FOCUSING ON THE ELEMENTAL CONTENTS, ANTIOXIDANT ACTIVITY AND CYTOTOXICITY OF *RHODODENDRON ARBORETUM* W.W.SM (TAWNG ZA LAT NI) FLOWER

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Abstract

Rhododendron arboreum W.W.Sm (Tawng Za Lat Ni) is one of the landmarks of Chin State. Local people use it as a salad, wine and other kinds of native foods. This research focused on phytoconstituents, elemental contents, antioxidant activity and cytotoxicity of flowers of *R. arboreum* (Tawng Za Lat Ni) that were investigated by UV -Visible spectrophotometer, animal test model (*Artemia salina*) and some modern techniques. Moreover, the elemental contents of these flowers were analyzed by Energy Dispersive X ray Fluorescence (EDXRF) Method that showed the presence of K, Si, Ca, Fe, Mn, Cu and Zn. According to antioxidant activity test, IC_{50} of Tawng Za Lat Ni flower was 4.57μg/mL indicating the presence of antioxidant substances that can bond with and stabilize free radicals helping to prevent the damage they cause to the body, thereby reducing the risk of cancer. The cytotoxic effect of ethanol extract of flowers was examined by brine shrimp assay (*Artemia salina*) and Tawng Za Lat flowers were not found to have cytotoxic effect (and considered to be safe for consumption.

Keywords: *R. arboreum,* antioxidant activity, cytotoxic effect, brine shrimp assay, *Artemia salina*

Introduction

Traditional medicines are made from plants, animal products and minerals (Still, 2003; Healing Base, 2011). However, medicinal plants and plant derived medicines are widely used in traditional cultures all over the world and they are becoming increasingly popular in modern society. Plants have been particularly used as medicine for health and survival of man (Petrovska, 2012). The knowledge of the development of ideas related to the usage of medicinal plants as well as the evolution of awareness has increased the ability of pharmacists and physicians to respond to the challenges that have emerged with the spreading of professional services in facilitation of man's life. Myanmar traditional knowledge and medicine is believed to be able to cure all of these diseases by using ingredients such as fresh or dried roots, stems, leaves, buds and flowers. Traditional medicine continues to be widely practiced by the majority of the population, partly as a supplement and partly as an alternative to modern medicine.

Scientists all over the country had to test various herbs on modern scientific lines with a view to proving claims of proper selection of medicinal plant. The general research methods includes proper selection of medicinal plants, preparation of crude extracts, biological screening, detailed chemopharmacological investigations, toxicological and clinical studies, standardization and use of active moiety as the lead molecule for drug design. *Rhododendron* species have long been used in traditional medicine. Animal studies and in vitro research has identified possible

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anti-inflammatory and hepatoprotective activities which may be due to the antioxidant effects of flavonoids or other phenolic compounds and saponins.

Rhododendron species are found in Sino Himalayas, southern and northeastern China, Japan, Myanmar, Thailand, Indonesia, Malaysia, Phillipines and New Guinea (Bhattacharyya, 2011) . In Myanmar, *Rhododendron arboreum* flower (white and red) is the famous landmark of Mt. Victoria as well as Chin State (Ong *et al*., 2018). Most species have brightly coloured flowers which bloom from late winter through to early summer (Turner and Wasson, 1997). Rhododendron species have long been used in traditional medicine (Popescu and Kopp, 2013). Scientific classification of *R. arboreum* (Tawng Za Lat Ni) (Figure 1) is as follows (Madhvi *et al*., 2019).

Family name	-	Ericaceae
Scientific name	-	*Rhododendron arboreum* W.W.Sm
Myanmar name	-	Tawng Za Lat Ni
English name	-	Rose Apple
Medicinal uses	-	Anti-inflammatory, hepatoprotective activities
Distribution	-	Hilly region (Chin State) in Myanmar
Part used	-	Flowers

(a) (b)

Figure 1 (a) Tawng Za Lat tree and (b) Flowers of Tawng Za Lat Ni

This research focused on the phytochemicals, elemental contents, antioxidant activity and cytotoxicity of the flowers of *Rhododendron arboreum* W.W.Sm (Tawng Za Lat Ni) that is suitable for local traditional food of Chin ethnic group.

