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In vitro Antioxidant Potential of Methanol Extract of Jamaica cherry, *Muntingia calabura* (L.)

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The *in vitro* antioxidant capability of methanolic leaf and root extracts of jamaica cherry, *Muntingia calabura* (L.) Willd (Elaeocarpaceae) was evaluated. The *in vitro* antioxidant activity was evaluated for ferric reducing antioxidant power, 1,1-diphenyl -2-picrylhydrazyl (DPPH) radical scavenging, reducing power activity and superoxide anion radical scavenging activity. Ascorbic acid and butylated hydroxy amide (BHA) were kept as standards. The study species posseses an DPPH activity of 29.21 & 21.46%, ferric reducing antioxidant power of 1.47 & 0.63 µM/g, reducing power activity of 1.27 & 2.43% and superoxide anion radical scavenging activity of 21.15 & 16.27 % at 10 mg/ml concentration of leaves and root respectively. The methanolic leaf and root extracts showed effectiveness in scavenging free radicals and has the potential to be a powerful antioxidant.

Key words: *Muntingia calabura,* Methanol extract, Antioxidant activity,radical scavenging.

Various forms of activated oxygen, generally known reactive oxygen species (ROS), have been implicated in many diseases, i.e. cancer, diabetes, artherosclerosis and heart disease (Hertog *et al*., 1993). Reactive oxygen species which can be classified into free radicals (i.e. superoxide ions, hydroxyl radicals and non-free-radicals (hydrogen peroxide (Halliwell, 1995; Squadriato and Pelor, 1998) are produced from endogenous sources within the living organisms *via* various mechanisms (i.e. normal aerobic respiration, stimulated poly-morphonuclear leukocytes and macrophages, and peroxisomes) (Halliwell and Gutteridge, 1989) or from exogenous sources (tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides) (Davies, 1994; Yildirim *et al.,* 2001). Free radicals, generated *in vivo* due to the various biochemical reactions occurring in the living tissues, are chemical species that have tendency to rob electrons from other molecules in the immediate surroundings in order to replace their own losses. This process will lead to damage of crucial bio-molecules including those present in cell membranes, mitochondria, DNA, etc. and thus predisposing of various patho physiological states if not effectively scavenged. Although tissue injury leads to the generation of ROS, the ROS can also cause tissue injury, when present in high concentration within the tissues or cells (Robinson *et al.,* 1997).

Muntingia calbura. L. (also known as Jamaica cherry) is selected as the target plant for the present study that belongs to the family Elaeocarpaceae (Morton, 1987). It is native to the American continent and is widely cultivated in warm areas of Asian region (Chin, 1989). Its leaves, barks and flowers are believed to possess medicinal value as reported in Peru folklore medicinal uses. This plant is rich in flavonoids, flavones and flavanones, contributting to its potent antitumor activities (Nshimo *et al.,* 1993). The roots have been employed as an emmenogogue in Vietnam and as an abortifacient in Malaysia. In the Philippines, the flowers of this species have been used to treat headaches, incipient colds, as an antidyspeptic, anti-spasmodic and diaphoretic. Infusions of the flowers of this plant is used as a tranquillizer and tonic in Colombia (Perez, 1975). Scientifically, this plant has been proven to possess anti-tumor (Kaneda *et al.,* 1991; Su *et al.,* 2003), anti-inflammatory, anti -pyretic properties (Zakaria *et al.,* 2007b; Zakaria *et al.,* 2008), anti-bacterial activity (Zakaria *et al.,* 2006) and anti-staphyloccocal activity (Zakaria *et al.,* 2007a). Thus, research to find on finding new sources of natural anti-oxidants is important. Also, in recent years, attention has been directed towards the possible therapeutic potential of anti-oxidants in controlling degenerative diseases associated with marked oxidative damage. The plant has been shown to possess significant anti-oxidant property and different classes of phytochemicals have been demonstrated to be responsible for the plant's anti-oxidant activity. The aim of the present study is to determine the anti-oxidant activity of the methanol extracts of *M.calabura*.

Materials and Methods

Plant collection

The plant samples were collected from Eastern Block Farm in Tamil Nadu Agricultural University, Coimbatore, India. The medicinal plant sample was certified through Botanical Survey of India (BSI), TNAU, Coimbatore-3, Tamil Nadu.

