BIOCHEMICAL STUDIES AND HEPATOPROTECTIVE POTENTIALITY OF *SMALLANTHUS SONCHIFOLIUS* (POEPP. AND ENDL.) H. ROBINSON (YACON) LEAVES

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Abstract

The present work focused on the studies of chemical constituents from the leaves of *Smallanthus sonchifolius* (Poepp. and Endl.) H.Robinson (Yacon) and some biological activities. Yacon is one of the edible plants and it is collected from Ywar Ngan Township, Southern Shan State. Phytochemical investigation of Yacon leaves was performed and it was found that alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, tannins, terpenoids, and organic acids were present, however, cyanogenic glycosides and starch were absent. Nutritional values were observed to compose by moisture (14.35 %), fiber (11.67 %), protein (11.43 %), ash (9.09 %), fat (5.31 %), carbohydrate (40.7 %) and energy value is 313.55 kcal/100 g of Yacon leaves were found to be determined by using the respective methods. In addition, the elements such as K (0.908 %), Ca (0. 801 %), S (0.120 %), Fe (0.028 %), Ba (0.008 %), Mn (0.006 %), P (0.002 %), Cu (0.002 %), Zn (0.001 %) were examined by ED XRF method. The antioxidant activities of watery and ethanol extracts of the leaves sample were determined by DPPH assay method. The IC_{50} value of watery and ethanol extracts were found to be 786.56 and 466.92 μg/mL, respectively. The ethanol extract is more effective than the watery extract. However, the two extracts show the mild activity when compared to the standard antioxidant ascorbic acid (IC_{50} = 4.57 μg/mL). *In vitro* screening of antimicrobial activity was examined by nutrient agar well diffusion method on eight different microorganisms (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aerugino, Bacillus pumilus, Candida albicans, Escherichia coli, Aspergillus flavus and Aspergillus niger*). Ethyl acetate and 95 % ethanol extracts of Yacon leaves showed antimicrobial activity on eight strains of microorganisms (inhibition zone diameter 12 mm to 30 mm). Watery extract against other strains expect *A. flavus* (inhibition zone diameter 13 mm to 20 mm). In addition, petroleum ether and methanol extracts against other strains except *P. aeruginosa* (inhibition zone diameter 11 mm to 30 mm). The cytotoxicity of methanol extract from Yacon leaf against hepatoma liver cancer cell HepG2 was evaluated by MTT assay. The IC_{50} value of methanol extract was found to be 53.68 μg/mL for 24 h treated time. The results of this study scientifically validate the traditional use of Yacon leaves for the treatment of liver diseases.

Keywords: *Smallanthus sonchifolius*, phytochemical, nutritional values, antioxidant activity, antimicrobial activity, cytotoxicity, MTT assay

Introduction

Plant is an important source of medicine and plays a key role in world health. Medicinal plants may be defined as those plants that are commonly used in treating and preventing specific ailments and diseases and that are generally considered to be harmful to humans (Schulz *et.al*., 2001). Medicinal plants have provided mankind a large variety of potent drugs to alleviate or eradicate infections and suffering from diseases in spite of advancement in synthetic drugs, some of the plant-derived drugs still retained their importance and relevance. The use of plant-based drugs all over world is increasing. Modern medicines and herbal medicines are complimentarily being used in areas for health care program in several developing countries such as countries in Africa, Asia and some part of Europe. Due to different outcomes on herbal plants, plants products surfaces all over the world due to the belief that many herbal medicines are known to be free from health and environmental effects (Angell and Kassirer, 1998). The world health organization

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estimates that the plant extracts or their active constituents are used as folk medicine in traditional therapies of 80 % of the world's population (Baker *et.al*., 2005).

