ANTIOXIDATIVE CONSTITUENTS OF SELECTED MALAYSIAN 'ULAM'

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***ABSTRACT*:** *Ten selected Malaysian `ulam' namely Sauropus androgynus (cekur manis), Piper sarmentosum (kaduk), Polygonum minus (kesum), Morinda citrifolia (mengkudu; pucuk), Centella asiatica (pegaga), Oenanthe javanica (selom), Manihot esculenta (ubi kayu;pucuk), Cosmos caudatus (ulam raja), Carica papaya (betik;pucuk) and Kaempferia galangal (cekur) were analyzed for α-tocopherol, ascorbic acid and carotenoids as well as catalase, ascorbate peroxidase and peroxidase specific activities. The antioxidant production varies between the selected 'ulam'. Results indicated that for the non-enzymatic antioxidants, the highest production of α-tocopherol and ascorbic acid were observed in O. javanica and the lowest was in C. papaya shoots and C. asiatica, respectively. P. sarmentosum exhibited significantly higher concentration of carotenoid and the least concentration was observed in C. asiatica and S. androgynus. Of all the 'ulam' studied, M. citrifolia produced the highest specific activity of ascorbate peroxidase and the lowest was observed in K. galangal. Catalase specific activity was 30-fold higher in S. androgynus compared to other 'ulam' while P. sarmentosum produced the highest specific activity of peroxidase. The results revealed that enzymatic and non-enzymatic antioxidants have central and interrelated roles acting both chemically and as substrates in detoxification reaction of reactive oxygen species. The combination action of these antioxidants might be useful for a better protection against the development of chronic diseases.*

Keywords: Malaysian 'ulam', non-enzymatic antioxidants, enzymatic antioxidants, free radicals, reactive oxygen species.

1. INTRODUCTION

Reactive Oxygen Species (ROS) including hydroxyl radicals, superoxide anion radical and hydrogen peroxide is continuously formed during life as a result of the oxygen metabolism. These ROS can harm healthy cells, create harmful molecules and contribute to the degenerative processes related to ageing and diseases [1,2]. The damaging effects of ROS have caused plant cells to develop complex redox homeostatic mechanisms to cope with oxidative stress by using ROS-scavenging enzymes. These ROS-scavenging enzymes include superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD), catalase (CAT), and low molecular weight antioxidants such as ascorbic acid, tocopherols, carotenoids, glutathione, and phenolic compounds [3].

Previous epidemiological studies have consistently shown that consumption of fruits and vegetables has been positively correlated with reduced risk of chronic diseases, such as cardiovascular diseases and cancers [4,5,6] and neurodegenerative diseases, including Parkinson's and Alzheimer's diseases [7]. Typical compounds in fruits and vegetables that exhibit antioxidant activity include vitamins, carotenoids and other phenolic constituents. Therefore, a general recommendation to the consumer is to increase the intake of foods rich in antioxidant compounds due to their well-known healthy effects.

In the present study, ten selected 'Ulam' that are widely consumed in Malaysia were analyzed for the content of six antioxidant compounds including ascorbic acid, α-tocopherol, carotenoids, ascorbate peroxidase, catalase and guaiacol peroxidase specific activities.

2. MATERIALS AND METHODS

Plant materials: Ten selected Malaysian `ulam' (Table 1) were purchased from a local market at Kampung Tok Jembal, Kuala Terengganu, Terengganu. Fresh, fully-expanded young leaves of samples were used in the determination of the antioxidants.**Non-enzymatic antioxidant assays**: Ascorbate was extracted according to the procedure of Jagota and Dani [8]. The absorbance of the mixture was then measured at 760 nm. The amounts of ascorbic acid in the samples were calculated based on the standard curve prepared at 0-60 µg/ml. α-Tocopherol was extracted based on the method by Hodges *et al*. [9]. The assay mixture was prepared as described by Kanno and Yamauchi [10]. A standard curve was prepared using α-tocopherol (Sigma, type V) at 0-1.4 µ g/ml. Carotenoid content was analyzed based on the method used by Lichtenthaler [11]. A fresh sample was ground up with 80% (v/v) acetone and were centrifuged at 10,000 rpm for 10 min. The absorbance of the supernatant obtained was measured at 663.2, 646.8 and 470nm. 80% acetone was used as a blank. **Enzymatic antioxidant assays**: Ascorbate peroxidase (APX) specific activity was assayed according to the method of Sairam *et al*. [12] and Nakano and Asada [13]. The changes in absorbance were monitored at 290nm using a spectrophotometer for 3 minutes. APX specific activity was expressed as µmol ascorbate oxidized per hour per mg protein. Catalase (CAT) specific activity was assayed following the method of Claiborne [14]. The reaction mixture contained 3ml of 19mM hydrogen peroxide in 50mM phosphate buffer (pH 7.0) and 100µl enzyme extract was added to start the reaction. The changes in absorbance of the reaction mixture were monitored using a spectrophotometer at 240nm for 3 minutes. CAT specific activity was expressed in µmoles of hydrogen peroxide consumed per minutes per mg protein. Guaiacol peroxidase (POD) specific activity was extracted based on the method of Agrawal and Patwanadhan [15]. The reaction mixture consists of 3ml of a solution containing 1ml 50mM phosphate buffer (pH 7.5), 1ml 20mM Guaiacol, 1ml 30mM hydrogen peroxide and 100µl enzyme extract. The changes in absorbance were monitored at 470nm using a spectrophotometer for 3 minutes. Specific activity for POD was expressed as µmoles of hydrogen peroxide consumed per minutes per mg protein.

