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The Protective Effect of *ettlingera coccinea* (TUHAU) against Autoxidation-induced Ox Brain Homogenate

(Kesan Perlindungan Ekstrak Akueus Daun *ettlingera coccinea* (TUHAU) terhadap Homogenat Otak Lembu Aruhan Autoksidasi)

NUR NAJMI MOHAMAD ANUAR, JAMALUDIN MOHAMED, ERNI NORFARDILA ABU HANIPAH, NOR JANNA YAHYA, ESTHER MATHIAS AJIK & IZATUS SHIMA TAIB

ABSTRACT

Oxidative stress involved in various pathological conditions. Plants have been proven to act as a natural exogenous antioxidant. The aim of this research is to investigate the protective effects of Etlingera coccinea leaves aqueous extract on autoxidation-induced ox brain homogenate. The brain homogenate was divided into 7 groups: control group with PBS solution, positive control group with 100 µg/ml ascorbic acid, test group with 25, 50, 100, 200 and 400 µg/ml of E. coccinea. The antioxidant potential of E. coccinea aqueous extract has been evaluated by antioxidant capacity assay such as Total phenolic content (TPC), radical scavenging assay (DPPH) and ferric reducing antioxidant power (FRAP). Malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH) were also measured at 0 hr and 1 hr after 37°C water bath incubation to determine the antioxidant status and oxidative damage. TPC assay showed (4.85 ± 0.28) mg GAE/g of dry weight of E. coccinea leaves. Based on DPPH and FRAP assay, E. coccinea aqueous extract showed a dose-dependent antioxidant activity. MDA level in the 50 µg/ml E. coccinea was significantly lower compared to the other groups ($p < 0.05$). The SOD activity was significantly increase in 400 µg/ml E. coccinea ($p < 0.05$) compared to other groups. E. coccinea at the dose of 25 µg/ml and 50 µg/ml showed significant increase in GSH level compared to other groups ($p < 0.05$). In conclusion, 25 µg/ml and 50 µg/ml of E. coccinea leave aqueous extracts exhibited a potential protective effect on autoxidation-induced ox brain homogenate.

Keywords: Antioxidant; autoxidation; E. coccinea; oxidative stress

ABSTRAK

Tekanan oksidatif terlibat dalam pelbagai keadaaan patologi. Tumbuhan telah dikenalpasti sebagai antioksidan eksogenus semulajadi. Kajian ini bertujuan untuk mengenalpasti kesan perlindungan esktrak akueus daun Etlingera. coccinea terhadap homogenat otak lembu teraruh autoksidasi. Homogenat otak lembu telah dibahagikan kepada 7 kumpulan: kumpulan kawalan dengan larutan PBS, kumpulan kawalan positif dengan 100 µg/ml asid askorbik, kumpulan ujian dengan 25, 50, 100, 200 dan 400µg/ml E.coccinea. Potensi antioksidan ekstrak akueus E. coccinea telah dinilai melalui asai kapasiti antioksidan seperti kandungan jumlah fenolik (TPC), asai perangkap radikal (DPPH) dan kuasa antioksidan penurunan ferik (FRAP). Malondialdehid (MDA), superoksida dismutase (SOD) dan glutation (GSH) turut diukur pada masa 0 dan 1 jam selepas diinkubasi dalam mandian berair 37°C bagi penentuan status antioksidan dan kerosakan oksidatif. Asai TPC menunjukkan (4.85 ± 0.28 mg) GAE/g daripada berat kering daun E. coccinea. Berdasarkan asai DPPH dan FRAP, akueus ekstrak E. coccinea menunjukkan peningkatan aktiviti antioksidan selari dengan peningkatan kepekatannya. Aras MDA pada kepekatan 50 µg/ml E. coccinea adalah lebih tinggi secara signifikan berbanding kumpulan rawatan yang lain ($p < 0.05$). Aktiviti SOD yang lebih tinggi secara signifikan dikesan pada kumpulan 400 µg/ml E. coccinea ($p < 0.05$) berbanding kumpulan rawatan yang lain. Selain itu, E. coccinea pada dos 25 µg/ml dan 50 µg/ml menunjukkan aras GSH yang lebih tinggi secara signifikan berbanding kumpulan yang lain ($p < 0.05$). Kesimpulannya, ekstrak akueus daun E. coccinea pada dos 25 µg/ml dan 50 µg/ml menunjukkan potensi kesan perlindungan terhadap homogenat otak lembu teraruh autoksidasi.