Smallanthus sonchifolius (Poepp. and Endl.) H. Robinson. (Yacon) is a tuber plant that is native to the Andean region. Yacon has been consumed commonly by diabeties and persons suffering from digestive disorders. Yacon also possesses the properties to treat kidney complaints and skin-rejuvenating activity. Fructooligosaccharides are the products recognized and used as food ingredients and prebiotics (Pedreschi *et al.,* 2002). Dried yacon leaves were used to prepare a medicinal infusion or mixed with common tea leaves in Japan (Aybar *et al*., 2001). In the present work, *S. sonchifolius* (Yacon) leaves were selected to investigate phytochemical constituents and some biological activities.

Materials and Methods

Collection and Preparation of Sample

The leaves sample of *S. sonchifolius* (Yacon) was collected from Ywar Ngan Township, Southern Shan State. After collection, the botanical name of the sample was identified and confirmed as *S. sonchifolius* (Yacon) leaves at Botany Department, Dagon University. The collected fresh sample was cleaned by washing thoroughly with water and air dried. After drying, the leaf sample was cut into small pieces and ground using grinding machine. And then this powdered sample was kept in the sealed air-tight container to prevent moisture changes and other contamination. It was then used without further purification or refining.

Phytochemical investigation

The dried powdered samples were used to chemical tests for the determination if the presence or absence of the major types of phytochemical constituents such as alkaloids, αamino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, starch, saponins, steroids, tannins, terpenoids, organic acids, cyanogenic glycosides using standard procedure (Finar, 1968; M-Tin Wa, 1972; Marini *et al*., 1981; Robinson, 1983; Shriner *et al.,* 1980).

Determination of Nutritional Values

Some nutritional values such as moisture, ash, protein, fiber, fat and energy values were quantitatively determined according to AOAC methods (AOAC, 2000) and total carbohydrate contents were also quantitatively determined by phenol-sulphuric acid method (Neeru *et al*., 2015).

Elemental Analysis of Leaves Sample by ED XRF

In order to determine the heavy toxic metals and micronutrient elements in leaves sample, elemental contents in the leaves of *S. sonchifolius* were determined by ED XRF method at the Universities' Research Center, Yangon. The major advantage of X-ray spectrometry is that it offers a satisfactory compromise among economy, speed and ease of operation (Ertel, 1991).

Determination of Antioxidant Activity

DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of plant materials. This assay has been widely used of plant materials to evaluate the free radical scavenging effectiveness of various flavonoids and polyphenols in food system (Leea *et al*, 2002). In this experiment, the antioxidant activity was studied on 95 % ethanol and aqueous extract from selected leaf sample by DPPH free radical scavenging assay. DPPH

(7 mg) was thoroughly dissolved in 100 mL of 95 % ethanol. This 180 µM DPPH solution was freshly prepared in the brown coloured bottle.

The control solution was prepared by mixing the 1.5 mL of 180 µM DPPH solution and 1.5 mL of 95 % ethanol in brown bottle. Blank solution was prepared by mixing the 1.5 mL of test sample solution with 1.5 mL of 95 % ethanol. Each respective H_2O and ethanol extracts (30 mg) and 30 mL of 95 % ethanol were thoroughly mixed by shaker. The mixture solution was filtered and stock solution was obtained. Desired concentration 1000 µg/mL, 800 µg/mL, 600 µg/mL, 400 µg/mL, 200 µg/mL and 100 µg/mL of each solutions were prepared from this stock solution by dilution with appropriate amount of 95 % ethanol.

4 mg of standard ascorbic acid was dissolved in 20 mL of 95 % ethanol to get the 200 µg/mL stock solution. Desired concentrations of 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL and 3.125 µg/mL solution were prepared by two-fold serially diluted with ethanol. These bottles were incubated at room temperature and were shaken on shaker for 30 min. After 30 min, the absorbance of these solution was measures at 517 nm by using spectrophotometer (UV-KWF, China). Absorbance measurements were done in triplicate for each solution and the mean values obtained were used to calculate % inhibition of oxidation. Then IC_{50} (50 % inhibitory concentration) values were also calculated by linear regressive excel program.

