Antioxidant Capacity of *Manilkara zapota* L. Leaves Extracts Evaluated by Four *in vitro* Methods

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Abstract: The antioxidant capacity of the *Manilkara zapota* L. leaves extracts, obtained by sequential extraction with different polarities of solvents, was evaluated by four different *in vitro* methods: DPPH, superoxide and hydroxyl radical scavenging activity and reducing capacity assessment assay. The acetone extract showed best DPPH radical scavenging activity; IC_{50} value 20 µg/ml almost equal to that of standard ascorbic acid IC_{50} value 11.4 µg/ml. The superoxide anion scavenging activity of acetone extract (IC_{50} = 140 µg/ml) was better than that of standard gallic acid (IC_{50} = 185 µg/ml). It showed good reducing capacity assessment. The antioxidant capacity of acetone extract may be due to its high phenolic content. The high antioxidant capacity observed for acetone extract suggested that this plant could be used as an additive in the food industry providing good protection against oxidative damage. However, conformation of its activity in *in vivo* models should be carried out. The work suggests that there is no universal criteria for presence or absence of antioxidant activity in different plants. It is imperative that one should evaluate more than one antioxidant methods and in more than one solvent in a single plant. [Nature and Science 2010;8(10):260-266]. (ISSN: 1545-0740).

Key words: antioxidant capacity, *Manilkara zapota,* DPPH, polar solvents, *in vitro* methods

1. Introduction

Antioxidants may be defined as compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Antioxidants can also protect the human body from free radicals and ROS (Reactive Oxygen Species) effects (Gulcin *et al.* 2010). Free radical is a chemical compound which contains an unpaired electron spinning on the peripheral layer around the nucleus. The family of free radicals generated from the oxygen is called ROS which cause damage to other molecules by extracting electrons from them in order to attain stability. ROS are ions, atoms or molecules that have the ability to oxidize reduced molecules. Oxidative damages caused by free radicals to living cells mediate the pathogenesis of many chronic diseases, such as Parkinson's disease, Alzheimer's disease (Bolton et al. 2000), cancers, aging, coronary, heart ailments cardiovascular diseases (Sun *et al.* 2004), atherosclerosis, cataracts and chronic inflammatory diseases, and other degenerative diseases (Ali *et al.* 2008).

Medicinal plants contain many antioxidants such as vitamins (A, C, E, K), carotenoids, flavonoids (flavones, isoflavones, flavonones, anthocyanins, catechins, isocatechins), polyphenols (ellagic acid, gallic acid, tannins), saponins, enzymes and minerals (selenium, copper, manganese, zinc, chromium, iodine, etc) (Ray and Hussan 2002). Natural antioxidants tend to be safer and also possess anti-viral, anti-inflammatory, anti-cancer, anti-mutagenic, anti-tumour, and hepatoprotective properties. The source of natural antioxidants may be all or any part of plants such as fruits, vegetables, nuts, seeds, leaves, roots, barks, peels, plant, etc. (Baravalia et al. 2009; Kaneria *et al.* 2009; Locatelli *et al.* 2010). However, it has been reported that generally leaves are selected for antioxidant studies (Chanda and Dave 2009).

ROS can be produced from both endogenous and exogenous substances. Potential endogenous sources include mitochondria, cytochrome P450 metabolism, peroxisomes, and inflammatory cell activation (Inoue et al. 2003). Exogenous sources of free radicals include tobacco, smoke, ionizing radiation, certain pollutants, organic solvents and pesticides. There are many synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which are commonly used, but they are reported to have side effects and are carcinogenic (Namiki 1990). Therefore, there is an increased interest in the use of natural antioxidants due to their presumed safety, nutritional and therapeutic value (Ajila *et al.* 2007). This may explain the interest in examining plant extracts as a source of cheaper and effective antioxidants and the growing interest in nutraceuticals.

There is no single, widely acceptable assay method for evaluating antioxidant capacity applicable to different compounds and different plant extracts, but the most commonly used methods for measuring antioxidant activity are those that involve the generation of free radical species which are then neutralized by antioxidant compounds (Arno *et al.* 2001). However, it is imperative that more than one antioxidant assay has to be performed for evaluating the antioxidant properties of any plant material (Chanda and Dave 2009).