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Plant material and extraction M. calabura

The shade dried leaf and root were made into a fine powder of 40 mesh size using the pulverizer. Following that, 100 g of the powder was filled in the filter paper and successively extracted using 500 mL methanol using the soxhlet extractor for 8 - 10 hours. The extraction was carried out for leaves and roots separately. Then the extract was filtered through Whatman No.1 filter paper and then subjected to the free radical scavenging assays.

Determination of antioxidant activity

The antioxidant activity was evaluated by four methods as below.

Ferric reducing anti-oxidant power(FRAP)

Total anti-oxidant activity was measured by FRAP assay (Benzie and Strain, 1996) . FRAP assay uses anti-oxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess.

FRAP assay was performed using a Varian Spectrophotometer. About 3 ml of freshly prepared FRAP reagent (redox indicator) was warmed to 37°C to which 100 µl distilled water was added and served as blank. In another cuvette, same amount of FRAP reagent was taken and added with 100 µl leaves and root samples. Absorbance readings at 593 nm were recorded from one minute till completion of reaction i.e., up to 8 minutes rather than the early reports of 4 minutes. The change in absorbance between final reading and initial readings was noted for each sample and related to the change in absorbance of the ascorbic acid standard. All tests were analyzed in triplicate.

Determination of DPPH scavenging activity

The free radical scavenging activities of *M. calabura* leaves and root samples were assayed using a stable DPPH, following a standard method (Blois, 1958). This method is based on the reduction of the free radical DPPH (2, 2 -diphenyl- 1-picrylhydrazyl). The reaction takes place when 1ml of DPPH (0.1mM solution of DPPH in methanol) was mixed with 3 ml of the leaf and root samples at room temperature. After a reaction time of 30 minutes, absorbance values at 517 nm were measured. The percent inhibition of the DPPH radical by the samples was calculated according to the formula:

Percentage inhibition = [(Ac- As) /Ac] x 100

Where,

Ac - absorbance of the control;

As - absorbance of the sample.

Reducing power assay

The reducing power was determined according to the method of Oyaizu (1986). About 1ml of leaves and root extract was mixed with 2.5 ml of 20 mM PO_4 buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50˚C for 20 minutes. After incubation, 2.5 ml of 10% trichloroacetic acid (w/v) was added and the mixture was centrifuged at 3000 rpm for 20 min. The upper layer (2.5 ml) was mixed with 2.5 ml deionized water and 0.5 ml of 0.1% ferric chloride and the absorbance was measured at 700 nm. Butylated hydroxy amide was used as a standard for comparison. Percentage reducing power was calculated as per the equation:

Percentage reducing power = 1-[1- (As/Ac)]

x100. Where,

Ac - absorbance of the control;

As - absorbance of the sample.

Superoxide anion radical scavenging activity

The superoxide scavenging ability of the leaves and root samples was assessed by the method of Nishikimi *et al*. (1972) with slight modification. About 1 ml of nitro blue tetrazolium (NBT) solution (156 µM NBT in 100 mM phosphate buffer, pH 7.4), 1 ml NADH solution (468 µM in 100 mM phosphate buffer, pH 7.4) and 0.1 ml of leaf/root samples were mixed. The reaction started by adding 100 µl of phenazine methosulphate (PMS) solution (60 µM PMS in 100 mM phosphate buffer, pH 7.4), to the mixture. The reaction mixture was incubated at 25°C for 5 min, and the absorbance at 560 nm was measured against blank sample. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The capability of scavenging the superoxide radical was calculated using the following equation:

Scavenging effect % = [(Ab-As)/ Ac] x

100 Where,

Ab- Absorbance of the blank, As - Absorbance

of the leaves and root sample.

Results and Discussion

***In vitro* antioxidant activity**

The methanol extract of *M. calabura* leaves and root showed good anti-oxidant activity in the tested assays.

