

REPORT

Assessment of total phenolic compounds and *in vitro* free radical scavenging potentials of water extracts of ten selected species of *Zingiberaceae* rhizomes use in folkloric medicine

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Abstract: The use of herbal medicine and traditional healing practices for diagnosis, treatment and prevention of illness and ailment continue to have more awareness among the scientific community due to their safety and also as a source of alternatives to synthetic products. This research assessed the total phenolic compounds and *in vitro* total antioxidant potentials of water extracts in selected species of *Zingiberaceae* rhizomes use as spice, drinks and medicine. DPPH and FRAP were used to determine the antioxidant capacity, total flavonoid, phenolic acids and polyphenol contents assays to evaluate the quality of the antioxidant activity and the control was ascorbic acid. The results showed that all extracts contain significant antioxidant activity with *Zingiber officinale* having the highest activity in all assays. DPPH (222.30mg/TE/g DW), FRAP (98.04mg/TE/g DW), Flavonoid (38.58mg/NGN/g DW) phenolic acid (10.78mg/GAE/g DW) and polyphenols (22.84mg/GAE/g DW). Significant and positive linear correlation were found in DPPH, FRAP and total flavonoid, phenolic acids and polyphenol contents. This study reveals some phytochemicals present in *Zingiberaceae* species, which might be responsible for their biological activities and reason for it use in folkloric medicine in Southeast Asia.

Keywords: *Zingiberaceae*, Antioxidant activity, Folkloric medicine, Phenolic compounds.

INTRODUCTION

Folk medicine is the combination of both beliefs that uses herbal medicine and traditional healing practices for diagnosis, treatment and prevention of an illness or ailment. The World Health Organization (WHO) estimated that 80% of the world habitants solely rely on traditional medicines, mainly herbal treatment, for their primary health care needs (Farnsworth *et al.*, 1985). This practice is mostly done by the indigenous people of Asia and Africa for their primary health care. Records from ancients Egypt, Assyria, China and India show that the use of plants for medicinal purposes dated back to the earliest recorded history.

Zingiberaceae species are known for their high medicinal values and also for their antioxidative properties, The members of *Zingiberaceae* are distributed mainly in the tropics and subtropics including the Indo-Malayan region which is the centre of the distribution and extending through tropical Africa to central and South America. In peninsular Malaysia, there are 150 species of *Zingiberaceae*s belonging to 23 genera. Some are found to contain oils like Limonine, Eugenol, Geraniol and Pinene (Habsah *et al.*, 2005). The rhizomes are used in dietary intake (Ibrahim *et al.*, 2007) and most are used as

spices or condiments. *Zingiber officinale* is rich in antioxidants (Kikuzaki and Nakatani, 1993), it is also found to contain antioxidants such as beta-carotene, ascorbic acid, terpenoids, alkaloids and polyphenols (Aruoma *et al.*, 1997).

In Malaysia, leaves of *Curcuma longa* are used in wrapping fish before steaming or baking while leaves of *Kaempferia pulchra* and *Curcuma longa* are ingredients of curries (Lim, 2003). Lee *et al.*, 2007 also report on tyrosinase inhibition property in rhizomes of *Zingiberaceae*. They have been new skin-lightening cosmetics developed from rhizomes of *Zingiberaceae*. *Curcuma longa*, *Curcuma xanthorrhiza* and *Kaempferiapandurata* protect the skin against the effects of UV-B damage and possible skin wrinkle (Pulok *et al.*, 2011). *Alpinia* is the largest genus in *Zingiberaceae* family. It has sharp odour, used for treating rheumatism, headache, renal disease and sore throat. *Alpinia* contain flavonoids such as Kaempferol-3-rutinoside, Kaempferol-3-olucronide and rutin. These flavonoids have biological activities such as antimicrobial and also serve as protective compounds against plants diseases (Wang *et al.*, 1989). *Zingiber officinale* is used in the treatment of high blood pressure, cancer and diabetes (Tepe *et al.*, 2006). *Zingiberaceae*s contain compounds such as terpenes, flavonoids, alcohols, ketones and

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phytoestrogens (Habsah *et al.*, 2000; Mau *et al.*, 2003; Suhaj, 2006). "Jamu" a popular Malaysia and Indonesia tonic drink is made from *Zingiberaceae*s but little or no findings have been documented to support all these mentioned facts.

