STUDY ON MORPHOLOGICAL, PHYSICOCHEMICAL INVESTIGATION AND ANTIMICROBIAL ACTIVITIES OF *SANSEVIERIA TRIFASCIATA* HORT. EX PRAIN. (NA-GAR-SET-GAMON)

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Abstract

The plant *Sansevieria trifasciata* Hort. ex Prain. (Na-gar-set-gamon) (or) snake plant belongs to family Asparagaceae It was naturally grown and found abundantly in Myanmar. They were collected from Dagon University, East Dagon Township, Yangon Region during the flowering and fruiting periods. The morphological characters, preliminary phytochemical test, physicochemical investigation and antimicrobial activities were studied. In morphological study, the plant was evergreen herb, stem less and rhizome horizontal. Preliminary phytochemical test showed the presence of alkaloid, α-amino acid, glycoside, carbohydrates, reducing sugar, phenolic compound, flavonoid, steroids and trace of terpenoids. In physicochemical investigation, the water soluble ash content of leaves was the most highest than the other solvents. In antimicrobial activities, the ethanol extract of leaves showed the most significant inhibition on *Vibrio cholerae*.

Keywords: Phytochemical test, Physicochemical test, Antimicrobial activities

Introduction

In Myanmar, there are two types of medicinal plants: wild plants and cultivated plants. Traditional medicine is an important part of human health care in developing countries.

Herbal medicine also called botanical medicine or phytopharmacy is a plant derived material or preparation with therapeutic or other human health benefits which contains either raw or processed ingredients from one or more plants. This is a major remedy in traditional medicine system, which is largely based on the use of vegetative and reproductive parts of the plants (Federico,

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1989). The medicinal plants, *Sansevieria trifasciata* Hort. ex Prain belonging to the family Asparagaceae, which are found abundantly in Myanmar.

Genus *Sansevieria* is native to tropical Africa; in Java, from the plains up to 1000, not rarely cultivated on a small scale, in gardens, fences and hedges also on or around groves (Backer, 1968). The family Asparagaceae comprises about 153 genera and some 2,500 species of flowering plants. Distributed nearly worldwide, the family is extremely diverse, and its members are united primarily by genetic and evolutionary relationships rather than morphological similarities (Melissa, 1993).

Sansevieria trifasciata Hort. ex Prain. grown of West Africa, it is cultivated as an ornamental pot plant. It is also distributed in Southern India. It grown dry region. Dry rocky and sandy places, lowlands and mid-country. Flowering seasons are January-June. Flowers fainty fragrant. Becoming depleted due to over-exploitation. English name of this plants is Bow-string hemp. Fibers extracted from the leaves were formerly used for making bow-strings and fishing lines. Now used in the Central Province for making reed mats and dusters. Roots used in indigenous medicine (Dassanayake and Clayton, 2000). *Sansevieria trifasciata* Hort. ex Prain. is called na-ga-set in Myanmar, snake plant, mother-in-law's tongue and bow-string hemp in English. This plant distributed in Yangon of Myanmar Region (Hundley and Chit Ko Ko, 1987 and Kress and Yin Yin Kyi, 2003).

In Myanmar, *Sansevieria trifasciata* Hort. ex Prain. root juice with honey is used for chronic cough and leaf juice treats mucous in throat for children. Leaf fibers used as rope. Leaf with white stripe is called Nagaset and leaf margin yellow is Nagakhoe (Ashin Nagathein, 1983). This plant has analgesic activity and antipyretic activity. Traditional used of this plant is the treatment of ear pain, swellings, boils, fever and inflammatory disordered (file://G:download/downloadflle-lhtm).

Several species are popular houseplants in temperate regions, with *Sansevieria trifasciata* Hort. ex Prain. is the most widely sold; numerous cultivars are available. The Chinese usually keep this plant potted in a pot

often ornamented with dragons and phoenixes. As a houseplant *Sansevieria* thrives on warmth and bright light, but will also tolerate shade. In Korea, potted *Sansevieria* is commonly presented as a gift during opening ceremonies of businesses or other auspicious events. *Sansevieria* use the crassulacean acid metabolism process, which absorbs carbon dioxide and releases oxygen at night. This purportedly makes them suitable bedroom plants. However, since the leaves are potentially poisonous if ingested, *Sansevieria* is not usually recommended for children's bedrooms. Some believe that having *Sansevieria* near children (such as in the study room) helps reduce coarseness, while others recommend placing pots near the toilet tank to counter the drain-down vibrations (file:///G:/ download/ Sansevieria. htm).

