In vitro anti oxidant activity and total phenolic content of *Dillenia indica* and *Garcinia penducalata*, commonly used fruits in Assamese cuisine

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ABSTRACT

Introduction: Ingredients of Indian cuisine are well known for their antioxidant properties which can help prevent many diseases. The fruits of *Dillenia indica* and *Garcinia penducalata* are commonly used by the Assamese speaking population of Assam, India, but their antioxidant properties have not been investigated. **Methods:** Methanol, petroleum ether and water extracts of the shade dried fruits of *Dillenia indica* and *Garcinia penducalata* were obtained and the IC_{50} values of their DPPH, hydroxyl, oxygen and nitric oxide scavenging activities were estimated along with their reductive ability, vitamin C and total phenolic content. Vitamin C was used as a standard reference for the antioxidant scavenging activities. **Result:** The IC_{50} values for DPPH, hydroxyl, oxygen and nitric oxide inhibition of vitamin C were 43.7 ug/ml, 50.44 ug/ml, 61.04 ug/ml and 41.82 ug/ml, respectively while the IC_{50} value for its reductive ability was 47.17 ug/ml. The IC_{50} values for the DPPH, hydroxyl, oxygen, nitric oxide and reductive ability of the methanolic extract of *Dillenia indica* were 31.25 ug/ml, 51.82 ug/ml, 51.44 ug/ml, 39.73 ug/ml and 40.18 ug/ml, respectively. These IC_{50} values were superior in comparison to its petroleum ether and water extracts. The methanolic extract of *Garcinia penducalata* had the highest amount of phenolic content and its IC_{50} values for DPPH, oxygen and nitric oxide scavenging activities were 50.23 ug/ml, 66.06 ug/ml and 63.02 ug/ml, respectively. **Conclusion:** The higher amount of phenolic content in the methanolic extract of *Dillenia indica* may contribute to its better *in vitro* anti oxidant property.

Keywords: antioxidant activity, Assam, *Dillenia indica*, *Garcinia penducalata*, total phenolic content.

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INTRODUCTION

Free radicals are paramagnetic molecular species which possess unpaired electron in its outer orbital.[1] The free radicals increase in the body when the body's antioxidant system weakens. An oxidative stress is caused when the free radicals exceed the homeostasis level, leading to diseases.[2–6] Oxidative stress is initiated by reactive oxygen species (ROS) such as hydrogen peroxide and superoxide anions, that react

with the living tissues and cause harmful effects such as lipid peroxidation, DNA fragmentation and cross linking of proteins and enzymes in them thereby causing diseases.[7]

Human diet can be a major source of antioxidants. Polyphenols which are organic compounds present in regular human diet have an antioxidant property.[8–10] Early men lived in forests and his food habits provided him dietary antioxidants to cope up with ROS but after the initiation of organized farming his food habits changed and now the food habits are completely different with very little dietary derived antioxidants. Moreover human beings are constantly facing environmental toxins. This might be one of the major reasons for the increase in the metabolic disorders in the present population.[11,12]

Although a number of commercial antioxidants are available they may not be enough to cure diseases arising due to increase in free radicals or decrease in the efficacy of antioxidant system in our body. One of the major sources of free radicals is the mitochondria and the mitochondrial mutation can be the possible cause of many diseases.[13–15]

Most of the diseases are detected too late that cause irreversible damage to the body. Therefore food that forms the natural source of antioxidants, can prevent free radicals from damaging our body tissues.[16,17]

Dillenia indica and *Garcinia penducalata* are two major fruits, easily available in Assam, North eastern India and its decoction is a major part of Assamese cuisine. They are sour in taste and are rich in vitamin C. There are many components of Indian cuisine which provide protection against free radicals and also enhance our body's antioxidant system.[18,19] In the present study, the *in vitro* antioxidant properties of the fruits of these plants are compared.

MATERIALS AND METHODS

Plant material

The fruits of *G. penducalata* and *D. indica* were collected in the month of June, 2011. The fruits were washed in distilled water. They were cut into small pieces and shade dried for a week. Later the dried fruits were mechanically grounded into a coarse powder. The samples of the same are deposited in the Centre for Biocultural Diversity (CBD), Chennai and the voucher receipt for the *G. penducalata* Roxb. and *D. indica* L. are MK1108201129 and MK1108201128, respectively.