Antioxidant capacity is compared with compounds that have the ability to protect a biological system against the potential harmful effects of processes or reactions involving reactive oxygen and nitrogen species (ROS and RNS) (Ayse *et al.*, 2008). Various ROS such as superoxide ion (O_2^-), hydroxide radical (OH \cdot), peroxy ($ROO\cdot$), nitric oxide (NO \cdot), peroxynitrite (ONOO \cdot) and hydrogen peroxide (H_2O_2) are generated through oxidative stress during daily activities (Abdel-Hameed, 2008). ROS compounds can destroy cellular lipids and proteins or become DNA adducts that lead to carcinogenic activity when they are present in high levels in living cells (Seifried *et al.*, 2007). The redox properties of phenolics compounds allow them to act as reducing agent, hydrogen donors and quenchers of singlet oxygen (Gulcin *et al.*, 2010). Flavonoids belong to group of low molecular phenolics and can be classified into flavanones, isoflavans, isoflavones, pterocarpan, flavanols, anthocyanins, coumestans and flavonols (Sultana and Anwar, 2008). Antioxidants are classified to synthetic, which are compound with phenolics structures that have different alkyl substitution while natural antioxidant can be phenolic compound (flavonoids and phenolics), nitrogen compound, carotenoid or ascorbic acid (Larson, 1988; Hudson, 1990; Hall and Cuppett, 1997). Synthetic antioxidants like butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA) have long being used as antioxidant and these compounds have been found to be carcinogenic and there have been restrictions on their use (Branen, 1975; Ito *et al.*, 1983).

Therefore, there is need to find safe and cheaper antioxidants from natural product source and it was then considered necessary to investigate the antioxidative properties of some common *Zingiberaceae* species use in folkloric medicine in Southeast Asia and identify the flavonoids present in them using water as the extraction solvent.

MATERIALS AND METHODS

Chemicals and reagents

DPPH, 2, 4, 6-tri (2-pyridyl)-S-triazine (TPTZ), Trolox, Folin-Ciocalteu's phenol reagent, Apigenin, Catechin, Kaempferol, Naringin, Quercetin, Rutin and Naringenin were purchased from Sigma Co. St. Louis, Missouri, USA while Sodium carbonate, Methanol, Aluminum Chloride, Iron (III) chloride hexahydrate, Gallic acid, Sodium nitrite, Ethyl acetate, Hexane and Acetic acid and Sodium hydroxide were purchased from Merck, Darmstadt, Germany. All chemicals and reagents were of analytical grade.

Plants

Ten different common *Zingiberaceae* species (ginger) rhizomes used as spices and for folkloric medicine in Southeast Asia were planted at Tanam Pertanian University, University Putra Malaysia (University Agricultural Park) and the rhizomes were harvested after one month of growth. The harvested rhizomes were identified and confirmed by the Herbal Garden Department, University Putra Malaysia (UPM).

Sample preparation

The harvested samples were washed in clean water, sliced into smaller pieces and dried in an oven at 70°C till a constant weight was achieved. Samples were then pounded into powder form using laboratory pestle and mortar and kept in an airtight container and stored in -20°C refrigerator till when needed.

Extraction

0.5g of each sample was weighed into a round bottom flask. 25mL of distilled water was added and the mixtures were refluxed at 60°C. Whatman No.1 filter paper was then used to filter the mixture and filtrate was stored in 15mL amber bottle in -20°C refrigerator (Norhaiza *et al.*, 2009).

Determination of total antioxidant capacity

The method of (Wong *et al.*, 2006) was used to determine the total antioxidant capacity of ten different samples. Stable 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) was used to determine the free radical scavenging capacity of each sample. 0.1mM methanolic DPPH solution was made and at 515nm the absorbance was taken using UV-2602 spectrophotometer. From each sample 60uL was added to 3mL of 0.1mM methanolic DPPH solution and incubated for 30min in the dark at room temperature. At 515nm, the difference in absorbance was measured and this process was repeated thrice. Calculation of percentage inhibition was measured using the formula (%) inhibition = $[(A_{515} \text{ control} - A_{515} \text{ sample}) / A_{515} \text{ control}] \times 100$ where A_{515} is absorbance at 515nm. This was expressed as mg Trolox equivalent per gram of dry weight of sample. Vitamin C serves as the control.

