SCREENING OF PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF THE RHIZOMES AND LEAVES OF *Hedychium coronarium* J.Koenig (Ngwe-Pan)

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Abstract

In the present study, the rhizomes and leaves of *Hedychium coronarium* J.Koenig (Ngwe-pan) were collected from Hmawbi Township, Yangon Region. The relative abundance of elemental composition was determined by ED-XRF method resulting the major mineral elements such as potassium, sulphur, calcium and phosphorus in the Ngwe-pan rhizomes and potassium, chlorine, calcium, sulphur in the Ngwe-pan leaves. In addition, the nutrient values of Ngwe-pan rhizomes and leaves were determined by AOAC method showing the moisture (13.08 %), ash (7.34 %), protein (3.36 %), crude fiber (14.81 %), crude fat (4.27 %), carbohydrates (25.17 %) and energy value (152.55 kcal/100 g) in Ngwe-pan rhizomes and moisture (13.89 %), ash (8.21 %), protein (7.46 %), crude fiber (35.42 %), crude fat (4.10 %), carbohydrates(12.93 %) and energy value (118.46 kcal/ 100g) in Ngwe-pan leaves. The preliminary phytohchemical constituents of both samples were examined by using the Test Tube method. According to the phytochemical tests, Ngwe-pan rhizomes and leaves extracts showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, starch, saponins, steroids, terpenoids and tannins. However, cyanogenic glycosides were not found in these samples. The screening of antioxidant activities of Ngwe-pan rhizomes and leaves was carried out by DPPH method. Ascorbic acid was used as the standard. From the screening, among the extracts of rhizomes and leaves samples, the EtOH and H_2O extract of Ngwe-pan leaves showed the more potent antioxidant activity than that of the rhizome extracts.

Keywords: *Hedychium coronarium*, nutrient values, elemental composition, phytochemical constituents, antioxidant activity

Introduction

Hedychium coronarium is a monocotyledon perennial herb which belongs to family Zingiberaceae. It is commonly known as white ginger or butterfly ginger because its flower looks like a flying butterfly. *H. coronarium*

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is an aromatic rhizomatous plant which possesses important medicinal properties and its various parts are used in traditional as well as modern medicine (Vaidyaratnam, 2006).

The medicinal value of this plant in the therapeutic field is mentioned in Ayurveda, Charaka Samhita and Sushruta Samhita. The all parts of this plant are utilized as medicine as well as other daily uses, although its applications varies by region. In the Ayurvedic system of Indian traditional medicine it has used as a febrifuge, tonic and excitant and also used as medicine in the treatment of tonsillitis, infected nostrils and tumor. In Chinese medicine it has been prescribed in treatment of headache, lancinating pain, contusion, inflammatory and intense pain due to rheumatism etc (Mishra, 2013). *H. coronarium* is also reported for its anti-cancerous, antioxidant, anti-hypertensive, diuretic, and leishmanicidal, anti-malarial activities it is also used in irregular menstruation, piles bleeding and stone in urinary tract. The essential oil extracted from leaves, flowers and rhizome of this plant possesses cercaricidal properties, molluscicidal activity, potent inhibitory action, antimicrobial activities, anti-inflammatory and analgesic effects. The paste prepared from rhizome is applied externally in cases of snakebite (Ray *et al*., 2011). The flowers and stems are also stand for Commercial importance as used in the manufacturing of perfume and paper respectively. Both of the flowers and rhizomes are also consumed as vegetables (Sarangthem *et al.*, 2012).

Aim

To perform the phytochemical constituents, elemental composition, evaluation of nutrient values and the determination of antioxidant activities of *H.coronarium* (Ngwe-pan) rhizomes and leaves.

Materials and Methods

Collection and Preparation of Plant Samples

The Ngwe-pan rhizomes and the leaves were collected from Hmawbi Township, Yangon Region, Myanmar, in the month of September-October, 2017. The plants were identified and authenticated at the Department of Botany, West Yangon University.

After collection, the rhizomes and the leaves were cleaned thoroughly with distilled water to remove any type of contamination. The washed rhizomes and leaves were air dried in shade for about two weeks and ground into the coarse powder with the help of a mechanical grinder. The powders of the samples were separately stored in air tight bottles.