Manilkara zapota L. belongs to the family *Sapotaceae.* It is an evergreen, glabrous tree, 8-15 m in height. It is cultivated throughout India, though it is native to Mexico and Central America. The seeds are aperients, diuretic tonic and febrifuge. Bark is antibiotic, astringent and febrifuge. Chicle from bark is used in dental surgery. Fruits are edible, sweet with rich fine flavour. Bark is used as tonic and the decoction is given in diarrhoea and peludism (Anjaria *et al.* 2002). The leaves are used to treat cough, cold, and diarrhoea (Mohiddin *et al.* 1992). Bark is used to treat diarrhoea and dysentery (Mohiddin *et al.* 1992). Antimicrobial and antioxidant activities are also reported from the leaves (Nair and Chanda 2008; Kaneria *et al.* 2009**)**. The aim of the present work was to determine the *in vitro* antioxidant capacity of *M. zapota* leaves extract obtained by cold percolation method sequentially, in solvents with different polarities, applying four different methods: 2, 2-diphenyl-1picrylhydrazyl (DPPH), super oxide and hydroxyl radical scavenging assays and reducing capacity assessment.

2. Procedure

2.1 Collection of Plant material

Fresh leaves of *Manilkara zapota* L. were collected in the month of August, 2009, from Jam-jodhpur, Jamnagar, Gujarat, India. The plant was compared with voucher specimen (voucher specimen No. PSN429) deposited at Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The leaves were separated, washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in air tight bottles.

2.2 Extraction

The dried powder of the leaves was extracted sequentially by cold percolation method (Wiart *et al.* 2004**)**, using different polarities of solvents like petroleum ether, toluene, ethyl acetate, acetone and water (aqueous). 10 g of dried powder was taken in 100 ml of petroleum ether in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. After 24 h, the extract was filtered with 8 layers of muslin cloth, centrifuged at 5000 rpm for 10 min. The supernatant was collected and the solvent was evaporated. The residue was then taken in 100 ml of (toluene, ethyl acetate, acetone distilled water successively) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. Then the procedure followed was same as above, and the dry extract was stored at 4^{o}C in air tight bottles. The residues were weighed to obtain the extractive yield.

2.3 Chemicals

2, 2-diphenyl-1picrylhydrazyl (DPPH), Nitroblue tetrazolium (NBT), Phenazine methosulfate (PMS), Nicotinamide Adenine Dinucleotide reduced (NADH), gallic acid, ascorbic acid, quercetin, aluminium chloride, 2-deoxy-2-D-ribose, thiobarbituric acid, trichloroacetic acid, potassium ferricyanide, Tris-HCl were obtained from Hi Media, Mumbai; petroleum ether, toluene, acetone, ethyl acetate, methanol, etc were obtained from Merck, India.

2.4 Quantitative phytochemical analysis

2.4.1 Determination of total phenol content

The amount of total phenol content, in various solvent extracts of leaves was determined by Folin-Ciocalteu's reagent method (Mc Donald *et al.* 2001). 0.5 ml of extract and 0.1 ml (0.5 N) Folin-Ciocalteu's reagent was mixed and the mixture was incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate solution was added and further incubated for 30 min at room temperature and the absorbance was measured at 760 nm. Gallic acid was used as a positive control. Total phenol values are expressed in terms of gallic acid equivalent (mg/g of extracted compounds).The assay was carried out in triplicate and the mean values with ± SEM is presented.

2.4.2 Determination of total flavonoid content

The amount of flavonoid content in various solvent extracts of leaves was determined by aluminium chloride colorimetric method (Chang *et al.* 2002). The reaction mixture 3 ml consisted of 1 ml of sample (1 mg/ml) and 0.5 ml of (1.2%) aluminium chloride and 0.5 ml (120 mM) potassium acetate was incubated at room temperature for 30 min. The absorbance of all samples was measured at 415 nm. Quercetin was used as positive control. The flavonoid content is expressed in terms of quercetin equivalent (mg/g of extracted compound). The assay was carried out in triplicate and the mean values with ± SEM are presented.