Kata kunci: Antioksidan; autoksidasi; E. coccinea; tekanan oksidatif

INTRODUCTION

Autoxidation process is a mechanism induced by the air through direct or indirect reaction with oxygen molecule which includes the usage or production of peroxidation radical also known as free radicals (Simic 1981). Increased production of free radicals and overwhelming antioxidant ability to eliminate the free radical causes oxidative stress (Kohen & Nyska 2002). When the free radicals are no longer manageable, they will attack the macromolecules such as lipid, protein and DNA in order to achieve stability (Goodman & Hochstein 1977). This will lead to chain reaction of free radical production and oxidative damage. Oxidative stress is the basic of various pathological conditions such as degenerative, cardiovascular and metabolic diseases as well as cancer (Valko et al. 2006).

Involvement of free radicals in the pathogenesis of various pathological diseases contribute to the vast growing of research regarding the potential of exogenous antioxidant in the body. The ability of plants to produce antioxidant and protection for their cellular membrane lead to the initiative of using plants as potential source of exogenous antioxidant (Shao et al. 2008). Besides the total phenolic content which is high in plants, other secondary metabolite such as flavonoid and polyphenolic compound also plays an important role as non-enzymatic antioxidants (Kasote et al. 2015). Therefore, consuming of plants with high antioxidant properties as food may become one of the ways in enhancing the activity of endogenous antioxidant, thus protecting the body from oxidative stress (Kasote et al. 2015). *E. coccinea* is a plant species from the Etlingera genus and Zingerberaceae ginger family (Shahid-Ud-Daula et al. 2015). It is known as Tuhau and widely consumed by the native people of Sabah (Kulip 2007). It is also being utilized as a traditional medication for stomachache, food poisoning and gastritis (Poulsen 2006). The high total of phenolic content discovered in the methanolic extract of *E. coccinea* leaves indicates its potential antioxidant capacity (Shahid-Ud-Daula et al. 2015). The antioxidant potential of the phenolic compound is contributed from its chemical structure. Phenolic compound contains aromatic hydroxyl group in its structure which is able to donate hydrogen atom to free radicals and thus terminate the chain reaction that promotes radicals' production. Therefore, the high phenolic content of *E. coccinea* reported in previous findings, directs the present study to investigate the effect of *E. coccinea* on autoxidation-induced ox brain homogenate.

MATERIAL AND METHODS

SAMPLING

Brain of freshly slaughtered ox was purchased in local market of Chow Kit, Kuala Lumpur and was carried in ice box into the laboratory. The meninges and the brain stem were stripped off and the cerebrum was washed using cold normal saline solution. The tissue was then chopped off and was homogenized in phosphate buffer saline (PBS) at 1:4 ratio and was kept at -20°C (Stocks et al. 1974).

ETLINGERA COCCINEA

Leaves of *E. coccinea* were purchased from local seller in Sabah and were identified by Herbarium UKM, with voucher number 40347. The leaves were then air-dried in the laboratory for further aqueous extract using protocols described previously (Mohamed et al. 2013).

HOMOGENATE TREATMENT

The brain homogenate was measured for total protein by using Bradford method (Zor & Selinger 1996). The concentration of protein was adjusted to 1 mg/ml for each group. The procedure for homogenate treatment was conducted based on the published study (Stocks et al. 1974). Negative control group was treated with 50 µl of PBS solution, whereby positive control group with 50 µl of 100 µg/ml ascorbic acid, and test groups with 50 µl of *E. coccinea* aqueous extract at 5 different concentrations which are 25, 50, 100, 200 and 400 µg/ml.

ANTIOXIDANT CAPACITY ASSAY

Total phenolic content of *E. coccinea* was measured by using Folin-Ciocalteu method (Müller et al. 2010). The presence of phenol compound was spectrophotometrically determined at 740 nm and was calculated at mg GAE/g of dry weight. The radical scavenging potential of *E. coccinea* was determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Braca et al. 2001). The discolouration of the radical was measured at 517 nm. The discoloration indicates the ability of antioxidant to scavenge radical through donation of hydrogen atom. Ferric reducing antioxidant (FRAP) assay was conducted to determine the ability of antioxidant presence in the plant to donate electron and reduced Fe^{3+} to Fe^{2+} (Sherikar & Mahanthesh 2015). The resulting blue colour production was measured at 593 nm using spectrophotometer.