Qualitative Elemental Analysis by Energy Dispersive X- Ray Fluorescence Method

Shimadzu EDX-8000 spectrometer can analyze the elements from Na to U under vacuum condition. In this research work relative abundance of elements present in Ngwe-pan rhizomes and leaves was determined by EDXRF spectrometer.

Determination of Nutritional Values

The nutritional values such as moisture, ash, crude protein, crude fiber, crude fat, carbohydrate contents and energy value of Ngwe-pan rhizomes and leaves were determined according to AOAC method (AOAC, 2000) at the Department of Research and Innovation Analysis Department (DRI), The Government of the Republic of the Union of Myanmar Ministry of Education (GRUMME), Yangon, Myanmar.

Preliminary Phytochemical Screening

The Ngwe-pan rhizomes and leaves were subjected to qualitative phytochemical tests for the identification of various bioactive constituents. Phytochemical screenings were carried out by using standard procedures to detect the presence of alkaloids, carbohydrates, glycosides, flavonoids, organic acids, phenolic compounds, reducing sugars, steroids, saponins, starch, terpenoids and tannins. After the addition of specific reagents to the test solution presence of phytoconstituents was examined by virtual observation of colour change or by precipitate formation.

Screening of Antioxidant Activity by DPPH Radical Scavenging Assay

The crude extracts of Ngwe-pan rhizomes and leaves were prepared by extracting the sample with different solvents like ethanol and water by cold percolation method. All of these extracts were kept for the determination of antioxidant activity. The antioxidant activity of 95 % EtOH and H_2O extracts was studied by DPPH Assay Method. DPPH radical scavenging activity was determined by spectrophotometric method. The control solution was prepared by mixing 1.5 mL of 60 µM DPPH solution and 1.5 mL of 95 % ethanol with vortex mixer. The sample solution was also prepared by mixing thoroughly 1.5 mL of 60 µM DPPH solutions and 1.5 mL of test sample solution with concentrations of (3.125, 6.25, 12.5, 25 and 50 µg/mL). The solutions were allowed to stand at room temperature for 30 min. After 30 min, measurement of absorbance at 517 nm was made by using spectrophotometer UV1800, Shimadzu, Japan. Absorbance measurements were done in triplicate for each solution and the mean value was obtained, and then used to calculate % inhibition of oxidation by the following equation,

$$\% \text{ oxidative inhibition } = \frac{A_c - (A - A_b)}{A_c} \times 100\ \%$$

% oxidative inhibition = % oxidative inhibition of test sample

A_c = absorbance of the control (DPPH alone)

A_b = absorbance of the sample + ethanol

A = absorbance of the DPPH radical + sample extract

Then IC_{50} (50 % inhibitory concentration) values were also calculated by linear regressive excel program (Brand-Williams *et al.,* 1995).

Results and Discussion

Relative Abundances of some Elements in Ngwe-pan Rhizomes and Leaves

As shown in Tables 1 and 2, by EDXRF data, the mineral elements such as K, S, Ca and P are major constituents in the rhizomes and the K, Cl, Ca and S are contained as main constituents in the leaves. Moreover, the

organic compounds are contained as predominant composition between (94-97%).

Table 1: Relative Abundance of Some Elements in Ngwe-pan Rhizomes

Element	Relative Abundance Percent (%)
K	1.222
S	0.283
Ca	0.251
P	0.184
Mn	0.101
Fe	0.005
Zn	0.002
Cu	0.001
Sr	0.001
Br	0.001
COH	97.948

Table 2: Relative Abundance of Some Elements in Ngwe-pan Leaves

Element	Relative Abundance Percent (%)
K	1.222
Cl	0.283
Ca	0.251
S	0.184
Mn	0.101
P	0.005
Fe	0.002
Zn	0.001
Cu	0.001
COH	94.788

Nutritional Values of Ngwe-pan Rhizomes and Leaves

The nutrient values of Ngwe-pan rhizomes and leaves such as moisture, ash, crude protein, crude fiber, crude fat, carbohydrates and energy value were determined by using standard methods for food analysis (AOAC, 2000) and the nutritional composition of the samples are described in Table 3.The rhizomes powdered samples were found to have higher energy values than in the leaves powdered samples.