2.5 Antioxidant capacity determination

2.5.1 DPPH free radical scavenging activity

The free radical scavenging activity of various solvent extracts of leaves was measured by using 2, 2-diphenyl-1picrylhydrazyl (DPPH) by the modified method of McCune and Johns (2002). The reaction mixture 3 ml consisted of 1.0 ml of DPPH (0.3 mM), 1.0 ml of extract (different concentrations) and 1.0 ml of methanol, was incubated for 10 min, in dark, after which the absorbance was measured at 517 nm. Ascorbic acid was used as positive control. The assay was carried out in triplicate and the mean values with ± SEM are presented. The percentage inhibition was determined by comparing the results of the test and the control. Percentage of inhibition was calculated using the formula

$$\% \text{ Inhibition} = [(B-A)/B] \times 100$$

Where, B = absorbance of blank (DPPH, plus methanol)
A = the absorbance of sample (DPPH, methanol plus sample)

2.5.2 Superoxide anion scavenging activity

The superoxide radical scavenging activity of various solvent extracts of leaves was measured by the method as described by Robak and Gryglewski (1988). Superoxide radicals are generated by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). The reaction mixture 3 ml consisted of 0.5 ml of NBT (0.3 mM) , 0.5 ml of Tris-HCl buffer (16 mM, pH 8), 0.5 ml NADH (0.936 mM), 0.5 ml PMS (0.12 mM) and 1 ml of different concentrations of different solvent extracts. The superoxide radical generating reaction was started by the addition of 0.5 ml of phenazine methosulfate (PMS) solution to the mixture. The reaction mixture was incubated at 25^{o}C for 5 min and then the absorbance was measured at 560 nm against a blank sample. Gallic acid was used as a positive control. The assay was carried out in triplicate and the mean values with ±SEM are presented. The percentage inhibition was determined by comparing the results of the test and the control.

2.5.3 Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of different solvent extracts of leaves was measured by studying the competition between deoxyribose and test compound for hydroxyl radicals generated by Fe^{+3}-Ascorbic acid-EDTA-H_2O_2 system (Fenton reaction) according to the method of Kunchandy and Rao (1990).

The reaction mixture 1 ml consisted of 100 µl of 2-deoxy-2-D-ribose (28 mM in 20 mM KH_2PO_4-KOH buffer, pH 7.4), 500 µl of the various solvent extracts, 200 µl EDTA (1.04 mM) and 200 µm $FeCl_3$ (1:1 v/v), 100 µl 1.0 mM H_2O_2 and 100 µl ascorbic acid (1.0 mM), was incubated at 37°C for 1 h. 1.0 ml of thiobarbituric acid (1%) and 1 ml of trichloroacetic acid (2.8%) was added and incubated at 100°C for 20 min. After cooling, the absorbance of pink colour was measured at 532 nm against a blank sample. Gallic acid was used as a positive control. The assay was carried out in triplicate and the mean values with ± SEM are presented. The percentage inhibition was determined by comparing the results of the test and the control.

2.5.4 Reducing capacity assessment

The reducing capacity assessment of different solvent extracts of leaves was determined using method as described by Athukorala *et al.* (2006). 1 ml of different concentrations of solvent extracts was mixed with 2.5 ml of potassium phosphate buffer (200 mM, pH 6.6) and potassium ferricyanide (2.5 ml, 30 mM). The mixture was then incubated at 50°C for 20 min. There after 2.5 ml of trichloroacetic acid (600 mM) was added to the reaction mixture and then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml of $FeCl_3$ (6 mM) and the absorbance was measured at 700 nm. Ascorbic acid was used as positive control. The assay was carried out in triplicate and the mean values with ± SEM are presented.

3. Results and Discussion

The extractive yield of 5 solvent extracts are given in Table 1.The extractive yield varied from 0.77g to 8.28g and can be ranked from high to low in the following order: Aqueous extract > Petroleum ether > Acetone extract > Toluene extract > Ethyl acetate extract. The extractive yield was more in aqueous extract and minimum in ethyl acetate extract. The extraction yield depends on solvents, time and temperature of extraction as well as the chemical nature of the sample. Under the same time and temperature conditions, the solvent used and the chemical property of sample are the two most important factors (Shimada *et al.* 1992).

