Investigation of Phytochemical Constituents and Antioxidant Activity of Rhizome from *Alpinia officiarum* Hance (Pa-De-Kaw)

Zin Win Win Htet[1], Phyo Phyo San[2], Tin Mar Ang[3] and Mon Mon Thu[4]

Abstract

The rhizomes of *Alpinia officinarum* Hance (Pa-de-kaw) were collected from Pathein Township. The phytochemical constituents of selected sample were found to be present the alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, tannins, steroids, starch and terpenoids and reducing sugars was absent in the *A. officinarum* Hance rhizomes. Antioxidant activity of *Alpinia officinarum* Hance was investigated by using DPPH radical scavenging assay. The IC_{50} values of ethanol and watery extracts from *Alpinia officinarum* Hance rhizomes were observed to be 80.44 and 45.74 μg mL^{-1} respectively. Therefore, watery extract of *A. officinarum* Hance is higher antioxidant activity than ethanol extract. The total phenolic content was found to be higher content in the 95 % ethanol extract (313.23 μg GAE/ mg) than that of watery extract (100.32 μg GAE/ mg). So, *Alpinia officinarum* Hance rhizomes should be used as food source as well as medicinal uses.

Keywords*:* *Alpinia officinarum* Hance rhizomes, phytochemical constituents, total phenolic content, antioxidant activity

Introduction

Plants are one of the most important sources of medicines. *Alpinia officinarum* Hance is a species of ginger. *Alpinia officinarum* Hance belonging to the family, *Zingiberaceae*, cultivated in South East Asia. The common names for *Alpinia officinarum* Hance is lesser galangal in English, Myanmar is Pa-de-kaw. Medical plants and derived medicine are widely used in traditional cultures all over the world and they are becoming increasingly popular in modern society as natural alternatives to synthetic chemicals.

This rhizome is characterized by dark reddish brown colour which has a strong aromatic odour. Aromatic and rhizomatous plant, *A. officinarum* Hance used in various biological activities. *A. officinarum* Hance are rich in phytochemical it is an important source of various types of active compounds that poses many biological activities and insecticidal activities. (Partban *et al*.,2015)

The most important of these bioactive constituents of plants are alkaloids, tannins and flavonoids components. *A. officinarum* Hance provides strong biological activity. Different parts of the plant are used in the treatment of many diseases for its anti-fungal, anti-tumor, antimicrobial, anti-inflammatory, anti-diabetic, antioxidant anti-ulcer and many other properties (*Biswas et al., 2010).*

Materials and Methods

Sampling of Rhizomes Materials

The rhizome of *Alpinia officinarum* Hance (Pa-de-kaw) from Pathein Township. After collection, the botanical name of the sample was identified by authorized botanist at Department of Botany, Pathein University. The sample was washed with distilled water and allowed to shade dry at room temperature. The dry sample was cut into small pieces and powdered with mixer grinder. After grinding, powder samples were stored in air tight container.

[1] Demonstrator, Department of Chemistry, University of Mandalay
[2] Demonstrator, Department of Chemistry, University of Mandalay
[3] Lecture, Dr, Department of Chemistry, Dagon University
[4] Professor, Dr, Department of Chemistry, University of Mandalay

Figure 1. Nature of *Alpinia officinarum* Hance

Investigation of Phytochemical constituents of *A. officinarum* Hance rhizome

Phytochemical investigation of *A. officinarum* Hance was carried out according to the reported methods to investigate the presence or absence of phytoconstituents such as alkaloids, α-amino acids, carbohydrates, glycosides, phenolic compounds, reducing sugar, saponins, starch, steroids, tannins, terpenoids and flavonoids (Vogel, 1966; Harborne 1983; Marini-Bettolo *et al*., 1981).

Determination of Total Phenol Content (TPC) of *Alpinia officinarum* Hance

(i) Construction of gallic acid standard curve

First, 0.5 mL each of different concentration of gallic acid concentration (100, 50, 25, 12.5 and 6.25 μg/ mL) was mixed with 5 mL of 10 % FC reagent in the test tubes and incubated for 5 min. To each tube, 4 mL of 1 M Na_2CO_3 was added and the tubes were kept at room temperature for 30 min and the absorbance of reaction mixture was read of 760 nm. A standard curve was prepared by plotting the absorbance against concentration of gallic acid (Sheikh, 2000).

