A Comparative Study of Phytochemical Constituents and Antioxidant Activities of Rhizomes of *Curcuma aeruginosa* Roxb. and *Kaempferia parviflora* Wall.

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Abstract

This research has been focused on the elucidation of chemical constituents and antioxidant activities of rhizomes of *Curcuma aeruginosa* Roxb. and *Kaempferia parviflora* Wall.The preliminary phytochemical investigations by test tube methods revealed the presence of alkaloids, carbohydrates, amino acids, flavonoids, phenolic compounds, glycosides, reducing sugars, starch, steroids, terpenoids, and tannins. However, cyanogenic glycosides and saponins were not detected in both plants. *In vitro* antioxidant activities of watery and ethanol extracts of rhizomes of *C. aeruginosa* and *K. parviflora* were assessed by DPPH method. The IC_{50} of ethanol and watery extracts of rhizomes of *C aeruginosa* were 16.02 µg/mL and 19.26 µg/mL respectively and that of ethanol and watery extracts of *K. parviflora* were 13.94 µg/mL and 14.49 µg/mL respectively. The total phenol contents were determined by FCR method. Total phenol contents (TPC) of ethanol and watery extract of *C. aeruginosa* were (52.31 ±8.70 µg GAE/mg) and (16.92 ± 0.20 µg GAE/mg) and that of *K. parviflora* were (107 ±9.27 µg GAE/mg) and (81.35± 0.20 µg GAE/mg).

Keywords: antioxidant activity, total phenol content, DPPH, FCR

INTRODUCTION

Nature has been a source of medicinal agent for thousands of years. Herbal medicine represents one of the most important fields of traditional medicine all over the world. Different extracts from traditional medicinal plants have been tested to identify the source of therapeutic effects. Developing countries still depend mainly on medicinal herbs due to their cheaper cost and their effectiveness in the treatment of various infectious diseases with lesser side effects (Butkhup *et al*, 2011). Plant extracts for the treatment of various ailments were highly regarded by the ancient civilizations. Today, plant materials remain an important resource for combating illnesses, including infectious diseases and many of these plants have been investigated for novel drugs or templates for the development of new therapeutic agents (Kumarasamy *et al*., 2002). Searches for medicinal plants that are more potent and efficient antibiotic agents have accelerated in recent years. In Myanmar most medicinal plants are traditionally used in folk medicine to treat several diseases. It has been established that oxidative stress is among the major causative factors in more than 100 diseases such as malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes and cancer (Alho and Leinonen, 1999). In recent years, many studies evidenced that medicinal plants with high content of antioxidants can be effective in prevention of the free radical formation by scavenging, thus playing an important role in the prevention of these diseases (Al-Fatimi *et al*., 2007). The natural active compounds found in medicinal plants belong to various chemical structures including terpenes, alkaloids, coumarins, lignans, quinines, flavonoids, tannins, stilbenes,

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curcuminoids, polysaccharides, etc. and some of these compounds have anticancer, antioxidant and antimicrobial activity (Kumar *et al*., 2007). Medicinal plants,

which form the backbone of traditional medicine, have in the last few decades been the subject for very intense pharmacological studies; this has been brought about by the acknowledgment of the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development. In developing countries, it is estimated that about 80% of the population rely on traditional medicine for their primary health care (Taylor and Attaur, 1994). There arises a need therefore to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies. The intensive search for novel types of antioxidants has been carried out from numerous plant materials (Lee *et al*., 2003; Al-Fatimi *et al*., 2007; Kumar *et al*., 2007). The different botanical varieties and geographical origin may affect the qualitative and quantitative phytochemicals in the interested plant. Therefore, the search for antioxidant activity of some selected medicinal plants, which can be applied to synthesize new drugs in order to cure infectious disease. Moreover, the determination of potential antioxidant activities in plant extracts may provide information for further use in food industry. Plants in the family Zingiberaceae are considered as important sources of food, spices, medicines, dyes, perfumes and cosmetics. Many of them are also cultivated for their economic uses or as ornamental plants. Several species have been used ethnomedical in various countries. Consequently, the aims of the present study were to determine the free radical scavenging activity of rhizomes of *Curcuma aeruginosa* Roxb. and *Kaempferia parviflora* Wall.