The results of total phenol content and flavonoid are given in Table 1. Different solvent extracts showed different levels of phenolic and flavonoid content. Phenolic content was maximum in acetone extract and minimum in toluene extract. Flavonoid content was maximum in ethyl acetate extract and minimum in aqueous extract. These results are slightly different than earlier reports (Kaneria *et al.* 2009), may be because of difference in the order of solvents used. Phenolic and polyphenols like flavonoid are secondary plant metabolites that are ubiquitously present in plants. They have been

suggested to play a role in the antioxidant function. Phenolic compounds have antioxidant properties because of their ability to scavenge free radicals and active oxygen species such as singlet oxygen, hydroxyl radicals. Estimation of phenolic content is important because there are many reports that phenols and antioxidant activity is directly correlated. Phenolic content can be an indicator of the antioxidant capacity of the extract.

Table 1. Total phenol, flavonoid and extractive yield of *Manilkara zapota* L. leaves

Sample	Extractive yield (%)	Total Phenol (mg/g)	Total flavonoid (mg/g)
Petroleum ether extract	4.29	ND	ND
Toluene extract	1.98	6.68 ± 0.57	22.93 ± 0.34
Ethyl acetate extract	0.77	82.21 ± 3.60	91.60 ± 0.56
Acetone extract	2.12	110.50 ± 0.66	76.70 ± 0.27
Aqueous extract	8.28	102.22 ± 3.13	19.14 ± 0.17

ND: Not done, Data presented as the mean ± SEM of three measurements.

The measurement of radical scavenging activity of any antioxidant is commonly associated with the using of DPPH method because it is quick, reliable and reproducible method. It is widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts (Mosquera *et al.* 2007). In the DPPH assay, the antioxidants reduce the stable radical DPPH to the yellow coloured diphenyl-picrylhydrazine which has maximum absorption at 517 nm.

Different solvent extracts showed different levels of DPPH activity (Table 2)**.** The toluene extract showed DPPH free radical scavenging activity in a concentration range of 30-180 µg/ml and its inhibition ranged from 15-84%; while aqueous extract showed activity in a concentration range of 40-240 µg/ml and its inhibition ranged from 11 -73%. Acetone extract on the other hand, showed scavenging activity in a concentration range of 10-35 µg/ml and its inhibition ranged from 23-83%. Ethyl acetate extract showed a very low DPPH free radical scavenging activity. Its IC50 value was more than 1000 µg/ml. This is in contrast to earlier report where the same extract showed IC50value equal to 320 µg/ml (Kaneria et al. 2009). This may be because of the use of solvents in different order. These results prove that solvents of different polarities play a very important role in extracting phytoconstituents.

Table 2. DPPH free radical scavenging activity of *Manilkara zapota* L. leaves

Solvents						Standard	
Toluene extract		Acetone extract		Aqueous extract		Ascorbic acid	
Concentration (µg/ml)	% Inhibition	Concentration (µg/ml)	% Inhibition	Concentration (µg/ml)	% Inhibition	Concentration (µg/ml)	% Inhibition
30	15.0 ± 0.004	10	23.10 ± 0.008	40	11.5 ± 0.005	2	8.60
60	30.2 ± 0.008	15	36.01 ± 0.005	80	24.4 ± 0.003	4	16.60
90	46.6 ± 0.008	20	49.80 ± 0.005	120	38.7 ± 0.002	8	31.33
120	65.1 ± 0.006	25	61.01 ± 0.000	160	51.2 ± 0.002	12	48.65
150	73.7 ± 0.009	30	71.66 ± 0.006	200	62.4 ± 0.001	14	59.70
180	84.1 ± 0.000	35	83.66 ± 0.000	240	73.1 ± 0.008	16	69.79
IC_{50} = 93 µg/ml		IC_{50} = 20 µg/ml		IC_{50} = 160 µg/ml		IC_{50} = 11.4 g/ml	

Data presented as the mean ± SEM of three measurements.

The IC_{50} value, a measure of the extract concentration which is required for 50% inhibition of the free radical DPPH, was determined. The IC_{50} value for toluene extract was 93 µg/ml for acetone extract 20 µg/ml aqueous extract was 160 µg/ml and that of standard ascorbic acid was 11.4 µg/ml. The IC_{50} value of acetone extract was very much near to that of standard. The involvement of free radical, especially their increased production, is a common feature of many dreadful human diseases, including cardiovascular diseases and cancer. Therefore such antioxidants from leaves of common fruits may be important in fighting these diseases by conferring protection against free radical damage to cellular DNA, lipids and proteins.