Ferric reducing antioxidant power activity (FRAP)

The results of anti-oxidant activity of the leaf and root extract of *M. calabura* based on ferric reducing anti-oxidant power activity are presented in Table 1. The FRAP assay was selected to be used as the first step in this investigation, because intrinsic anti-oxidant potential of an extract could be measured, taking anti-oxidant constituents as reductants in a redox-linked colorimetric reaction. The anti-oxidant power of extracts was compared with ascorbic acid as a reference standard. The ferric reducing anti-oxidant power activity was determined to be increased with the increase in the concentration of extract from 5 to 30 mg/mL. The inhibition of the FRAP was varying

Table 1. Ferric reducing antioxidant power activity of methanol extract of *M. calabura*

Sample concentration	FRAP activity (µM/g)(n=6)	
(mg/ml)	Leaf samples	Root samples
5	0.86 (± 0.03)$_e$	0.23 (± 0.02)$_e$
10	1.47 (± 0.01)$_d$	0.63 (± 0.02)$_d$
15	2.12 (± 0.58)$_c$	1.07 (± 0.04)$_c$
20	2.85 (± 0.06)$_c$	1.78 (± 0.03)$_c$
25	3.46 (± 0.01)$_b$	2.54 (± 0.02)$_b$
30	4.05 (± 0.06)$_a$	3.16 (± 0.58)$_a$

Values are mean (± SE) and values followed by the same letter in each column are not significantly different from each other by Duncan's multiple range test ($p \leq 0.05$)

from 0.86 µM/g in 5 mg/mL of extract to 4.05 µM/g in 30 mg/mL extract in *M. calabura* leaf extract. The FRAP values of methanolic extract of root varied from 0.23 µM/g in 5mg/mL of extract to 3.16 µM/g in 30mg/mL extract.

DPPH radical scavenging activity

The method of scavenging DPPH free radicals can be used to evaluate the anti-oxidant activity of specific compounds or extracts in a short time. The anti-oxidant activity was evaluated by the ability of investigated extract to reduce the stable DPPH free radicals. Due to its unpaired electron, DPPH radical gives a strong absorption band at 517 nm (deep violet colour). As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes and the resulting decolorization is stoichiometric with respect to the number of electrons taken up. To evaluate the anti-oxidant activity of the methanol leaf and root extract, the radical scavenging capacity based on DPPH assay was determined and the results are shown in Table

2. The percentage of scavenging effect on the DPPH radical was increased with the increase in the concentrations of the extract from 5 -30 mg/mL. The percentage of inhibition of the DPPH radical varied

Table 2. DPPH radical scavenging activity of methanol extract of *M. calabura*

Sample concentration	Percentage activity (n=6)	
(mg/ml)	Leaf samples	Root samples
5	21.58 (± 0.58)$_d$	15.42 (± 0.58)$_f$
10	29.21 (± 1.15)$_e$	21.46 (± 1.73)$_e$
15	34.20 (± 1.15)$_d$	24.31 (± 1.15)$_d$
20	39.78 (± 1.15)$_c$	29.46 (± 0.01)$_c$
25	44.12 (± 0.01)$_b$	35.08 (± 0.60)$_b$
30	49.45 (± 0.01)$_a$	42.17 (± 0.02)$_a$

Values are mean (± SE) and values followed by the same letter in each column are not significantly different from each other by Duncan's multiple range test ($p \leq 0.05$)

from 21.58% in 5 mg/mL of the extract to 49.45% in 30 mg/mL of methanolic leaf extract. DPPH scavenging activity of root extract varied from 15.42% in 5 mg/ mL of the extract to 42.17% in 30 mg/mL of extract.

Reducing power assay

In the reducing power assay, the yellow colour of the test solution changed to various shades of green and blue, depending on the reducing power of each compound. The presence of reducers (*i.e*. anti-oxidants) caused the reduction of the Fe_{3+}/ ferricyanide complex to the ferrous form. Therefore, measuring the formation of Perl's Prussian blue at 700 nm can monitor the Fe_{2+}concentration. The results of anti-oxidant activity of the leaf and root extract of *M. calabura* based on reducing power activity are presented in Table 3. The percentage of reducing power activity increased with the increase in the concentrations of the extract from 5 -30 mg/mL. The percentage of inhibition of the reducing power varied

Table 3. Reducing power assay of methanol extract of *M. calabura*

Samples concentration	Percentage activity (n=6)	
(mg/ml)	Leaf samples	Root samples
5	0.84 (± 0.06)$_f$	1.93 (± 0.33)$_f$
10	1.27 (± 0.01)$_e$	2.43 (± 0.02)$_e$
15	1.63 (± 0.58)$_d$	2.91 (± 0.58)$_d$
20	2.27 (± 0.01)$_c$	3.45 (± 0.03)$_c$
25	2.58 (± 0.05)$_b$	3.87 (± 0.01)$_b$
30	2.96 (± 0.58)$_a$	4.26 (± 0.02)$_a$