Botanical Aspect of *Curcuma aeruginosa* Robx.

Family	-	Zingiberaceae
Genus	-	Curcuma
Species	-	*C. aeruginosa*
Botanical name	-	*Curcuma aeruginosa* Roxb.
English name	-	Pink and Blue Ginger
Part used	-	Rhizome

Figure 1(a) Photographs of *C. aeruginosa* Roxb.

Description and distribution of of *C. aeruginosa* Roxb.

Curcuma aeruginosa Roxb., (Figure 1a) which belongs to the family Zingiberaceae, is an aromatic perennial herb with 30-40 cm in height. Its leaves are glabrous, alternate, elliptic or elliptic oblong, entire, reddish-purple leaf sheath and

midrib. The rhizome is bullish violet, ellipsoid ovate shape. The inflorescences are scape from the apex of the rhizome. Bracts are green. Coma bracts are pink. Corolla is yellow. The ethnomedical uses of this plant are treatment of dysentery, gastritis, dyspepsia and flatulence (Neamsuvan *et al.*, 2012; Nanda *et al.*, 2013). To the best of our knowledge, there are very little information available on chemical compositions and biological activities of the essential oil from *C. aeruginosa* fresh rhizome. *C. aeruginosa* is a native tropical plant of Southeast Asia, including Myanmar, Cambodia, Vietnam, Malaysia, Indonesia, Thailand and Western Ghats of South India.

Botanical Aspect of *Kaempferia parviflora* Wall.

Family	-	Zingiberaceae
Genus	-	Kaempferia
Species	-	*K. parviflora*
Botanical name	-	*Kaempferia parviflora* Wall.
Myanmar name	-	Na -Nwin - Net
English name	-	Black Ginger
Part used	-	Rhizome

Figure 1(b) Photographs of *Kaempferia parviflora* Wall.

Description and distribution of of *K. parviflora* Wall

The plant *K. parviflora* Wall. Figure 1(b) is a medicinal plant naturally distributed throughout Southeast Asia, In Thailand, it is widely grown in the country's northeastern region, especially in Leoi province where there are suitable conditions for growth and production of the active compounds. It is widely used as material for organic red wine and traditional medicine. The rhizomes of *K. parviflora* Wall. have been known to be effective against some diseases, including stomachic, hypertension, diuretic and diabetics. A few studies on pharmacological activities of the *K. parviflora* Wall. extract have been scientifically investigated. It has been reported that the extract of *K. parviflora* Wall. has health-promoting for longevity, treatment for colic disorder, duodenal ulcer, gastric ulcer and allergy (Yenjai *et al.*, 2004; Rujjanawate *et al.*, 2005; Tewtrakul and Subhadhirasakul, 2007). In addition, the alcoholic infusion of its rhizome has been used as a tonic for rectifying male impotence, body pains and gastrointestinal disorders. The result of

this study suggests that the *K. parviflora* Wall. can be used as a potential source of natural antioxidants, with pharmaceutical applications.

Sample Collection of Preparation

Rhizome of *Curcuma aeruginosa* Roxb was collected from Kachin state and *Kaempferia parviflora* Wall. was collected from Pyin Oo Lwin Township, Mandalay Region. The collected sample was washed with water, chopped into small pieces using a stainless knife and air dried to a constant weight at room temperature for one month. The dried sample was subsequently milled into coarse powder using a grinding mill and then stored in an airtight plastic container for the experimental works.