Chemicals

All analytical grade chemicals used for the experiments were purchased from SRL, India Pvt. Ltd.

Preparation of extract

Soxlet extraction was done for 72 hours to prepare the methanol and petroleum extracts of the coarse fruit powders of the two plants. The emulsions obtained were powdered by using a rotary evaporator to obtain the dry extracts. A part of the coarse dry powder of the fruits was soaked in water overnight and the supernatants obtained were filtered and lyophilized. This lyophilized powders formed the water extracts of the plants. The methanolic and petroleum ether extracts and the lyophilized water extract were used for *in vitro* antioxidant analysis. The per cent concentrations of the methanolic, petroleum ether and water extracts of *G. penducalata* were 15%, 10% and 8% while they were 4%, 2% and 6%, respectively for *D. indica*.

DPPH RADICAL SCAVENGING ACTIVITY

DPPH free radical scavenging activity of the methanol, petroleum ether and water extracts of *G. penducalata* and *D. indica* fruits were carried out by the methods of Cotelle A et al. (1996).[20] Different concentrations ranging from 10–110 ug/ml of the extracts were added to 100 uM of DPPH (2,2, Diphenyl-2-picryl hydrazyl) and the absorbance was read at 517 nm after incubation. Ascorbic acid was used as the standard.

NITRIC OXIDE RADICAL SCAVENGING ACTIVITY

Different concentrations of the methanol, petroleum ether and water extracts of *G. penducalata* and *D. indica* fruits were

Table 1 Levels of phenolic content and ascorbic acid in the extracts of *D. Indica* and *G. penducalata*

	Extracts		
	Methanol	Petroleum ether	Water
	D. indica		
Phenolic content	8%	3%	0.8%
Ascorbic acid	3%	0.5%	8%
	G. penduculata		
Phenolic content	5%	1%	0.6%
Ascorbic acid	1%	0.2%	6%

mixed with sodium nitroprusside and incubated. Griess reagent was added to the mixtures and their optical density was read at 546 nm following the method of Marcocci et al. (1994).[21] Ascorbic acid was used as the standard.

HYDROXYL RADICAL SCAVENGING ACTIVITY

In order to find the scavenging activity of the extracts against hydroxyl radicals, the method of Kunchandy & Ohkawa (1990)[22] was followed. The reaction mixture consisted of 28 mM 2-deoxy-D-ribose, 1.04 mM EDTA, 0.2 mM $FeCl_3$,1.0 mM hydrogen peroxide along with different concentrations of the methanol, petroleum ether and water extracts of *G. penducalata* and *D. indica* fruits. Ascorbic acid was used as a standard drug. After 60 min of incubation, DNA damage was assessed by the method of Ohkawa et al. (1979).[23]

SUPEROXIDE RADICAL SCAVENGING ACTIVITY

The superoxide scavenging activity of the methanol, petroleum ether and water extracts of the fruits was determined by the method of Robak & Gryglewski (1998).[24] Different concentrations of the extracts were mixed with 156 uM nitro blue tetrazolium (NBT), 468 uM reduced nicotinamide adenine dinucleotide (NADH) and 60 uM phenazine methosulphate (PMS). After incubation, the optical density of the reaction mixtures was measured at 560 nm and the percentage inhibition calculated.

DETERMINATION OF REDUCING ABILITY

The reducing ability of methanol, petroleum ether and water extracts of the fruits was analyzed using the method of Jayprakash et al. (2001).[25] The extracts in different concentrations were mixed with 1% potassium ferricyanide and incubated. The reactions were stopped using 10% TCA and the reaction mixtures were centrifuged. Equal volumes of water and 0.1% ferric chloride were added to the supernatants. Absorbance was measured at 700 nm.

DETERMINATION OF TOTAL PHENOLIC COMPOUNDS

The total phenolic content was analyzed by the method of Slinkard & Singleton (1977).[26] An aliquot of 1.5 ml of 2N Folin-Ciocalteau was added to different concentrations gallic acid and incubated at room temperature. Sodium carbonate (20% aqueous) solution was added and the volume of all the mixtures was made up to 10 mL using distlled water. After incubation for 30 minutes, the absorbance was read at 760 nm. Known standard extracts (0.1 mL) from the fruits of *G. penducalata* and *D. indica* were processed in the same way.