Values are mean (± SE) and values followed by the same letter in each column are not significantly different from each other as determined by Duncan's multiple range test ($p \leq 0.05$)

from 0.84% in 5 mg/mL of the extract to 2.96% in 30 mg/mL of methanolic leaf extract. The reducing power activity of root extract varied from 1.93% in 5 mg/mL of the extract to 4.26% in 30 mg/mL of extract.

Superoxide anion radical scavenging assay

The superoxide scavenging activity of *M. calabura* leaves and root was evaluated by NBT (O_2 scavenging) assay. To evaluate the anti-oxidant activity of the methanolic extract of leaf and root the radical scavenging capacity based on superoxide anion radical scavenging assay was determined and the results are shown in Table 4 for the species, *M. calabura*. The percentage of superoxide anion radical scavenging effect increased with the increase in the concentrations of the extract from 5 -30 mg/ mL. The percentage of inhibition of the superoxide

Table 4. Superoxide anion radical scavenging activity of methanol extract of *M. calabura*

Samples concentration	Percentage activity (n=6)	
(mg/ml)	Leaf samples	Root samples
5	14.23 (± 0.58)$_f$	12.18 (± 0.59)$_f$
10	21.15 (± 1.15)$_e$	16.27 (± 1.15)$_e$
15	26.74 (± 0.59)$_d$	21.59 (± 0.01)$_d$
20	31.07 (± 0.01)$_c$	26.45 (± 0.01)$_c$
25	35.26 (± 0.02)$_b$	30.06 (± 0.03)$_b$
30	40.82 (± 0.58)$_a$	37.12 (± 0.58)$_a$

Values are mean (± SE) and values followed by the same letter in each column are not significantly different from each other by Duncan's multiple range test ($p \leq 0.05$)

anion radical varied from 14.23% (in 5 mg/mL of the extract) to 40.82% in 30 mg/mL of methanolic leaf extract. The superoxide anion radical scavenging activity of root extract varied from 12.18% in 5 mg/ mL of the extract to 37.12% in 30 mg/mL of extract.

The present study demonstrated the radical scavenging property of *M. calabura* by *in vitro* assays.

The methanol extracts of the plant leaves and root assayed against the DPPH radical scavenging and superoxide assays were found to exhibit remarkable radical scavenging activities with the percentage of inhibition recorded.

It is generally known that the anti-oxidant activities of putative anti-oxidants involves various mechanisms, such as radical scavenging, decomposition of peroxides, binding of transition metal ion catalysts, prevention of chain initiation and prevention of continued hydrogen abstraction (Diplock *et al.,* 1998). Hence, the free radical scavenging capacity of an extract may serve as a significant indicator of its potential anti-oxidant activity. Increasing evidences have suggested that many age-related human diseases (i.e. cancer, inflammation and brain dysfunction) are the result of cellular damage caused by free radicals (Perry *et al*., 2000; Carr and Frei, 2000). Antioxidants have been shown to play an important role in preventing such diseases. For example, several cancer chemo preventive agents exhibit anti-oxidant activity through their ability to scavenge oxygen radicals (Ito *et al.,* 1999; Wei and Frenkel, 1993).

Oxidative stress can arise from an imbalance between the generation and elimination of reactive oxygen species, leading to excess ROS levels, that inflicts indiscriminate damage to virtually all biomolecules, leading, in turn, to various diseases and cell death (Scandalios, 2005). Reactive species can be eliminated by a number of enzymatic and non-enzymatic anti-oxidant defence mechanisms (Boullier *et al.,* 2001).

Free radicals (super oxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and proxynitrite) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA (Gutteridge, 1995). It has been established that oxidative stress is among the major causative factors in the induction of many plant and human diseases (Squadriato and Pelor, 1998). The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is anti -oxidative defense mechanisms. Anti-oxidants are those substances which possess free radical chain reaction breaking properties. Recently, there has been an upsurge of interest in the therapeutic potential medicinal plants as anti-oxidants in reducing oxidative stress-induced tissue injury (Pourmorad *et al.,* 2006).