Materials and Methods

Sample Collection

Flowers of Tawng Za Lat Ni were collected from Chin State, Myanmar. The flowers were identified at Department of Botany, University of Mandalay. The collected flowers were dried in the shade for two weeks and then, 300 g of the flower samples were dissolved in 2.5 L of ethanol.

Phytochemical Tests of *R. arboreum* (Tawng Za Lat Ni)

In order to know the type of chemical constituents consisting in the Tawng Za Lat Ni flower, phytochemical tests were carried out according to the reported methods (Evans,1996).

Elemental Analysis of *R. arboreum* (Tawng Za Lat Ni)

The elements present in Tawng Za Lat Ni flowers were examined by Energy Dispersive X-ray Fluorescence Spectroscopy (EDXRF) (SPECTRO XEPOS EDXRF Spectrometer, Germany) at the Department of Chemistry, Monywa University.

Determination of Antioxidant Activity of *R. arboreum* (Tawng Za Lat Ni)

In this study, DPPH (2,2-diphenyl-1-1-picryl hydrazyl) free radical scavenging assay has been chosen to evaluate the free radical scavenging effectiveness of crude extract of Tawng Za Lat Ni flower (Marinova and Batchvarov, 2011) at Department of Biotechnology, Mandalay Technological University.

Sample solutions of Tawng Za Lat Ni flowers extract were prepared by dissolving 3.125 mg of flower extract in 100 mL of ethanol to obtain 31.25 μg/mL and then it was serially diluted to obtain 15.625, 7.812, 3.906, 1.953 μg/mL solutions.

The control solution prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of ethanol, and the sample solution prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of test sample solution were incubated at room temperature and shaken on a shaker for 30 min. After incubation, the absorbance values of different concentrations of tested samples were measured at 517 nm. Absorbance measurements were used to calculate the percentage of radical scavenging activity (% RSA) by the following equation:

$$\% \text{ RSA} = [A_{DPPH} - (A_{sample} - A_{blank})/ A_{DPPH}] \times 100$$

where,

$\%$ RSA = % radical scavenging activity of test sample
A_{DPPH} = absorbance of DPPH in EtOH solution
A_{sample} = absorbance of sample + DPPH solution
A_{blank} = absorbance of sample + EtOH solution.

Ascorbic acid was taken as a standard for preparation of standard curve with different concentrations (2.000,1.000, 0.500,0.250 and 0.125 μg/ mL).

Determination of Cytotoxicity by Hatching of Brine Shrimp Larvae

Dried cysts of *Artemia salina* (brine shrimp) were hydrated for 30 min in sterile distilled water and then cysts were suspended in 400 mL of sterile artificial sea water with salinity 0.33 ppt. A 60 watt lamp was positioned upon it to provide a direct light and warmth throughout the embryogenesis. Filtered air was passed through the cooled solution to reoxygenate the medium.

Free swimming nauplii started to appear after 12 h and most of the eggs became hatched into free swimming forms by 24 h. The nauplii were collected using a Pasteur pipette with a nozzle of at least 1 mm in diameter.

Sample solutions of Tawng Za Lat Ni flowers extract were prepared by dissolving 100 mg of flower extract in 100 mL of ethanol to obtain 1000 μg/mL and then it was serially diluted to obtain 500, 250, 125 and 62.5 μg/mL solutions.

Brine shrimp toxicity test was determined by the methods of Mayer *et al* (1982). Firstly, 1 mL each of the diluted extract solutions (test solution) was added to vials and thirty nauplii were collected with Pasteur pipette from the hatching container and were transferred to each vial carrying over the minimum amount of sea water. The vials with solvent and potassium dichromate solutions were also filled with thirty nauplii as controls.

The vials were restored in the dark room while the temperature was controlled at25 ± 1^{o}C. After 24 h incubation in the dark room, the vials were taken out for counting of nauplii. Nauplii were considered dead if they immobilized at the bottom of the vials. Counting of dead and alive nauplii in each vial was made to get LD_{50} for each flower ethanol extract (Sahgal *et al*., 2010). Based on the percentage of the mortality, the 50% lethality dose (LD_{50}) to the nauplii was determined by using the graph of mean percentage mortality versus the log of concentration.

