# TRADITIONAL MALAYSIAN SALADS (*ULAM*) AS A SOURCE OF ANTIOXIDANTS

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## ABSTRACT

This paper reviews some important antioxidant enzymes i.e. catalase, glutathione peroxidase, superoxide dismutase, as well as nonenzymatic antioxidants such as total polyphenol and vitamin C, and their function in protecting skin against oxidative stress. Since Malaysia is a tropical country rich in a variety of plants, this paper also discusses new findings on antioxidant contents of traditional salads, in particular the antioxidant enzymes, vitamin C and total polyphenol. The selected traditional vegetables studied were grouped as the leafy vegetables : ulam raja (*Cosmos caudatus*), minth (*Mentha arvensis*), pennywort (*Centella asiatica*), basil (*Ocimum basilicium*), sweet basil (*Persicaria tenella*), and poddy vegetables : yardlong bean (*Vigna sesquipedalis*), wing bean (*Psophocarpus tetragonolobus*), "petai" (*Parkia speciosa*) dan "jering" (*Archidendron jiringa*). Besides antioxidant contents, their anti-tyrosinase activities were also determined.

***Keyword :*** *antioxidants, anti-tyrosinase, oxidative stress, ROS, traditional vegetables*

## INTRODUCTION

Skin is the largest organ in our body, endowed with important physiological and protective functions. Because the skin is the outermost layer of protection, it is constantly exposed to environmental oxidative stress such as ultraviolet radiation (UVR), air pollutants and chemical oxidants, resulted in the production of reactive oxygen species (ROS) (Zai & Maibach 2006). The skin is supplied with an antioxidant defense system that includes enzymatic and nonenzymatic components. However, excessive free radical attack (such as overexposure to UVR) can overwhelm cutaneous antioxidant capacity, leading to oxidative damage and ultimately to skin cancer, immunosuppression and premature skin aging (Dawson et al. 2007). To prevent or diminish oxidative-stress-induced skin damage, topical antioxidants have been widely used. Certain botanicals, such as French rose petal extract, can protect skin cells from oxidative damage (Dawson et al. 2007). Supplementing the endogenous antioxidant system may prevent or minimize ROS-induced photoaging. This can be accomplishd by induction or transdermal delivery of various enzymatic and nonenzymatic antioxidants (Zai & Maibach 2006).

Malaysia, being a tropical country, is blessed with a variety of plants which according to ethnobotanical record, can be categorized as medicinal plants. Some of the plants are used as vegetables and spices. Those which are consumed raw or after a short blanching are collectively called "ulam". According to Khairiah et al. (2004), about 109 plant species from 52 families have been recorded to be consumed as "ulam". Most of work on local herbs, vegetables and "ulam" were concentrated on the total polyphenol content and antioxidant activities (Amin et al. 2004; Asmah et al. 2000; Faridah et al. 2006; Jayamalar & Suhaila 1998; Noriham et al. 2005) which can be used to substantiate their health-promoting claims. However, other aspects of antioxidation such as the presence of antioxidant enzymes i.e.superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase(GSH-px) and anti-tyrosinase activities in the "ulam" extracts, have not been fully studied. So far, the only antioxidant enzyme reported in local plants was SOD in kaduk (*Piper sarmentosum*) and mengkudu (*Morinda elliptica*) (Subramanium et al. 2003). Similarly their potential as a source of anti-tyrosinase components useful in the formulation of skin-lightening products, is still obscure.

## ROS AND OXIDATIVE STRESS

Reactive oxygen species (ROC) are considered a major contributer to skin aging, cancer and certain skin disorders. Although ROS normally have a short half-life, they can react with DNA, proteins and unsaturated fatty acids (Dreher & Maibach 2001; Steenvoorden & van Henegouwen 1997). Healthy skin possesses an antioxidant defense system against oxidative stress. However, excessive free radical attack (such as overexposure to UVR) can overwhelm cutaneous antioxidant capacity, leading to oxidative damage and ultimately to skin cancer, immunosuppression and premature skin aging (Thiele et al. 2000; Dreher & Maibach 2001; Steenvoorden & van Henegouwen 1997; Scharffetter-Kochanek et al. 2000).