Crude extracts of the various parts (leaves, fruits, roots, stem and trunk bark) of *Garcina atroviridis* showed strong anti-oxidant activity exceeding that of the standard vitamin (Mackeen *et al.,* 2000). Aqueous extracts from the different parts of the four medicinal plants, *Momordica charantia, Glycyrrhiza glabra, Acacia catechu* and *Terminalia chebula* were found to be rich sources of enzymatic and non-enzymatic antioxidants (Naik *et al.,* 2005).

The results of the present investigation showed that the DPPH scavenging activity of the *M. calabura* leaf extract was significantly higher (29%) and this is due to the fact that, in the presence of an anti-oxidant, DPPH radical obtains one more electron and the absorbance decreases. The superoxide dismutase (SOD) activity was also found to be higher (21%). From this, it is evident that the *M. calabura* possesses anti-oxidant and capable of counteracting reactive oxygen species (ROS), which are responsible for various oxidative damages in the living system. The methanolic extract of *Muntingia calabura* root exhibited 21% DPPH activity and 20% SOD activity.

In summary, the anti-oxidant property of *M. calabura* was tested by four different *in vitro* assays. The methanolic extracts of root and leaves found to possess moderate anti-oxidant activity. From this it is evident that the methanolic extract of *M. calabura* is capable of counteracting reactive oxygen species (ROS), which are responsible for various oxidative damages in the living system.

References

Benzie, I.F. and Strain, J.J. 1996. The ferric reducing ability of plasma as a measure of antioxidant power the FRAP assay. *Anal. Biochem*., **239**: 70-76.

Blois, M.S. 1958. Anti-oxidant determinations by the use of a stable free radical. *Nature*, **29**: 1199-1200.

Boullier, A.M., Ohtani, T., Fujimoto, K., Ito, H. and Dubois, M. 2001. Fluid inclusions in pseudotachylytes from the Nojima fault, Japan. *J. Geophys. Res*., **106**: 21965-21977.

Carr, A. and Frei, B. 2000. The role of natural anti-oxidants in preserving the biological activity of endothelium-derived nitric oxide. *Free Rad Biol Med*., **28**: 1806-1814.

Chin, W.Y. 1989. A Guide to the wayside trees of Singapore. BP Singapore Science Centre, 145. **p**

Davies, K.J.A. 1994. Oxidative stress the paradox of aerobic life. *Biochem Symp*., **61**: 1-34.

Diplock, A.T., Charleux, J.L. and Crozier W.G. 1998. Functional food science and defence against reactive oxygen species. *Br. J. Nutr*., **80**: 77-112.

Gutteridge, J.M.C. 1995. Lipid peroxidation and anti-oxidants as biomarkers of tissue damage. *Clin. Chem*., **41**: 1819–28.

Halliwell, B. 1995. How to characterize an antioxidant: an update. *Biochem Soc Symp*., **61**: 73-101.

Halliwell, B. and Gutteridge, J.M. 1989. Free radicals in biology and medicine. Clarendon Press Oxford: 23-30.

Hertog, M.G.L., Feskens, E.J.M. and Hollman, P.C.H. 1993. Dietary anti-oxidant flavonoids and risk of coronary heart disease: The zupthen elderly study. *The Lancet*, **342**: 1007-1014.

Ito, H., Miyake, M. and Nishitani, E. 1999. Anti-tumor promoting activity of polyphenols from *Cowania mexicana* and *Coleogyne ramosissima*. *Cancer Lett*., **143**: 5-13.

Kaneda, N.J.M., Pezzuto, D.D., Soejarto, A.D., Kinghom. N. R., Farnworth, T., Santisuk P., Tuchinda, J., Udchachon and Reutrakul, V. 1991. Plant anti-cancer agents, XLVIII. New cytotoxic flavonoids from *Muntingia calabura* roots. *J. Natural. Prod*., **54**:196-206.

Mackeen, M.M., Ali, A.M., Lajis, N.H., Kawazu, K., Hassan, Z., Amran, M., Habsah, M., Mooi, L.Y. and Mohamed, S.M. 2000. Anti-microbial, anti- oxidant, anti-tumor-promoting and cytotoxic activities of different plant part extracts of *Garcinia atroviridis*. *J. Ethanopharmacol*., **72**: 395-402.