Results and Discussion

Phytochemicals Present in *R. arboreum* (Tawng Za Lat Ni) Flower

The results of the phytochemical screening of *Rhododendro**n** arboreum* (Tawng Za Lat Ni) flowers are shown in Table 1. According to above table, flowers of Tawng Za Lat Ni contains alkaloids, tannins, flavonoids, terpenes, glycosides, phenolic compounds, polyphenols and reducing sugars

Table 1 Phytochemicals present in *R. arboreum* (Tawng Za Lat Ni) Flowers

No	Test	Reagent	Observation	Result
1	Alkaloids	(1) Wagner's reagent	Brown ppt	+
		(2) Dragendorff's reagent	Orange ppt	+
2	Tannins	10% $FeCl_3$,dil H_2SO_4	Yellow brown colour solution	+
3	Saponins	Distilled water	No froth like comb	-
4	Flavonoids	Conc: HCl, Mg turning,heat	Pink colour solution	+
5	Terpenes	$CHCl_3$, Acetic anhydride, conc: H_2SO_4	Deep pink colour solution	+
6	Glycosides	10% Lead acetate	White ppt	+
7	Steroids	Acetic anhydride, conc: H_2SO_4	No green colour solution	_
8	Phenolic compounds	10% $FeCl_3$	Greenish blue colour	+
9	Polyphenols	1% $FeCl_3$, 1% $K_3Fe(CN)_6$	Greenish blue colour	+
10	Reducing sugars	Benedict solution	Orange	+

(+) = present of constituent, (-) = absence of constituent

Elemental Analysis of *R. arboreum* (Tawng Za Lat Ni) Flowers by EDXRF Method

The relative abundance of some elements present in flowers of Tawng Za Lat Ni was analyzed by EDXRF method. The results obtained are listed in Table 2. According to the EDXRF report, there are eleven elements found in the flowers of Tawng Za Lat Ni. Among them, potassium is the most abundant element that is very suitable for human body that decreases the risk of stroke, lowers blood pressure, protects against loss of muscle mass, preserves bone mineral density and reduces the formation of kidney stones.

Antioxidant Activity of *R. arboreum* (Tawng Za Lat Ni) Flowers

The antioxidant activity of ethanolic extract of sample was determined by DPPH free radical scavenging assay. Ascorbic acid was used as standard and the result of IC_{50} value of the standard ascorbic acid (0.39 μg/mL) is shown in Table 3. According to Table 4 and Figure 2 it was found that the ethanolic extract scavenged DPPH radical . In DPPH screening assay that IC_{50} value of sample was found to be 4.57 μg/mL which was a little higher than that of standard ascorbic acid (IC_{50} 0.39 μg/mL). So, the sample extract has less antioxidant activity than the standard ascorbic acid. Tawng Za Lat Ni flowers contained phenolic compounds providing them the potential of scavenging free radicals. The sample has a suitable antioxidant activity for the treatment of cancer and hepatic diseases.

Table 2 Relative Abundance of Some Elements Present in Tawng Za Lat Ni Flowers

No	Elements	Symbols	Relative abundance (%)
1	Potassium	K	0.678
2	Silicon	Si	0.158
3	Phosphorus	P	0.109
4	Sulphur	S	0.105
5	Calcium	Ca	0.084
6	Iron	Fe	0.006
7	Manganese	Mn	0.004
8	Copper	Cu	0.002
9	Titanium	Ti	0.001
10	Zinc	Zn	0.001
11	Gold	Au	0.001

Table 3 Results of IC_{50} value of the standard Ascorbic Acid

Concentration (μg/mL)	Mean Absorbance	Mean % Inhibition	IC_{50} (μg/mL)
0.125	0.3593	41.21401	
0.250	0.3204	47.57853	
0.500	0.2871	53.02683	0.39
1.000	0.2541	58.42605	
2.000	0.2189	64.18521	

Table 4 Results of IC_{50} Value of the Tawng Za Lat Ni Flowers

Concentration (μg/mL)	Mean Absorbance	Mean % Inhibition	IC_{50} (μg/mL)
1.950	0.2250	45.21549	
3.900	0.2184	46.82250	
7.800	0.1908	53.54273	4.57
15.625	0.0951	76.84441	
31.250	0.0194	95.27636	