Preliminary Phytochemical Investigation of *C. aeruginosa* and *K. parviflora*

A 1 g each of Rhizome of *Curcuma aeruginosa* Roxb and *Kaempferia parviflora* Wall. powder were subjected to the test of alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins and terpenoids as the preliminary phytochemical test according to reported methods (Marini-Bettolo *et al*., 1981 and M-Tin-Wa, 1972).

Preparation of Various Crude Extracts from *C. aeruginosa* and *K. parviflora*

The dried powder (100 g) of each sample was percolated with 95% ethanol (500 mL) for one week and filtered. This procedure was repeated for three times. The combined filtrate containing plant constituents were evaporated under reduced pressure by means of a rotary evaporator. Consequently, 95% ethanol soluble extract was obtained. The 95 % ethanol extract was then partitioned with pet-ether (60-80 °C) (500 mL) by using separatory funnel. The pet-ether fraction was removed under reduced pressure in a rotary evaporator. The pet-ether extract was obtained. The defatted residue was then extracted 3 times with 95 % ethanol (500 mL) for one week by percolation. Removal of the solvent from combined ethanol fractions provided ethanol crude extract. Watery extract of two samples was prepared by boiling 100 g of each sample with 500 mL of distilled water for 6 h and filtered. It was repeated three times and the filtrates were combined followed by heating on water bath and sand bath to give watery extract. Each extract was stored in refrigerator for screening of biological activities.

Screening of *In Vitro* Antioxidant Activity

DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging assay was chosen to evaluate the antioxidant activity of the sample. This assay has been widely used to evaluate the free radical scavenging effectiveness of various flavonoids and poly phenols in food sources. In this experiment, the antioxidant activity was studied on ethanol and watery extracts of selected samples by DPPH radical scavenging assay.

(a) Preparation of 60 μM DPPH solutions

DPPH (2.364 mg) was thoroughly dissolved in EtOH (100 mL). This solution was freshly prepared in the brown colored reagent bottle and stored in the fridge for no longer than 24 hours.

(b) Test sample solutions

Accurately weighed 4 mg of each test sample and 100 ml of 95 % ethanol were thoroughly mixed by shaker. The mixture solution was filtered and the stock solution was obtained. The desired concentrations (40 μg/ml, 20 μg/ml, 10 μg/ml, 5

μg/ml, 2.5 μg/ml, 1.25 μg/ml) of each sample solution was prepared by serial dilution with 95 % ethanol.

(c) Blank solution

Blank solution was prepared by mixing the test solution (1.5 ml) with 95 % ethanol (1.5ml).

(b) Determination of *in vitro* antioxidant activity

The DPPH radical scavenging activity was determined by UV-visible spectroscopic method (Ashokkumar and Ramaswamy, 2013). The control solution was prepared by mixing 1.5 mL of 60 μM DPPH solution and 1.5 mL of 95 % EtOH using vortex mixer. The sample solution was also prepared by mixing thoroughly 1.5 mL of 60 μM DPPH solution and 1.5 mL of each sample solution. The mixture solutions were allowed to stand at room temperature for 30 min. After 30 min, measurement of absorbance of these solutions at 517 nm were made by using a UV-7504 UV-visible spectrophotometer. Absorbance measurements were done in triplicate for each concentration and then mean values so obtained were used to calculate percent inhibition of oxidation by the following equation.

$$\% \text{ Inhibition} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100$$

where, $A_{Control}$ = absorbance of control solution

A_{Sample} = absorbance of tested sample solution.

The 50 % antioxidant inhibition concentration (IC_{50}) of tested samples and positive control were determined by linear regressive excel programme.