(ii) Determination of gallic acid equivalent in crude extract sample

The total phenolic content in the crude extract was estimated by Folin-Ciocalteu method. Each crude extract samples (0.5 mL) was added into 5 mL of 10 % FC reagent and incubated for 5 min. To each tube, 4 mL of 1 M Na_2CO_3 was added and the tubes were kept at room temperature for 30 min and the absorbance of reaction mixture was read of 760 nm. The blank solution was prepared as above procedure by using distilled water instead of sample solution. Total phenolic content was estimated as μg gallic acid equivalents per milliliter (μg GAE/ mL) of crude extract (Sheikh, 2000).

Antioxidant Activity of *Alpinia officinarum* Hance by DPPH Assay Method

DPPH free radical scavenging activity was determined by UV-visible spectrophotometric method.

The antioxidant power (IC_{50}) is expressed as the test substances concentration (μg/mL) that result in a 50 % reduction of initial absorbance of DPPH solution and that allows to determine the concentration. IC_{50} (50 % inhibition concentration) values were calculated by linear regressive excel program (Sekiwa *et al.*, 2000).

Results and Discussion

Phytochemical Investigation for Chemical Constituents of *Alpinia officinarum* Hance

The preliminary phytochemical tests revealed the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins,

tannins, steroids, starch and terpenoids. The reducing sugars were not found in the rhizomes of *A.officinarum* Hance. These results are shown in Table 1.

Table 1. Results of Phytochemical Analysis of *Alpinia officinarum* Hance

No	Types of compounds	Extracts	Test reagents	Observation	Remark
1	Alkaloids	1 % HCl	Dragendroff's reagent Mayer's reagent Wagner's reagent	Orange ppt. White ppt. Brown ppt.	+ + +
2	α-amino acid	H_2O	Ninhydrin reagent	Purple spot	+
3	Carbohydrates	H_2O	10 % α-naphthol Conc:H_2SO_4	Red ring	+
4	Flavonoids	EtOH	Mg ribbon conc: HCl	Pink color	+
5	Glycosides	H_2O	10 % lead acetate solution	White ppt.	+
6	Phenolic compounds	H_2O	5 % $FeCl_3$ sol^n $K_3Fe(CN)_6$	Deep blue color	+
7	Reducing sugars	Dil. H_2SO_4 NaOH Sol^n:	Benedict's solution	No Brick red ppt.	-
8	Saponins	H_2O	Distilled water	Frothing	+
9	Starch	H_2O	I_2 solution	Deep blue color	+
10	Steroids	PE	Acetic anhydride and conc: H_2SO_4	Blue/blue green	+
11	Tannins	EtOH	1 % Gelatin, 5 % $FeCl_3$, solution	Green color	+
12	Terpenoids	$CHCl_3$	Acetic anhydride and conc: H_2SO_4	Reddish brown spot	+

(+) = presence (-) = absence ppt.= precipitate

Total Phenolic Content of Some Crude Extracts from the Rhizomes of *Alpinia officinarum* Hance

The total phenolic content of *Alpinia officinarum* Hance was estimated by Folin-Ciocalteu method. Gallic acid was used to construct standard calibration curve for total phenol. Total phenol content (TPC) was expressed as microgram of gallic acid equivalent per milligram of crude extract (μg GAE/ mg).

The results of total phenolic content of 95 % EtOH and watery extracts of *Alpinia officinarum* Hance were presented in Table 3. Bar graphs of total phenolic content of 95 % EtOH and watery extracts of *Alpinia officinarum* Hance are shown in Figure 3. In the case of the TPC was found to be higher content in the 95 % EtOH extract (313.23 μg GAE/ mg) than that in watery extract (100.32 μg GAE/ mg).

