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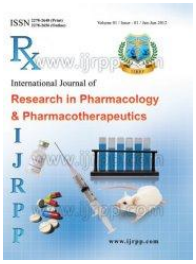

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In vitro studies on phytochemical analysis and antioxidant activity of *Manilkara zapota*

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ABSTRACT

Sapota is one of the prominent fruits and belongs to family sapotaceae. India is the largest producer of sapota. In this present study, phytochemicals were analysed and antioxidant activity was determined for sapota fruit. The antioxidant capacity of the *Manilkara zapota* L fruit extract was evaluated by three different in vitro methods: DPPH activity, hydrogen peroxide scavenging activity and total antioxidant activity. The ethanolic extract showed best antioxidant activity almost equal to that of standard ascorbic acid. The antioxidant capacity of ethanolic extract may be due to its high phytochemical constituents. The high antioxidant capacity observed in aqueous and ethanolic extracts suggested that this plant could be used as an additive in the food industry providing good protection against oxidative damage. Results indicate that nutrient composition, phytochemical and antioxidant activity of sapota was promising. It is also a good source of antioxidants. These antioxidants are important in all of our diets, especially in infant diets. Thus sapota fruit can be used as a potent therapeutic agent.

Keywords: Antioxidant Capacity, *Manilkara zapota*, DPPH, In Vitro Methods.

INTRODUCTION

Fruits are identified as rich sources of antioxidants and copiously used to overcome oxidative stress. The fact behind the health beneficial property of fruits is the large number of nutraceutical phytochemicals that they contain viz., polyphenols, carotenoids, sterols, saponins, terpenes and vitamins. Phytochemical components like phenolics, ascorbic acid and carotenoids may have direct influence over the radical-scavenging potential. Antioxidants that help in lowering incidence of degenerative diseases such as cancer, arthritis, arteriosclerosis, heart disease, brain dysfunction and acceleration of the ageing process. [1]

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). A 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 damage 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). Epidemiological studies all over the world have shown that vegetable-rich diets are associated with higher life expectancy. The reason for this could be due to the higher antioxidants presents in such diets. Higher fruit and vegetable intake was associated with a lower risk of myocardial infarction and other cardiovascular diseases, cancers of the mouth, pharynx, larynx, stomach, colon, etc. [2]

Antioxidants are substances which when present at low concentration are able to prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species. These reactive oxygen species, mainly are reactive free radicals such as superoxide, hydroxyl, peroxyl, alkoxyl and non-radicals such as hydrogen peroxide, hypochlorous. Sapota fruit is rich in carbohydrates and provides a good amount of proteins and minerals like calcium, phosphorus and iron. The fruits are tonic, enrich blood, increase muscular strength, cooling, sedative to the heart and relieve vomiting. [1]

Antioxidants have been reported to prevent oxidative damage by free radical and ROS; any may prevent the occurrence of disease, cancer and aging. It can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers. Natural antioxidants, particularly in fruits, vegetables and beverages have gained interest among consumers9. Antioxidants are dietary substance that protects body cells from the oxidative damage to a target molecule caused by oxidation from free radicals by reactive oxygen species (ROS). During the last two decades, there has been in search for new plant derived drugs containing the medically useful alkaloids, glycosides, polyphenolics, steroids and terpenoids derivatives, which contributes to the antioxidant property 11. Dietary phenolic compounds and flavonoids have generally been considered, as non-nutrients and their possible beneficial effect on human health have only recently been recognized. Flavonoids are known to possess antioxidant and anticarcinogenic activities 12. Therefore a search for natural antioxidants of plant origin gained momentum in recent years. [3]