Ferric reducing antioxidant potential (FRAP)

Method of Benzie and Strain (1996) was used to measure the ability of each sample to reduce yellow 2, 4, 6-tri (2-pyridyl)-S-triazine (TPTZ) complex. The FRAP reagent was made in ratio 10:1:1 from 300mM sodium acetate buffer at P_H 3.6, 10mM TPTZ solution and 20mM $FeCl_3 \cdot 6H_2O$ respectively. 20uL of extract was added to 3mL of FRAP reagent. The mixture was incubated at 37°C for 30 min in a water bath. This was done in triplicate. Absorbance was measured at 593nm with UV-2602 spectrophotometer. The ferric reducing capacity was calculated using the formula antioxidant (%) = $[(A_{593} \text{ sample} - A_{593} \text{ control}) / A_{593} \text{ sample}] \times 100$, where A_{593} is

absorbance at 593nm and vitamin c was use as control. Ferric reducing capacity was expressed as mg Trolox equivalents per gram of dry weight of sample.

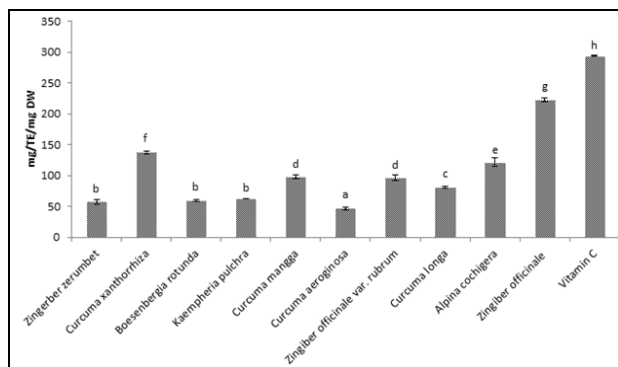


Fig. 1: DPPH method for the determination of total antioxidant capacity of ten selected *Zingiberaceae* species. Values are means \pm SD (N=3). Bar represents standard error.

Determination of phenolic compounds

Total flavonoid assay

Aluminum chloride method by (Marinova *et al.*, 2005) was used to measure total flavonoid contents of each samples. 4mL of distilled water was added to 200uL of extracts, 0.3mL 5% NaNO₃ was then added. After 5 min, 0.3mL of 10% AlCl₃ was added, at 6th minute, 2mL of 1M NaOH was added and the whole solution was made up to 10mL by adding 3.2mL of distilled water. The mixture was vigorously mixed and absorbance read at 510nm. Total flavonoid was expressed as mg Naringenin equivalents (NGN)/g sample.

Total phenolic acids assay.

Folin – Ciocalteu method by Singleton and Rossi (1995) was used to measure total phenolic acids content in each extract. 9mL of distilled water was added to 100uL of extracts, and then 1mL of Folin-Ciocalteu's phenol reagent was added, 10mL of 7% Na₂CO₃ after 5 min. The whole mixture was made to 25mL by adding 4.9mL distilled water. It was then incubated for 90 min at room temperature. Absorbance was taken at 750nm and total phenolic content was expressed as mg gallic acid equivalents (GAE)/g of dry sample.

Total polyphenols assay.

Folin-Ciocalteu's method by (Hakiman *et al.*, 2012) was used to measure the total polyphenol content. 10 times dilution of Folin-Ciocalteu's was used. 2.5mL of Folin-Ciocalteu's phenol reagent was added to 100uL of extract. After 5mins, 2.5mL of 7% of Na₂CO₃ was added. The mixture was incubated for 1hr at room temperature. Absorbance was taken at 725nm and mg gallic acid equivalents per gram of dry sample (GAE)/g of dry sample was used to express total polyphenol content.

STATISTICAL ANALYSIS

Results were presented as mean \pm standard deviation (SD) of three replicates and analyzed using One-way ANOVA, the difference between samples were determined by Duncan's Multiple Range test ($p < 0.05$) using SPSS Statistics version 21. Correlation coefficients (R) were calculated using Microsoft Excel 2010.

RESULTS

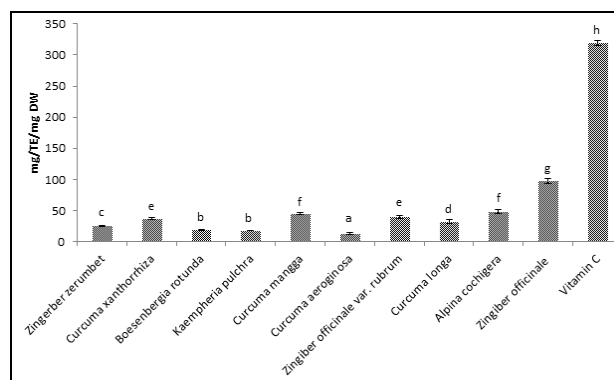


Fig. 2: FRAP determination of the ferric reducing power of ten selected *Zingiberaceae* species. Values are means \pm SD (n = 3). Bar represents standard error.

