Investigation on Antimicrobial Activities of Leaves of *Panaxpseudoginseng* Wall.

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Abstract

The main aim of this research paper is to investigate the antimicrobial activities of leaves of Panax pseudoginseng Wall. Panax pseudoginseng Wall. is not only a well-known medicinal herb but also a very good sex tonic. The qualitative phytochemical screening of leaves of Panax pseudoginseng Wall. was performed by using standard methods. The mineral elements of leaves of Panax pseudoginseng Wall. were analyzed by Energy Dispersive X-ray Fluorescence, EDXRF Spectrometer. Seven minerals were found in leaves of Panax pseudoginseng Wall. The antimicrobial activities of the crude extract of leaves of Panax pseudoginseng Wall. in various solvent systems were tested by agar well diffusion method on six selected organisms, such as Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and Escherichia coli. The ethanol crude extract of these leaves showed high activities on all tested organisms except n-hexane and ethyl acetate crude extracts. These antimicrobial activities of leaves of Panax pseudoginseng Wall. can be a significance of antimicrobial medicines to treat infection.

Keywords: leaves of *Panax pseudoginseng* Wall, phytochemical screening, mineral elements, antimicrobial activities

1. Introduction

Plants are the main sources of new pharmaceuticals and healthcare products because of bioactive compounds such as phytochemicals. The phytochemicals are basically divided into two groups, i.e., primary and secondary constituents. Primary constituents comprises common sugars, amino acid, proteins, chlorophyll, fats and lipids while secondary constituents consists of alkaloids, terpenoids, steroids and flavonoids, etc [1].

Minerals cannot be synthesized biochemically by living organisms. Plants get minerals from soil. Most of the minerals in a human diet come from eating plants and animals or from drinking water. Minerals are classified as either major minerals (calcium, phosphorus, potassium, sodium and magnesium) needed in amount greater than 100 milligram per day or trace minerals (sulphur, iron, chloride and copper) needed in amounts less than 20 mg daily. And the antimicrobial medicines can be divided into antibotics that are used against bacteria and antifungals that are used against fungi [2].

Among the medicinal plants, *Panax pseudoginseng* Wall. is known to be rich in medicinal and nutritional values. Ginseng is any one of the eleven species of slow-growing perennial plants with fleshy roots, belonging to the family Araliaceae. Most researchers believe that ginseng was first used as a food. The most popular ginsengs are Asian ginseng, American ginseng and Korea ginseng in the world. Ginseng has been used as a medicine for over two thousand years. In Latin, the word *panax* means "cure-all", and the family of ginseng plants is one of the best-known herbs.

Panax pseudoginseng Wall. flowers in May to June and fruits in July to October. *Panax pseudoginseng* Wall. is economically important for food and medicine. Its young leaves and shoots are eaten as vegetables while root is used as medicine. It is distributed in Northern Myanmar, Northern India, Bhutan, Southeastern Tibet, Western China and Thailand [5].

The leaves and the flowers of *Panax pseudoginseng* Wall. are antibacterial, anti-inflammatory, antiseptic, aphrodisiac, cardiotonic, diuretic, expectorant, haemostatic, hypoglycaemic and stimulant. The flowers are also used to treat vertigo and dizziness. The root is used internally in the treatment of indigestion, vomiting, coronary heart disease, angina and nosebleeds [3].

1.1. Botanical Description of *Panax pseudoginseng* Wall.

Scientific name : *Panax pseudoginseng* Wall.
Family : Araliaceae
English name : Himalayan ginseng
Myanmar name : Myanmar ginseng
Locality : Putao, Kachin State
Habit : A perennial herb
Parts used : Leaves

The plant, leaves, flowers and roots of *Panax pseudoginseng* Wall. are shown in Figure 1.

Figure 1. Plant, leaves, flowers and roots of *Panax pseudoginseng* Wall.

