

STUDY ON ANTIOXIDANT ACTIVITY, ANTIMICROBIAL ACTIVITY AND ACUTE TOXICITY OF PLUKENETIA VOLUBILIS L. (SACHA INCHI) LEAVES

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Abstract

This research focused on the investigation of phytochemical constituent, mineral content, antioxidant, antimicrobial activities and acute toxicity of sacha inchi leaves. Firstly, the preliminary phytochemical screening was done. The leaves give positive test for alkaloid, flavonoid, glycoside, terpene, steroid, reducing sugar, saponin, tannin and phenolic compound but cyanogenic glycoside was not found. The mineral content was determined by EDXRF spectroscopic technique. The main minerals present in sacha inchi leaves are calcium and potassium. The antioxidant activity of ethanol extract was determined by DPPH assay. The IC₅₀ value of ethanol extract was found to be 179.60 µg mL⁻¹. Moreover, the antimicrobial activity of ethanol extract was investigated by agar well diffusion method on seven selected microorganisms. Among selected microorganisms, the ethanol extract showed the highest activity on *Bacillus pumilus* and high activities on *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescens* and *Staphylococcus aureus*. In addition, oral acute toxicity test of ethanol extract was studied by Organization of Economic Cooperation and Development (OECD) guideline (425). According to oral acute toxicity test, the tested sacha inchi leaves can be considered relatively safe to the dose level of 5000 mg/kg body weight. No toxicity effects after oral acute exposure of ethanol extract to mice were observed.

Keywords: phytochemicals, antioxidant activity, antimicrobial activity, acute toxicity

Introduction

Plants have been a valuable source of natural products for a long period of time to maintain human health. Natural products have been used in the treatment of several diseases for centuries, among them, wound healing. To heal, the wound or burn needs to free of infection. Human beings have relied on natural products as a resource of drug for thousands of years. Herbal drugs constitute only those traditional medicines, which primarily use medicinal plant preparation for therapy (Nascimento *et al.*, 2013). According to world health organization, traditional medicine is the synthesis of therapeutic experience of the generation of indigenous systems of medicine. In literature, sacha inchi leaves make a wonderful, aromatic tea that not only tastes delicious but is gluten free and contains antioxidants, leading to health benefits which include helping to reduce blood sugar levels, cholesterol and blood. Among different medicinal plants, *Plukenetia volubilis* L., a perennial oilseed vine belonging to the Euphorbiaceae family native to the rainforests of South America was selected for chemical analysis.

Botanical Aspect of *P. volubilis* L.

Scientific name	-	<i>Plukenetia volubilis</i> L.
Family	-	Euphorbiaceae
Genus	-	<i>Plukenetia</i>
Species	-	<i>volubilis</i>
English name	-	Sacha inchi, mountain peanut
Myanmar name	-	Kyalpe
Part used	-	Leaves



Figure 1 Sacha inchi leaves

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Medicinal Uses of Sacha inchi Leaves

Sacha inchi botanical extract is supposed to support the healthy functioning of the brain, heart and nervous system and to help maintain healthy levels of cholesterol and blood pressure. Encourages weight loss. It regenerates the nervous system's cells, enhancing the mood and communication abilities. It relieves the pain caused by arthritis. It cures certain skin condition. It prevent the onset of inflammation, distribute essential nutrients throughout the body, balance the mood (Health benefits times, 2020).

Materials and Methods

Sample Collection and Preparation

The sample sachu inchi leaves to be analyzed was collected from Kyaukpadaung Township, Mandalay Region. The sachu inchi leaves were cut into small pieces and air dried. And then, the sample was powdered by mortar and pestle. It was stored in well stoppered bottle and used throughout the experiment.

Preliminary Phytochemical Test of Sacha inchi Leaves

Phytochemical investigation of sachu inchi leaves powder was done according to standard procedure (Harbone, 1984).

Qualitative Elemental Analysis of Sacha inchi Leaves by EDXRF Spectrometry

The determination of minerals containing in sachu inchi leaves was studied by Energy Dispersive X-ray Fluorescence (EDXRF) spectrometer. The measurement was carried out at University of Research Centre (URC), Yangon.

Screening of Antioxidant Activity of Ethanol Extract of Sacha inchi Leaves by DPPH Assay

The antioxidant activity of ethanol extract of sachu inchi leaves was studied by DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay method. This assay has been widely used to evaluate the free radical scavenging effectiveness of various flavonoids and polyphenols in food system (Marinova *et al.*, 2011).