Determination of protein content: Protein concentration was determined following the method of Bradford [16]. The protein concentration was calculated according to a standard

curve prepared with various concentrations 0-1.0 mg/ml protein of Bovine Serum Albumin (BSA).

3. RESULTS AND DISCUSSION

Ascorbic acid, α-tocopherol, carotenoids and phenolic compounds are antioxidants which work both singly and synergistically to prevent or delay oxidative reactions that lead to degenerative diseases [17,18]. Results revealed that the concentrations of all antioxidants studied were varied between 'ulam'. Similar findings were reported by Jeffery *et al*. [19]; Kurilich *et al*. [20]; Lisiewska & Kmiecik [21]; Vallejo *et al*. [22] and Van der Berg *et al*. [23]. The authors observed that variation in the antioxidant contents of Brassica vegetables is caused by many factors: variety, maturity at harvest, growing condition, soil state and condition of post-harvest storage. In addition, Podsedek [24] reported that the content of polyphenols in vegetables can be influenced by climatic conditions and cultural practices.

α-Tocopherol, in cooperation with other antioxidants, plays a part in reducing ROS levels (mainly singlet oxygen, $^1O_2$ and hydroxyl radical, $OH^{\cdot}$) in photosynthetic membranes and limiting the extent of lipid peroxidation by reducing the lipid peroxyl radicals to the corresponding hydroperoxides. The reaction between α-tocopherol and lipid radical occurs in the membrane-water interphase where α-tocopherol donates a hydrogen ion to lipid radical with consequent tocopheroxyl formation [25]. As shown in Figure (1), the highest concentrations of α-tocopherol were found in *O. javanica* followed by *M. esculenta*. Only low concentration of α-tocopherol was found in other 'ulam'. Strong variations in α-tocopherol contents were found to depend on the developmental stage of the leaves and the environmental conditions, such as light intensity, temperature [26], drought and pollutants [27]. The ascorbic acid content varied from 0.01 ± 0.001 to 1157.814 ± 15.285 μg/g.fwt. The highest amount of ascorbic acid was also observed in *O. javanica*. A high content of ascorbic acid was also present in *P. sarmentosum* and *M. esculenta*. No significant differences ($p>0.05$) were observed between the ascorbic acid content in *P. minus* and *S. androgynus* while *K. Galangal* exhibited lower concentration of ascorbic acid. Only low activities of ascorbic acid were detected in the other ulam with the lowest was in *C. asiatica* [Figure (2)]. Subramaniam *et al*. [28] also reported that *O. javanica* and *P. sarmentosum* were among those exhibited the highest activities of antioxidant compared to the other species of Malaysian Ulam.

Apart from being high in the ascorbic acid concentrations, *P. sarmentosum* was also very rich in carotenoids content compared to other Ulam [Figure (3)]. A study by Chanwitheesuk *et al.* [29] also noted that *P. sarmentosum* was among the best sources of total carotenes and total xantophyll of 43 edible plants in Thailand. However, in their study, the carotenes values were lower compared to our study. These differences are probably caused by the differing varieties and growing conditions [20]. In addition, environmental differences, such as temperature, soil and solar intensity can affect the total nutrient values in plants of Brazil [26]. The high temperature should be the major reason for the significantly higher carotenoid concentration as carotenoid biosynthesis is slow at low temperatures [30].