Antimicrobial Activity Screening by Agar Well Diffusion Method

Antimicrobial activity of different crude extracts such as (pet ether, ethyl acetate, ethanol, methanol, water extracts) of leaves were screened in *in vitro* by agar well diffusion method (Dorman and Deans, 2000). Test microorganisms are *Bacillus subtilis*, *Staphylococus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albican*, *Escherichia coli*, *Aspergillus flavus* and *Aspergillus niger* species. This experiment was carried out at Pharmaceutical Research Department (PRD), Ministry of Industry, Yangon.

Examination of *in vitro* Cytotoxic Activity by MTT Reduction Assay method

The cytotoxicity of methanol extract of the sample was examined by using MTT reduction assay method. HepG2 cells were seeded in a 96 well flat-bottomed microliter plate at a density of 1×104 cells/ well and allowed to adhere for 24 h at 37 °C in a CO_2 incubator. After 24 h the cell were then treated with 40 to 100 µg/mL of methanol extract for 24 h at 37 °C in a CO_2 incubator. Subsequently, 10 µL of MTT solution (5 mg/mL in phosphate buffer solution) were added to each well and incubated for 4 h at 37 °C. The culture medium was discarded, and 100 µL of DMSO solution was added into each well and mixed by gently shaking for 10min.Absorbance (the interesting of the dissolved formazan crystal (purple color) was quantified using the ELISA plate (microplate reader) at 595 nm (Padhya et al., 2013). Cell viability was calculated from the mean values of the data from three wells and cytotoxic activity was expressed as the IC50 (50 % inhibitory concentration) value.

$$(\%)\ \text{Cell viability} = 100 \times \frac{A_{bs}(\text{test sample}) - A_{bs}(\text{Blank})}{A_{bs}(\text{control}) - A_{bs}(\text{Blank})}$$

Results and Discussion

Phytochemical Profile of Yacon Leaves

According to the phytochemical test results, α-amino acids, alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, tannins, terpenoids and organic acids are present in the sample but cyanogenic glycosides and starch are absent. Alkaloids, as reported by Elekwa *et al*., 2008, have been seen to interfere with cell division which makes them an important plant part to possibly be used as remedy in the treatment of cancer. Saponins and glycosides have been reported to have hypertensive and cardiac depressive properties (Trease and Evans, 1985). The primary role of carbohydrate is to provide energy to all cells (Slavin and Carlson, 2014). Flavonoids and many other phenolic components have been reported on their effective antioxidants, anticancer, antibacteria, cardioprotective agents, anti-inflammation, immune system promoting, skin protection from UV radiation (Dzialo *et al.*, 2016).

Some Nutritional Values and Elemental Analysis of Yacon Leaves

The determination of some nutritional values such as moisture, ash, protein, fat, fiber, carbohydrates and energy value contents were determined by reported methods. The moisture content of sample was determined by oven dried method and was found 14.35 %. The fat content was determined by the soxhlet extraction method and 5.31 % was obtained. In addition, the sample was also studied for fiber content by acid alkali treatment, protein content by AOAC method and ash content by using muffle furnace. The total ash in the sample is the inorganic residue remaining after the organic matter has been burnt away. The fiber, protein, ash, carbohydrates contents and energy value for Yacon leaves were found to be 11.67 %, 11.43 %, 9.09 % and 40.7 % and 313.55 kcal/100 g, respectively. The results are shown in Table 1.

X-ray spectrometer permits simultaneous analysis of light element to heavy metal. Shimadzu EDX-720 spectrometer can analyze the elements from $_{11}$Na and $_{92}$U under vacuum condition. The ED XRF spectrum of the sample results was reported in Table 2. It can be seen that essential minerals for human health such as potassium and calcium in leaves were the most predominant. The primary functions of potassium in the body include regulating fluid balance and controlling the electrical activity of the heart and other muscle strength. Calcium is key for the health of bone and teeth, but it also affects muscles, hormones and nerve function. According to ED XRF, no toxic element was found in leaves sample.