2. Materials and Methods

2.1. Sample Collection and Preparation

The leaves of *Panax pseudoginseng* Wall. were collected from Putao, Kachin State, in October 2018. The leaves of *Panax pseudoginseng* Wall. were washed with tap water, cut into small pieces and air dried for one month.

The fresh leaves, air dried leaves and ground leaves of *Panax pseudoginseng* Wall. are shown in Figure 2.

Figure 2. Fresh, air dried and ground leaves of *Panax pseudoginseng* Wall.

2.2. Phytochemical Screening of Leaves of *Panax pseudoginseng* Wall.

Phytochemical Screening of leaves of *Panax pseudoginseng* Wall. was carried out by standard methods [4].

2.3. Determination of Mineral Contents in Leaves of *Panax pseudoginseng* Wall.

The minerals of leaves of *Panax pseudoginseng* Wall. were measured at Research Center, Monywa University by Energy Dispersive X-ray Fluorescence Spectrometer (EDX-700), Shimadzu, Japan.

2.4. Determination of Antimicrobial Activities of Leaves of *Panax pseudoginseng* Wall.

The antimicrobial activities of leaves of *Panax pseudoginseng* Wall. were tested at Pharmaceutical Research Department, Yangon by agar well diffusion method on six selected organisms *Bacillus subtilis, Bacillus pumilus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*.

3. Results and Discussion

3.1. Phytochemical Screening of Leaves of *Panax pseudoginseng* Wall.

Phytochemical screening of leaves of *Panax pseudoginseng* Wall.is shown in Figure 3 and Table 1.

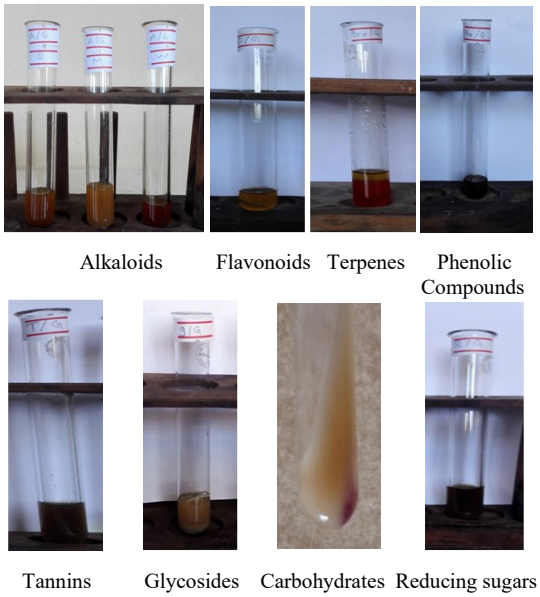

Figure 3. Phytochemical analysis of leaves of *Panax pseudoginseng* Wall.

Table 1. Results of phytochemical test on leaves of *Panax Pseudoginseng* Wall.

No.	Tests	Extracts	Test reagents	Observation	Results
1.	Alkaloids	1 % HCl	Dragendroff's reagent	Orange ppt	+
			Wagner's reagent	Reddish brown ppt	+
			Mayer's reagent	White ppt	+
2.	Flavonoids	EtOH	Mg turning, conc: HCl	Yellow color solution	+
3.	Terpenes	$CHCl_3$	Acetic anhydride, conc: H_2SO_4	Red color solution	+
4.	Phenolic compounds	EtOH	10 % $FeCl_3$	Black color solution	+
5.	Steroids	$CHCl_3$	Acetic anhydride, conc: H_2SO_4	No Green coloration	–
6.	Saponins	H_2O	Distilled water	Frothing	+
7.	Tannins	H_2O	10 % $FeCl_3$	Dark brown color solution	+
8.	Glycosides	H_2O	10 % lead acetate	White ppt	+
9.	Carbohydrates	H_2O	10 % α -naphthol, conc: H_2SO_4	Violet color ring of the interface of the two layers	+
10.	Reducing sugar	H_2O	Benedict's solution	Brick-red ppt	+