An increased concentration of end products of lipid peroxidation is the evidence most frequently quoted for the involvement of free radicals in human diseases. Several studies support the hypothesis that lipid oxidation products ingested with food or produced endogenously represent a health risk. It is well established that free radicals and reactive oxygen species are continuously produced *in vivo*. Numerous detoxifying mechanisms in the body protect the cells from the toxic effects of free radicals and the associated peroxidation of lipids. These include protective enzymes (superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione transferase, catalase, etc.) and several low molecular weight water and lipid soluble antioxidants (reduced glutathione, uric acid, Ubiquinol-10, etc). Apart from these endogenous antioxidants, there are several essential antioxidants, which include vitamins E, A, C and carotenoids. There is increasing evidence that under certain circumstances, these protective systems may be overwhelmed. Free radical mediated oxidation of lipids particularly affects the polyunsaturated fatty acids (PUFA). The primary products of free radical oxidation undergo rapid and spontaneous fragmentation. Some have considerable lethal potential. The peroxidation of lipids has gained significance in recent times because of its implication in the causation of a number of diseases. [2]

ROS can be produced from both endogenous and exogenous substances. Potential endogenous sources include mitochondria, cytochrome P450 metabolism, peroxisomes, and inflammatory cell activation. 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. Therefore, there is an increased interest in the use of natural antioxidants due to their presumed safety, nutritional and therapeutic value. This may explain the interest in examining plant extracts as a source of cheaper and effective antioxidants and the growing interest in nutraceuticals. [4]

The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of a potentially damaging

molecules commonly called "free radicals." Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction. Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating. Free radicals are electrically charged molecules, i.e., they have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves. Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur. And until subsequent free radicals are deactivated, thousands of free radical reactions can occur within seconds of the initial reaction. Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being. [5]

Sapota is also a good source of antioxidants. These antioxidants are important in all of our diets, especially in infant diets. The amount of free radicals that are produced during metabolism can be countered very effectively by some vitamins and particularly by antioxidants. [1]

Hence, the present study attempts to investigate the total phytochemicals and antioxidant activity of fresh juice from the fruit of *manilkara sapota.*

MATERIALS AND METHODS

Collection of plant materials

The zapota fruit was obtained from a local supermarket of Mayiladuthurai, Nagai district, Tamilnadu, South India. The length of fruit is up to 2-2.5 inches. Its skin is mild and brown. The sapota is round or oval, measures about 10cm in diameter, and weight about 150g.

Preparation of fruit juice

About 10 fruits of sapota were taken. The peel of the fruit and the pulp were collected. Those pulps were mashed by grinding method and the fruit juice was extracted.

Preparation of fruit extract

About 50ml of fresh fruit juice of sapota fruit exhaustively extracted in 50ml of 60% aqueous, ethanol the resultant extract was boiled in a water bath until a syrupy consistency was obtained.

Preliminary phytochemical screening

Phytochemical analysis of all extracts was carried out by standard procedure. [19]

ANTIOXIDANT SCREENING

DPPH radical scavenging activity

Briefly, a 2ml aliquot of DPPH (2,2-diphenylpicrylhydrazyl) 1ml methanol solution (25 µg/ml) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. The lower absorbance of the reaction mixture indicated higher free-radical scavenging activity [6]

Total antioxidant capacity

The Total antioxidant was evaluated by the phosphor molybdenum method.[8] To 1 ml of extract of different concentrations was treated with 1ml of reagent solution (0.6mm sulphuric acid, 28mm sodium phosphate and 4mm ammonium molybdate) in eppendeff tube. Capped tubes were incubated in the thermal block at 95 C for 90 mins. After cooling to room temperature, the absorbance was measured at 695nm against the blank. The activity was compared with ascorbic acid as standard.

Hydrogen peroxide scavenging activity

Scavenging activity of extract was evaluated by hydrogen peroxide. [9]1ml of sample is mixed with 3ml of phosphate buffer 1ml of H_2O_2 and incubated for 10mins at 37 C after incubation the absorbance value of the reaction mixture was recorded at 230nm. Ascorbic acid used as standard.