Antioxidant Activity of crude extracts from leaves of yacon

DPPH (1, 1-diphenyl-2-picryl-hydrazyl) method is the most widely reported method for screening of antioxidant activity on many plant drugs. This method is based on the reduction of colored free radical DPPH in 95 % ethanol solution by different concentration of the samples. The antioxidant activity was expressed as 50 % oxidative inhibitory concentration (IC_{50}). The percent oxidative inhibition values of crude extracts measured at different concentration and the results are summarized in Table 3. From these experimental results, it was found that as the concentrations increased, the absorbance values decreased i.e. increase in radical scavenging activity of crude extracts usually expressed in term of % inhibition. From the average values of % inhibition, IC_{50} (50 % inhibition concentration) values in μg/mL were calculated by linear regressive excel program.

From these results, it can be clearly seen that IC_{50} values were found to be 466.92 μg/mL for ethanol extract and 786.56 μg/mL for water extract. The lower the IC_{50} showed the higher the free radical scavenging activity. Ethanol extract was found to be more effective than watery extract in free radical scavenging activity. However, it was observed that all of these extracts have the lower antioxidant activity than standard ascorbic acid (IC_{50} = 4.57 μg/mL).

Table 1 Some Nutritional Value of Yacon Leaf

No.	Parameters	Contents
	Moisture (%)	11.43
2.	Carbohydrates (%)	14.35
3.	Fat (%)	5.31
4.	Protein (%)	11.67
5.	Ash (%)	9.09
6.	Crude fiber (%)	40.7
7.	Energy value (kcal/100 g)	313.55

Table 2 Relative Abundance of Element in Yacon Leaf

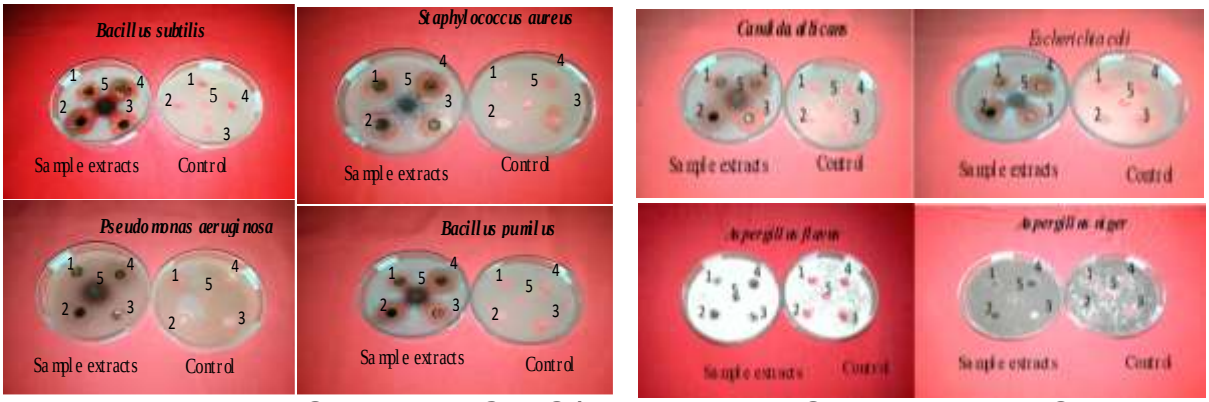
No.	Element	Relative abundance (%)
1	K	0.908
2	Ca	0.801
3	S	0.120
4	Fe	0.028
5	Ba	0.008
6	Mn	0.006
7	P	0.002
8	Cu	0.002
9	Zn	0.001
10	C H	98.122

Table 3 Percent Oxidative Inhibition and IC_{50} Values of 95 % Ethanol and Watery Extracts of Yacon Leaves