(+) = the presence of constituents
(–) = the absence of constituents

According to these results, the leaves of *Panax pseudoginseng* Wall. contained alkaloids, flavonoids, terpenes, phenolic compounds, saponins, tannins, glycosides, carbohydrates and reducing sugars. However, steroids were absent. Alkaloids affect the

central nervous system and have antibacterial and analgesic properties. Natural phenolic compounds including flavonoids and tannins play an important role in cancer prevention and treatment. Their various bioactivities are responsible for chemopreventive properties e.g antioxidant, anticarcinogenic, anti-diabetic and anti-inflammatory effects. Terpenes have been found to be useful in the prevention and therapy of several diseases, including cancer because of their antimicrobial, anti-allergenic and anti-inflammatory activities. Glycosides help in weight loss, lower blood pressure and reduce blood sugar. They can be used in the treatment of heart disease due to their action on cardic muscle (steroids and aglycone). Plant containing carbohydrates and reducing sugar are known to exert a beneficial action on immune system by increasing body strength and hence are valuable as dietary supplements. Carbohydrates also provide major source of energy. The presence of saponins can control human cardiovascular disease, and reduce cholesterol level and cancer risk. The presence of these phytochemicals in leaves of *Panax pseudoginseng* Wall. plays a role in local therapeutic applications.

3.2. Elemental Analysis of Leaves of *Panax pseudoginseng* Wall.

The results of mineral content in leaves of *Panax pseudoginseng* Wall. Vent. are shown in Figure 4 and Table 2.

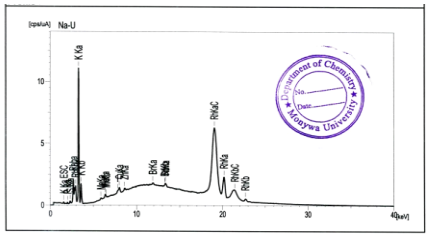

Figure 4. EDXRF spectrum of leaves of *Panax pseudoginseng* Wall.

Table 2. Results of qualitative analysis of mineral contents in leaves of *Panax pseudoginseng* Wall.

No	Analysis		Relative abundance (%)
1	Potassium	(K)	0.901
2	Phosphorus	(P)	0.106
3	Sulfur	(S)	0.070
4	Iron	(Fe)	0.002
5	Copper	(Cu)	0.002
6	Manganese	(Mn)	0.001
7	Zinc	(Zn)	0.001
8	Carbon, hydrogen	(C H)	98.916

According to these results, the amount of potassium (0.901%) is significantly higher than that of other minerals. Causes for low levels of potassium include chronic diarrhea, and vomiting. Phosphorus (0.106%) works together with calcium to aid bone health in the body and is useful in healing diseases such as rickets and brittle bones. Sulfur (0.070%) can also create healthy skin and heal scars. Iron (0.002%) plays a role in oxygenating human's blood and preventing anemia. Copper (0.002%) plays a role in making red blood cells and maintaining nerve cell. It is essential for respiratory enzyme formation and important for nerve. Manganese (0.001%) is useful for healthy skin, nails and hair. Zinc (0.001%) is required for gene expression and immune function in human body. In fact, zinc is the second-most-abundant trace mineral— after iron — and is present in every cell. In addition, 98.916% of carbon and hydrogen was also found in these leaves.

The minerals in human body are related to each other and indirectly play a role in the growth process. These minerals are all important for the human body. However, the varieties of minerals are required in different amounts for human' health.

3.3. Antimicrobial Activities *of* *Panax pseudoginseng* Wall. Leaves

The antimicrobial activities of leaves of *Panax pseudoginseng* Wall. are given in Figure 5 and Table 3.