Determination of Total Phenolic Content of Rhizomes *C. aeruginosa* and *K. parviflora* by FCR Method

One of the antioxidative factors, total phenolic content (TPC) was measured by spectrophotometrically according to the Folin-Ciocalteu method (Reynertson, 2007). Frist, 1 mL of different concentration of Gallic acid solution (20, 10, 5, 2.5, 1.25 and 0.625 μg/mL) was mixed with 5 mL of diluted F-C reagent (FCR: H_2O, 1:10) and incubated for 5 min. To each tube, 4 mL of 1M sodium carbonate was added and the tubes were kept in room temperature for 15 min and the UV absorbance of reaction mixture was read at λ_{max} 765 nm. A standard curve was prepared by plotting the absorbance against concentration of Gallic acid. The phenolic content in each sample was estimated by Folin–Ciocalteu method. Each extract (1 mg) was mixed with 1 mL of distilled water. To this, 5 mL of F-C reagent (1:10) was added and incubated for 5 min. To each tube, 4 mL of 1M sodium carbonate was added and the tubes were kept in room temperature for 15 min and the UV absorbance of reaction mixture was read at λ_{max}765 nm. The blank solution was prepared as the above procedure by using distilled water instead of sample solution. Total phenolic content was estimated as μg Gallic acid equivalents (GAE)/mL of different extracts.

RESULTS AND DISCUSSION

Preliminary phytochemical tests indicated the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins and terpenoids. However cyanogenic glycosides were not detected in the rhizomes of *C. aeruginosa* and *K. parviflora*. The

presence of alkaloids, flavonoids and phenolic compounds in *C. aeruginosa* and *K. parviflora* indicate the antioxidant activity of that plant.

***In vitro* Antioxidant Activity by DPPH Free Radical Scavenging Assay**

The presence of phenolic compounds like polyphenols, flavonoids, tannins, and terpenes in plant extracts shows significant antioxidant effect due to their free radical scavenging activity (Rahman and Moon, 2007). Free radicals such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and superoxide anion radical are often generated by various biological oxidation reactions. These oxidative mediators can lead to the damage of important biomolecules such as proteins, nucleic acid, and lipids. The antioxidant activities of ethanolic and water extracts of *C. aeruginosa* and *K .parviflora* rhizomes were measured as DPPH free radical scavenging activity and exhibited significant variations According to the procedure as described (Ashokkumar and Ramaswamy, 2013), ascorbic acid was used as standard. The decolouration of the initial color is proportional to the test substances having anti-radicalizing power. Preliminary test for radical scavenging activity by DPPH method based on the change in color of crude extracts. The absorbance of different concentrations (40, 20, 10, 5, 2.5, 1.25 µg/mL) of tested samples were measured at maximum absorption of wavelength 517 nm by using UV-7504 spectrometer. Absorbance was measured in triplicates for each solution. These results are shown in Table 1 and Figure 2. Since the lower the IC_{50} values, the higher the antioxidant activity of the sample have, the *K. parviflora* ethanol extract (IC_{50} = 13.94 µg/mL) possessed the highest radical scavenging property among the extracts. The IC_{50} values were found to be *C. aeruginosa* (H_2O)(IC_{50} = 19.26 µg/mL)< (Ethanol) (IC_{50} = 16.02 µg/mL) < *K. parviflora* (H_2O)(IC_{50} = 14.49.26 µg/mL)< (Ethanol) (IC_{50} = 13.94 µg/mL). However the antioxidant potency was found to be weaker than that of standard ascorbic acid (IC_{50} = 1.21 µg/mL).

Table 1 Radical Scavenging Activity (Percent Inhibition and IC_{50} Values) of Crude Extracts of Rhizomes of *C. aeruginosa, K. parviflora* and Standard Ascorbic Acid