Extracts	% Inhibition (mean ±SD) in different concentrations (µg/mL)						IC_{50}(µg/mL)
	100	200	400	600	800	1000	
Watery	16.78 ± 3.78	22.72 ± 0.88	29.89 ± 0.43	41.04 ± 1.37	51.17 ± 0.85	67.62 ± 3.18	786.56
Ethanol	11.396 ± 1.79	22.61 ± 1.27	37.94 ± 4.63	73.91 ± 2.47	82.598 ± 0.609	85.48 ± 0.618	466.92

Screening of Antimicrobial Activity

Five crude extracts (PE, MeOH, EtOAc, EtOH and H_2O) were screened for antimicrobial activity against eight different microorganisms using agar well diffusion method. Larger the zone diameter, the more activity is on the test bacteria. According to the results in the Table 4. *In vitro* screening of antimicrobial activity was examined by nutrient agar well diffusion method on eight different microorganisms (*B. subtilis, S. aureus, P. aerugino, B. pumilus, C. albicans, E. coli, A. flavus and A. niger).* EtOAc and 95 % EtOH extracts of Yacon leaves showed antimicrobial activity on eight strains of microorganisms (inhibition zone diameter 12 mm to 30 mm). H_2O extract against other strains expect *A. flavus* (inhibition zone diameter 13 mm to 20 mm). In addition, PE and MeOH extracts against other strains except *P. aeruginosa* (inhibition zone diameter 11 mm to 30 mm). *In vitro* antimicrobial assays showed appreciable antibacterial activity against eight microorganisms. These results indicate that it can significantly decreases their representative diseases (such as, food poisoning, dizziness, head ache, stomach cramps, skin infections, respiratory infection, failure of heart, kidney and liver, pneumonia, diabetes, abdominal pain, fever, vomiting fatigue, joint pain, live cancer.



1. PE extract 2. MeOH extract 3. EtOAc extract 4. EtOH extract 5. H_2O extract

Figure 1 Inhibition zone of various crude extracts of yacon leaves againsts *B. subtilis, S. aureus, P. aeruginosa, B. pumilus, C. albicans, E. coli, A. flavus, A. niger*

Table 4 Inhibition Zone Diameters of Various Crude Extracts against Eight Microorganisms by Agar Well Diffusion Method

Samples	Inhibition Zone Diameters (mm) of Crude Extracts					
	Pet-ether	**MeOH**	**EtOAc**	**EtOH**	**H_2O**	**Control**
B .subtilis	23 (+++)	30 (+++)	30 (+++)	30 (+++)	20 (++)	-
S. aureus	24 (+++)	30 (+++)	30 (+++)	30 (+++)	20 (++)	-
P. aeruginosa	-		15 (+)	15 (+)	17 (++)	-
B. pumilus	20 (++)	25 (+++)	25 (+++)	30 (+++)	20 (++)	-
C. albicans	20 (++)	30 (+++)	25 (+++)	28 (+++)	20 (++)	-
E. coli	15 (+)	25 (+++)	24 (++)	20 (++)	15 (+)	-
A. flavus	11 (+)	12 (+)	13 (+)	13 (+)	-	-
A. niger	11 (+)	13 (+)	12 (+)	13 (+)	13 (+)	-

Acceptance Criteria

	Susceptible	Intermediate	Resistant
Disc Diffusion (mm)	≥ 21 (+++)	17 to 20 (++)	≤ 16 (+)

Cytotoxicity of Methanol Extract of yacon leaf

The cytotoxicity of methanol extract of Yacon leaf was evaluated by MTT assay. The cytotoxicity of methanol extract was expressed in terms of mean ± SD standard deviation and IC_{50} (50 % Inhibitory Concentration) and the results are shown in Table 5 and Figure 2**.** There are many plants extracts have been used as anticancer agents even vegetables and fruits many help reduce the risk of cancer in humans. Some Thai plants namely *Glochidion daltonii, Cladogynos orientalis, Catimbium speciosum, Acorus tatarinowii, Amomum villosum and Pinus kesiya* were also reported against the human hepatocarcinoma (HepG2) cell line (Machana *et al*., 2011).