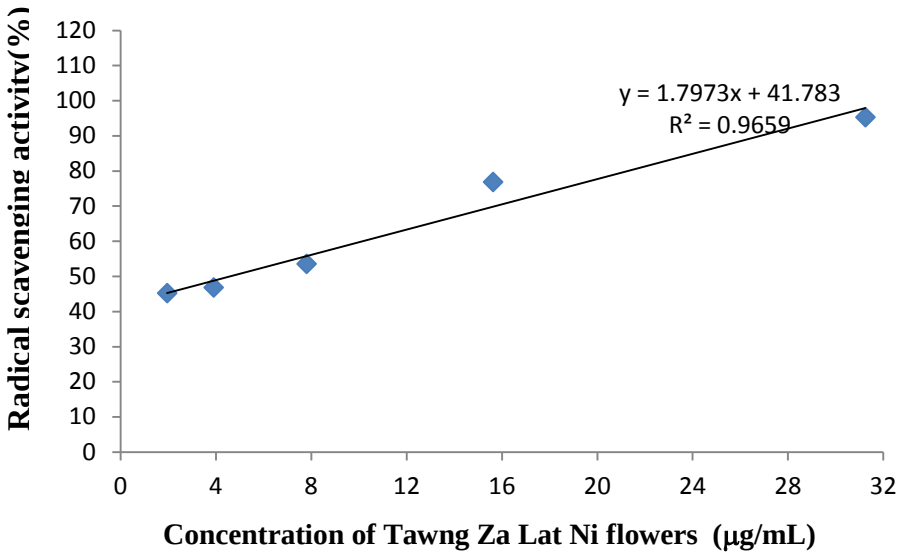

Figure 2 Plot of radical scavenging activity as a function of concentration of Tawng Za Lat Ni flowers

Toxicity Testing of the Tawng Za Lat Ni Flowers Using Brine Shrimp

Toxicity of the test samples was tested by using brine shrimp (*Artemia salina*) . Brine shrimp toxicity test results are shown in Table 5. Plotting of mortality percentage versus log of concentration of Taung Za Lat Ni flower extract demonstrates a linear correlation (Figure 3). Furthermore, there is a direct proportional relation between the concentration of the Taung Za Lat Ni flower extract and the degree of lethality (R^2 = 0.9917). LD_{50} value of Taung Za Lat Ni flower extract was calculated as > 1000 µg/mL. LD_{50} is the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals (brine shrimp). The LD_{50} is one way to measure the short-term poisoning potential (acute toxicity) of a material. According to Meyer's toxicity index, the sample with LD_{50} < 1000 µg/mL are considered as toxic, while the sample with LD_{50} > 1000 µg/mL are considered as non-toxic (Meyer *et al*., 1982). Thus, Taung Za Lat Ni flower were found to be noncytotoxic in the brine shrimp bioassay so that they can be used for human consumption.

Table 5 Mortality Percentage of *R. arboreum* (Tawng Za Lat Ni) Flowers by Brine Shrimp Test

Concentration C (µg/mL)	Log C	Alive	Dead	Accumulated Alive	Accumulated Dead	Ratio/ Death: Total	Mortality (%)
1000	3	1	29	1	75	75 : 76	0.987
500	2.69897	13	17	14	46	46 : 60	0.767
250	2.39794	18	12	32	29	29 : 61	0.475
125	2.09691	20	10	52	17	17 : 69	0.246
62.5	1.79588	23	7	75	7	7 : 82	0.085