DETERMINATION OF ASCORBIC ACID

The method for the estimation of total ascorbic acid in the extracts was done according to Indian Pharmacopia (1996).[27]

Solution containing 0.2 mg ascorbic acid/ml was prepared in water containing 3% w/v metaphosphoric acid. It was titrated against 0.5 mg/ml of 2,6-dichlorophenol indophenol (2,6-DCPIP) solution. End point was the development of permanent pink coloration. Sample solutions containing known quantities of the extracts of *G. penducalata* and *D. indica* were mixed and titrated to determine the amounts of ascorbic acid present in different extracts.

Per cent inhibition was calculated by using the formula,

$$\text{Per cent inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

$A_{control}$: Absorbance of the control sample.

A_{test}: Absorbance of the test sample.

STATISTICAL ANALYSIS

Six replicates were maintained in each of the experiments and their SEM was evaluated based on which, non linear regression (curve fit), exponential growth were derived to calculate the IC_{50} values of the SAWE and standard compounds. All the statistical analyses were done by using paired t test.

RESULTS

DPPH inhibition

As shown in Figure 1a, the IC_{50} values for DPPH inhibition of vit C, methanol, petroleum ether and water extracts of *D. indica* were 43.7 ug/ml, 31.25 ug/ml, 65.77 ug/ml and 74.73 ug/ml and the values for *G. penduculata* (Figure 1b) were 43.7 ug/ml, 50.23 ug/ml, 75.69 ug/ml and 106.95 ug/ml, respectively.

Hydroxyl radical

The IC_{50} values for hydroxyl radicals of vit C, methanol, petroleum ether and water extracts of *D. indica*

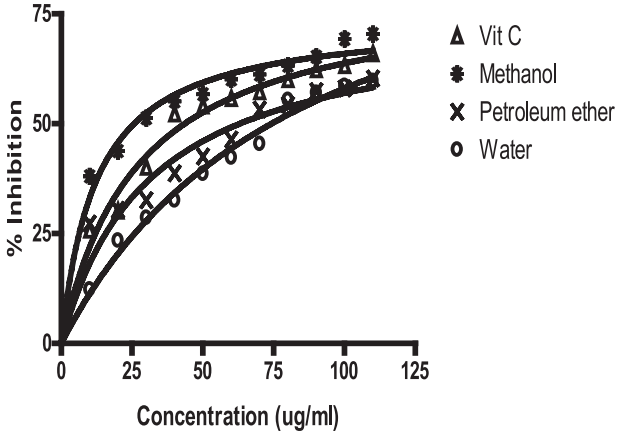

Figure 1a. DPPH Inhibitory activity of *D. indica.*

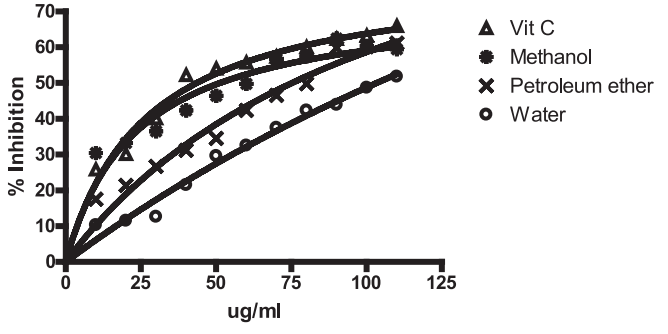

Figure 1b. DPPH Inhibitory activity of *G. penducalata.*

were 50.44 ug/ml, 51.82 ug/ml, 58.12 ug/ml and 75.1 ug/ml, respectively (Figure 2a). The IC_{50} values of the extracts of *G. penducalata* were > 110 ug/ml concentration (Figure 2b).