RESULTS AND DICUSSION

The present investigation was carried out to screen the phytochemical and antioxidant activity of *Manilkara zapota.*

Table 1: phytochemical screening of *Manilkara zapota*

S.No	Name of the test	Aqueous	Ethanol
1	Steroids	+	+
2	Phenols	-	-
3	Tannins	+	+
4	Flavonoid	+	+
5	Alkaloids	-	-
6	Glycosides	+	-
7	Terpenoids	+	+
8	Saponins	+	+
9	Quinine	+	+
10	Coumarins	+	-
11	Phlobatannins	-	-
12	Carbohydrate	+	+
13	Anthraquinones	+	+

(+) = Indicates presence

(-) = Indicates absence

The qualitative phytochemical analysis carried out in ethanol and aqueous extracts revealed the presence of carbohydrate, steroids, tannins, flavonoid, terpenoids, saponins, quinine, and anthraquinine.

In the ethanol extract of m. zapota the results were positive for eight compounds (steroids, tannins, flavonoids, terpenoids, saponins, quinine, carbohydrate, anthraquinine). The m.zapota aqueous extract shows the positive results are ten compounds (steroids, tannins, flavonoids, glycosides, terpenoids, saponins, quinine, coumarins. Carbohydrate, anthraquinine (table 1)

The phytochemical analysis has shown has presence the shown the presence of potent phytochemical substances such as tannins, flavonoids, terpenoids, saponins, carbohydrate, and steroids. [18]

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, antimutagenic, 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. [7]

Free radicals are the main culprit in lipid peroxidation, highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. Free radicals are fundamental to many biochemical processes and represent an essential part of aerobic life and metabolism. Free radical oxidative stress caused a wide variety of clinical disorders. A serious imbalance between the production of free radicals and the antioxidant defense system is responsible for oxidative stress. Antioxidants exert their mode of action by suppressing the formation of reactive oxygen species either by inhibition of enzymes or by chelating trace elements. [3]

Sapota fruit as moisture 73.7 per cent, carbohydrate 21.4 per cent, fiber 2.6 per cent, fat 1.1 per cent, protein 0.7 per cent, mineral 0.5 percent, vitamin C, 6 mg, thiamine 0.02 mg, riboflavin 0.03 mg, niacin 0.02 mg and mineral content calcium 28 mg, phosphorus 27 mg and iron 2 mg. [1]

The screening of aqueous and ethanolic extracts indicates the presence of steroids, tannins, flavonoid, terpenoids, saponins, quinine, carbohydrates, anthraquinine and absence of phenols, alkaloids, phlobatannins, glycosides and coumarins are absent in the ethanolic extract of fruit juice of *Manilkara zapota* is given in table 1. From the above results, the phytochemical analysis of aqueous extracts showed more phytonutrients than ethanolic extract.

Highest total saponins and flavonoid content was present in the ethanol extract followed by aqueous

extracts. Unripe fruit has high amounts of tannins, which can pucker mouth. [17]

Flavonoids are important for human because of their high pharmacological activities as radical scavengers. [3]

Table 2: Hydrogen peroxide scavenging activity of *Manilkara zapota*

S.No	Concentration	H_2O_2 (%)		
		Aqueous	Ethanol	Standard
1	50	18.8±0.65	22.9±0.5	20.6±0.8
2	100	21.2±0.5	25.5±1.05	28.9±0.3
3	150	24.9±0.5	28.5±0.83	32.7±1.2
4	200	26.9±0.4	32.8±2.5	40.0±2.3