Table 3: Nutritional Compositions of Rhizomes and Leaves of Ngwe-pan

Parameter	Nutritional Composition (%)	
	Rhizomes	Leaves
Moisture	13.08	13.89
Ash	7.34	8.21
Crude protein	3.36	7.46
Crude fiber	14.81	35.42
Crude fat	4.27	4.10
Carbohydrate	25.17	12.93
Energy value (kcal/100g)	152.55	118.46

Preliminary Phytochemical Screening of Ngwe-pan Rhizomes and Leaves

Phytochemical screening of the extracts of Ngwe-pan rhizomes and leaves were carried out to identify the secondary metabolites such as alkaloids, carbohydrates, flavonoids, glycosides, organic acid, phenolic compounds, reducing sugars, saponins, steroids, starch, tannins and terpenoids according to standard phytochemical methods. The phytochemical analysis revealed that alkaloids, carbohydrates, flavonoids, glycosides, organic acid, phenolic compounds, reducing sugars, saponins, steroids, starch, tannins and terpenoids were present in Ngwe-pan rhizomes and leaves. Cyanogenic glycoside was not detected in both samples (Table 4).

Table 4: Results of Phytochemical Investigation of Ngwe-pan Rhizomes and Leaves by Test Tube Method

Chemical Constituents	Test Reagent	Observation	Inference	
			R	L
Alkaloids	(i) Dragendorff's	Orange red precipitate	+	+
	(ii) Wagner's	Yellow ppt	+	+
	(iii) Mayer's	Creamy white precipitate	+	+
	(iv) Hager's	Yellow ppt	+	+
Carbohydrates	10% α-naphthol and Conc: H_2SO_4	Dull violet precipitate	+	+
Cyanogenic glycosides	Sodium picrate and Conc: H_2SO_4	No change	-	-
Flavonoids	Mg turning and Conc: HCl	Orange to red colour sol[n]	+	+
Glycosides	10% Lead acetate solution	yellow precipitate	+	+
Organic Acids	Bromocresol green indicator	Deep blue colour solution	+	+
Phenolic Compounds	5% Ferric chloride solution	Brown colour ppt	+	+
Reducing Sugars	Benedict's solution	Light green colour solution	+	+
Steroids	Liberman Burchard	red colour solution	+	+
Saponins	Foam test	Marked frothing	+	+
Starch	Iodine solution	Deep violet solution	+	+
Tannins	Ferrous sulphate	Greenish black precipitate	+	+
Terpenoids	Liberman Burchard	Brown ppt	+	+

(+) = present, (-) = absent, R = Rhizomes, L = Leaves, ppt = precipitate

Antioxidant Activity of Ngwe-pan Rhizomes and Leaves

Antioxidant activity was studied by DPPH free radical scavenging property by using UV spectroscopic method. Absorbance measurements were done in triplicate for each sample solution. Absorbance values obtained were used to calculate % inhibition, 50 % inhibitory concentration and standard deviation. The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search for the in *vitro* general antioxidant activity of pure compounds as well as plant extracts. The decrease in absorbance by the DPPH radical indicates increase in concentration of the extract which manifested in the rapid discoloration of the purple DPPH.

In the present work, the free radical scavenger activity of ethanol and water extracts of Ngwe-pan rhizomes and leaves and the standard ascorbic acid were assessed on the basis of the radical scavenging effect on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The various concentrations of each extract (50, 25, 12.5, 6.25 and 3.125 µg/mL) were prepared by dilution with

ethanol as the solvent. Ascorbic acid was used as standard in 3.125-50 μ g/mL solution. The absorbance of these solutions was measured at 517 nm by using UV spectrophotometer.

In the rhizomes extracts, the % free radical scavenging activities of ethanol and water extracts and ascorbic acid are presented in Table 5 and Figure 1. The % inhibition of ethanol extracts was 48.46 % and 56.29 % respectively at the concentration of 12.5 and 25 μ g/mL. And also, the % inhibition of water extract was 49.73 % and 52.46 % at 12.5 and 25 μ g/mL. The IC_{50} values of two extracts and standard ascorbic acid were calculated using linear regression method. Among two extracts tested for the in *vitro* antioxidant activity using the DPPH method, the water and ethanol extracts of rhizomes showed the highest antioxidant activities, with IC_{50} values of 14.06 and 20.11 μ g/mL. The IC_{50} value of standard ascorbic acid was 12.42 μg/mL. The IC_{50} value of Ngwe-pan rhizomes water and ethanol extracts were found to be higher than that of standard ascorbic acid.