Table (1) A list of Malaysian 'ulam' used in this study

Scientific name	Local name	Part used	Uses
Carica papaya	Betik	Shoots	Asthma relief, rheumatism, reducing cough, lacsative
Centella asiatica	Pegaga	Leaves	Poultice for wound, scar or ulcer
Cosmos caudatus	Ulam raja	Leaves	Improving blood circulation, blood cleansing
Kaempferia galanga	Cekur	Leaves	Carminative, relieve flatulence, haemagogic
Manihot esculenta	Ubi kayu	Shoots	Relieve rheumatism, fever, headache, diarrhoea, injury.
Morinda citrifolia	Mengkudu	Leaves	Digestive tonic, treat dysentery, diarrhoea, colic, nausea, antiseptic.
Oenanthe javanica	Selom	Leaves	Treating jaundice, hypertension, polydipsia diseases, antidiabetic effects, antianaphylactic, liver protective
Piper sarmentosum	Kaduk	Leaves	Treating coughs and asthma, flu, rheumatism, headaches, pleurosy and lumbago, antiseptic, analgesic, antimalaria
Polygonum minus	Kesum	Leaves	Post-natal tonic, digestive problem, anti-dandruff control, antiaging.
Sauropus androgenus	Cekur manis	Leaves	Against fever, urinary problem, stimulate milk production and recovery of the womb of women after childbirth

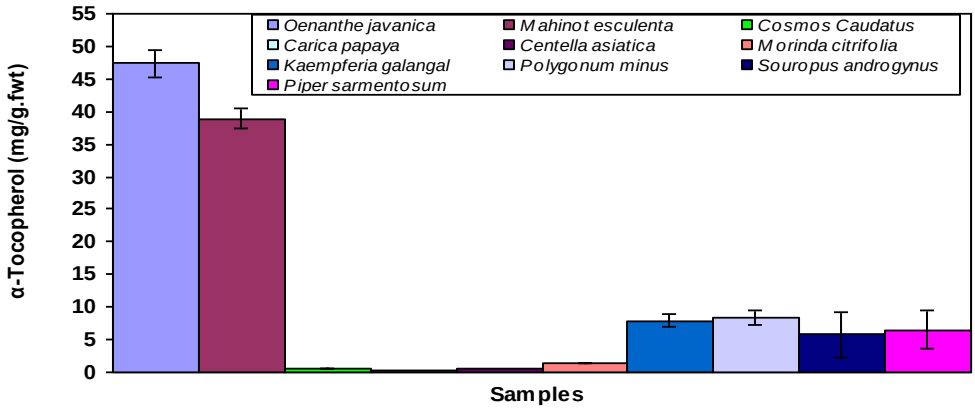

Figure (1) The α-tocopherol concentrations (mg/g.fwt) of ten Malaysian 'ulam'. Data are means ± standard errors, n=20.

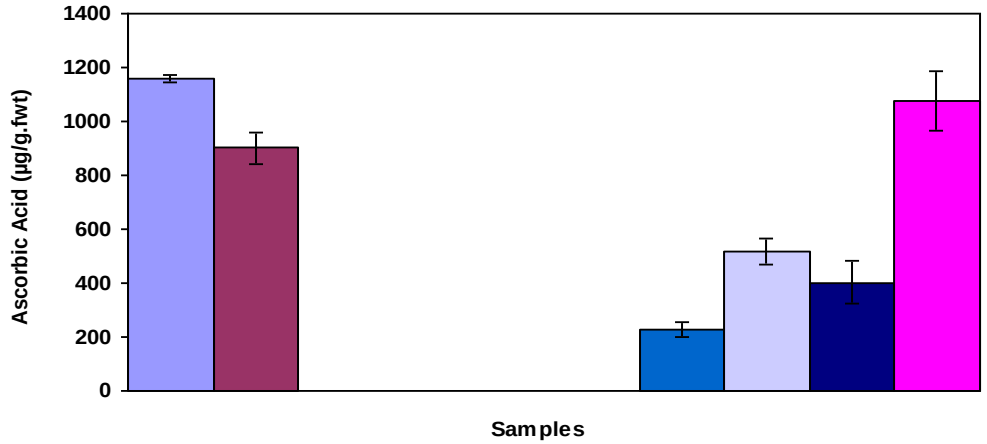

Figure (2) The ascorbic acid concentrations (µg/g.fwt) of ten Malaysian 'ulam'. Data are means ± standard errors, n=20.

