

Diagnostic Characters of Powdered Seeds and Antibacterial Activities of Seed Extract of *Plukenetia Volubilis* L. with Different Solvents

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Abstract

Plukenetia volubilis L. (Kyel pae) is also known as sacha inchi, star bean, Inca inchi, Inca peanut and mountain peanut belonging to the family Euphorbiaceae is grown around the world. This plant is a promising plant with high economic value; the seeds have great nutritional values and numerous health benefits. This plant was collected from Wet-Winn village, May-Myo Township and identified with the help of available literature. The morphological characters of these plants were studied and diagnostic characters of seed powdered were also examined under microscope. This plant is perennial plant and axillary raceme. Staminate flowers are cluster at the top and one or two pistillate flowers at the base. Many stamens, ovary superior, star shaped fruit and four to seven seeded per fruit, ovoid or globoid. Abundant of oil, pitted vessel, fiber, tracheid and fiber-tracheid are present in the seed powder. Preliminary phytochemical investigation of seeds of this plant shows the presence of alkaloids, glycosides, phenolic compounds, flavonoids, steroids, tannins, saponins, α -amino acids, protein, reducing sugars, starch and carbohydrates except the glycosides, flavonoids and steroids. The seven crude extracts were prepared in different concentration (0.1 μ L to 0.5 μ L) tested with *Escherichia coli* and *Staphylococcus aureus* by using agar-well diffusion method *in vitro*. Watery extract 0.5 μ L showed highest activity on *Escherichia coli* (22mm clear zone) and *Staphylococcus aureus* (24mm clear zone) than the other crude extracts.

Keywords: Morphological, Diagnostic characters, Phytochemical investigation and Antibacterial activities

Introduction

Sacha inchi (*Plukenetia volubilis* L.) belong to the family Euphorbiaceae. A very large family containing about 317 genera and 5000 species are widespread in the world, primarily in the tropics and subtropics, poorly represented in the temperate regions; about 70 genera and 470 species present in China; 30 genera and 95 species in Hong Kong (Qi-ming & De-lin, 2008). 300 genera and 750 species of cosmopolitan distribution, but best developed in the tropical and subtropical regions (Trease.W, and C. Evans, 2002). The genus *Plukenetia* is a pantropic genus of 19 species (Website-1).

In the Amazon Rainforest in Peru, sacha inchi has been cultivated by indigenous people for centuries, and will grow in warm climates up to altitudes of 1700 meters (5500 feet) as long as there is continued availability of water and good drainage it grows better in acidic soils and alluvial flats near river (Guillen. M, *et al.*, 2003). It is a perennial plant that is native to many tropical parts of South America including Peru, Bolivia, Colombia, Venezuela, Ecuador and Suriname and may be called by a number of names, including Inca Peanut and mountain Peanut, for hundreds of years. These simple fruiting bodies have been cultivated in south America by indigenous people, but the seeds are only beginning to be exported to other countries. These plants are also widely grown in Southeast Asia and have become a stap snack in Thailand. In Myanmar, it is mainly cultivated in Sagaing, Magway, Bago regions and Shan State (Website-2).

This plant is small and star-shaped, containing 4-7 pods. Its seeds have been widely called the newest superfood in certain natural healing circles. The oil derived from these seeds is also highly sought after for its concentration of antioxidants and active organic compound. These seeds do contain a high level of calories and other health benefits such as weight loss, hair care, skin care, lower blood pressure, improves digestion, prevent diabetes, lower LDL cholesterol, raise HDL cholesterol, prevent cognitive disorders, improve sleep quality, eye care, prevent diarrhea and cramping and hemorrhoids. Sacha inchi seeds are packed with nutrients, including and, as well as omega 7 (vacceinic acid), potassium, various other antioxidants and unsaturated fatty acid (FAS). These FAS include the ability to prevent cardiovascular disorders, lower glyceride levels and antithrombotic action (Website-3).