The aim of present research is to know the chemical compound and medicinal value of the plant. To fulfill this aim, the main objectives are (i) to verify the morphological characters of the plants (ii) to examine powdered sample of leaves for standardization of crude drug (iii) to investigate the qualitative analysis on phytochemical test (iv) to find out the presence or absence of chemical properties, physicochemical properties and (v) to examine the antimicrobial activities of leaves extracts.

Materials and Methods

Botanical Studies

The specimens used in this research were collected widely in Dagon University, East Dagon Township, Yangon Region. They were collected especially during the flowering and fruiting period from January to June in 2013. The collected fresh specimens of both vegetative and reproductive parts of the plants were identified by using literatures of Lawrences, 1964; Backer, 1965; Hundley and Chit Ko Ko, 1987; Dassanayake, 2000 and Kress *et al.*, 2003. Taxonomic descriptions were accompanied with the photograph of natural habitats, L.S of flower, T.S of ovary and parts of the plants with measurements.

Chemical Studies

The leaves of *Sansevieria trifasciata* Hort. ex Prain. were collected from Dagon University, East Dagon Township, Yangon Region. The leaves samples were washed with water and were cut slices by knife. Then these slices were dried at room temperature for 2-3 weeks. The dried leaves were pulverized by grinding with a blender to get fine powdered and stored in air tight container. For preliminary phytochemical test, the air-dried powdered of the leaves were tested for alkaloids, α-amino acid, glycoside, cyanogenic glycoside, carbohydrates, reducing sugar, starch, saponin, tannin, phenolic compound, flavonoid, steroids and terpenoids were carried out. The physicochemical properties solubility of powdered leaves were carried out using moisture content, total ash content, water soluble ash content, acid insoluble ash content, aqueous soluble matter content and various solvent such as methanol, ethanol, ethyl acetate, chloroform, petroleum ether and acetone.

Preliminary Phytochemical Test of Leaves of Sansevieria trifasciata Hort. ex Prain.

The preliminary phytochemical tests were carried out according to Vogel, 1956; British Pharmacopoeia 1968, Marini Bettolo *el. al.,* 1981; Robison 1983 and Central Council for Research in Unani Medicine, 1987.

Test for Alkaloid

One gram of powdered sample was boiled for about 20 minutes with 20ml of 10% HCl and filtered. The filtrate was divided into four portions and tested with Dragendroff's reagent, Sodium picrate, Wagener's reagent and Mayer's reagent. The precipitate formed an addition of the reagent indicated the presence of alkaloid (Robison, 1983).

Test for α-Amino acid

One gram of powdered sample was boiled with 10ml of distilled water for 20 minutes and then filtered. And then, a few drops of each filtrate was spotted on a filter paper using a capillary tube, allowed it to dry and sprayed

with ninhydrin reagent. The filter paper was dried at room temperature and then kept it in over at 110°C for a few minutes after which the purple colour appears due to the presence of α-amino acids (Marini Bettolo *et. al.,* 1981).

Test for Glycoside

One gram of powdered sample was heated in a glass test tube with 10ml of distilled water on the water-bath for 20 minutes. The mixture was filtered and 10% basic lead acetate solution was added drop-wise to the filtrate. Pale yellow precipitate was observed which showed the presence of glycoside (Marini Bettolo *et. al*.,1981).

Test for Cyanogenic glycoside

Two grams of powdered sample was boiled with 10ml of distilled water for 20 minutes and filtered. Then about 5 drops of concentrated sulphuric acid were added and sodium picrate paper was trapped in the neck of the test tube by means of a loosely closed cock. The resultant mixture was heated by using a spirit burner. Observation was made to see if the paper turned brick red which indicated the presence of cyanogenic glycoside (Marini Bettolo *et. al.,* 1981).

Test for Carbohydrate

One gram of powdered sample was boiled with 10ml of distilled water for 20 minutes and filtered. The filtrate was introduced into a test tube and a few drops of 10% α-naphthol was added shaken. The test tube was then inclined at an angle of 45° and concentrated sulphuric acid was added slowly along the side of the tube. A red ring was formed between the two layers, showing the presence of carbohydrate (Marini Bettolo *et. al.,* 1981).