Test samples	% inhibition (Mean ± SD) In Different Concentration (µg/mL)						
	1.25	2.5	5	10	20	40	IC_{50} (mg/mL)
Ethanol extract (*C. aeregonosa*)	22.83 ± 2.58	24.24 ± 0.32	31.14 ± 1.29	42.01 ± 2.58	57.53 ± 2.26	71.68 ± 1.29	16.02
Water extract (*C. aeregonosa*)	21.61 ± 0.30	24.44 ± 1.21	28.10 ± 1.51	40.17 ± 1.21	53.85 ± 0.60	63.68 ± 1.21	19.26
Ethanol extract (*K. parviflora*)	21.21 ± 0.00	25.95 ± 0.01	33.06 ± 0.03	45.23 ± 0.03	57.23 ± 0.04	75.29 ± 0.01	13.94
Water extract (*K. parviflora*)	20.46 ± 0.76	22.02 ± 0.92	30.07 ± 0.51	43.78 ± 0.43	54.95 ± 0.41	59.56 ± 0.31	14.49
Ascorbic acid	34.36 ± 0.01	53.86 ± 0.02	62.38 ± 0.02	66.87 ± 0.01	72.75 ± 0.01	82.50 ± 0.01	2.25

Data are expressed as means of triplicate determination ± standard deviation (SD). *****- Standard

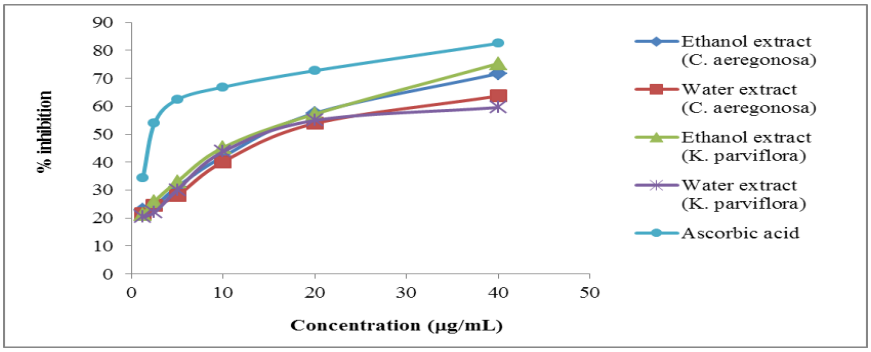

Figure 2 % Radical scavenging activity and IC_{50} values of ethanol and watery crude extracts of rhizomes of *C. aeruginosa* , *K. parviflora* and standard Ascorbic acid

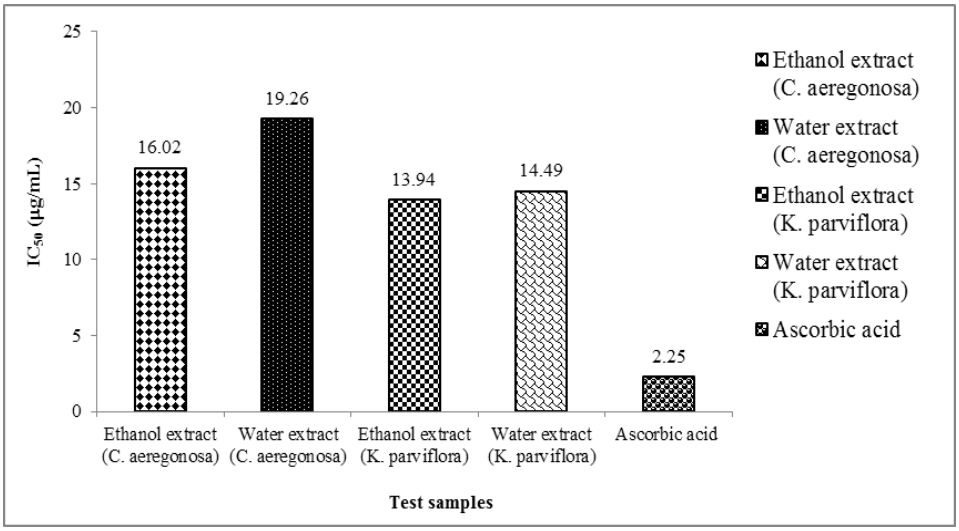

Figure 3. IC_{50} values of ethanol and watery crude extracts of rhizomes of *C. aeruginosa* ,
K. parviflora and standard Ascorbic acid