Acetone extract had maximum phenol content and also showed good DPPH scavenging activity, thus agreeing with the reports that there is a direct correlation between phenolic content and antioxidant activity

(Maisuthisakul *et al.* 2007). The other extracts did not show this correlation. i.e. ethyl acetate extract of the same plant had considerably good amount of phenols but did not posses DPPH radical scavenging activity. On the other hand, toluene extract had low amount of phenols (less than that of ethyl acetate) but had more DPPH radical scavenging activity. These results are in agreement with second group who report that there is no correlation between phenolic content and antioxidant activity (Locatelli *et al.* 2010). It appears from the present results that the same plant extracts in different solvents show different levels of antioxidant activity because different solvents extract different phytoconstituents. Therefore, it is essential to extract the plant material in different solvents for antioxidant evaluation.

Superoxide anions are a precursor to active free radicals that have potential of reacting with biological macromolecules and thereby inducing tissue damage (Halliwell and Gutteridge 1984). Superoxide anion radical ($O_2^{\cdot-}$) is a weak oxidant but it gives rise to the generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both free radicals contribute to oxidative stress (Elmastas *et al.* 2006). Superoxide anion radical scavenging activities of ethyl acetate, acetone and extracts are shown in Table 3. In ethyl acetate extract, 50% inhibition was achieved at 1000 µg concentration while, in acetone extract and aqueous extract, 50% inhibition was achieved at 250 µg concentration and 300 µg in the reaction mixture respectively. The IC_{50} value of ethyl acetate extract was 800 µg/ml, while that of acetone and aqueous extract was 140 µg/ml and 270 µg/ml respectively. The acetone extract appeared to be a better scavenger of superoxide anion radical than that of standard gallic acid (IC_{50} = 185 µg/ml). Toluene extract showed IC_{50} value more than 1000 µg/ml.

The phenolic rich leaf solvent extracts brought significant inhibition of superoxide radicals. The acetone extract was found to be a good scavenger of superoxide radicals. There was a direct correlation between phenolic content and superoxide free radical scavenging activity in the following order; phenolic content acetone >aqueous >ethyl acetate >toluene and superoxide free radical scavenging activity acetone >aqueous >ethyl acetate >toluene. It can be stated that acetone extract may prevent formation of other deleterious radicals such as hydroxyl and hydroperoxides, which initiate free radical chain reactions.

Table 3. Superoxide anion free radical scavenging activity of *Manilkara zapota* L. leaves
Data presented as the mean ± SEM of three measurements.

Solvents						Standard	
Ethyl acetate extract		Acetone extract		Aqueous extract		Gallic acid	
Conc. (µg/ml)	% Inhibition	Conc. (µg/ml)	% Inhibition	Conc. (µg/ml)	% Inhibition	Conc. (µg/ml)	% Inhibition
200	32.54 ± 0.008	50	17.19 ± 0.000	50	10.42 ± 0.009	50	11.88 ± 0.015
400	47.69 ± 0.005	100	42.68 ± 0.001	100	16.30 ± 0.005	100	24.95 ± 0.004
600	48.90 ± 0.004	150	56.80 ± 0.002	150	24.35 ± 0.005	150	41.38 ± 0.006
800	51.11 ± 0.001	200	69.08 ± 0.006	200	35.39 ± 0.004	200	52.18 ± 0.001
1000	63.86 ± 0.010	250	75.84 ± 0.003	250	44.58 ± 0.005	225	66.66 ± 0.002
IC_{50} = 800 µg/ml		IC_{50} = 140 µg/ml		IC_{50} = 270 µg/ml		IC_{50} = 185 µg/ml	

Hydroxyl radical scavenging activity of various solvents extracts did not show any type of hydroxyl radical scavenging activity. This was entirely different result from that of other antioxidant activities shown by the extracts of the same plant. This difference in the antioxidant activity may be due to different mechanism of action and various plants contain different phytoconstituents. The antioxidant effect exponentially increases as a function of the development of the reducing power, indicating that the antioxidant properties are concomitant with the development of reducing power (Oyaziu 1986). All the four extracts showed a concentration dependent reducing capacity assessment in the studied concentration range. However, acetone extract showed more reducing capacity as compared to other three extracts (Table 4).