In the leaves extracts, the % free radical scavenging activities of ethanol and water extracts are presented in Table 5 and Figure 2. The % inhibition of ethanol extracts was 44.64 % and 56.83 % at the concentration of 6.25 and 12.5 μ g/mL, respectively. And also, the % inhibition of water extract was 48.63 % and 50.82 % at 6.25and 12.5 μ g/mL, respectively. Among two extracts tested, the ethanol and water extracts of leaves showed the highest antioxidant activity, with IC_{50} values of 10.33 and 12.48 μ g/mL, respectively.

It indicates that the water and ethanol extracts of Ngwe-pan rhizomes and leaves have the potency of scavenging free radicals in *in vitro* and may provide leads in the ongoing search for natural antioxidants from medicinal plants to be used in treating diseases related to free radical reactions.

Table 5: Percent Oxidative Inhibition and IC_{50} Values of Crude Extracts of Ngwe-pan Rhizomes and Leaves

Test samples	%RSA±SD at Different concentration (µg/mL)					IC_{50} (µg/mL)
	3.125	6.25	12.5	25	50	
Rhizomes of Ngwe-pan (Ethanol)	23.66±0.001	39.93±0.002	48.46±0.003	56.29±0.001	78.08±0.004	20.11
Rhizomes of Ngwe-pan (H_2O)	46.56±0.002	48.08±0.005	49.73± 0.001	52.46±0.003	61.20±0.001	14.06
Leaves of Ngwe-pan (Ethanol)	42.98±0.002	44.64±0.003	56.83±0.011	90.93±0.001	67.49±0.006	10.33
Leaves of Ngwe-pan (H_2O)	46.88±0.001	48.63±0.002	50.82±0.004	53.55±0.002	57.63±0.001	12.48
Standard Ascorbic acid	35.26±0.217	40.35±0.218	50.12±0.150	66.61±0.122	80.71±0.070	12.42

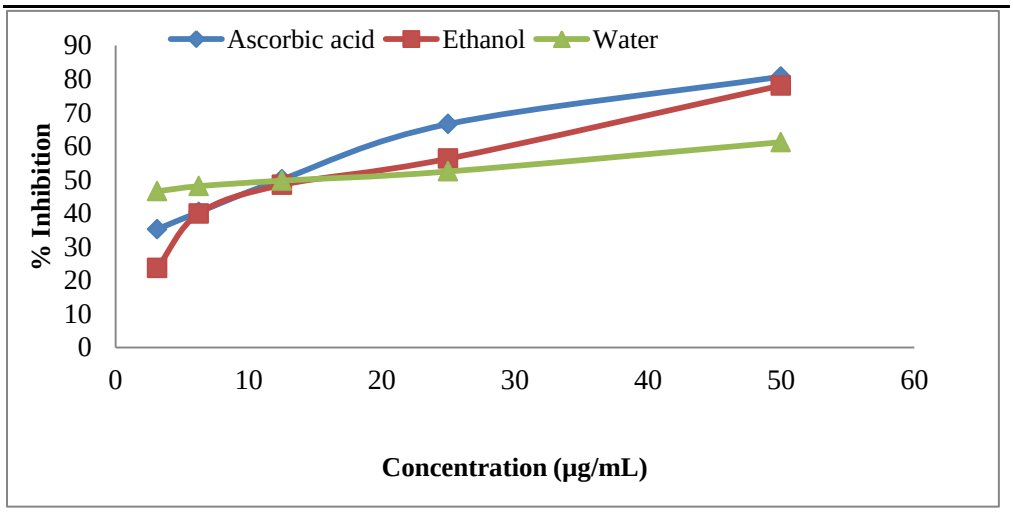

Figure 1: Antioxidant activity of ascorbic acid and different concentrations of Ngwe-pan rhizomes extract