Total Phenol Contents of Ethanol and Watery Extracts of Rhizomes of *C. aeruginosa* and *K. parviflora* by Folin-Ciocalteu Reagent (FCR)

In this study, the total phenolic contents of (Rambutan) seeds was measured at 765 nm colorimetrically by Folin-Ciocalteu method. The TPC content of ethanol and watery extract of *C. aeruginosa* were (52.31 ±8.70 µg GAE/mg) and (16.92 ± 0.20 µg GAE/mg) and that of *K. parviflora* were (107 ±9.27 µg GAE/mg) and (81.35± 0.20 µg GAE/mg). The total phenol content of ethanol extract of *K. parviflora* (107 ±9.27 µg GAE/mg) was higher than the other extracts. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds. They possessed biological properties such as antiaging, anticarcinogen and antiinflammation (Zainol, 2003). These correlations indicated that high total phenol contents contributed to high in antiradical scavenging activity.

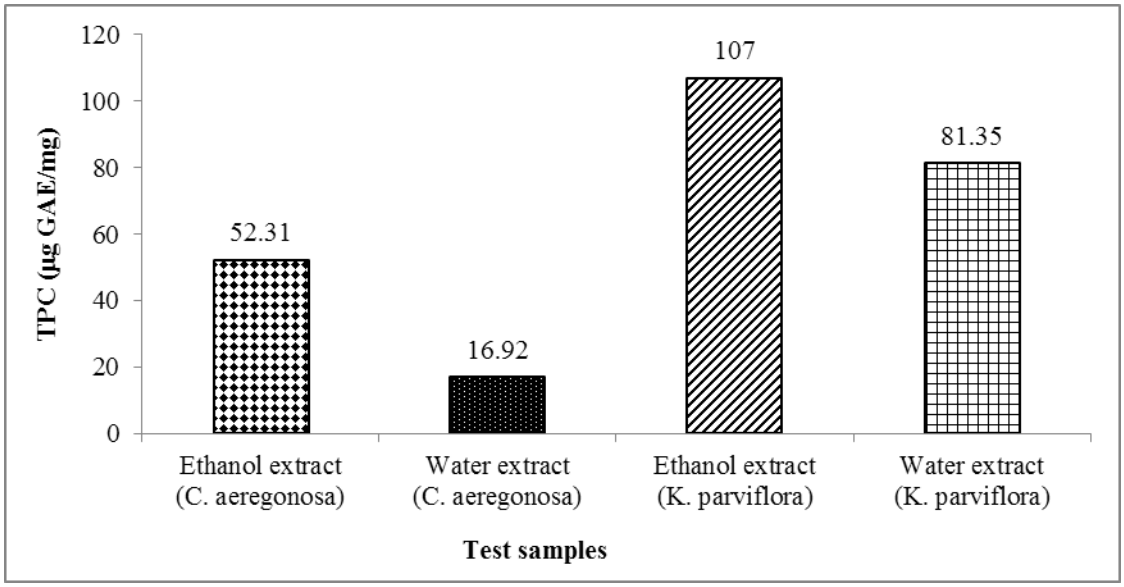

Figure 4. Total phenol content of rhizomes of *C. aeruginosa* and *K. parviflora*

CONCLUSION

In conclusion, the findings in the present study are in agreement to a certain degree with the traditional uses of the plants. The findings support the view that some Myanmar medicinal plants are promising sources of potential antioxidants activity, which may be efficient as preventive agents in the pathogenesis of some diseases. The highest antioxidant activities were detected in *K. parviflora* Wall., followed by *C. aeruginosa* Roxb. However, the characteristics of the phytochemicals and the pharmacological mechanisms of the extracts should be further studied to gain more understanding of their antioxidant activity in body and food systems, which may be further exploited to synthesize new drugs in order to cure infectious disease.

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