Determination of Antimicrobial Activity of Ethanol Extract of Sacha inchi Leaves

The antimicrobial activity of ethanol extract of leaves was tested by employing Agar well diffusion method in Meiktilar University. The tested microorganisms are *Agrobacterium tumefaciens*, *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescens* and *Staphylococcus aureus*.

Determination of Acute Toxicity of Ethanol Extract of Sacha inchi Leaves

The acute toxicity test on 95 % ethanol extract of the sachu inchi leaves could be carried out according to OECD (Organization of Economic Co-operation and Development) guidelines 425. The test substance 95 % ethanol extract of the sachu inchi leaves were administered orally in a single dose by using cannula. One group was served as the control and only vehicle distilled water was given orally. Three mice were used for each doses level. In this study, the starting dose 175 mg/kg body weight test substance was given to 3 mice. Mice were observed after dosing at least once during the first 30 min periodically during the first 24 h with special attention given during the first 4 h and daily up to 14 days.



Figure 2 Weighing fasted body weight of each female albino mice



Figure 3 Administration of leaves solution to the test mouse

Results and Discussion

Phytochemical of Sacha inchi Leaves

Preliminary phytochemical screening was carried out in order to know the different types of chemical constituents present in the leaves of *plukenetia volubilis* L. According to phytochemical tests, it gives positive tests for alkaloid, flavonoid, steroid, terpene, glycoside, reducing sugar, phenolic, saponin, tannin and cyanogenic glycoside is negative. These results are shown in Table 1. These phytochemical compounds are key micronutrients needed for the body immune system. These have a broad range of protective benefits from reducing inflammation and speeding healing to preventing infection and fighting cancer.

Table 1 Results of Phytochemical Tests of Sacha inchi Leaves

No.	Tests	Solvent Extract	Test Reagents	Observation	Results
1.	Alkaloid	1% HCl	Dragendorff's reagent Wagner's reagent	Orange ppt Brown ppt	+ +
2.	Flavonoid	95 % EtOH	Mg ribbon, Conc: HCl	pink colour	+
3.	Steroid	95 % EtOH	Acetic anhydride, Conc: H ₂ SO ₄	Green colour	+
4.	Terpene	95 % EtOH	Acetic anhydride, CHCl ₃ , Conc: H ₂ SO ₄	Reddish brown colour	+
5.	Glycoside	Distilled Water	10 % lead acetate	White ppt	+
6.	Reducing Sugar	Distilled Water	Benedict's solution	Brick red ppt	+
7.	Phenolic	Distilled Water	10 % FeCl ₃	Greenish blue colour	+
8.	Saponin	Distilled Water	Shaking	Permanent frothing	+
9.	Tannin	Distilled Water	Dil :H ₂ SO ₄ , 10 % FeCl ₃	Brown ppt	+
10.	Cyanogenic glycoside	Distilled Water	Sodium picrate solution	No brick red colour	-

(+) = presence (-) = absence ppt = precipitate

Qualitative Elemental Analysis of Sacha inchi Leaves

The elemental content of sacha inchi leaves were determined by EDXRF technique. The observed elements are shown in Figure 4 and Table 2. In accordance with Table 2, sacha inchi leaves contain significant amounts of calcium and potassium was the second most element. These minerals are considered to be essential in human nutrition to keep the blood pressure regulated.

Calcium is required for the development of bones and teeth, muscle contraction and nerve transmission. The primary functions of potassium in the body include regulating fluid balance and controlling the electrical activity of the heart and other muscles. These elements are essential because they play key roles in several body functions.

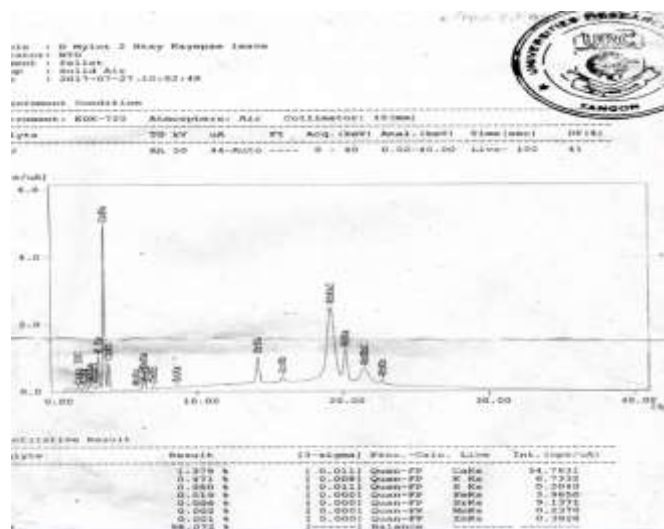


Figure 4 EDXRF spectrum of sach a inchi leaves

Table 2 Relative Abundance of Elements in Sacha inchi Leaves

No.	Symbols	Relative abundance
1	Ca	1.379
2	K	0.471
3	S	0.050
4	Fe	0.019
5	Sr	0.006
6	Mn	0.002
7	Zn	0.001
8	C,H	98.072

