Chemical Constituents in Leaves of *Polygonum chinense* L. (Ma-Har-Gar-Kyan-Sit)

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ABSTRACT

Polygonum chinense L. is commonly used in traditional treatment of many ailments. The leaves of *Polygonum chinense* L. were collected from Bago Township, Bago Region in month of December, 2017. It is also called Mway-Hauk-Ywet. The phytochemical constituents of samples were determined by test tube method. The moisture and ash contents were determined by oven dried method and ashing method. The extractable matter contents in leaves sample were observed that the amount of water extract (27.8065 %) and ethanol extract (5.695 %). In the elemental analysis in leaves sample by energy dispersive X- ray fluorescence spectrometry **(**EDXRF), Al (67.411 %) and S (26.267 %) are more significant than other elements such as Cu (2.014 %), Fe (1.563 %),Cr (1.467 %), Mn (0.649 %), Os (0.304 %), Zn (0.235 %), Mo (0.090 %). Agar well diffusion method was used to determine in the vitro antimicrobial activity on the different extracts such as EtOAc, PE, EtOH and water. It was found that the EtOH extract of *Polygonum chinense* L. exhibited the highest activity (17 - 13) mm against *Bacillus subtilis, Bacillus pumalis, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and Candida albicans*.

Keywords : *Polygonum chinense* L., phytochemical, extractable matter, EDXRF, Agar well diffusion method

1. INTRODUCTION

Most of the people use traditional medicine for the treatment of diseases. Although synthetic drugs and antibiotic brought about a revolution in controlling different diseases, plants occupy a very significant role as raw material for some important drugs (Medicinal Plants of Myanmar, 2000). In most countries, many plant parts, such as roots, leaves, barks, seeds, fruits and flowers are used in traditional medicine. Thus plants may save many lives if they are used correctly (WHO, 1990). In Myanmar most people depend on traditional medicines and herbal medicines rather than western synthetic drugs for the treatment of various diseases. Before the production of medicine, the human often used plants that were crude drugs. So in this research, the traditional medicinal plant was selected.

Polygonum chinense L. belonging to family Polygonaceae was chosen in the present study to investigate the pharmacognostic nature, especially isolation of bioactive compounds from the leaves and wound healing activity of leaf extract on animal models (Chevallier, 1996). The plant is commonly called Ma- Har- Gar- Kyan- Sit, Wetkyein or Boktaung in Myanmar (Kress *et al*., 2003) and Chinese knotweed in English. Members of the genus *Polygonum* are utilized in pharmaceutical preparation all over the world. Leaves are used for curing skin diseases and can be eaten as vegetable (Wealth of India, 1959). *Polygonum chinense* L. (Figure 1) is used as tonic and in the treatment of skin diseases in Myanmar (Nagathein, 1972).

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Family	:	Polygonaceae
Botanical Name	:	*Polygonum chinense* L.
Myanmar Name	:	Ma- Har- Gar- Kyan- Sit
English Name	:	Chinese Knotweed
Local Name	:	Kinn- ywet
Genus	:	*Polygonum*
Species	:	*P. chinense*
Part used	:	Leaves
Synonyms	:	*Persicaria chinensis* (L.) H Gross
	:	*Ampelygonum chinense* (L) Lindl.

Aim and Objectives

The aim of this research was to study the chemical constituents in leaves of *Polygonum chinense* L.(Ma- Har- Gar- Kyan- Sit).