OXIDATIVE DAMAGE AND ANTIOXIDANT STATUS

For oxidative damage, lipid peroxidation was measured by malondialdehyde (MDA) (Stocks & Dormandy 1971) while for the antioxidant status as assessed by measuring superoxide dismutase (SOD) activity (Beyer & Fridovich 1987) and glutathione (GSH) level (Ellman 1959).

STATISTICAL ANALYSIS

The differences between all of the normality groups were statistically evaluated using One-Way ANOVA and Independent Student's *t*-test (SPSS version 22.0). All data were expressed as mean ± SEM with significance value at $p < 0.05$.

RESULTS AND DISCUSSION

ANTIOXIDANT CAPACITY ASSAY

The total of phenolic content in the leaves of *E. coccinea* aqueous extract showed 4.85 ± 0.28 mg GAE/g per dry weight. The antioxidant capacity of *E. coccinea* aqueous extracts, DPPH and FRAP are presented in Figure 1 and 2, respectively. Previous research showed that high total phenol and flavonoid content were recorded in the leaves of *E. coccinea* methanolic extract (Shahid-Ud-Daula et al. 2015). Phytochemical compound in plants such as phenolic groups were proven to possess the ability to act as antioxidant (Rice-Evans et al. 1997). The result at the present study revealed that the total phenolic content in *E.*

coccinea aqueous extract was far lower than in methanolic extract reported in the previous study (Shahid-Ud-Daula et al. 2015) . However, it is reported that the total phenols in dry seaweed extracted using organic solvents were higher compared to aqueous extract (Hwang & Thi 2014). This is probably due to the properties of phenolic compounds that generally exhibit higher solubility in organic solvents compared to water. Furthermore, the total phenolic content also depends on the size of its molecules which suggest that phenol compound with higher molecular weight are more soluble in aqueous acetone (Dai & Mumper 2010). The phenols extracted in this study might possess smaller molecular weight and therefore are readily soluble in water.

The DPPH assay showed that the percentage of radical scavenging ability (RSA) of *E. coccinea* was dose-dependent and significantly different among concentrations ($P < 0.001$). There was no significant difference between RSA of acid ascorbic and 400 µg/ml of *E. coccinea*. From the data, ascorbic acid group had the highest percentage of RSA though there was no significant difference between the ascorbic acid group and 400 µg/ml *E. coccinea*. The assay also demonstrated that the *E. coccinea* aqueous extract able to scavenge radicals in dose-dependent manner. This is probably higher phenolic content at higher *E. coccinea* concentration thus, the capacity to scavenge DPPH radicals are better in the higher concentration. This positive correlation between the total phenolic content and its antioxidant capacity also has been shown in previous study (Ferreira et al. 2007). Similarly, the radical scavenging activity of *Bauhinia vahlii* leaves extract, a wild plant widely consumed in India and Pakistan, also showed a dose-dependent relation of its radical scavenging activity (Sowndhararajan & Kang 2013).

Similarly, FRAP assay also showed that there was a dose-dependent relationship between ferric reducing power and the concentration of *E. coccinea* aqueous extract. Ferric reducing power between each group was significantly different ($p < 0.001$). The higher the concentration, the higher its ferric reducing ability. Moreover, the ferric reducing ability of *E. coccinea* aqueous extract was found to be higher than its radical scavenging ability. The dose-dependent of ferric reducing ability was probably due to the presence of compounds that were able to terminate the radical production by donating hydrogen atom. These compounds might be more abundant at higher concentration and vice versa (Duh et al. 1999).