Screening on Antioxidant Activity of Ethanol Extract of Sacha inchi Leaves

Antioxidant activity of ethanol extract of sach a inchi leaves were studied by DPPH assay. The percent oxidative inhibition values of leaves extract measured at different concentration and the results are tabulated in Table 3. As the concentration increased, the absorbance value decreased i.e., increase in radical scavenging activity of each extract usually expressed in terms of % inhibition. The 50% inhibition concentration (IC₅₀) for leaves extract was calculated by linear regressive excel program.

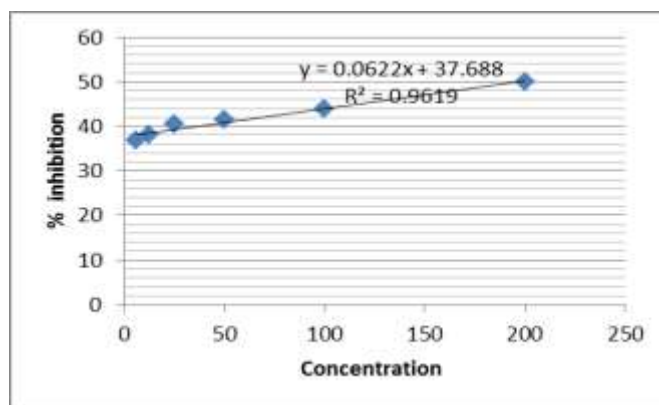


Figure 5 Percent inhibition activity of ethanol extract of Sacha inchi leaves

Table 3 Antioxidant Activity of Sacha inchi Leaves

Concentration of sample (µg/mL)	Mean absorbance	Mean % inhibition	IC ₅₀ (µg/mL)
200	0.205	49.85632	179.60
100	0.218	43.95887	
50	0.228	41.38817	
25	0.231	40.61697	
12.5	0.241	38.04627	
6.25	0.246	36.76093	

Antimicrobial Activity of Ethanol Extract of Sacha inchi Leaves

Antimicrobial activity of leaves have been investigated by Agar well diffusion method on seven selected microorganisms. Agar well diffusion method is based on the zone diameter in millimeter (mm) of Agar well. The larger the zone diameter is the more activity on the tested microorganisms. According to Table 4, the result informs that responds highest activity on *B. pumilus* and high activity on *C. albicans*, *S. aureus*, *A.tumefaciens*, *B. subtilis*, *E. coli* and *P. fluorescens*.

Table 4 Inhibition Zone Diameters of Ethanol Extract of Sacha inchi Leaves

No.	Microorganisms	Inhibition zone diameters
1	<i>A. tumefaciens</i>	13 mm (++)
2	<i>B. pumilus</i>	19 mm (+++)
3	<i>B. subtilis</i>	15 mm (++)
4	<i>C. albicans</i>	14 mm (++)
5	<i>E.coli</i>	16 mm (++)
6	<i>P.fluorescens</i>	15 mm (++)
7	<i>S.aureus</i>	15 mm (++)

Agar well- 8 mm, 8 mm -12 mm (+), 13 mm-17 mm (++) , 18mm - above (+++)