The important role of ROS in UVR-induced skin damage is well documented (Scharffetter-Kochanek et al. 2000). UVR-induced skin damage includes acute reactions such as erythema, edema and pain followed by exfoliation, tanning and epidermal thickening. ROS and other free radicals, particularly the highly damaging hydroxyl radical, can deplete the skin antioxidants and hence damage biomolecules such as lipids, protein and nucleic acids (Thiele et al. 2000; Dreher & Maibach 2001).

Overexposure to sunlight has been linked to cutaneous aging through the production of reactive oxygen species (ROS) that can activate matrix metalloproteinases (MMP), enzymes involved in the break-down of the dermal proteins, such as collagen and elastin. The degradation of these proteins is a primary contributor to wrinkle formation and loss of skin elasticity (Dawson et al. 2007).

## SKIN ANTIOXIDANT DEFENSE SYSTEMS

The skin is supplied with an antioxidant defense system that includes enzymatic and nonenzymatic components (Table 1) (Thiele et al. 2000). Because the skin is the outermost layer of protection, it is constantly exposed to environmental oxidative stress such as ultraviolet radiation (UVR), air pollutants and chemical oxidants. A skin antioxidant defense system is essential in protecting the epidermis from damage by free radicals generated by environmental and endogeneous factors (Thiele et al. 2000; Dreher & Maibach 2001); the antioxidants counteract free radicals by removing them from the body.

Table 1. Skin antioxidant defense systems.

| **Enzymatic Antioxidants** | **Nonenzymatic Antioxidants** |
|---|---|
| Glutathione peroxidase (GSH-Px) | Glutathione (GSH) |
| Catalase (CAT) | Vitamin E ($\alpha$-tocopherol) |
| Superoxide dismutase (SOD) | Vitamin C (ascorbate) |
| Haem-Oxygenase (HO) | $\beta$-Carotene |
| Thioprotein reductase | Melanin |
| Metallothionein (MT) | Ubiquinol (coenzyme Q) |
| | Mannitol, xanthine, sorbate, urate |

Source : Thiele et al. 2000

**Enzymatic Antioxidants :**

*Glutathione peroxidase :* glutathione peroxidase (GSH-Px) is considered an important antioxidant enzyme defense mechanism in skin (Steenvoorden & van Henegouwen 1997). Active against $H_2O_2$ and lipid peroxides, it catalyzes the reactions of GSH with these compounds. A small increase in GSH-Px activity may completely compensate for the total absence of catalase in fibroblasts from catalase-deficient patients (Shindo & Hashimoto 1995).

*Catalase* : Catalase (CAT) is a scanvenger of $H_2O_2$ in skin. It detoxifies $H_2O_2$ by decomposing two $H_2O_2$ molecules to two molecules of water and one oxygen. CAT is markedly reduced after UVR exposure (Steenvoorden & van Henegouwen 1997).

Compared to GSH-Px, CAT is thought to be less important as antioxidant enzymes because fibroblasts from CAT-deficient patients did not show decreased survival after a single dose of UVR when compared to normal cells (Shindo & Hashimoto 1995).

*Superoxidase dismutase* : Superoxide dismutase (SOD) catalyzes the reduction of superoxide anion, one of the ROS formed in irradiated skin, to a lesser reactive $H_2O_2$. Repeated skin exposure to UVR or other type of oxidative stress induces extra SOD activity in the long run. However, this is a minimal effect, usually not sufficient to compensate for the acute loss of activity after a single high UV dose (Steenvoorden & van Henegouwen 1997).

*Haeme-Oxygenase* : Haem-Oxygenase (HO) breaks down haem to biliverdin, which is then converted to bilirubin; both a powerful antioxidants. Interestingly, most antioxidants are decreased when encountering oxidative stress, but HO is increased. Therefore, low dose UVA might be used to induce this enzyme to provide axtra photoprotection (Steenvoorden & van Henegouwen 1997).