Table 2. The Absorbance of Gallic Acid Standard Solution at λ_{max} 760 nm

No.	Concentration (µg/mL)	Absorbance at λ_{max} 760 nm
1	6.25	0.163
2	12.5	0.184
3	25	0.215
4	50	0.286
5	100	0.454

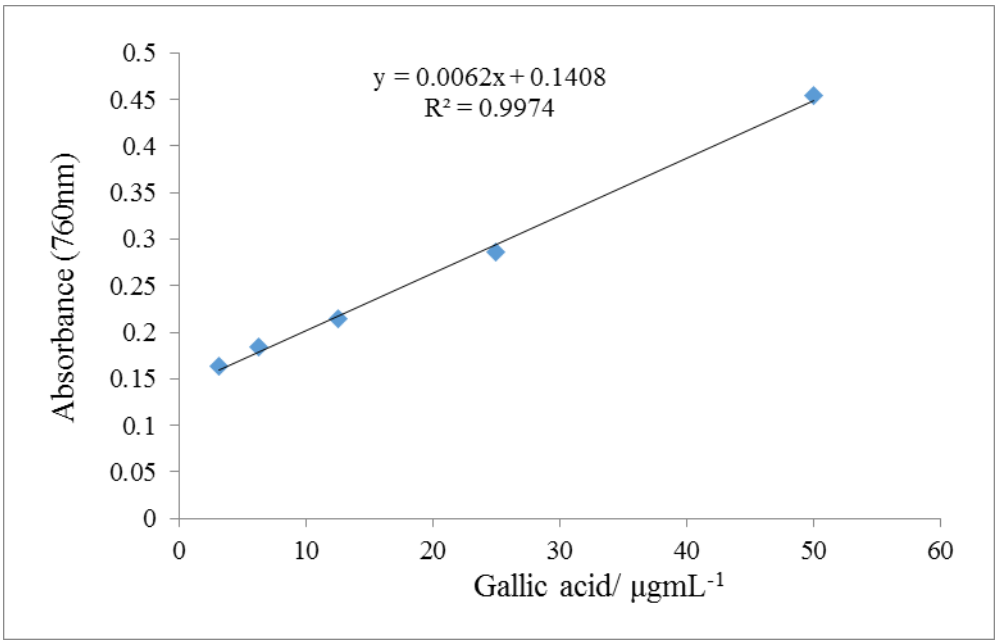

Figure 2. A plot of gallic acid standard curve

Table 3. Total Phenolic Content (TPC) of Ethanol and Watery Extracts from the Rhizomes of *Alpinia officinarum* Hance by Folin–Ciocaltue (FC) Method

No.	Extract	Absorbance	TPC (µg GAE /mg of Extract)
1	EtOH	0.335	313.23
2	Watery	0.203	100.32

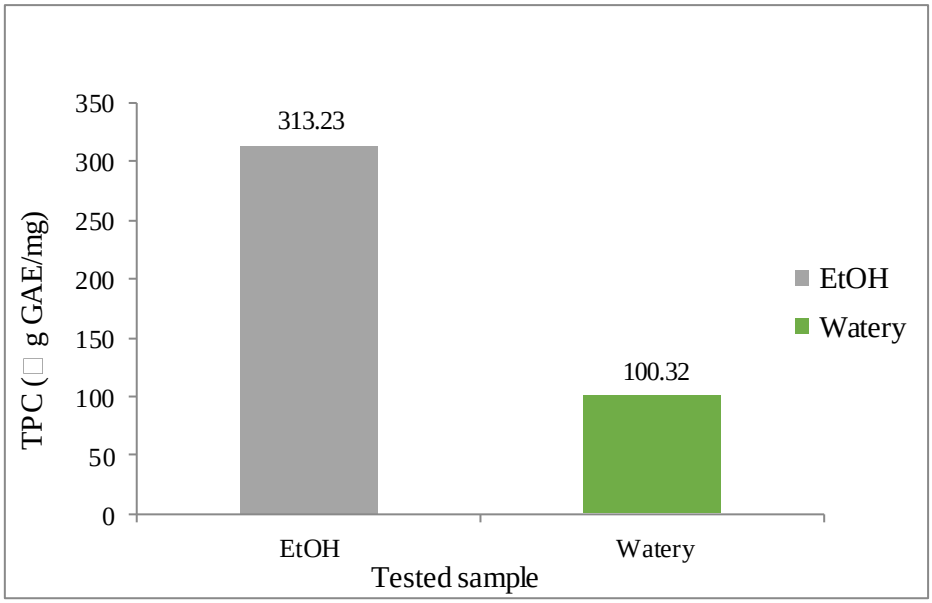

Figure 3. A bar graph of total phenolic content (TPC) of crude extracts from the rhizomes of *Alpinia officinarum* Hance