To achieve the aim, the following objectives were carried out:

- To select the leaves sample and confirm the botanical name of the *Polygonum chinense* L.
- To determine the phytochemical constituents of *Polygonum chinense* L. by test tube method
- To determine the ash content in *Polygonum chinense* L.(Ma- Har- Gar- Kyan- Sit) by ashing method
- To determine the moisture content in *Polygonum chinense* L.(Ma- Har- Gar- Kyan- Sit) by oven dried method
- To perform the qualitative elemental analysis by energy- dispersive X- ray fluorescence **(**EDXRF**)** spectrometer
- To prepare the different crude extracts such as EtOH and water extracts
- To evaluate the antimicrobial activity of the crude extracts by agar well diffusion method

2. MATERIALS AND METHODS

Selected medicinal plant used in this study is Polygonum chinense L.(Ma - Har-Gar- Kyan Sit). The leaves of Ma - Har - Gar- Kyan - Sit were collected from Bago Township in Bago Region (Figure 1) in month of December, 2017. After collection, *Polygonum chinense* L. was identified at Department of Botany, Bago University. The collected leaves samples were cleaned and air dried at room temperature. The dried sample was made powder (Figure 2) by using electric grinder and stored in the air-tight containers to prevent moisture and other contaminations.

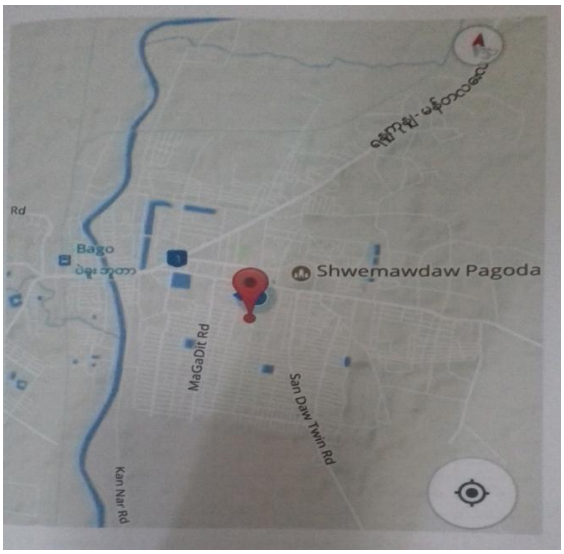
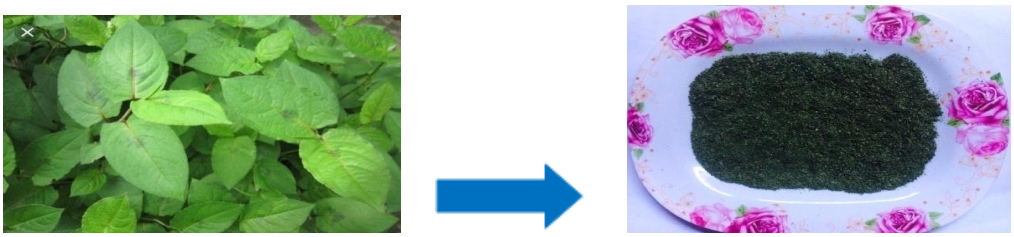


Figure 1. Sampling site of leaves of Polygonum chinense L.

(Ma-Har-Gar-Kyan-Sit)



(A) (B)

Figure 2. Photographs of selected *Polygonum chinense* L.(Ma- Har- Gar- Kyan-Sit) (A) Leaves (B) Powder

Phytochemical investigation was carried out by test-tube method to investigate the presence or absence of alkaloids, α -amino acids, carbohydrates, glycosides, flavonoids, phenolic compounds, reducing sugar, saponins, steroids, terpenoids and tannins. The moisture and ash contents in leaves of Ma- Har- Gar- Kyan- Sit were determined by using the AOAC method (AOAC,1990). Relative quantitative elemental determination of the sample was carried out by using the energy dispersive X- ray fluorescence **(**EDXRF) spectrometer. Antimicrobial activities of the different varieties of test sample extracts (PE, EtOH, H_2O, EtOAc) were investigated against six species of microorganisms by employing agar well diffusion method.

3. RESULTS AND DISSUSSION

The phytochemicals such as alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, glycosides, saponins, starch, tannins, steroids and terpenoids were present in sample by Test-tube method (Figure 3).

The moisture content was determined by oven drying method. The moisture content of food is of great importance for several reasons but its accurate determination is very difficult (Pearson, 1981). The powered sample was directly placed in oven at 105 º C. The moisture content of sample was found to be 8.8467 %.