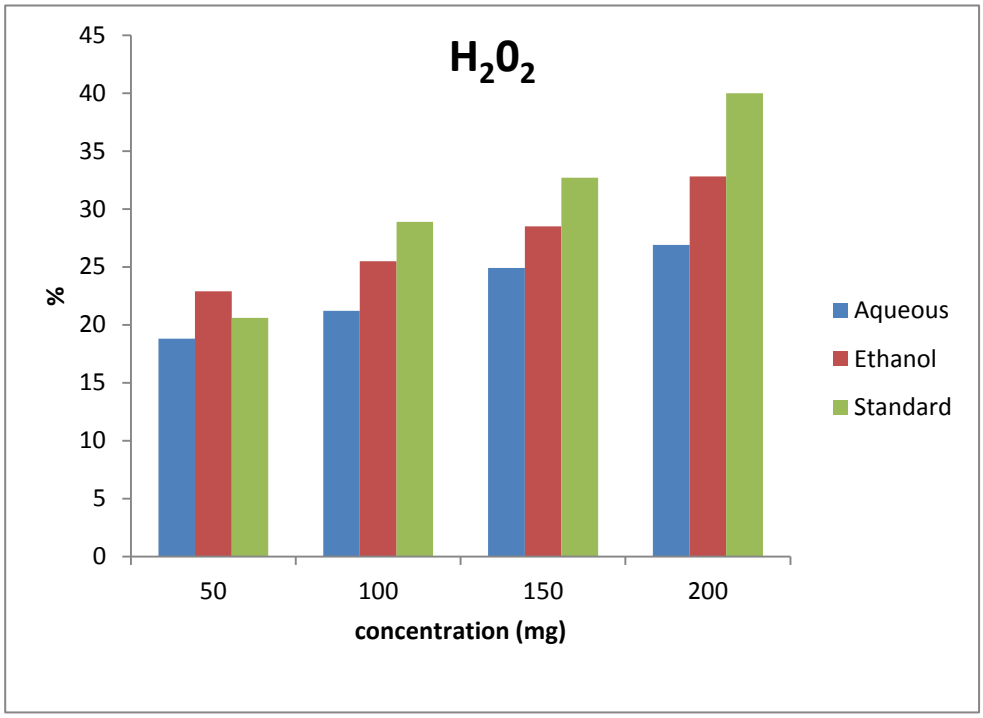

Figure 1

Figure 1 and table 2 shows the hydrogen peroxide scavenging activity of *Manilkara zapota* in comparison with ascorbic acid standard. It shows the Hydrogen peroxide scavenging activity of *Manilkara zapota* which is increased in ethanolic extracts when compared to aqueous extract.

Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with and Fe2+possibly Cu2+ ions to form hydroxyl radicals and this may be the origin of many of its oxide effects. From the results, it appeared that the H2O2 scavenging activity of the plant extract is significant compared to that of the standard ascorbic acid. Scavenging of H_2O_2 by extract may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water. The ability of the extracts to effectively scavenge hydrogen peroxide, determined according to the method where they are compared with that of ascorbic acid as standard. [10]

Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, microorganisms and food. H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (· OH) that can initiate lipid peroxidation and cause DNA damage. *Manilkara zapota* efficiently scavenged hydrogen peroxide which may be attributed to the presence of

phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralizing it into water. [14]

Table.3: DPPH scavenging activity of *Manilkara zapota*

S.no	Concentration	DPPH		
		Aqueous	Ethanol	Standard
1	50	27.9±0.4	27.1±1.3	20.6±0.8
2	100	35.9±0.6	35.3±0.26	28.9±0.3
3	150	43.4±2.7	42.1±1.4	32.7±1.2
4	200	48.9±1.3	46.5±2.05	40.0±2.3