The acetone extract had more phenolic content and also showed more reducing capacity assessment. The reducing properties are generally associated with the presence of reductones. It has been reported that the antioxidant action of reduction was based on the breaking of the free radical chain by donating a hydrogen atom. Reductones also react with certain precursors of peroxide, thus preventing peroxide formation. Results in the present study demonstrate that acetone extract exert a remarkable reducing activity indicating that some enzymaticprotein molecules may be involved in the ferricyanide reduction. In fact, several enzymes such as cytochrome c reductase (Rafferty and Malech 1996) or the lactate dehydrogenase may catalyze the ferricyanide reduction.

Table 4. Reducing capacity assessment of *Manilkara zapota* L. leaves

Solvents								Standard	
Toluene extract		Ethyl acetate		Acetone extract		Aqueous extract		Ascorbic acid	
Conc. (µg/ml)	Reducing activity (OD at 700nm)	Conc. (µg/ml)	Reducing activity (OD at 700nm)	Conc. (µg/ml)	Reducing activity (OD at 700nm)	Conc. (µg/ml)	Reducing activity (OD at 700nm)	Conc. (µg/ml)	Reducing activity (OD at 700nm)
20	0.001 ± 0.000	20	0.004 ± 0.000	20	0.042 ± 0.001	20	0.002 ± 0.000	20	0.187 ± 0.000
40	0.002 ± 0.002	40	0.018 ± 0.002	40	0.120 ± 0.020	40	0.023 ± 0.003	40	0.366 ± 0.001
60	0.004 ± 0.000	60	0.042 ± 0.000	60	0.285 ± 0.045	60	0.100 ± 0.015	60	0.543 ± 0.022
80	0.006 ± 0.000	80	0.081 ± 0.000	80	0.391 ± 0.002	80	0.149 ± 0.000	80	0.671 ± 0.005
100	0.008 ± 0.001	100	0.118 ± 0.006	100	0.409 ± 0.001	100	0.172 ± 0.007	100	0.762 ± 0.000
120	0.013 ± 0.000	120	0.138 ± 0.001	120	0.454 ± 0.006	120	0.153 ± 0.016	120	0.898 ± 0.007
140	0.015 ± 0.000	140	0.158 ± 0.002	140	0.552 ± 0.001	140	0.218 ± 0.003	140	1.083 ± 0.031
160	0.018 ± 0.000	160	0.176 ± 0.001	160	0.602 ± 0.000	160	0.239 ± 0.002	160	1.299 ± 0.001
180	0.026 ± 0.001	180	0.198 ± 0.001	180	0.651 ± 0.008	180	0.271 ± 0.000	180	1.429 ± 0.006

Data presented as the mean ± SEM of three measurements

The results of antioxidant assays showed that the sequential extraction with different polarities of solvents extracted compounds of different antioxidant capacities as measured by different *in vitro* methods. The acetone extracted compounds were capable of scavenging all the free radicals evaluated in the present study while it was not the same with other solvents.

Various solvent extracts of the same plant showed varied levels of antioxidant capacities. Acetone extract showed positive correlation between phenolic content and antioxidant activity. Ethyl acetate and toluene extract showed negative correlation i.e. the former had more phenolic content but less antioxidant activity while the latter had less phenolic content but more antioxidant activity. Thus there are no universal criteria for presence or absence of antioxidant activity in different plants. It is imperative that one should evaluate more than one antioxidant methods and in more than one solvent in a single plant. This is necessary because plant is rich in secondary metabolites and it is not known which one predominates; and also the mechanism of action of different antioxidant assays is different.

4. Conclusion

Searching plant sources may bring new natural products into pharmaceutical, cosmetic and food production. In the present work, the high antioxidant capacity observed for acetone extract of *M. zapota* leaves suggest that it may play a role in preventing human diseases in which free radicals are involved, such as cancer, ageing and cardiovascular diseases. Therefore, it is suggested that this plant could be used as an additive in the food industry providing good protection against oxidative damage. The plant is easy accessible in high quantities and therefore its application could be beneficial. However, conformation of its activity in *in vivo* models should be carried out. Such antioxidants could replace synthetic toxic antioxidant.

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