The ash content was measured by ashing in the muffle furnace. The sample was charred and heated in the muffle furnace at temperature of about 500 ºC for six hours. The ash content of sample was found to be 11.8433 %. The moisture and ash contents of leaves samples used in this work are shown in Table 1 and Figure 4.

Figure 3. Phytochemical Test for Leaves of *Polygonum chinense* L. (Ma- Har Gar- Kyan- Sit)

Table 1. The Moisture and Ash Contents in Leaves of *Polygonum chinese* L. (Ma- Har- Gar- Kyan- Sit)

No.	Parameters	Content (%)
1	Moisture	8.8467± 0.0474
2	Ash	11.8433± 0.0274

Figure 4. Histogram of Moisture and Ash Contents in Leaves of *Polygonum chinense* L. (Ma- Har- Gar- Kyan- Sit)

The percentage of crude extract yield was derived from the weight of dried powdered leaves sample. The extractable matter contents in leaves of Ma- Har- Gar- Kyan- Sit were shown in Table 2. According to the results, it was observed that the yield of water extract was higher than the ethanol extract.

Table 2. Extractable Matter Contents of *Polygonum chinense* L. (Ma- Har- Gar- Kyan- Sit)

Sample	Extractable Matter Contents (%)	
	EtOH	H_2O
*Polygonumchinense*L.	5.695	27.8065

Relative quantitative elemental determination of the sample was carried out by using EDXRF spectrometer. The EDXRF spectrum (Figure 5) of leaves sample, *Polygonum chinense* L. showed the inorganic mineral elements: such as Al, S, Cu, Cr, Fe, Mn, Zn, Mo, Os contents (Table 3). Long lasting uptakes of significant concentrations of aluminium can lead to serious health effects, such as: damage to the central nervous system, dementia, loss of memory, listlessness, severe trembling. The aluminum content was found to be 67.411 % in Ma- Har- Gar- Kyan- Sit.

Sulphur is needed for all living things. It is especially important for humans because it is a part of the amino acid methionine, which is an absolute dietary

requirement for us. The average person takes in around 900 mg of sulphur per day, mainly in the form of protein. Elemental sulphur is not toxic, but many simple sulphur derivatives are, such as sulphur dioxide (SO_2) and hydrogen sulphide. Sulphur is also acted as a laxative and has been known as the "beauty mineral," it helps the complexion and the skin to stay clean and youthful. The sulphur content was found to be 26.267 % in Ma- Har- Gar- Kyan- Sit.

Copper is a constituent of the blood pigment hemocyanin which is replaced by the iron-complexed haemoglobin. High concentrations can be toxic and lethal. A high and toxic concentration of the essential element causes adverse health effects, which include nausea, diarrhea, liver and kidney damage. The main areas where copper is found in humans are liver, muscle and bone. Copper helps the body to use iron. The copper content was found to be 2.014 % in Ma- Har- Gar- Kyan- Sit.

Chromium is a crucial trace element with many sites of action and has a vital biological activity which is necessary in glucose homeostasis. It regulates insulin and blood glucose levels by stimulating insulin signalling pathway and metabolism and thus it may improve insulin sensitivity. The chromium content was found to be 1.467 % in Ma- Har- Gar- Kyan- Sit.

Iron is important as a carrier of oxygen in blood (Kittle, 1956). It is essential to all cells. Function of iron include involvement in energy metabolism, gene regulation, cell growth and differentiation, oxygen binding and transport, muscle oxygen use and storage, enzyme reactions, neurotransmitter synthesis and protein synthesis. An adult male requires about 10 mg of iron a day and a woman during menstruation will require about 18 mg a day. The iron content was found to be 1.563 % in Ma- Har- Gar- Kyan- Sit.

Manganese is also indicated in stimulating growth of the connective tissue and also thought to be of importance in brain functioning. Mn deficiency can result in impaired glucosed tolerance, altered carbohydrate and lipid metabolism, impaired insulin secretion, and skeletal abnormalities. The manganese content was found to be 0.649 % in Ma- Har- Gar- Kyan- Sit.