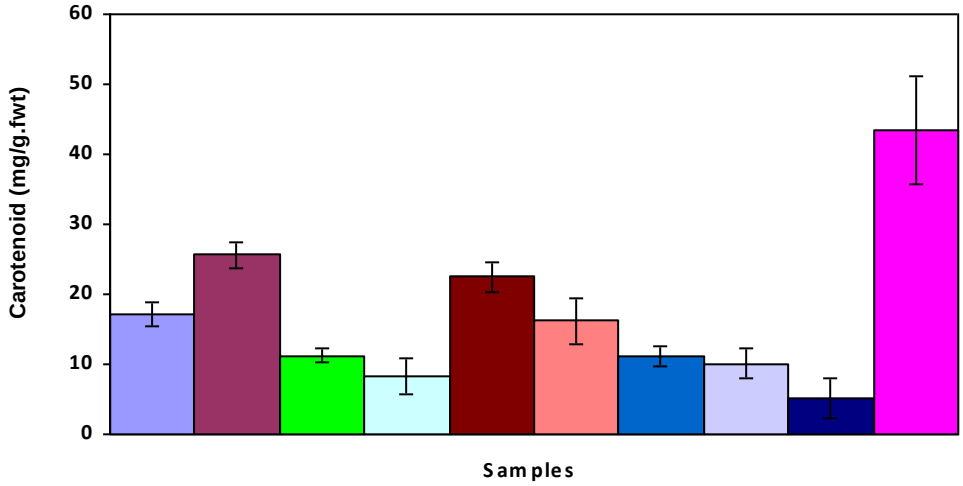

Figure (3) The carotenoid content (mg/g.fwt) of ten Malaysian 'ulam'. Data are means ± standard errors, n=20.

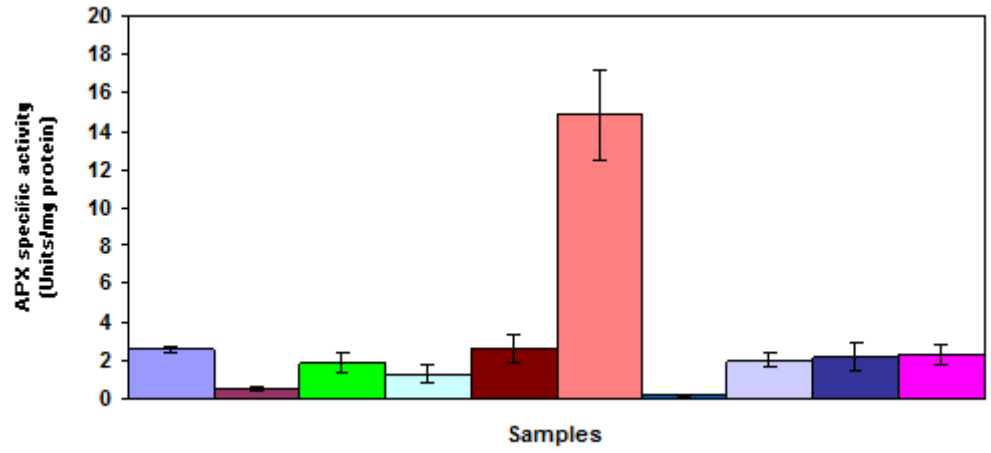

Figure (4) APX specific activity of ten Malaysian 'ulam'. Data are means ± standard errors, n=20.

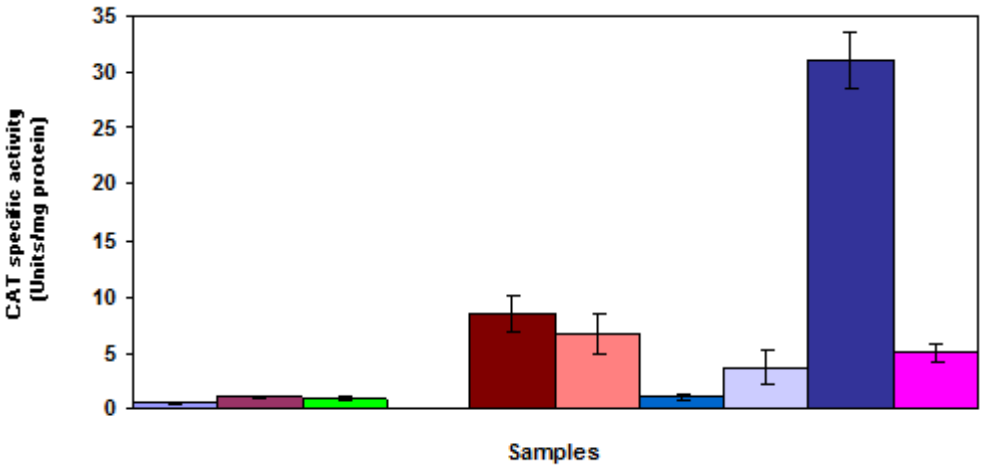

Figure (5) CAT specific activity of ten Malaysian 'ulam'. Data are means ± standard errors, n=20.