Antioxidant Activity of Ethanol and Watery Crude Extracts of *Alpinia officinarum* Hance Rhizomes

The antioxidant activities of ethanol and watery extracts of *A. officinarum* Hance were studied by DPPH free radical scavenging assay. Colorimetery with DPPH, a stable free radical, has been reported as a simple method for evaluation of the free radical scavenging activity. It tends to capture hydrogen from the antioxidant. Due to its free radical, the ethanolic DPPH solution is violet and absorbance at 517 nm. The colour changes upon neutralization of this free radical from violet to pale yellow by daylight. The decolouration of the initial colour is proportional to the test substances having antiradicalizing power.

From these experimental results, the rhizome of *Alpinia officinarum* Hance was found to have antioxidant activity. IC_{50} values of ethanol and watery extracts are 80.44 and 45.74 µg/ mL respectively. According to the result, the watery extract of *Alpinia officinarum* Hance was found to be more antioxidant potency than that of ethanol extract. Antioxidant potency of ethanol and watery extracts were concluded to be weak if compare with the potency of standard butylated hydroxytoluene (IC_{50} = 13.45 µg/ mL).

Table 4. Radical Scavenging Activity (% RSA) and IC_{50} Values of Watery and Ethanol Extracts of *Alpinia officinarum* Hance and Standard BHT

Extracts	% RSA ± SD of different concentrations of extracts (µg/mL)						IC_{50} (µg/mL)
	12.5	25	50	100	200	400	
Watery	32.97 ± 0.000	39.97 ± 0.002	52.06 ± 0.009	74.73 ± 0.001	95.47 ± 0.004	96.70 ± 0.005	45.74
EtOH	27.34 ± 0.002	30.91 ± 0.001	40.38 ± 0.001	56.18 ± 0.001	74.59 ± 0.001	93.41 ± 0.001	80.44
BHT (Std)	49.23 ± 0.002	59.36 ± 0.006	71.63 ± 0.002	85.58 ± 0.003	97.24 ± 0.001	98.47 ± 0.000	13.45

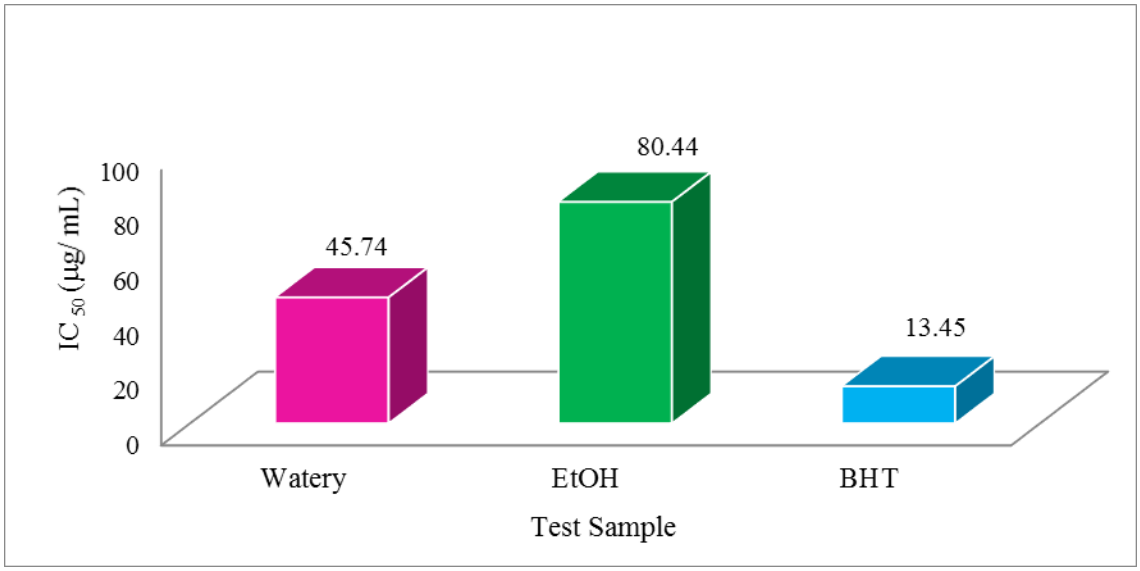

Figure 4. A bar graph representing IC_{50} values of crude extracts and standard BHT