In this study, the local plant Yacon leaves showed IC_{50} (53.68 µg/mL) for cytotoxicity against HepG2 cell line.

Table 5 Viability of HepG2 Cell by MeOH Extract of Yacon Leaves Using MTT Assay

Extract	Cell Viability (mean ±SD) in different concentrations (µg/mL)					IC_{50} (µg/mL)
	0	40	60	80	100	
MeOH	1.0 ±0.00	0.63 ±0.02	0.44 ±0.01	0.29 ±0.2	0.13 ±0.02	53.68

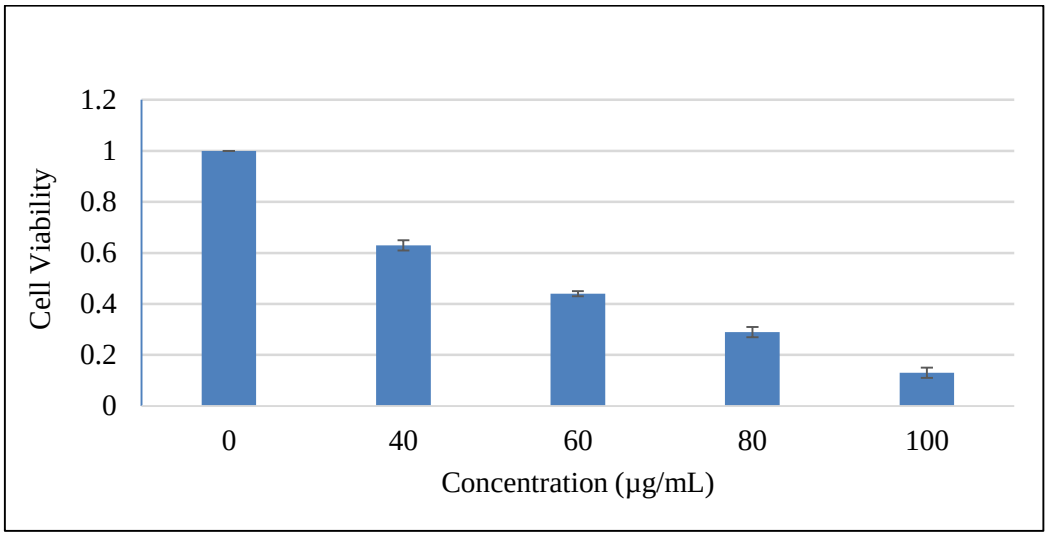


Figure 2 A bar graph of cell viability for 24 h treated by MeOH extract

Conclusion

From the overall assessments of the present work concerning with the phytochemical constituents and some biological activities of yacon leaves, the following inference could be deduced. The preliminary phytochemical investigation of yacon leaves were performed and it was found that alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, tannins, terpenoids, and organic acids were present, however, cyanogenic glycosides and starch were absent. Nutritional values were observed to compose by moisture. The nutritional values for yacon leaves were found to be good source of fiber, protein and carbohydrate. Qualitative elemental analysis of plant sample by ED XRF method showed that K and Ca were the highest amount of elements in the sample. The primary functions of potassium in the body include regulating fluid balance and controlling the electrical activity of the heart and other muscles. Calcium is one of the most important minerals for the human body. It helps form and maintain healthy teeth and bones. The radical scavenging activity of ethanol extract was found to be more effective than watery extract by DPPH assay. Antimicrobial activity showed significantly decreases population of pathogenic bacteria eight different microorganisms (*B. subtilis, S. aureus, P. aerugino, B. pumilus, C. albicans, E. coli, A. flavus and A. niger*). The IC_{50} value of MeOH extract against human liver cancer line (Hep G2) was observed 53.68 µg/mL. According to these observation, yacon leaves could be applied not only nutrition but also for therapy.

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