Morton, J.F. 1987. Jamaica cherry. In fruits of warm Climates Miami, 65-69.

Naik, S., Paul, B. and Callanan, P. J. 2005. Detection of hard x-ray pulsations and a strong iron k emission line during an extended low state of GX 1+4. *The Astrophysical J.*, **618**: 866–873.

Nishimiki, M., Rao, N.A. and Yagi, K. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. *Biochem. Biophys. Res. Comm*., **46**: 849:853.

Nshimo, C.M, Pezzuto, J.M, Kinghorn, A.D and Farnsworth, N.R. 1993. *International J. Pharmacognosy.,* **31**: 77.

Oyaizu, M. 1986. Studies on products of browning reactions: Anti-oxidative activities of product of browning reaction prepared from glucosamine. *Jpn. J. Nutr*., **44:** 307–315.

Perez, A.E. 1975. Plants medicinales y venenosas de Colombia, Hernando Salazar, Medellin, Colombia: 192.

Perry, G., Raina, A.K.L. and Nonomura, A. 2000. How important is oxidative damage? Lessons from Alzheimer's disease. *Free Rad Biol Med*., **28**: 831-834.

Pourmorad, F., Hosseinimehr, S.J. and Shahabimajd, N. 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African J. Biotechnol*., **5**: 1142-1145.

Robinson, E.E., Maxwell, S.R.J. and Thorpe, G.H.G. 1997. An investigation of the anti-oxidant activity of black tea using enhanced chemiluminescence. *Free Rad Res*., **26**: 291-302.

Scandalios, J.G. 2005. Oxidative stress: molecular perception and transduction of signals triggering anti-oxidant gene defences. *Braz. J. Med. Biol. Res*., **38**: 995-1014.

Squadriato, G.I. and Pelor, W.A. 1998. Oxidative chemistry of nitric oxide: The roles of superoxide, peroynitrite and carbon dioxide. *Biol. Med*., **25**: 392-403.

Su, B.N.E., Jung, P.J.S., Vigo, J.G., Graham, F., Cabiess, H.H., Fong, J.M., Pezzuto and Kingborn, A.D. 2003. Activity-guided isolation of the chemical constitutents of *Muntingia calabura* using a quinine reductase induction assay. *Phytochemistry*, **63**: 335-341.

Wei, H.C. and Frenkel, K. 1993. Relationship of oxidative events and DNA oxidation in SENCAE mice to *in vivo* promoting activity of phorbol ester-type tumor promoter. *Carcinogen*., **14**: 1195-1201.

Yildirim, A., Oktay, M. and Bulaloulu, V. 2001. The antioxidant activity of the leaves of *Cydonia vulgaris*. *J Med Sci*., **31**: 23-27.

Zakaria, Z.A., Fatimah, C.A., Mat, J.A.M., Zaiton, H., Henie, E.F.P., Sulaiman, M.R., Somchit, M.N., Thenamutha, M. and Kasthuri, D. 2006. The *in vitro* antibacterial activity of *Muntingia calabura* extracts. *Int. J. Pharmacol*., **2**: 439-442.

Zakaria, Z.A., Mat, J.A.M., Mastura, M., Mat. J.S.H., Mohamed, N.A., Mohd, S.N., Jamil, M.S., Rofiee. and Sulaiman, M.R. 2007a. *In vitro* anti-staphylococcal activity of the extracts of several neglected plants in Malaysia. *Int. J. Pharmacol*., **3**: 428-431.

Zakaria, Z.A., Mohamed, N.A., Hazalin, N., Mohd, S.N.H., Abdul, G.M., Hassan, M.H., Gopalan, H.K. and Sulaiman, M.R. 2007b. Antinociceptive, anti-inflammatory and antipyretic effects of *Muntingia calabura* aqueous extract in animal models. *J. Nat. Med.,* **61**: 443-448.

Zakaria, Z.A., Somchit, M.N., Sulaiman, M.R., Mat, J.A.M. and Fatimah, C.A. 2008. Effects of various receptor antagonist, pH and enzymes on *Muntingia calabura* antinoception in mice. *Res. J. Pharmacol.,* **3**: 31-37.

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