Molybdenum is likely safe when taken by mouth appropriately by adults. Molybdenum is safe in amounts that do not exceed 2 mg per day, the tolerable upper intake level. However, molybdenum is possibly unsafe when taken by mouth in high doses. Adult should avoid exceeding 2 mg daily. The molybdenum content was found to be 0.090 % in Ma- Har- Gar- Kyan- Sit.

Osmium tetroxide, OsO_4, is highly toxic. Concentrations in air as low as 10^{-7}g m^{-3} can cause lung congestion, skin damage, and severe eye damage. Osmium tetroxide can be absorbed into the body by inhalation of its vapour, by inhalation of its aerosol and by ingestion. A harmful contamination of the air can be reached very quickly on evaporation of this substance at 20°C. The substance is corrosive to the eyes, the skin, and the respiratory tract. Inhalation of this substance may cause lung oedema. Exposure to high concentrations may result into death. The effects may be delayed. Repeated or prolonged contact with skin may cause dermatitis. The substance may have effects on the kidneys. The osmium content was found to be 0.304 % in Ma- Har- Gar- Kyan- Sit.

High protein foods contain high amounts of zinc. Not only is a low dietary zinc intake incompatible with good health, but also excess intake of zinc is harmful. In man, the inhalation of zinc oxide fumes causes fever, malaria and depression and

coughing, which might induce vomiting, excessive salivation and headache. The zinc content was found to be 0.235 % in Ma- Har- Gar- Kyan- Sit.

Antimicrobial activities of the different varieties of test sample extracts (PE, EtOH, H_2O, EtOAc) were investigated against six species of microorganisms by employing agar well diffusion method .The sample were tested on six species of microorganisms including *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albican* and *E- coli*. The results were shown in Table 4. and Figure 6.

The highest antimicrobial activity of EtOH extract was observed against *Bacillus subtilis* zone of inhibition of 17 mm followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albican* and *E- coli* with zones of inhibition 13 mm respictively. The PE extract was less effective against *Bacillus pumilus* with zone of inhibition 12 mm followed by *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albican* and *E- coli* with zones of inhibition 13 mm. Water exhibited potent against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albican* and *E- coli*. So it may be inferred that the tested samples (extracts of PE, EtOAc, EtOH, H_2O) can be used in the formulation of medicine for the treatment of diseases related with these microorganisms.

Table 3. The relative abundance of elements in Leaves sample by EDXRF spectrometry

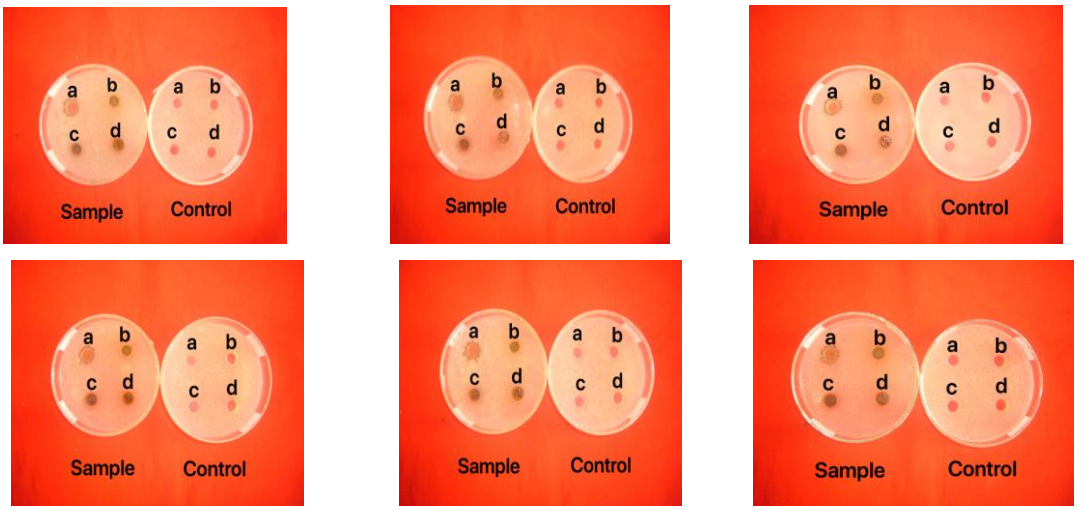
No.	Elements in leaves sample	Contents (%) in leaves sample
1	Al	67.411
2	S	26.267
3	Cu	2.014
4	Cr	1.467
5	Fe	1.563
6	Mn	0.649
7	Mo	0.090
8	Zn	0.235
9	Os	0.304