*Other enzymatic antioxidants* : The enzyme thioprotein reductase, involved in DNA synthesis, is an active scavenger of nitroxide and superoxide radicals. Metallothionein (MT), a cycteine-rich protein, has also been proven to have antioxidant properties (Steenvoorden & van Henegouwen 1997).

**Nonenzymatic Antioxidants:**

*Glutathione* : Glutathione (GSH) act as a direct free radical scavenger, and is thought to be the main protective effect of UVB wavelengths. It plays a pivotal role in the cellular defense against oxidative damage. Depletion of GSH in cultured human cells makes them sensitive to UVA- and UVB-induced mutations and cell death (Steenvoorden & van Henegouwen 1997).

*Vitamin E:* Vitamin E ($\alpha$-tocopherol, or $\alpha$-TOC) is a lipophilic endogenous antioxidant that provides protection against UV-induced oxidative membrane damage. It is believed that the broad biological activities of vitamin E are due to its ability to inhibit lipid peroxidation and stabilize biological membranes (Steenvoorden & van Henegouwen 1997).

*Vitamin C* : Vitamin C (ascorbate) is an efficient scavenger of superoxide for many free radicals. The biochemical importance of vitamin C is primarily based on its reducing potentials, which is required in a number of hydroxylation reactions (Thiele et al. 2000).

*$\beta$-Carotene* : $\beta$-Carotene is a vitamin A precursor and is an important member of carotenoid family of antioxidants. It is capable of quenching excited triplet states and singlet oxygen and scavenging lipid peroxide radicals (Steenvoorden & van Henegouwen 1997).

*Melanin* : The most obvious protective property of the two cutaneous pigments, pheomelanin and eumelanin, depends on their ability to absorb and scatter light. In this process, pheomelanin and melanin precursors 5-S-cysteinylDOPA (SCN) and 5,6-dihydroxyindole (DHI) generate free radical species that can subsequently damage DNA. However, eumelanin and especially DHI and SCN also have antioxidant potential (Steenvoorden & van Henegouwen 1997).

## "ULAM" AS A SOURCE OF NATURAL ANTIOXIDANTS

"Ulam' is a traditional Malay salads. In most cases, they are consumed raw, however, depending on the type of "ulam", short blanching is employed to improve palatability. In this study, selected "ulam" were analyzed for their antioxidant enzyme (CAT, GSH-Px and SOD) activities. Their vitamin C and total polyphenol contents activities were also determined to further explore their antioxidant capacity. The selected "ulam" studied were grouped as the leafy vegetables : ulam raja (*Cosmos caudatus*), minth (*Mentha arvensis*), pennywort (*Centella asiatica*), basil (*Ocimum basilicium*), sweet basil (*Persicaria tenella*), and poddy vegetables : yardlong bean (*Vigna sesquipedalis*), wing bean (*Psophocarpus tetragonolobus*), "petai" (*Parkia speciosa*) dan "jering" (*Archidendron jiringa*).

**Antioxidant Enzymes :**

The activities of CAT, GSH-Px and SOD in different parts of "ulam" are summarized in Figure 2. As shown in Figure 2, "jering" testa was the highest in CAT and SOD; while the highest GSH-Px activity was demonstrated by "jering" cotyledon.

Table 2. Activity of CAT, SOD and GSH-Px in selected "ulam" and effect of blanching on the activity

| Sample of "ulam" | Enzyme Activity (U/g) | | | |
|---|---|---|---|---|
| | CAT | SOD (Activity) | SOD ($IC_{50}$, mg) | GSH-Px |
| Yardlong bean | 860.00 | 40.0 | 24.46 | 22.24 |
| Winged bean | 301.00 | 40.0 | 23.21 | 37.96 |
| "Jering" – cotyledon | 3354.00 | 10.0 | 148.04 | 678.71 |
| "Jering" – testa | 8600.50 | 1460.0 | 0.069 | 384.96 |
| "Jering" - pod | | 370.0 | 2.71 | |
| "Petai" – cotyledon | 2232.00 | 30.0 | 40.13 | 531.38 |
| "Petai" – testa | 516.00 | 270.0 | 3.75 | 49.86 |
| "Petai" - pod | | 40.0 | 24.59 | |
| "Kerdas" – cotyledon | 3268.00 | | | 303.05 |
| "Kerdas" – testa | 172.00 | | | 254.86 |
| "Ulam raja" | 344.00 | 50.0 | 21.57 | 74.04 |
| Mint (pudina) | 946.00 | 40.0 | 172.00 | 48.77 |