Test for Reducing Sugar

One gram of powdered sample was boiled with 10ml of distilled water for 20 minutes and filtered. The filtrate was treated with Fehling's solution, then boiled about 20 minutes, it furnished green precipitates, indication the presence of a reducing sugar (Vogel, 1956).

Test for Starch

One gram of dried powdered sample was boiled with 10 ml of distilled water for about 20 minutes. It was then filtered and two drops of iodine solution were added to the filtrate. Blue black colour was formed which indicate the presence of starch (Marini Betolo *et. al.*, 1981).

Test for Saponin

One gram of powdered sample was boiled with 10 ml of distilled water for about 20 minutes and filtered. The filtered and the filtrate shaken vigorously with distilled water for a few minutes. Market forthing which lasted for about half an hour to take place, indicating the presence of saponin (Marini Betolo *et. al.*, 1981).

Test for Tannin

One gram of powdered sample was boiled with 10ml of distilled water for about 20 minutes and filtered. The filtrate was treated with a few drops of 1% ferric chloride solution. If a bluish black or yellowish brown colour resulted indicating the presence of tannins (Marini Betolo *el. al.*, 1981).

Test for Phenolic compound

One gram of powdered sample was boiled with 10 ml of distilled water for 20 minutes and filtered. The filtrate was treated with neutral *5%* ferric chloride solution, it gave deep blue colour, indicating the presence of phenol groups (Marini Betolo *et. al.*, 1981).

Test for Flavonoid

One gram of powdered sample was extracted with 95% ethanol for 20 minutes and filtered. Then, the ethanolic extract was treated with 5-10 drops of dilute hydrochloric acid which was followed by a small piece of zinc or magnesium. The solution was boiled for few minutes. The appearance of pink or brown colour indicates the presence of flavonoid (Robison, 1983).

Test for Steroids and Terpenoids

One gram of powdered sample was extracted with petroleum ether for 20 minutes and filtered. When the petroleum ether extract was dissolved in 1ml chloroform. The chloroform extract was treated with 3 drops acetic anhydride and concentrated sulphuric acid. The reenish colour was turns to blue green indicate the presence of steroids and deep pink of terpenoid (Central Council for Research in Unani Medicine, 1987).

Physicochemical Investigation of Leaves *Sansevieria trifasciata* Hort. ex Prain.

Physicochemical investigation was determined according to British Pharmacopoeia (1965) as follows.

Determination of Moisture Content

Five grams of powdered was weighed accurately in a beaker and dried in an oven at the temperature of 110°C for 1 hr. After drying the beaker was removed from the oven and cooled at room temperature and weighed. The procedure was repeated until a constant weight was obtained. Then, percentage of moisture content was calculated.

$$\% \text{ of moisture} = \frac{M-m}{W} \times 100$$

M = weight of sample of weighting beaker in g before drying

m = weight of sample and weighting beaker in g after drying

W = weight of taken in g.

Determination Weight of Total Ash

Ten grammes of powdered was weighed in a crucible and placed in a muffle-furnace at 500°C until substance turned into ash. After that, the crucible was cooled and weighted. This procedure was repeated until a constant weight was obtained and the percentage of total ash was calculated.

$$\% \text{ of total ash} = \frac{M\text{-}m}{W} \times 100$$

Determination of Water Soluble Ash

The total ash was boiled with 10ml of water for 15 mins. The insoluble matter was collected on an ashless filter paper, washed with water and ignited to a constant weight. This weight or insoluble matter was substrated from the weight of the total ash, to give weight of the water-soluble ash.

Determination of Acid Insoluble Ash

The total ash was boiled gently with 10ml of 6% dilute hydrochloric acid for 5 minutes and filtered using ashless filter paper. The insoluble matter was collected and washed with boiling water until free of acid. The acid insoluble residue was dried in an oven at 105° C and weighed. The percentage of the acid insoluble ash was calculated.