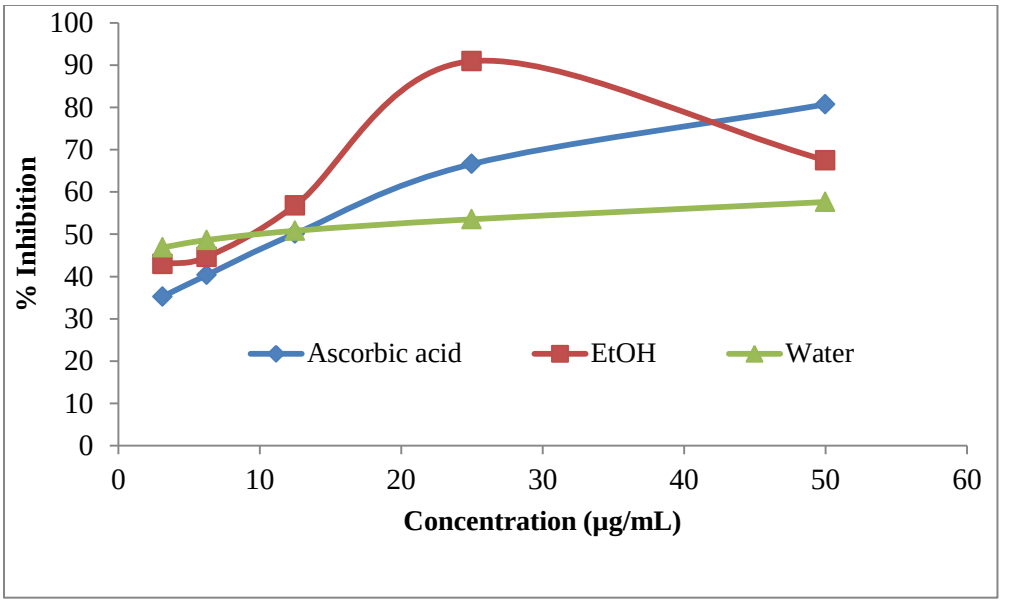

Figure 2: Antioxidant activity of ascorbic acid and different concentrations of Ngwe-pan leaves extract

Conclusion

In the present work, phytochemical analysis, nutritional values, EDXRF analysis and antioxidant activity of the Ngwe-pan rhizomes and leaves were studied. The phytochemical analysis of Ngwe-pan rhizomes and leaves powdered sample showed the presence of alkaloids, carbohydrates, glycosides, flavonoids, organic acids, phenolic compounds, reducing sugars, steroids, saponins, starch, terpenoids and tannin. Cyanogenic glycosides were absent both in the two samples. The presence of secondary metabolites in the rhizomes and leaves suggests that their consumption could have a preventive effect on human body and help to treat related diseases by providing indicated properties to the plant. By ED-XRF data, the mineral elements such as K, S, Ca and P are major constituents in the rhizomes and the K, Cl, Ca and S are also contained in the leaves. Therefore, Ngwe-pan rhizomes and leaves contained nutritionally important minerals. These mineral contents were well within permissible range for human consumption, therefore, recommended for safety and nutritious food. In addition, some nutrient values of rhizomes sample was found to contain of 13.08 % moisture, 7.34 % of ash, 3.36 % of

protein, 14.81 % of crude fiber, 4.27 % of crude fat, 25.17 % of carbohydrates and 152.55 kcal/100g of energy value, whereas the leaves sample was also contained of 13.89 % moisture, 8.21 % of ash, 7.46 % of protein, 35.42 % of crude fiber, 4.10 % of crude fat, 12.93 % of carbohydrates and 118.46 kcal/ 100g of energy value. These nutrient values of the sample support the diet supplement for the human health. *In vitro* antioxidant potential by DPPH free radical scavenging assay using ascorbic acid as the standard. The 95% EtOH and H_2O extracts of plant rhizomes and leaves were analyzed. Antioxidant activities in terms of IC_{50} values of H_2O and EtOH extracts of rhizomes were 14.06 µg/mL and 20.11 µg/mL respectively. Moreover, the IC_{50} values of leaves extract of H_2O and EtOH were 12.48 and 10.33 µg/mL, respectively. The smaller the IC_{50} values will greater the antioxidant activities. Therefore, the EtOH extract (10.33 µg/mL) and H_2O extract (12.48 µg/mL) of Ngwe-pan leaves and H_2O rhizome extract (14.06 µg/mL) have the highest antioxidant activity than the rhizome EtOH extract (20.11 µg/mL). The study revealed that *H.coronarium* rhizomes and leaves contained appreciable amounts of mineral elements, nutrients such as energy, protein and phytochemicals. According the findings, the water and ethanol extracts of *H.coronarium* leaves have more potent antioxidant activities than the *H.coronarium* rhizomes. Therefore, *H.coronarium* rhizomes and leaves possess the reasonable antioxidant activities and bioactive organic constituents will be beneficial for further studies on pharmacology activities.

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