Cultivated for centuries in South America, sacha inchi oil from seed has long been used for its health and medicinal properties. This plant seed contains Omega-3, 6 and 9 (essential fatty acid), vitamin E, protein (Website-3, 4 & 5), fat (good or healthy fat), dietary fiber and magnesium (Website-3 & 4), high vitamin A(Website-3 & 5),

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serotonin (hormone) (Website-4), essential amino acid (Website-5), sacha inchi oil has great potential economic value in cosmetics, pharmaceuticals, and food industries. Additionally, its oil is a promising resource for biofuel production and its shells can be used to synthesize a silver nanocatalyst (Website-5). Its seeds have a high anticancer potential that protects cells from oxidation to lipoproteins, a key factor in preventing cancer from spreading. Rich in omega-3 of seeds has an anti-cancer power that prevents the appearance of certain types of cancer such as colon, breast and prostate and even influences in reducing the size of tumor (Website-6).

Sacha inchi oil comes from organic seeds that can be used in cosmetic application, hair treatment, function food ingredient, food oil, and dietary/health supplement. Our research shows 10 major health benefits; they include promoting brain function and cognitive development, preventing and reducing high blood pressure, risk of stroke and heart diseases, decreasing cancer, preventing and reversing depression and other emotional and mental health challenges, developing eye vision, treating rheumatoid arthritis and side effects of chemotherapy (Website-5).

WHO, (1998) reported that plants have important effects due to the presence of secondary metabolites of the different chemical composition such as terpenes, steroid, flavonoid, alkaloids, arthocyanins, saponin, phenolic compounds, tannins, and quinones. Their medicinal value is based on their pharmacological action on the human and animal organism. *Escherichia coli* and *Staphylococcus aureus* are the most common bacteria found in the environment and the human body, opportunistic pathogens that cause severe and life-threatening infections in immunocompromised patients. Thus, the control of these two bacteria in the food industry is required (Hammer K.A., *et al.*, 1999 & Islam K., *et al.*, 2014).

Escherichia coli bacteria normally live in the intestines of people and animals. Most are harmless and actually are an important part of a healthy human intestinal tract. However, some are pathogenic, meaning they can cause illness, either diarrhea or illness outside of the intestinal tract (Website-7). *Staphylococcus aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis (Website-1). Therefore, in this research, medicinal valuables of sacha inchi are selected. The present research includes morphology, diagnostic characters of powdered seeds and antibacterial activities of sacha inchi seed were investigated on *Escherichia coli* and *Staphylococcus aureus*.

Materials and Methods

Plukenetia volubilis L. (sacha inchi) were collected from Wet-winn village in May Myo Township during flowering and fruiting period from March to July, 2019. The plants specimen were identified at the Botany Department, Yangon University of Distance Education with the help of literatures of Nath Niar, 1962; Hooker, 1885; Guillen, *et al.*, 2003; Dawn Berkeloar & Tim Motis, 2018.

The seeds of *Plukenetia volubilis* L. were dried in room temperature for one month and then pulverized by using grinding mill for microscopical characters, sensory characters, phytochemical investigations and anti-bacterial activity tests. Microscopical studies of powdered seeds were examined by the method of B.P Pandey, 2002; S.N. Pandey, A. Chadha, 1996; Metcalfe & Chalk, 1950; Trease & Evans, 2009 and the sensory characters of powdered seeds were carried out at Botany Department, Yangon University of Distance Education. For preliminary phytochemical investigations, tests for alkaloids, glycosides, phenolic compounds, flavonoids, steroids, terpenoids, tannins, saponins, α -amino acids, protein, reducing sugars, starch and carbohydrates were done by using various solvents according to the British Pharmacopoeia, 1968; Marrini Bettalo *et al.*, 1981; Central Council for Research in Unani Medicine, 1987 and Trease & Evans, 2002 at Botany Department, Yangon University. In addition, antibacterial activities of powdered seeds were tested with two pathogenic organisms such as *Escherichia coli* and *Staphylococcus aureus* by using agar well diffusion assay methods (Cruickshank, 1975) at Botany Department, Dagon University.

Results

Scientific Name	- <i>Plukenetia volubilis</i> L.
Myanmar Name	- Kyel-pae
Common Name	- Star bean, Sacha inchi, Sacha peanut, mountain peanut, Inca nut, Inca peanut
Family	- Euphorbiaceae