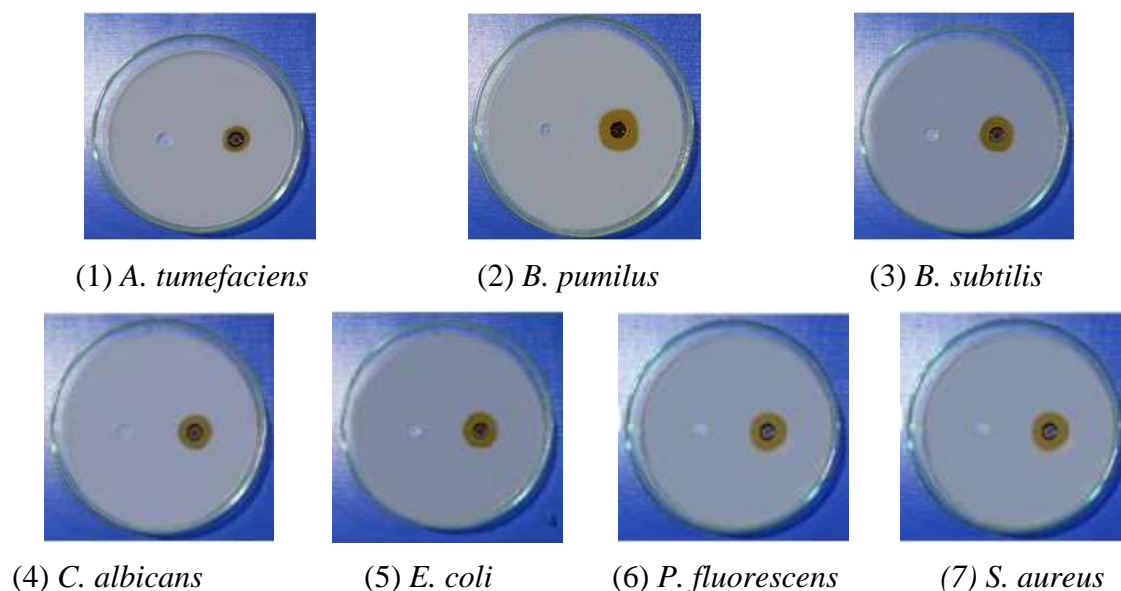


Figure 6 Inhibition zones of ethanol extract of sacha inchi leaves

Acute Toxicity of Ethanol Extract of Sacha inchi Leaves

For safety profile, the ethanol extracts of sacha inchi leaves were tested for acute toxicity study on albino mice. This tested was done according to OECD guidelines (425). In this experiment different groups of mice were used by calculating of AOT-425. The different groups of mice were administered with 4 different doses (175 mg/kg, 550 mg/kg, 1750 mg/kg, 5000 mg/kg) of ethanol extract of leaves of sacha inchi and vehicle (distilled water) 10 mL/kg body weight (control) were kept under observation for two weeks. The resultant data based on body weights record and cage side observation are presented in Table 5 and Table 6.

Table 5 Acute Toxicity Study of Sacha inchi Leaves Based on Daily Body Weight Record and Mortality Record

Test dose	Dosage of extract (mg/kg)	Sex	Body weight of mice (g)		Mortality up to 14 days
			Day 0	Day 14	
1	175	Female	35	37	Nil
2	550	Female	37	43	Nil
3	1750	Female	27	29	Nil
4	5000	Female	26	26	Nil
5	5000	Female	30	31	Nil
6	5000	Female	27	33	Nil

Table 6 Acute Toxicity Study of Ethanol Extract of Sacha inchi Leaves Based on Cage Side Observations

No.	Parameters	Observations
1	Condition of the fur	Normal
2	Skin	Normal
3	Subcutaneous swellings	Nil
4	Abdominal distension	Nil
5	Eyes-dullness	Nil
6	Eyes-opacities	Nil
7	Pupil-diameter	Normal
8	Ptosis	Nil
9	Colour and consistency of the faeces	Normal
10	Wetness of soiling of the perineum	Nil
11	Condition of teeth	Normal
12	Breathing abnormalities	Nil
13	Gait	Nil

Conclusion

In this research work, sachu inchi leaves were selected for preliminary phytochemical examination. It was found that alkaloid, flavonoid, glycoside, steroid, terpene, phenolic, reducing sugar, saponin and tannin but cyanogenic glycoside was not found. The minerals, Ca, K, S, Fe, Sr, Mn and Zn were observed in sachu inchi leaves. Among these elements calcium concentration is distinctly higher than other elements. According to the antioxidant activity study of ethanol extract of leaves using DPPH assay, the IC_{50} value was found to be $179.60 \mu\text{g mL}^{-1}$. Furthermore, the antimicrobial activity of ethanol extract of sachu inchi leaves were also investigated by employing Agar well diffusion method against seven selected microorganisms. It was observed that the ethanol extract of the leaves exhibited the highest activity on *B. pumilus* and high activity on remaining microorganisms. Moreover, from the determination of oral acute toxicity, all the tested mice from treated groups increased body weight for all the 14 days as compared with 0 day body weight. From the daily body weight record the tested mice at all dose levels showed no death. The LD_{50} value of the test substance was found to be more than 5000 mg/kg based on body weight. From the cage-side observations record, the tested animals at all dose levels showed no significant changes in behaviors before and after administration. Thus the ethanol extract of sachu inchi leaves can be considered free from toxic effects up to the dose level of 5000 mg/kg for oral administration. Therefore, the sachu inchi leaves can be used in medicinal and pharmaceutical industries.

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Online Materials

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