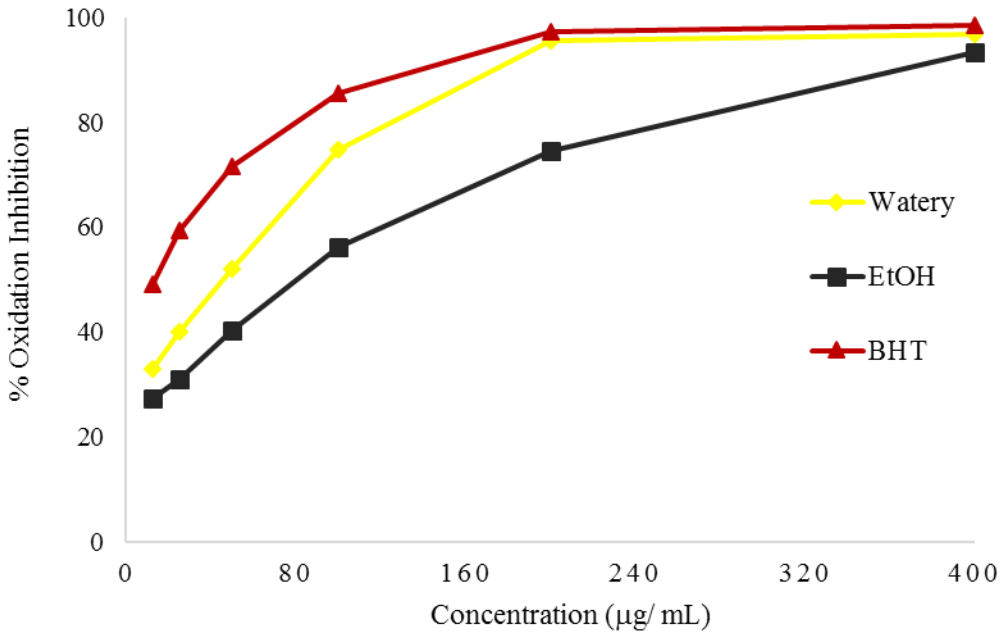

Figure 5. Plot of % radical scavenging activity vs. concentration (µg/ mL) of *Alpinia officinarum* Hance and standard BHT

Conclusion

The significant finding may be described as follows. Preliminary phytochemical tests revealed that the secondary metabolites such as alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, tannins, steroids, starch and terpenoids were found in the *A. officinarum* Hance rhizomes. Reducing sugars were not found in the *Alpinia officinarum* Hance.

The total phenolic content (TPC) in 95 % EtOH and watery extracts of *Alpinia officinarum* Hance were estimated by Folin-Ciocaiteu method. In the case of the TPC was found to be higher content in the 95 % EtOH extract (313.23 µg GAE/ mg) than that of watery extract (100.32 µg GAE/ mg).

The antioxidant activities of *Alpinia officinarum* Hance crude extracts were investigated by using DPPH assay. The IC_{50} values of EtOH and watery extracts of *Alpinia officinarum* Hance were to be observed 80.44 and 45.74 µg mL^{-1} respectively. The watery extract of *A. officinarum* Hance is higher antioxidant activity than EtOH extract. Antioxidant activity of the selected sample is higher in watery extract but total phenolic content is higher activity in ethanol extract. Therefore, these results showed that the selected sample extracted with the polar solvents possessed both antioxidant activity and total phenolic content. The present work reveals that *Alpinia officinarum* Hance rhizomes may be used as a source of medicine for treatment of diseases related to microbial infection.

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