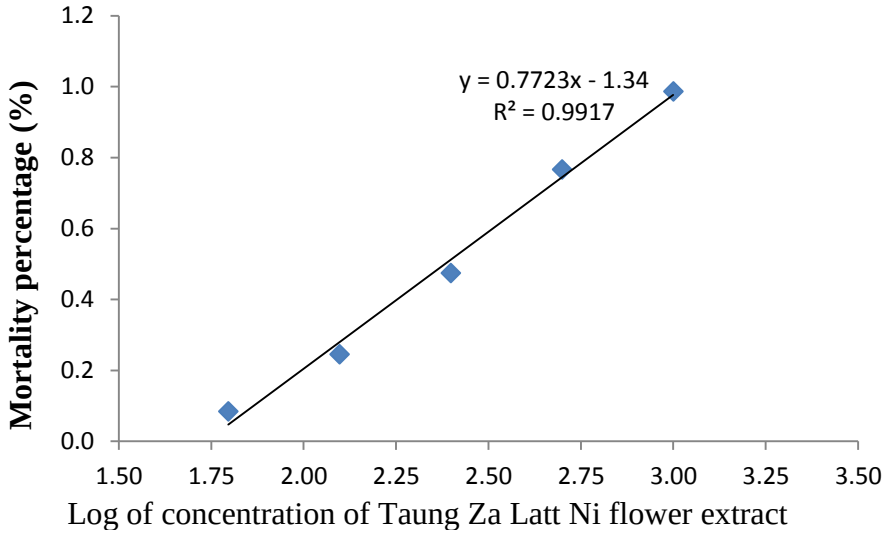

Figure 3 Plot of mortality percentage as a function of log concentration of Tawng Za Lat Ni flowers extract

Conclusion

In this research work, the phytochemical constituents of *R. arboreum* (Tawng Za Lat Ni) flower consists of secondary plant products such as alkaloids, tannins, flavonoids, terpenes, glycosides, phenolic compounds, polyphenols and reducing sugars by phytochemical assays. According to EDXRF report, there are eleven elements that are found in these sample flowers in which potassium constituent is the highest percentage among elements. IC_{50} value of Tawng Za Lat Ni flower is 4.57 μg/mL and thus, it has less antioxidant activity than the standard ascorbic acid (IC_{50} =μg/mL). However, it has a moderate amount of antioxidant activity. Brine shrimp assay showed that *R. arboretum* (Tawng Za Lat Ni) flower was noncytotoxic. *Rhododendron* flowers have a number of health benefits and have full potency to be utilized in the food and beverage industry.

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References

Bhattacharyya, D. (2011**).** "Rhododendron Species and Their Uses with Special Reference to Himalayas- A Review". *Assam University Journal of Science & Technology: Biological and Environmental Sciences*. vol. 7 (1), pp. 161–167

Evans, W.C.(1966) . *Trease Evans Pharmacognosy*. London: 14th Ed, WB Saunders Ltd, pp.119- 159

Healing Base. (2011). *The Use of Metals and Minerals in Ayurvedic and Siddha Medicine.* www.healingbase.com/the-use-of-metals-minerals-in. (Accessed 28 July 2019)

Marinova, G. and Batchvarov, V. (2011). "Evaluation of the Methods for Determination of the Free Radical Scavenging Activity by DPPH". *Bulgarian Journal of Agricultural Science*, 17 (1) , pp.11-24

Meyer, B. N., Ferrigni, N. R., Putman, J. E., Jacbsen, L. B., Nicols, D. E. and McLaughlin, J. L. (1982) . "Brine Shrimp : A Convenient General Bioassay For Active Plant Constituents**".** *Plant Medica*., vol. 45, pp. 31- 34

Ong, H.G., Ling, S.M., Win, T.T.M., Kang, D.H., Lee, J.H. and Kim,Y.D.(2018). "Ethnobotany of Wild

Medicinal Plants Used by the Müün Ethnic People: A Quantitative Survey in Southern Chin State, Myanmar". *Journal of herbal medicine*, vol. 13, pp. 91-96

Petrovska, B.B.(2012). "Historical Review of Medicinal Plants' Usage". *Pharmacogn Rev.* , vol.,6(11), pp. 1–5

Popescu, R. and Kopp, B. (May 2013). "The Genus Rhododendron: An Ethnopharmacological and Toxicological Review". *J Ethnopharmacol.*, vol.147 (1), pp. 42–62

Sahgal, G., Ramanathan, S. , Sasidlharan, S. M., Mordi, S., Ismail, S. and Mansor, S. M. (2010). "Brine Shrimp Lethality and Acute Oral Toxicity Studies on *Swietenia mahagoni* (Linn.) Jacq. Seed Methanolic Extract". *Pharmacognosy Research*, vol. 2, pp. 215-220

Still, J.(2003). "Use of Animal Products in Traditional Chinese Medicine: Environmental Impact and Health Hazards". *Complement Ther Med*., vol.11(2), pp.118-122

Turner Jr., R.J. and Wasson, E. (1997). *Botanica: The Illustrated A-Z of Over 10,000 Garden Plants and How to Cultivate Them.* USA: 1st ed., Better World Books, p. 742