Determination of Soluble Matter Content in Different Solvents

Soluble matter content was determined by the method of British Pharmacopoeia, 1968. Five grams of powder was soaked with 50ml of distilled water in flask, closed for 72 hrs. The mixture was filtered and evaporated in a weighed beaker then placed on a boiling water-bath until it was completely evaporated. The percentage of soluble matter was calculated. Similarly the same procedure was repeated for the determination of aqueous, methanol, ethanol, ethyl acetate, chloroform, petroleum ether and acetone.

Antimicrobial Activities of Different Solvent Extracts from Leaves of *Sansevieria trifasciata* Hort. ex Prain.

Apparatus Used

Autoclave, clean bench, conical flask, hot air sterilizer, measuring cylinders, micropipettes, steam-drying oven, petridishes, pipettes, water bath and loops.

Test Microorganisms

The test organisms, used in this research work were obtained from the Central Research and Development Center (CRDC) for determination of antimicrobial activities.

Preparation of Plates for Antimicrobial Activities Test

The antimicrobial activities were performed by agar-well diffusion method. Nutrient agar was prepared according to method described by Cruikshank, 1975. Nutrient agar was boiled and 20-25 ml of the medium was poured into a test- tube and plugged with cotton wool and autoclaves at 121 °C for 15 minutes. Then the tubes were cooled down to 30-35°C and poured into sterilized petridishes and 0.01 ml of spore suspension were also added into the dishes. The agar was allowed to set for 30 minutes after with 5mm plate agar well was made with the help of sterilized cork borer. After that, about 0.1ml of sample was introduced into the agar-well and incubated at 37°C for 24-48 hrs. The inhibition zone appeared around the agar-well indicating the presence of anti-microbial activity. The extent of antimicrobial activity was measured from the zone of inhibition diameter. The results were shown in Table 2, Figures 2 to 4.

Results

Morphological Characters of *Sansevieria trifasciata* Hort. ex Prain.

Scientific name - *Sansevieria trifasciaia* Hort. ex Prain.

Myanmar name - Nagar-set-gamon

English name - Snake plant, Mother – in law's tongue, Bow-string hemp.

Family - Asparagaceae

Flowering and fruiting period - January to June

Part used - Leaves

Perennial herbs, evergreen. Stemless; rhizome horizontal; sympodial, producing leafy shoots at intervals, 0.5-1.0m in height, aerial shoots in a single clump. Leaves simple, tuft thick, upright fleshy to rigidly coriaceous, both surfaces shinning smooth, dark green, with numerous very conspicuous, light or greyish green irregularly confeined transverse bands, a narrow dark green margin, tapering to apex, acute, apiculate, linear-laceolate or ensiform and channeled, and 52.5-76.9cm in length and 3.5-5.5cm in breadth. Inflorescences (peduncle included) racemes, 51.0-57.0cm in length and 6.0-7.0cm in breadth penduncle length and breadth. Flowers in fassicles of 3-7, membranous bract, 5.0-7.0mm in length and 2.0-3.0mm in breadth, each flower with of a minute bract, 2.0-2.5mm in length and 0.5-1.0 mm in breadth; pedicel cylinder, 0.5-1.0mm in length and 0.5-0.8mm in breadth, actinomorphic, regular, bisexual, trimerous, hypogynous. Tapels 3+3, united at the base, tapel tube short 1.5-1.8mm in length and 2.0-3.0mm in breadth, cylindrical, pale yellowish green; limbs linear or narrowly lanceolate, revolute, pale greenish white, 1.5-2.0cm in length and 1.0-2.0mm in breadth, inferior. Stamens 3+3, epipetalous, filaments filiform, pale yellowish green, 1.9-2.1cm in long; anther dithecous, dorsifixed, longitudinal dehiscence, sagittate at base, 2.5-3.0mm in length and 0.3-0.5mm in breadth, inferior. Ovary 3-carples, 3.0-3.5mm in length and 1.0-2.0mm in breadth, 3 loculi, with anatropous ovule in each locule, axile placentation; style filiform, 2.0mm- 3.0mm in length, pale yellowish green, stigma 3 lobed, exserted, 1.0-1.5mm in diameter, superior. Fruit berry globose, orange in colour when ripe. Seeds broadly ovoid, with horny endosperm.