Oxygen radical

The IC_{50} values for oxygen radical scavenging activity of vit C, methanolic, petroleum and water extracts of *D. indica* were 61.04 ug/ml, 51.44 ug/ml, 78.42 ug/ml and 92.44 ug/ml, respectively (Figure 3a). The IC_{50} value

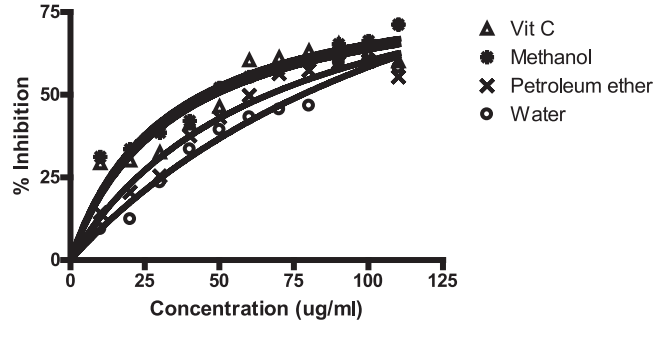

Figure 2a. Inhibition of Hydroxyl radical by *D. indica.*

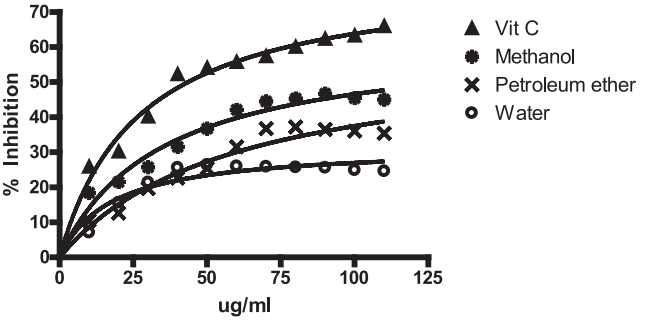

Figure 2b. Inhibition of Hydroxyl radical by *G. penducalata.*

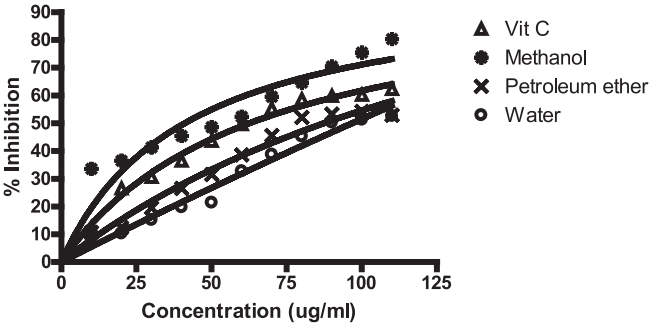

Figure 3a. Inhibition of. Free Oxygen radical by *D. indica.*

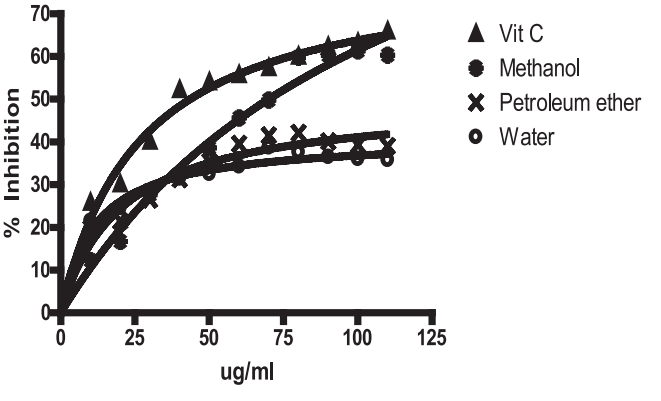

Figure 3b. Inhibition of Free Oxygen radical by *G. penducalata.*

of the methanolic extract of *G. penduculata* were 66.06 while that of the petroleum ether and water extract was t > 110 ug/ml (Figure 3b).

Nitric oxide

The IC_{50} values of the scavenging activity of nitric oxide radicals for vit C, methanol, petroleum ether and water extracts of *D. indica* were 41.82 ug/ml, 39.73 ug/ml, 47.37 ug/ml and 71.87 ug/ml, respectively (Figure 4a). On the other hand, the IC_{50} values for nitric oxide free radical scavenging activity of the methanolic and petroleum ether extracts of