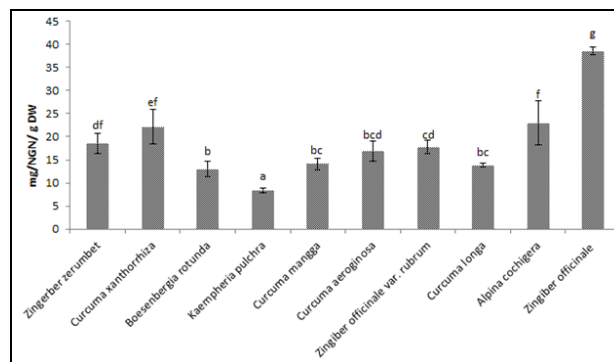


Fig. 3: Total flavonoid determination using aluminum chloride method of ten selected *Zingiberaceae* species. Values are means \pm SD (n=3). Bar represents standard error.

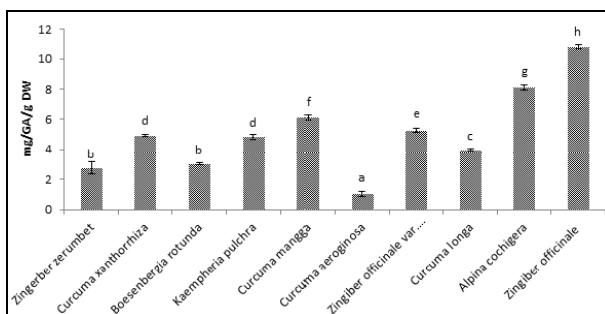


Fig. 4: Total phenolic acids determination of ten selected *Zingiberaceae* species. Values are means \pm SD (n=3). Bar represents standard error.

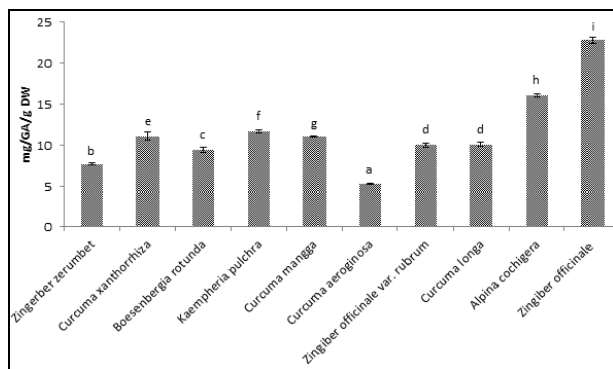


Fig. 5: Total polyphenol determination of ten selected *Zingiberaceae*. Values are means \pm SD (n=3). Bar represents standard error.

DISCUSSION

Scientific researches continue to support traditional uses of many plants for treatment of many diseases and ailments. These treatments in folkloric medicine uses plants and *Zingiberaceae* is not an exception because it has been used in the management and treatment of swelling, headache, toothache, stomach ache, rheumatism and arthritis (Mitra *et al.*, 2007). In this research, the total antioxidant capacity of aqueous extracts of ten selected *Zingiberaceae* species used in folkloric medicine in Southeast Asia were evaluated using DPPH and FRAP assay and phenolic contents (Total flavonoid, phenolic acids and polyphenol) were also assayed as medicines, spices and drink in Southeast Asia.

In general all the extracts showed significant antioxidant activities in fig. 1 and 2. In fig. 1, free radical scavenging activities of the ten selected species of *Zingiberaceae* shows significant results with extracts of *Zingiber officinale* and *Curcuma longa* having the highest free radical scavenging activities of 222.30mg/TE/g DW and 137.68mg/TE/g DW. No significant difference in the activities of *Z. zerumbet*, *B. rotunda* and *K. pulchra*. This might be due to similar antioxidant capacity of the three species. Vitamin C was used as control with scavenging activity value of 293.61mg/TE/g DW. This is in agreement with the previous work of (Aruoma *et al.*, 1997) that *Zingiber officinale* contains high antioxidants such as β -carotene, ascorbic acid (Vitamin C), terpenoids and polyphenols. (Kikuzaki and Nakatani, 1993). This corroborated this research work that *Zingiber officinale* is rich in antioxidant.