| | | | | |
|---|---|---|---|---|
| Pennyworth (pegaga) | 1462.00 | 60.0 | 16.67 | 36.98 |
| Basil (selasih) | 1204.00 | 80.0 | 12.70 | 56.13 |
| Sweet basil (kesum) | 1462.00 | 40.0 | 23.06 | 43.41 |

**Vitamin C:**

All the samples studied contained moderate amount of vitamin C, however their contents were diminished by blanching (Table 3). As expected, blanching also causes reduction in the activity of enzymes (data not shown). Whenever possible, it is therefore advisable to consume the "ulam" raw in order to retain their bioactivities.

**Anti-tyrosinase Activity:**

Table 4 shows the anti-tyrosinase activities ($IC_{50}$) of the selected ulam. As indicated by the lowest $IC_{50}$, "ulam raja" extract has potential to be incorporated in skin-lightening cream. In general, leafy "ulam" is higher in anti-tyrosinase activity compared to the poddy ones.

Table 3. Content of Vitamin C in selected "ulam" and the effect of blanching on the residual content.

| Sample of "ulam" | Vitamin C (mg/g dry basis) | | |
|---|---|---|---|
| | Before Blanched | After Blanched | Reduction (%) |
| Yardlong bean | 1.786 | 1.757 | 16.28 |
| Winged bean | 1.911 | 1.504 | 21.30 |
| "Jering" – cotyledon | 1.878 | 1.661 | 11.56 |
| "Jering" – testa | 0.381 | 0.257 | 32.55 |
| "Petai" – cotyledon | 1.206 | 0.214 | 88.26 |
| "Petai" – testa | 0.657 | 0.563 | 14.31 |
| "Kerdas" – cotyledon | 1.146 | 1.022 | 10.82 |
| "Kerdas" – testa | 0.533 | 0.387 | 27.39 |
| "Ulam raja" | 1.853 | 0.876 | 52.72 |
| Mint (pudina) | 1.521 | 0.401 | 73.64 |
| Pennyworth (pegaga) | 2.043 | 0.898 | 56.04 |
| Basil (selasih) | 1.527 | 0.703 | 53.96 |
| Sweet basil (kesum) | 2.836 | 0.771 | 72.81 |

**Total Polyphenol :**

Polyphenols are also contributing to the antioxidant capacity of plant extracts. As shown in Table 4. testa are generally higher but the edible cotyledons are lower in total polyphenol.

## CONCLUSION

The results from the studies have clearly shown the potential of "ulam" as a natural sources of antioxidants. Based on their antioxidant enzyme activities and anti-tyrosinase activities, some have shown potential to be incorporated in cosmetic products.

Table 4. Activity of anti-tyrosinase and total polyphenol content of selected "ulam"

| **Type of "ulam"** | **$IC_{50}$ (mg)** | **Total Polyphenol (%)** |
|---|---|---|
| Yardlong bean-pod | 128.96 | 2.53 |
| Yardlong bean - testa | | 34.73 |
| Yardlong bean - cotyledon | | 3.29 |
| Winged bean – pod | 252.37 | 33.79 |
| Winged bean – testa | | 67.31 |
| Winged bean - cotyledon | | 7.86 |
| "Jering "– pod | | 36.06 |
| "Jering" – testa | | 82.24 |
| "Jering" – cotyledon | 84.89 | 6.15 |
| "Petai" - pod | | 14.97 |
| "Petai" – testa | | 27.55 |
| "Petai "– cotyledon | 67.44 | 5.30 |
| "Kerdas" – cotyledon | 152.44 | |
| "Ulam raja" | 30.47 | 19.41 |
| Mint (pudina) | 174.18 | 8.37 |
| Pennyworth (pegaga) | 192.73 | 5.18 |
| Sweet basil (kesum) | 58.47 | 21.94 |

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