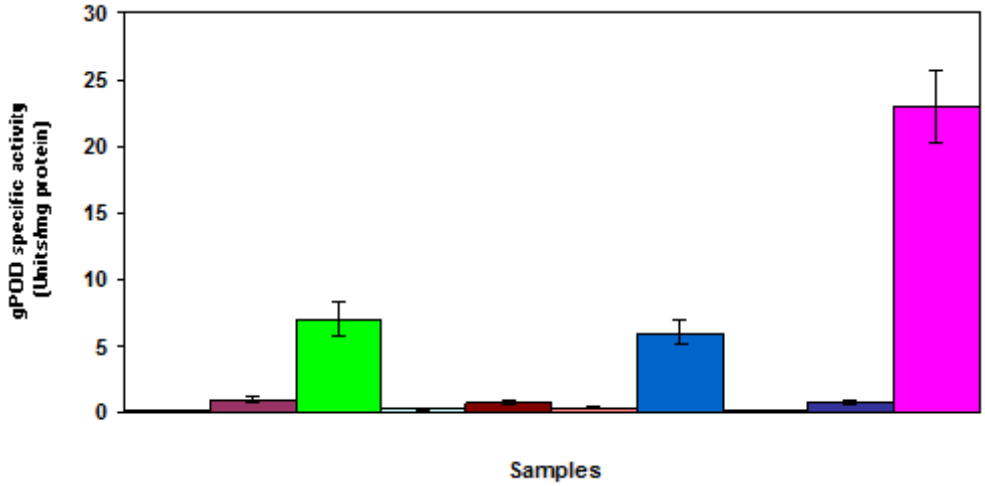

Figure (6) POD specific activity of ten Malaysian 'ulam'. Data are means ± standard errors, n=20.

Major enzymatic ROS-scavenging mechanisms of plants include SOD, APX and CAT. The balance between SOD and APX or CAT activities in cells is crucial for determining the steady-state level of superoxide radicals and hydrogen peroxide [31]. It should be noted that the highest APX and CAT specific activities were observed in *M. citrifolia* and *S. androgynus*, respectively [Figures (4) and (5)]. As mentioned earlier, both 'ulam's contained only low amount of non-enzymatic antioxidants. Lower concentration of molecular antioxidants might be balanced with higher activities of APX in *M. citrifolia* and CAT in *S. androgynus*. The finding of APX in almost all cellular compartments as well as the high

affinity of APX for H_2O_2 suggests that this enzyme plays a crucial role in controlling the level of ROS in *M. citrifolia*. By contrast, CAT is only present in peroxisomes, but it is indispensable for ROS detoxification during stress, when high levels of ROS are produced [32].

Peroxidases are widely distributed in the plants. Thus, peroxidases have been associated with an ever-increasing number of physiological processes especially in plant oxidative stress, lignification and auxin metabolism [33]. Results indicated that higher gPOD specific activity in the *P. sarmentosum* leaves [Figure (6)] was accompanied by a dense of CAT and at the same time decrease in the APX specific activity. In contrast, Willekens *et al*. [32] reported that a deficiency in CAT resulted in the induction of APX and glutathione peroxidase (GPX), suggesting that these enzymes were induced to compensate for CAT suppression. Rizhsky *et al*. [34] also demonstrated that APX deficiency results in the induction of CAT, SOD, and GR. The increase of POD specific activity in *P. sarmentosum* leads to enhance the oxidative stress protection in this plant. Thus, results suggest that gPOD may be an efficient enzymatic antioxidant in scavenging the ROS such as superoxide and hydrogen peroxide in this 'ulam'.

4. CONCLUSION

The antioxidants production varies between 'ulam'. *P. sarmentosum* can be considered as a good source of natural antioxidants since it possesses a high concentration of ascorbic acid, carotenoid as well as POD specific activity. In addition, *O. javanica*, *M. esculenta*, *M. citrifolia* and *S. androgynus* were also observed higher in certain antioxidant compounds. Enzymatic and non-enzymatic antioxidants may act together to reduce ROS level more effectively than single dietary antioxidants. The combination action of both antioxidants might be better approaches for protection against the development of chronic diseases.

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