ANTIOXIDANT AND OXIDATIVE DAMAGE STATUS

The MDA, SOD and GSH levels are as demonstrated in Figure 3-5, respectively. At the concentration of 50 µg/ml, the levels of MDA were significantly lower compared to negative control group, 100 µg/ml, 200 µg/ml, and 400 µg/ml *E. coccinea* aqueous extracts ($p < 0.05$). MDA levels detected in this study indicate the ability of *E. coccinea* to inhibit lipid peroxidation. The present data showed that lower concentration of *E. coccinea* aqueous extract possessed the ability to inhibit lipid peroxidation. Most probably, the phenolic compounds in the lower concentration were more efficient to inhibit the lipid peroxidation process in the brain homogenate compared to the higher concentration groups. The MDA production with 400 µg/ml E. coccinea aqueous extracts were comparable the untreated group, which suggest that at higher concentration of *E. coccinea*, it acts as a pro-oxidant agent. Phenolic compounds can act both as an antioxidant and also as pro-oxidant depending on the concentration. At higher concentration, it exhibited pro-oxidant properties by increasing the superoxide radical activity (De Marchi et al. 2009), reducing total antioxidant capacity (Robaszkiewicz et al. 2007), and producing phenoxyl radical that can cause cellular damage (Galati & O'brien 2004). An in vitro study on erythrocytes showed that lower concentration *Ocimum sanctum* extract possessed higher MDA inhibition activity compared to the higher concentration (Geetha & Vasudevan 2004).

The SOD activities recorded in the ascorbic acid group and 400 µg/ml of *E. coccinea* were significantly higher compared to other groups ($p < 0.05$). The results from this study showed both higher and lower SOD activity which probably happened due to exogenous antioxidant. Exogenous antioxidant can increase SOD activity as part of the feedback mechanism to protect the cellular system, however, it can also lower the SOD activity when the demands for the SOD enzyme decreased as there is an increase in other antioxidant mechanism facilitated by the exogenous antioxidant (Surai 2016). The increase in SOD activity can also contribute to the increased in hydrogen peroxide production that able to react with superoxide radicals to form reactive and dangerous hydroxyl radicals (Mikuni et al. 1985). This is because, the efficiency of SOD enzyme depends on the activity of CAT and GPx which both are responsible to eliminate hydrogen peroxide formed from the reaction of superoxide radical with SOD enzyme (Lee et al. 2003). This present study did not measure the level of CAT nor GPx and thus the increment in the SOD activity in the 400 µg/ml *E. coccinea* aqueous extracts might indicate that *E. coccinea* potentially act as antioxidant or pro-oxidant. However, previous research showed that there was increased SOD activity in in vitro rat brain homogenate treated with *Annona squamosa* Linn. extract, when the concentration increased (Shirwaikar et al. 2004).

The GSH level in the ascorbic acid group, 25 µg/ml, and 50 µg/ml *E. coccinea* were significantly increased ($p < 0.05$) compared to other groups. GSH is an important substrate for detoxification of free radicals and in the regeneration of vitamin C (Traber & Stevens 2011). Furthermore, it is also an important radical scavenging antioxidant in the brain (Gawryluk et al. 2011). Therefore, the increment of GSH level in the ascorbic acid group and 25 µg/ml E. coccinea aqueous extracts suggests that the lower concentration of *E. coccinea* might possessed

potential antioxidant activity that able to protect the brain homogenate from lipid peroxidation. The decreased in GSH level may lead to the damage of mitochondria in the brain that ultimately causes increased ROS level (Jain et al. 1991). Increase in ROS level causes the cellular system to be more susceptible towards oxidative damage that can cause pathogenesis of multiple pathological conditions (Ballatori et al. 2009). The brain homogenate treated with higher concentration *E. coccinea* aqueous extract were shown to exhibit decreased GSH level which might suggest that *E. coccinea* at 400 µg/ml can act as a pro-oxidant agent. Previously, it has been demonstrated that an in vitro cellular model treated with leaves of *Moringa stenopetala*, showed low GSH level at the highest concentration of the extract (Mekonnen et al. 2005).

CONCLUSION

Low concentrations (25 and 50 µg/ml) of *E. coccinea* aqueous extract of leaves showed potential antioxidant properties in protecting the ox brain homogenate induced by autoxidation. However, higher concentrations exhibited its potential as pro-oxidant. Further study on the potential of *E. coccinea* aqueous extract as antioxidant should be done in vivo to investigate the mechanism and effectiveness of the extract in systemic.

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Nur Najmi Mohamad Anuar
Jamaludin Mohamed
Erni Norfardila Abu Hanipah
Nor Janna Yahya
Esther Mathias Ajik
Izatus Shima Taib
Programme of Biomedical Science
School of Diagnostic and Applied Health Sciences
Faculty of Health Sciences
Universiti Kebangsaan Malaysia
50300 Jalan Raja Muda Abdul Aziz
Kuala Lumpur, Malaysia.

Corresponding author: Dr Izatus Shima Taib
Email: izatusshima@ukm.edu.my
Tel: +603-9289 7608
Fax: +603-2692 9032

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