Table 4. Results for Antibacterial Activity of *Polygonum chinense* L. (Ma- Har- Gar- Kyan- Sit) by Agar Well Diffusion Method

Sample	Solvent	Organisms					
		1	**2**	**3**	**4**	**5**	**6**
Polygonum chinense L.	Pet-ether	13mm (+)	13mm (+)	13mm (+)	12mm (+)	13mm (+)	13mm (+)
	EtOAc	16mm (++)	14mm (+)	12mm (+)	14mm (+)	15mm (++)	14mm (+)
	EtOH	17mm (++)	13mm (+)	13mm (+)	13mm (+)	13mm (+)	13mm (+)
	Water	Nd	Nd	Nd	Nd	Nd	Nd
Control	Pet-ether	-	-	-	-	-	-
	EtoAc	-	-	-	-	-	-
	EtOH	-	-	-	-	-	-
	Water	-	-	-	-	-	-

Agar well -10mm
10mm~ 14mm(+)
15mm~ 19mm(++)
20mm above(+++)
ND - Not Detectable

Organisms
(1) *Bacillus subtilis*
(2) *Staphylococcus aureus*
(3) *Pseudomonas aeruginosa*
(4) *Bacillus pumilus*
(5) *Candida albican*
(6) *E- coli*



1-6 Microgamisms, a. P.E, b. H_2O, c. EtOAc, d. EtOH

Figure 6. Antimicrobial activity of PE, H_2O, EtOAc, EtOH extracts of (Ma- Har- Gar- Kyan- Sit) against six microorganisms

4. CONCLUSION

The preliminary phytochemical constituents of *Polygonum chinense* L. (Ma- Har- Gar- Kyan -Sit) were investigated by test tube method. According to this method, alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, glycosides, saponins, starch, tannins, steroids and terpenoids were present in leaves sample. The phenolic compounds such as flavonoids, phenolic acids and tannins have antioxidant activity. Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They often have pharmacological effect and are used as medications and recreational drugs. Flavonoids enhance the effects of vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, virus and other microbes. Terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal medicines and are under investigation for Antibacterial, Antineoplastic and other Pharmaceutical functions. Tannins have shown potential Antiviral, Antibacterial and Antiparasitic effects. Saponins cause hemolysis of red blood cells. In the leaves sample of *Polygonum chinense* L. (Ma- Har- Gar- Kyan -Sit), moisture content (8.8467 %) and ash content (11.8433 %) were observed. The extractable matter contents of leaves of *Polygonum chinense* L were observed that water extract was the higher amount of crude extract (27.8065 %) than that of ethanol extract (5.695 %). In the elemental analysis in leaves of *Polygonum chinense* L. by EDXRF, Aluminum (Al) and Sulfur (S) are more significant than other elements Copper (Cu), Iron (Fe), Chromium (Cr), Manganese (Mn), Osmium (Os), Zinc (Zn), Molybdenum (Mo).

The antimicrobial activities of the different varieties of test sample extracts (PE, EtOH, H_2O, EtOAc) were investigated against six species of microorganisms by employing agar well diffusion method. The screening was done against six bacterial strains namely *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albican* and *E- coli*. Among the leaves extracts, the highest antimicrobial activity was observed in EtOH extract whereas H_2O extract did not show antimicrobial activity.

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