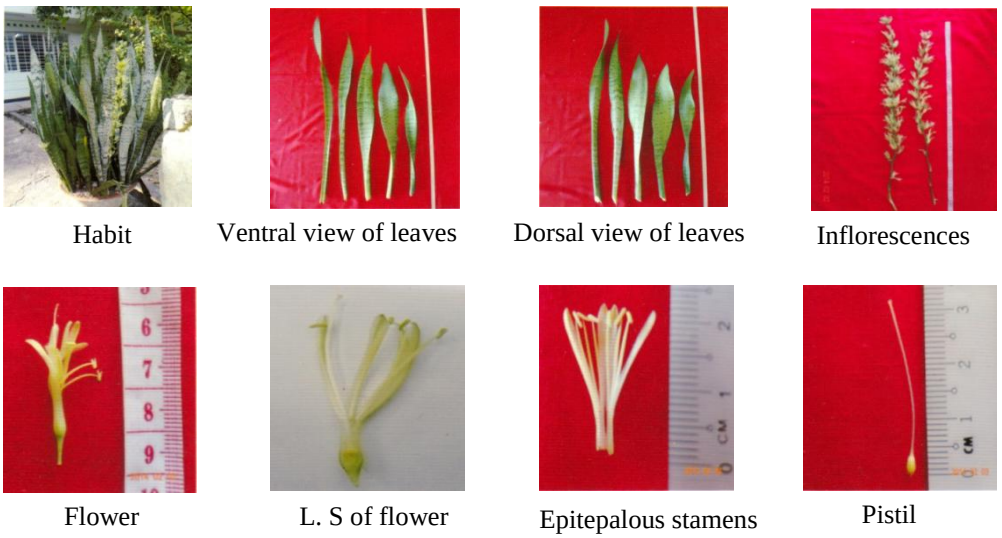


Figure 1. Morphological characters of *Sanseviveria trifasciata* Hort. ex Prain

Chemical Studies

Preliminary Phytochemical Test of Leaves from Sansevieria trifasciata Hort. ex Prain.

The results of preliminary phytochemical test of air-dried powdered leaves from *Sansevieria trifasciata* Hort. ex Prain. indicated that alkaloid, α-amino acid, glycoside, carbohydrate, reducing sugar, starch, phenolic compound, flavonoid, steroids and terpenoids (trace) are found to be present and cyanogenic glycoside, starch, saponin and tannin are absent. The results are shown in Table 1.

Table 1. Preliminary Phytochcmical Test of Leaves from *Sansevieria trifasciata* Hort. ex Prain.

No.	Test	Extract	Test reagent	Observation	Results
1.	Alkaloid	10% HCl	Dragendroffs reagent Sodium picrate solution Wagner's reagent Mayer's reagent	Orange ppt Yellow ppt Brown ppt White ppt	+ + + +
2.	α-amino acid	H_2O	Ninhydrin reagent	Purple spot	+
3.	Glycoside	H_2O	10% lead acetate solution	Pale yellow ppt	+++
4.	Cyanogenic glycoside	H_2O	H_2S0_4 (Conc:) & sodium picrate paper	No colour change	–
5.	Carbohydrates	H_2O	10% α-napthol + H_2S0_4 (Conc:)	Red ring	+
6.	Reducing sugar	H_2O	Fehling's solution	Greenish yellow ppt	+
7.	Starch	H_2O	Iodine solution	Brown ppt	–
8.	Saponin	H_2O	Distilled water	No foaming	–
9.	Tannin	H_2O	1% $FeCl_3$ solution	Brown ppt	–
10.	Phenolic compound	H_2O	5% $FeCl_3$ solution	Bluish black ppt	+ + +
11.	Flavonoid	95% Ethanol	Mg/HCL (Conc:)	Brown colour	+
12.	Steroids	Petroleum ether	Acetic anhydride	Green colour	+
13.	Terpenoids	Petroleum ether	Acetic anhydride and H_2S0_4 (Conc:)	Pink colour (trace)	(trace)

Key to the table (+ + +) = prominent (+) = present (-) = absent (ppt.) = precipitate

Physicochemical Investigation of Leaves of *Sansevieria trifasciata* Hort. ex Prain

The results of the physicochemical investigation, the moisture content were determined and recorded. The solubility of leaves powdered in

petroleumether, acetone, ethyl acetate, methanol, distilled water, ethanol and chloroform were carried out to determine the amount of total solids soluble in an individual solvent. *Sansevieria trifasciata* Hort. ex Prain. leaves were found to be significantly soluble in aqueous were highest than those of other solvents and the least soluble in petroleum- ether. The results were shown in Table 2.