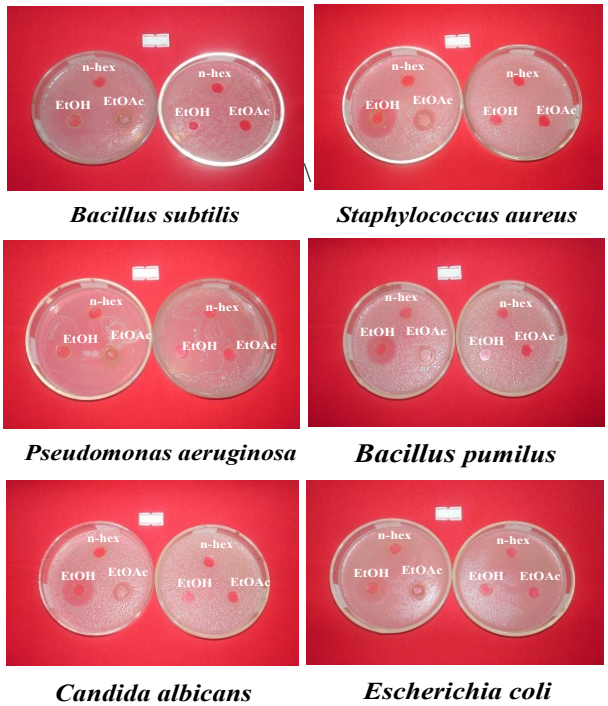

Figure 5. Antimicrobial activities of leaves of *Panax pseudoginseng* Wall.

Table 3. Results of Antimicrobial Activities of *Panax pseudoginseng* Wall. Leaves

Sample	Solvents	Inhibition zone diameters of Leaves of *Panax pseudoginseng* Wall. against six microorganisms (mm)					
		I	II	III	IV	V	VI
Leaves of Myanmar ginseng	n-hexane	–	–	–	–	–	–
	EtOAc	–	18 (++)	–	17 (++)	16 (++)	–
	EtOH	28 (+++)	29 (+++)	28 (+++)	25 (+++)	25 (+++)	25 (+++)

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

Organisms
I. *Bacillus subtilis*
II. *Staphylococcus aureus*
III. *Pseudomonas aeruginosa*
IV. *Bacillus pumilus*
V. *Candida albicans*
VI. *Escherichia coli*

According to these results, n-hexane crude extract of leaves of *Panax pseudoginseng* Wall. showed no activities on all tested organisms. The ethyl acetate crude extract responded medium activities on *Staphylococcus aureus, Bacillus pumilus* and *Candida albicans*, and no activities on *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. The ethanol crude extract responded high activities on all tested organisms. It showed that the leaves of *Panax pseudoginseng* Wall. is a source of antimicrobial drugs.

4. Conclusion

In this research work, the leaves of *Panax pseudoginseng* Wall. contained alkaloids, flavonoids, phenolic compounds, glycosides, terpenes, saponins, tannins, carbohydrates and reducing sugars while steroids were absent. These phytochemical compounds are the key candidates in the medicinal values. The leaves of *Panax pseudoginseng* Wall. contained macrominerals such as, potassium, phosphorus, sulfur, and trace minerals that include iron, copper, manganese and zinc. It was found that potassium is richer than other minerals. Despite the small amounts of minerals needed by the body, their lack can cause serious health problems. The n-hexane crude extract of leaves of *Panax pseudoginseng* Wall. showed no activities on all tested organisms. The ethyl acetate crude extract responded medium activities on *Staphylococcus aureus, Bacillus pumilus* and *Candida albicans*. The ethanol crude extract responded high activities on all tested organisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albicans* and *Escherichia coli*. It proves that the leaves of *Panax pseudoginseng* Wall. could have the great potential as antimicrobial agents in the treatment of infectious organisms.

Further works are needed to isolate, elucidate and characterize the structures of the bioactive compounds in these leaves for medicine. Therefore, it is recommended that the leaves of *Panax pseudoginseng* Wall. can be used as a good source for active antibiotic drugs. And it is a very important point for main active ingredients which can be extracted from the leaves of *Panax pseudoginseng* Wall.

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