Morphological Characters

Perennial woody climber and monoecious plant. Leaves are alternate, triangular, ovate, heart-shaped, apex acute and base cordate, serrate, densely pubescent present lower surface than the upper ones, petiolate and stipulate. Inflorescences are axillary raceme. Staminate flowers are cluster at the top and pistillate flowers at the base. Staminate flowers are cream colour, bracteates (minute and caducous), bracteolate, pedicillate, unisexual, actinomorphic. Perianths are 4 or 5, aposepalous, valvate, petaloid, and ovate. Stamens are many attached on the convex receptacle, apostemonous exerted, the filament filiform, anther ditheous, longitudinal dehiscence, dorsifixed. Pistillate flowers are one or two, bracteate, bracteolate, pedicellate, unisexual, actinomorphic, perianth are 4, valvate, sepalous (green), triangular, ovary superior. Carpels are four to seven, syncarpous, tetralocular, axile placentation, one ovule in each locule, the style long and the stigma 4-7 lobed. Fruits are capsule, star-shaped, 4-7 lobed, green in juvenile, blackish brown when mature. Seeds are 4-7 seeded per fruit, ovoid or globoid.



Figure 1. Habit

Figure 2. Inflorescence

Figure 3. Male Flower

Figure 4. Female Flower



Figure 5. L.S. of Female Flower



Figure 6. T.S of Ovary



Figure 7. Fruits

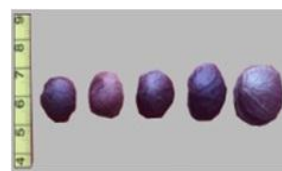


Figure 8. Seeds

Sensory Characters of the powdered seeds of *Plukenetia volubilis* L.

The color of seeds was cream color and the dour was aromatic. The tastes of powdered seeds were slightly acrid. The textures of seeds were fibrous. The results were shown in Figure 9 and 10.



Figure 9. Seeds



Figure 10. Powdered Seeds

Microscopical characters of the powdered seeds of *Plukenetia volubilis* L.

The pitted vessel was present with long tail. The fiber was hollow lumen and long with pointed of both ended. Tracheid and fiber-tracheid were long and thickened at the end. The oils were abundant and druses of calcium oxalate crystals were present in the seeds. There were shown in figure 11-15.



Figure 11. Pitted Vessel, Oil and Druses Crystals



Figure 12. Fiber, Oil and Druses Crystals



Figure 13. Tracheid and Druses Crystals

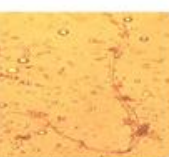


Figure 14. Fiber-tracheid, Oil and Druses Crystals



Figure 15. Oil and Druses Crystals



Figure 16. Preliminary Phytochemical test for alkaloids, glycosides, flavonoids, phenolic compounds, steroids, terpenoids, tannins, saponins, α -amino acids, protein, reducing sugars, starch and carbohydrates

Preliminary Phytochemical Test of the Powdered Seeds of *Plukenetia volubilis* L.

In this study, preliminary phytochemical investigation of seeds of this plant showed the presence of alkaloids, phenolic compounds, steroids, tannins, saponins, α -amino acids, protein, reducing sugars, starch and carbohydrates except the glycosides, flavonoids and terpenoids. The results were shown in Table 1 and Figures 16.

Table (1) Preliminary Phytochemical Test of *Plukenetia volubilis* L. Seed

No.	Tests	Extracts	Test Reagents	Observation	Results
1	Alkaloid	Methanol Methanol Methanol	1% HCL + Mayer's reagent 1% HCL + Wagner's reagent 1% HCL + Hager's reagent	White ppt. Brown or reddis brown ppt. Yellow ppt.	+ + +
2	Glycosides	Methanol	1ml H ₂ O + NaOH	White color	-
3	Phenolic compounds	Methanol	2ml H ₂ O + 10% FeCl ₃	Blackish/green color	+
4	Flavonoids	Methanol	Mg coil+HCl(dil)	White color	-
5	Steroids/Terpenoid	Methanol	CHCl ₃ +H ₂ SO ₄ (con)	reddish brown/ White color	+/-
6	Tannins	Water	5% FeCl ₃ +H ₂ SO ₄ (dil)	Yellow brown ppt./Reddish brown ppt.	+
7	Saponin	Water	Shaken with 2mlH ₂ O	Forthing	+
8	α -Amino acid	Water	Ninhydrin reagent	Pink spot	+
9	Proteins	Water	Million's reagent (heated)	White ppt. turned red when heated	+
10	Reducing sugar	Water	1ml H ₂ O and mixture equal part fehling's A and B	Brick red ppt.	+
11	Starch	Water	Iodine	Blue black/brown ppt.	+
12	Carbohydrates	Water	1ml benedict's reagent and boil for few minutes	Brick red ppt./red ppt.	+

(+) Present (-) Absent

Antibacterial activities from the seeds of *Plukenetia volubilis* L.