Table 2. Physicochemical Examination of Leaves of *Sansevieria trifasciata* Hort ex Prain.

No.	Physicochemical characters	Average (%)
1.	Moisture content	30.39
2.	Total ash content	11.10
3.	Water soluble ash content	30.17
4.	Acid insoluble ash content	11.59
5.	Aqueous soluble matter content	3.18
6.	Methanol soluble matter content	2.92
7.	Ethanol soluble matter content	2.52
8.	Ethyl acetate soluble matter content	2.26
9.	Chloroform soluble matter content	1.26
10.	Petroleum ether soluble matter content	1.10
11.	Acetone soluble matter content	2.68

Antimicrobial Activities of Different Solvent Extracts of Leaves of *Sansevieria trifasciata* Hort. ex. Prain.

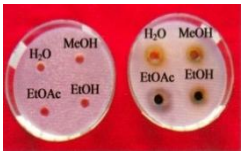
In this study, methanol, chloroform and petether extract of leaves showed activities against on nine test organisms. Aqueous extract of leaves did not showed against on *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Bacillus pumalis*. Ethanol extract of leaves showed activities against *Vibrio cholerae, Proteus mirabilis and Bacillus subtilis.* Ethyl acetate extract of leaves showed against on *Vibrio cholerae*, *Pseudomonas aeruginosa and Klebsiella pneumoniae.* The acetone extract of leaves did not showed against on *Pseudomonas aeruginosa.* Among them, ethanol extract of leaves showed the highest activity against on *Vibrio cholerae.* These results were shown in Table 2 and Figure 2.

Table 3. Inhibition Zone Exhibited by Different Solvent Extract of Leaves of ***Sansevieria trifasciata*** Hort. ex Prain.

No.	Microorganisms	Inhibitions Zone (mm)						
		H_2O	MeOH	EtOH	EtOAC	$CHC1_3$	P.E	Ace
1.	*Vibrio cholerae*	20 +	20 +	35 + + +	30 + +	15 +	15 +	10 +
2.	*Pseudomonas aeruginosa*	-	25 +++	-	10 +	15 +	15 +	-
3.	*Staphylococcus aureus*	15 +	15 +	-	-	20 +	15 +	10 +
4.	*Klebsiella pheumoniae*	20 +	20 +	-	15 +	15 +	10 +	12 +
5.	*Proteus mirabilis*	-	20 +	20 +	-	20 +	15 +	15 +
6.	*Candida albicans*	20 +	25 ++	15 +	-	15 +	10 +	12 +
7.	*Esherichia coli*	15 +	20 +	10 +	-	10 +	12 +	10 +
8	*Bacillus subtilis*	10 +	10 +	-	-	20 +	15 +	10 +
9.	*Bacillus pumalis*	-	20 +	15 +	-	15 +	13 +	10 +

Key to the table

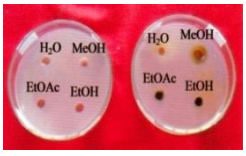
(+) = 10mm-20mm (++) = 21mm-30mm (+++) = 31mm above(5 mm) = Agar well



Control

Antimicrobial activity

Vibrio cholerae



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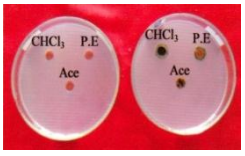
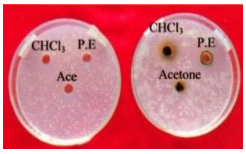


Antimicrobial activity

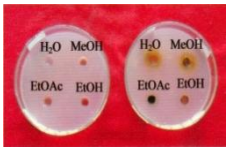
Pseudomonas aeruginosa

Control



Antimicrobial activity

Staphylococcus aureus



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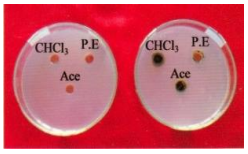

