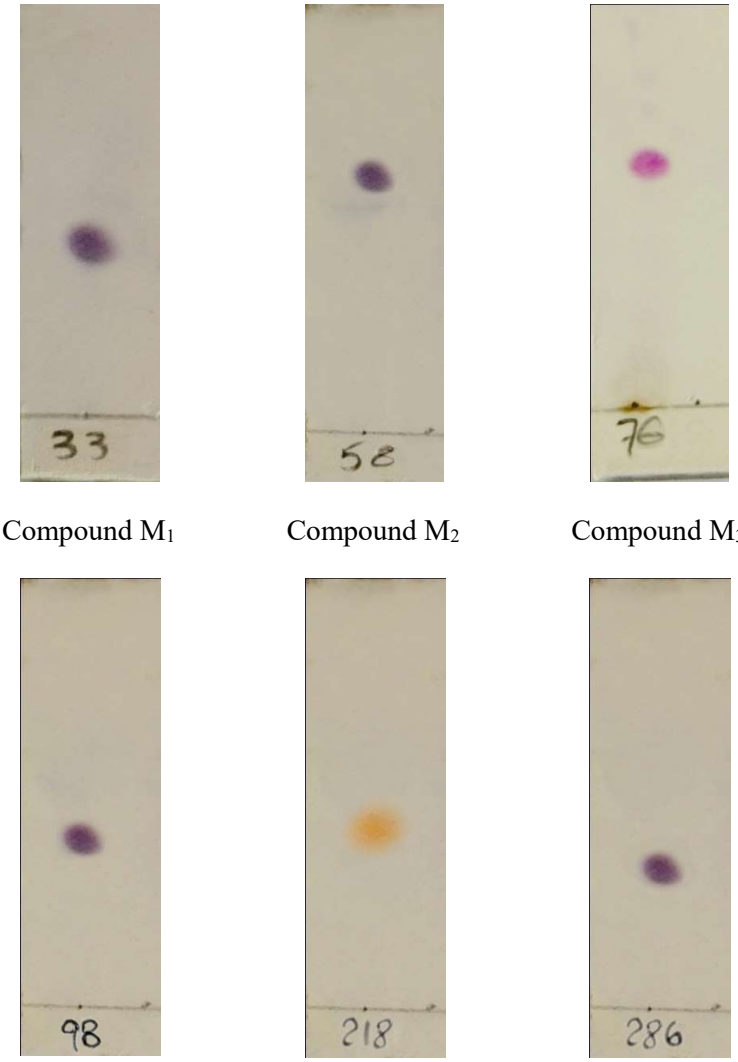
Table 3 UV Spectral Data Assignment of Compound M_5

Reagents	Observed λ_{max}(nm)	Assignment
Sample + MeOH	270.50	$\pi \rightarrow \pi^*$ transition
Sample + MeOH + NaOH	315.00	$n \rightarrow \pi^*$ transition

Table 4 Comparison of FT IR Spectral Data of Compound M_6 and β-sitosterol β-D-glucoside

Wavenumber (cm^{-1})		Vibrational mode	Group assignment
Isolated Compound	β-sitosterol β-D-glucoside*		
3414	3395	ν O-H	$-OH$
2960-2850	2934, 2870	ν C-H	$-CH_3$, $>CH_2$, $\geq CH$
1462	1461	δCH_2 & δ_{as} CH_3	$>CH_2$, $-CH_3$
1381, 1368	1373	δ_{sy} CH_3	$-CH_3$
1167, 1107, 1070, 1024	1072, 1024	ν C-O	2° alcohol & ether
839, 800	-	δ_{oop} = C-H	trisubstituted double bond

*Arunachalam *et al.*, 2009



Compound M_1 Compound M_2 Compound M_3

Compound M_4 Compound M_5 Compound M_6

Spraying regent – Anisaldehyde – conc:H_2SO_4 and 10% $FeCl_3$
Solvent system – PE : EA – 99:1, 99:1, 9:1, 5:1, 1:4, EA only
R_f value of M_1, M_2, M_3, M_4, M_5, M_6 – 0.43, 0.63, 0.65, 0.44, 0.42, 0.34

Figure 8 Thin layer chromatograms of six isolated compounds from EA extract of yacon tuber

Conclusion

The results of the present study established the presence of phytochemicals in the yacon tuber extract. Qualitative phytochemical screening was carried out to establish the presence of alkaloids, flavonoids, polyphenols, α-amino acids, glycosides, steroids, terpenoids, phenolic compounds, carbohydrate, saponins, tannins, protein and reducing sugars in the yacon tuber. Starch and cyanogenic glycosides were not detected.The experimental data also suggested the presence of significant amounts of stigmasteryl acetate (0.003%), β-sitosteryl acetate (0.005 %), hexadecanoic acid (0.009%), β-sitosterol (0.02%), chlorogenic acid (0.12 %) and β-sitosterol β-D-glucoside (0.8%) in the ethyl acetate fraction of the 70 % ethanol extract of the yacon tuber. Thus yacon, *i.e.Smallanthus sonchifolius* tubers has great potential for the human health.

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