Two bacterial namely *Escherichia coli* and *Staphylococcus aureus* were subjected to test bacterial activities. In this experiment, watery extract 0.5 μ L showed highest activity on *Escherichia coli* (22mm clear zone) and *Staphylococcus aureus* (24mm clear zone) than the other crude extracts. The results were shown in Table 2 and Figures 17, 18.

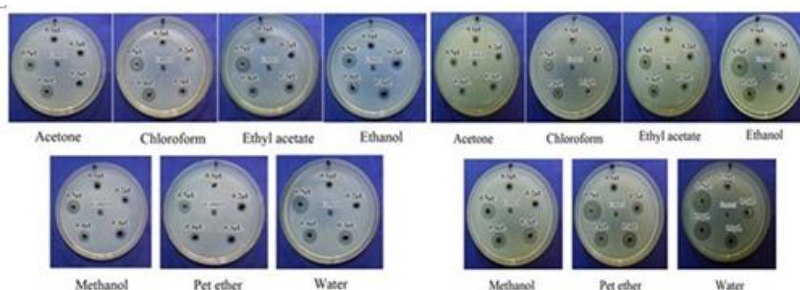


Figure 17. *Escherichia coli*

Figure 18. *Staphylococcus aureus*

Table 2. Different concentration on antibacterial activities of seed extracts of *Plukenetia volubilis* L.

Sample	Test organisms	<i>Escherichia coli</i> (N.C.I.B-8134)					<i>Staphylococcus aureus</i> (N.C.P.C-6371)				
		0.1µL	0.2µL	0.3µL	0.4µL	0.5µL	0.1µL	0.2µL	0.3µL	0.4µL	0.5µL
Sacha inchi Seed	Solvents Extracts										
	Acetone	10 mm	10 mm	10 mm	14 mm	14 Mm	10 mm	10 mm	10 mm	10 mm	10 mm
	Choloroform	10 mm	10 mm	12 mm	16 mm	16 Mm	10 mm	10 mm	10 mm	12 mm	12 mm
	Ethyl acetate	10 mm	10 mm	12 mm	12 mm	16 Mm	10 mm	10 mm	10 mm	12 mm	14 mm
	Ethanol	10 Mm	10 mm	10 mm	10 mm	14 Mm	10 mm	10 mm	10 mm	14 mm	18 mm
	Methanol	10 Mm	14 mm	14 mm	14 mm	18 Mm	10 mm	10 mm	14 mm	14 mm	14 mm
	Petether	10 Mm	10 mm	12 mm	12 mm	14 Mm	10 mm	14 mm	16 mm	16 mm	20 mm
	Water	10 Mm	10 mm	18 mm	18 mm	22 Mm	12 mm	14 mm	16 mm	20 mm	24 mm
Control	Solvents	0.2µL					0.2µL				
	Acetone	-					-				
	Choloroform	-					-				
	Ethyl acetate	-					-				
	Ethanol	-					-				
	Methanol	-					-				
	Petether	-					-				
	Water	-					-				

Agar well – 8mm

DISSCUSSION AND CONCLUSION

Plukenetia volubilis L. (sacha inchi) belong to the family Euphorbiaceae. This plant is an important new crop with application in the food and pharmaceutical industries (Website-7).

According to Hooker (1885), genus *Plukenetia* was twining shrubs. Leaves are alternate, often cordate, entire or toothed. Flowers are axillary, leaf opposed male flower are usually minute and fascicled. Female flower presents lower on the raceme. Male flowers are sepals 4-5, valvate, filament short, free, anther erect. Female flowers, sepals are valvate, ovary 3-4 celled, cell-one ovuled, capsule subglobose or depressed. Seeds are globose. This genus characters are the same with the studied morphological characters in the research.

The sacha inchi plant reaches a height of 2 meters with alternate, heart-shaped, serrated leaves. The male flowers are small, white and arranged in clusters. Two female flowers are located at the base of the inflorescence. The fruits are capsule, green and ripen blackish brown, four to five lobes, some may have up to seven, and seeds are oval. These characters are agreement with those described by Guillen, *et al.*, 2003; Dawn B & T, Motis, 2018.