Antimicrobial activity

Klebsiella pneumoniae

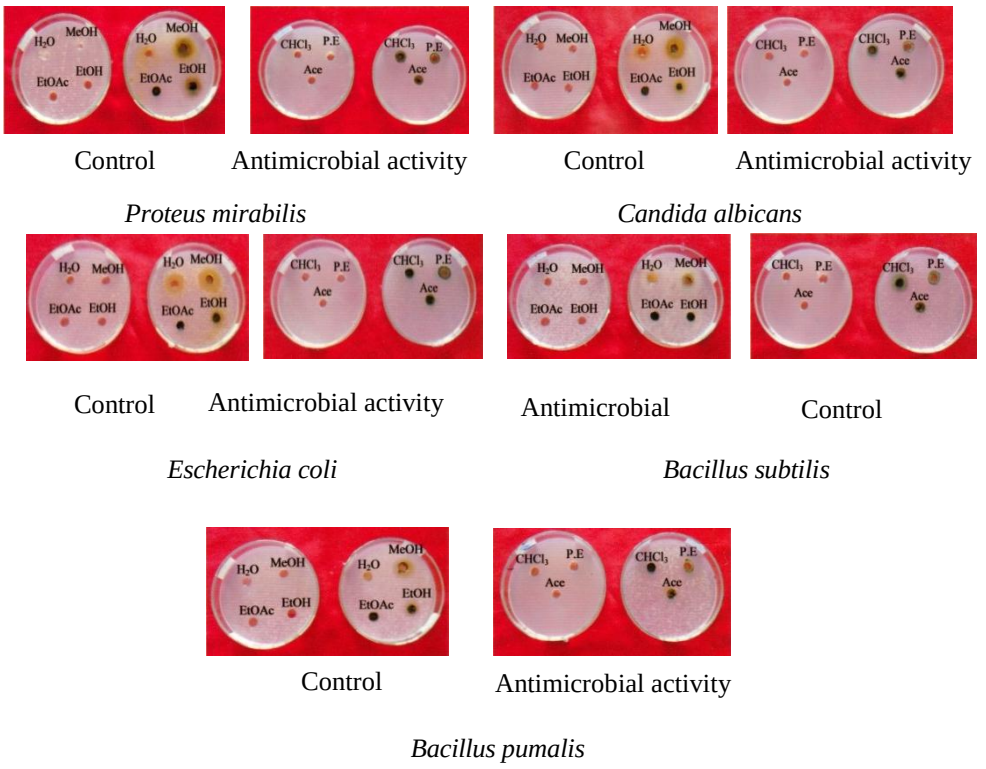

Figure 2. Antimicrobial activities of leaves of *Sansevieria trifasciata* Hort. ex Prain.

Discussion and Conclusion

In this research, the medicinal plant *Sansevieria trifasciata* Hort. ex Prain. belonging to family Asparagaceae has been studied. This plant was collected as wild and cultivated plants which are found abundantly in Dagon University, East Dagon Township, Yangon Region. In morphological studies, they are perennial herbs, evergreen; stemless, rhizome horizontal. Leaves

simple, tuft thick, upright fleshy to rigidly coriaceous, with light or grayish green transverse bands, dark green margin, linear-lanceolate. Inflorescences racemes, flowers in fassicles, actinomorphic, regular, bisexual, hypogynous. Tepal tube short, limbs linear. Epitepalous stamens. Ovary 3 carpels, one ovule in each locule, axile placentation. Fruit berry. Seed with horny endosperm. These results were agreed with Michael, 2004).

The preliminary phytochemical test was carried out on the powders of *Sansevieria trifasciata* Hot. ex Prain. According to these results, alkaloid, glycoside, carbohydrates, flavonoid and terpenoids were present the described by Trease and Evans, 2002, file:// G:/ download/downloadfile-1.htm.

In the physicochemical examination, the powdered leaves were soluble in seven solvents. Among them, the highest yield was obtained from aqueous extract of leaves and moderately soluble in petroleum ether.

In test of antimicrobial activities, the different solvent extracts of leaves inhibited *Vibrio cholerae, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia, Proteus mirabilis, Candida albicans, Escherichia coli, Bacillus subtilis* and *B. pumalis.* Methanol extract of leaves showed higher antimicrobial activities than other extract.

Therefore, the present study focused chemical compounds by using phytochemical test, physicochemical properties and antimicrobial activities of this plant which could be assumed to be beneficial for human health, especially in Myanmar traditional medicine.

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