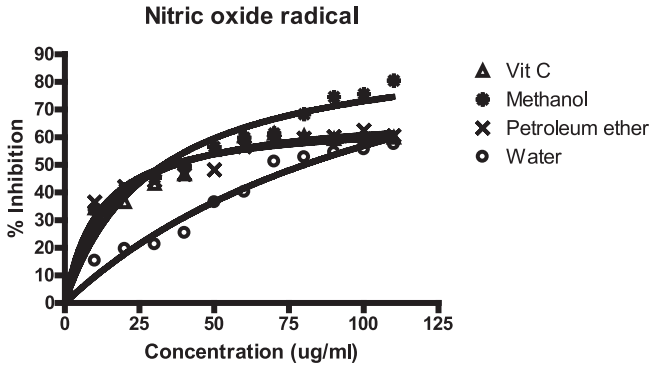

Figure 4a. Inhibition of Nitric oxide radical by *D. indica.*

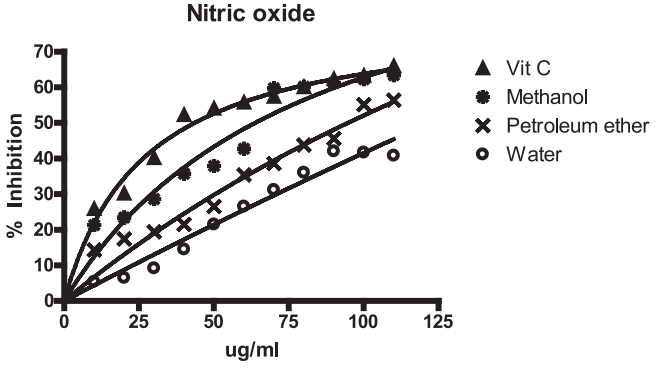

Figure 4b. Inhibition of Nitric radical by *G. penducalata.*

G. penducalata were 63.02 and 95.5 ug/ml, respectively, while that of the water extract was >110 ug/ml concentration (Figure 4b).

Reducting ability

The IC_{50} values for the reducing ability of vit C, methanol, petroleum ether and water extracts of *D. indica* were 47.17 ug/ml, 40.18 ug/ml, 65.52 ug/ml and 102.45 ug/ml, respectively (Figure 5a) while IC_{50} value for the extracts of *G. penduculata* was >110 ug/ml (Figure 5b).

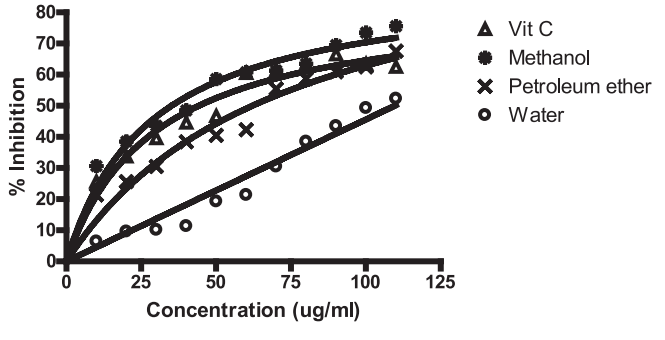

Figure 5a. Reductive ability of *D. indica.*

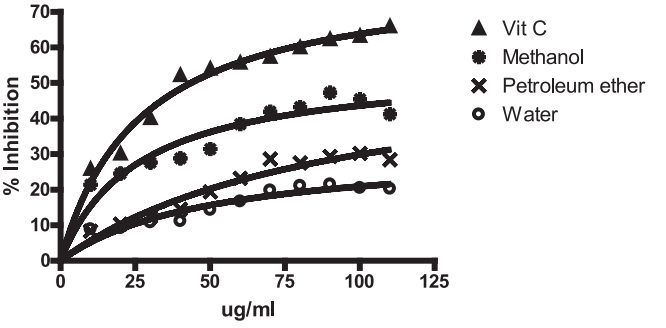

Figure 5b. Reductive ability of *G. penducalata.*

Phenolic content

The phenolic content of the methanolic, petroleum ether and water extracts of *D. indica* was 8%, 3% and 0.8%, respectively while it was 5%, 1% and 0.6% for the respective extracts of *G. penduculata.*

Ascorbic acid content

The percentage of ascorbic acid estimated in the methanolic, petroleum ether and water extracts of the fruits of *D. indica* was 3%, 0.5% and 8%, respectively while those of the fruits of *G. penduculata* were 1%, 0.2% and 6%, respectively.

DISCUSSION

Free radicals are produced at very high levels during illness that damage human tissues. Right dietary sources can provide the much needed antioxidants to control the free radicals from damaging the affected tissues.[28] During oxidative phosphorylation in mitochondria, hydrogen peroxide which is formed from super oxide anions by super oxide dismutase is detoxified to molecular oxygen and water by catalase.