In fig. 2, the ferric reducing capacity of the ten *Zingiberaceae* species also shows *Zingiber officinale* having the highest FRAP activity (98.04mg/TE/g DW) while *Curcuma aeruginosa* has the lowest activity (13.34mg/TE/g DW). No significant difference was observed in the activities of *B. rotunda* and *K. pulchra*, which suggest that both plants have similar antioxidant

activity. The high FRAP activity of *Zingiber officinale* also suggests that it is rich in antioxidants and this might be the reason for its biological activities seen in traditional uses. Thaipong *et al.*, 2006 also reported that antioxidant activity can be determined using FRAP method.

When fig. 1 and 2 were compared, similar trends and patterns were observed with *Zingiber officinale* having the highest value and *Curcuma aeruginosa* having the lowest value in both FRAP and DPPH methods. The DPPH values for all the extracts were observed to be higher compared to values of extracts FRAP. The reasons for this could be that the compounds have more affinity toward DPPH free radical compared to ferric ion, which suggests that antioxidant activities of *Zingiberaceae* species might be due to the donation of hydrogen or electron (Liu *et al.*, 2008).

In fig. 3, total flavonoid content for the ten *Zingiberaceae* species also reveals *Zingiber officinale* with the highest total flavonoid content (38.58mg/NGN/g DW) and *Kaempheria pulchra* with the lowest value (8.31mg/NGN/g DW). This could also be the reason for its biological activities. There is a positive correlation between flavonoid and the DPPH and FRAP antioxidant activities. Flavonoid antioxidant activities are influenced by hydroxylation and presence of sugar moiety (Bravo, 1998). Flavonoids can form complexes with metal and prevent metal-initiated lipid oxidation (Hendrich *et al.*, 1999).

In fig. 4, the total phenolic acids for the ten selected species of *Zingiberaceae* extracts shows *Zingiber officinale* with the highest value of 10.78mg/GA/g DW while *Curcuma aeruginosa* has the lowest value of 1.04mg/GA/g DW. No significant difference between *Z. zerumbet* and *B. rotunda* is also suggesting the presence of related biological activity in both species while in fig. 5, total polyphenol content for the ten selected species of *Zingiberaceae* extracts also shows *Zingiber officinale* with the highest total polyphenol content (22.84mg/GA/g DW) and *Curcuma aeruginosa* has the lowest value of 5.31mg/GA/g DW. No significant difference between *Zingiber officinale* variety *rubrum* and *Curcuma longa* indicating similar total polyphenol content in both extracts.

From the table, there is a positive correlation between antioxidant activities and phenolic compounds (total flavonoids, total phenolic acids and total polyphenols). The highest correlation is between polyphenol and phenolic acids ($r=0.97$) and other highly correlated values are DPPH and FRAP ($r=0.96$), FRAP and phenolic acids ($r=0.92$), FRAP and polyphenols ($r=0.91$) and DPPH and polyphenol ($r=0.90$).

Table 1: Correlation between total antioxidant content (DPPH and FRAP) against Phenolic compounds in water extracts of ten-selected sp. of *Zingiberaceae*. All values of correlation are significant at P<0.05

	FRAP	FLAVONOID	POLYPHENOL	PHENOLIC ACID
DPPH	0.96	0.89	0.90	0.89
FRAP	---	0.88	0.91	0.92
FLAVONOID	---	---	0.76	0.71
POLYPHENOL	---	---	---	0.97

Total phenolic acid and total polyphenols content also shows similarity and very high positive correlations with both DPPH and FRAP methods. Same pattern and trend were observed in the activities of all extracts. *Zingiber officinale* have highest activity confirming the high antioxidants capacity seen in all the assays. From the correlation table, all phenolics content of all extracts have strong positive correlation. The work of (Maisuthisakul *et al.*, 2007) reveals that phenolic compounds and its derivatives are strongly correlated with antioxidant activities. In this study, total polyphenol, phenolic acids and flavonoid content correlated positively and strongly with the total antioxidant activities using both DPPH and FRAP.

In conclusion, this research revealed some of the phytochemicals composition present in selected *Zingiberaceae* species and all extracts have significant antioxidant activity with *Zingiber officinale* having the highest antioxidant activity. The presence of these phytochemicals might be responsible for *Zingiberaceae* species biological activities and the reason for its use in folkloric medicine in Southeast Asia.

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