In microscopical study; pitted vessel, fiber, tracheid and fiber-tracheid, abundant oil and druses of calcium oxalate crystals are present in the powdered seeds.

The seeds of sacha inchi are of great interest because of their high oil content (35-60%) (Follegatti – Romero, *et al.*, 2009), oil (41-54%) (Crutierr, *et al.*, 2011; Hamaker, *et al.*, 1992). The oil was evaluated in a 4 month ingestion study (10-15ml per day) by adults, showing it was safe and tended to increased blood level of HDL cholesterol. (Gonzales GF, Gonzales, C (2014)). star bean oil has a high content of polyunsaturated fatty acid, alpha-linolenic acid (omega-3) and linoleic acid (omega 6) have beneficial effects on human health by preventing several diseases like arthritis, cancer, diabetes, attention deficit hyperactivity dis-order and inflammatory skin diseases (Gogus and Smith, 2010; Hanssen and Schmity H, 2011), coronary heart disease and hypertension (Gogus and Smith, 2010; Hanssen and Schmity H, 2011, Follegatti – Romero, *et al.*, 2009).

Metcalfe & Chalk (1950) described that family Euphorbiaceae possess crystals. Website-8, the main minerals present in sacha inchi seeds were calcium (2406ppm). Calcium plays a role in many of your body's basic functions. Your body needs calcium in order to circulate blood, move muscles, and release hormones.

Calcium also helps carry messages from your brain to other parts of your body (Website- 9).

In this study, preliminary phytochemical investigation of seeds of star bean shows the presence of alkaloids, phenolic compounds, steroids, tannins, saponins, α -amino acids, protein, reducing sugars, starch and carbohydrates except the glycosides, flavonoids and terpenoids. Several chemical composition studies have shown that star bean (*Sacha inchi*) seeds contain various health-promoting compound such as alkaloids, phenolic compound, phytosterol (Chirinos, *et al.*, 2013; Kornsteiner, *et al.*, 2006), saponins (Srichamnong W., *et al.*, 2018), protein (Crutierrer, *et al.*, 2011; Hamaker, *et al.*, 1992) and carbohydrate (Website-7). Therefore, *sacha inchi* seeds should be considered as an important dietary source of health promoting phytochemicals.

In Gonzalez, *et al.*, 2015 reported that *sacha inchi* is also used traditionally for skin care, in order to maintain skin softness, and for the treatment of wounds, insect bites and skin infections, in a tropical context where the skin is frequently damaged. Laboratory testing with certified seed oil, determined antibacterial activity against radio labelled *Staphylococcus aureus*. According to Friedman, *et al.*, 2013, it was almost five times more active on the detachment of *S.aureus* from human skin explants. *Sacha inchi* oil possessed antimicrobial activity on both *E.coli* and *S.aureus*.

In this research of two organisms namely *Staphylococcus aureus* and *Escherichia coli* were subjected to test bacterial activity. In the present study, the powdered seeds were extracted by using different organic solvents such as acetone, chloroform, ethyl acetate, ethanol, methanol, pet ether and distilled-water. Among them, it was recorded that watery extract (0.5 μ L) concentration on *Escherichia coli* (22mm) and *Staphylococcus aureus* (24mm) showed most activities.

Therefore, *sacha inchi* seed has been well known in the treatment of intestinal tract infections and skin diseases. In addition, the watery extracts (0.5 μ L) concentration provided widest clear zone of 24 mm on *Staphylococcus aureus* which can more affect the skin diseases. The present results also supported finding of Gonzalez *et al.*, 2015 and Friedman, *et al.*, 2013.

Thus, it can be concluded the *sacha inchi* plant has significant medicinal values and makes people healthy. The findings of this study provide useful information on the diagnostic characters, phytochemical tests and antibacterial activities. It may also help to human get more medicinal values from the consumption of *sacha inchi* seed in Myanmar.

Acknowledgement

We have to recognize the special thanks to Dr. Khin Thant Sin, Pro-rector, Yangon University of Distance Education for her permission to make in this research work. Our grateful thanks go to Dr. Nu Nu Swe, Professor and Head, Department of Botany, Yangon University of Distance Education, for her experience and advice in this research. We also thank to Dr. Kay Thi Mya, Professor, Department of Botany, Yangon University of Distance Education, for her suggestion.

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