Although the hydroxyl radical scavenging activity of the various extracts of *D. indica* exhibited IC_{50} values <110 ug/ml concentration, the scavenging activity of the extracts of *G. penducalata* was surprisingly >110 ug/ml.

Similarly the oxygen free radical scavenging activity of the different extracts of *D. indica* exhibited an IC_{50} value of <110 ug/ml but the methanolic extract of the same exhibited a better free radical scavenging activity compared to that of vitamin C. Clearly, the methanolic extract of *D. indica* exhibited an IC_{50} value better than that of vitamin C.

Nitric oxide (NO) is found in abundance in the nervous[29] and muscular system.[30] Excess of NO can cause tissue injury

leading to neuronal and muscular diseases like Parkinson's disease, stroke, Huntington's disease, amyotrophic lateral sclerosis and Duchenne muscular dystrophy.[31,32] Production of high amount of NO in the large cerebral blood vessels leads to vasoconstriction and migraine[33,34] along with erectile dysfunction which is one of the major complications of diabetes.[35] NO can inhibit glycolysis by competing with oxygen at cytochrome oxidase and cis acotinase.[36]

NO can react with superoxide to produce peroxynitrite which is neurotoxic. These peroxynitrites formed can also react with superoxide dismutase leading to nitration of tyrosine residues in skeletal muscles thereby causing muscle toxicity.[37]

The IC_{50} value for the nitric oxide scavenging activity of the methanolic extract of *D. indica* was lesser than that of vitamin C which was used as a standard reference. Even though the IC_{50} values of petroleum ether and water extracts of *D. indica* was higher than that of vitamin C, these values were lesser than that of the similar solvent extracts obtained from the fruits of *G. penducalata*.

The DPPH scavenging activities of the various extracts of *D. indica* and *G. penducalata* were <110 ug/ml but the methanolic extract of *D. indica* exhibited an IC_{50} value better than that of vitaminc C.

Similarly the reducing ability of the methanolic extract of *D. indica* was found to be better than that of vitamin C. The concentration at which various extracts of *D. indica* exhibited its reductive property to be <110 ug/ml whereas the same for *G. penducalata* was >110 ug/ml concentration.

The phenolic content was found maximum in the methanolic extracts of both the plants. But the phenolic content of the methanolic extract of *D. indica* was the highest amongst all the extracts obtained from the fruits of *D. indica* and *G. penducalata*. Ascorbic acid being polar was maximum in the water extracts of both the plants. The phenolic and ascorbic acid contents of the petroleum ether extracts of both the plants was found to be intermediary between the methanolic and water extracts.

Polyphenols and vitamin C are important diet-derived antioxidants that provide enormous antioxidants to protect the body tissues against free radicals.[38–41] These antioxidants react with the highly reactive ROS thereby preventing a chain reaction which formins highly reactive products resulting in lipid peroxidation.[42]

Therefore the higher amounts of phenolic content in the methanolic extract of *D. indica* might be one of the major causes for its enhanced *in vitro* antioxidant activity. Although the considerable level of ascorbic acid in the water extracts of the plants could not match the *in vitro* free radical scavenging activity of vitamin C, the high levels of phenols in the methanolic extracts exhibited better free radical-scavenging activity compared to the ascorbic acid level of water extracts.

Traditionally *D. Indica* is used against stomach ache,[43] fever, dysentery and constipation[44,45]. And it possess antidiabetic, hypolipidemic,[46] hepatoprotective, anti-inflammatory, antileukemic and antimicrobial properties.[47]. These diseases can harm the living tissues and the antioxidant property of *D. indica* can protect the tissues against free radical damage initiated during the diseased state.

Vitamin C is a strong antioxidant but naturally eliminated from the body by renal excretion.

The decoctions of the fruits of both the plants are used in the Assamese cuisine. Even though the water extracts of both the plants exhibited IC_{50} values at higher concentrations, data obtained from our study affirms that the decoction of *D. indica* would be an ideal source of antioxidants to prevent the harmful activities of the free radicals generated in our body. Therefore the traditional food habits are potential sources of antioxidants to combat the deleterious effects produced by modern life-style diseases.

CONCLUSION

The present study concludes that *Dillenia indica* and *Garcinia penducalata* both possesses anti oxidant activities. The anti oxidant activity of *Dillenia indica* is superior to that of *Garcinia penducalata* due to its higher phenolic content.

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