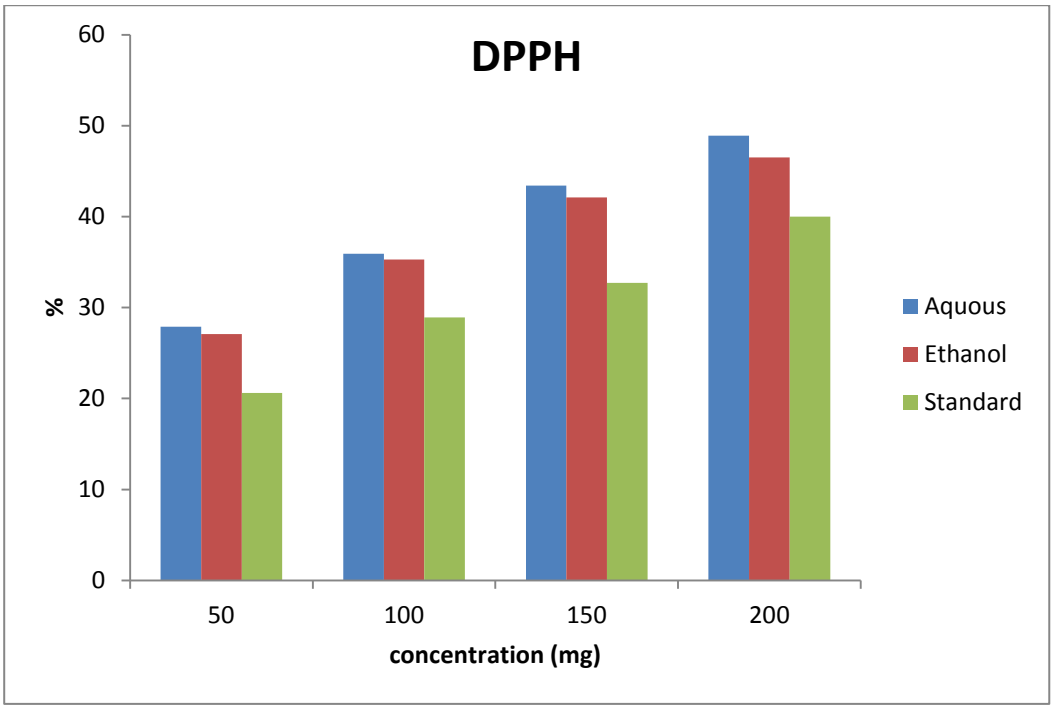

Figure 2

Figure 2 and table 3 shows the DPPH activity of *Manilkara zapota* in comparison with ascorbic acid standard. It indicates the level of DPPH activity of *Manilkara zapota* which is increased in aqueous extract when compared to ethanolic extracts.

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts. DPPH radical scavenging activities of the extracts depended not only on plant type, but also upon the extraction solvent. In general, DPPH scavenging activities increased with increasing phenolic components such as flavonoids, phenolic acids and phenolic diterpenes. These phenolic components possess many hydroxyl groups, including a o - dihydroxy group which have very strong radical scavenging effect and antioxidant power. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 1, 1-diphenyl-1, 2-picryl hydrazine is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecules a whole. The delocalisation also gives rise to the deep violet colour, characterized by an absorption band in methanol solution centered at 517 nm. [11]

The dose response curve of DPPH radical scavenging activity of crude extracts of the plant was observed, when compared with standard ascorbic acid. Highest antioxidant activity was given by Heliotropium indicum extract at the concentration of 200mg/ml among all the methanolic leafs which is found to be more than even the ascorbic acid. Thus, it is clear that polyphenolic antioxidants in leaves of

selected plants play an important role as bioactive principles and the scavenging effect can be attributed to the presence of active phyto constituents in them. [11]

The electron donation ability of natural products can be measured by 2,2′-diphenyl-1- picrylhydrazyl radical (DPPH) purple-coloured solution bleaching . The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test. In the present study among all the fractions tested, n-butanol, chloroform and ethyl acetate showed significantly higher inhibition percentage and positively correlated with total phenolic content. The results of this study suggest that the plant extract contains phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage. [15]

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples. DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers. Usually, higher total phenol and flavonoids contents lead to better DPPH-scavenging activity. [12]

Table 4: Total antioxidant capacity of *Manilkara zapota*

S.NO	Concentration	TAC (%)		
		Aqueous	**Ethanol**	**Standard**
1	50	22.5±0.2	23.8±0.58	20.6±0.8
2	100	24.9±0.5	25.9±0.6	28.9±0.3
3	150	27.7±0.3	29.5±1.1	32.7±1.2
4	200	32.2±1.6	35.9±1.2	40.0±2.3

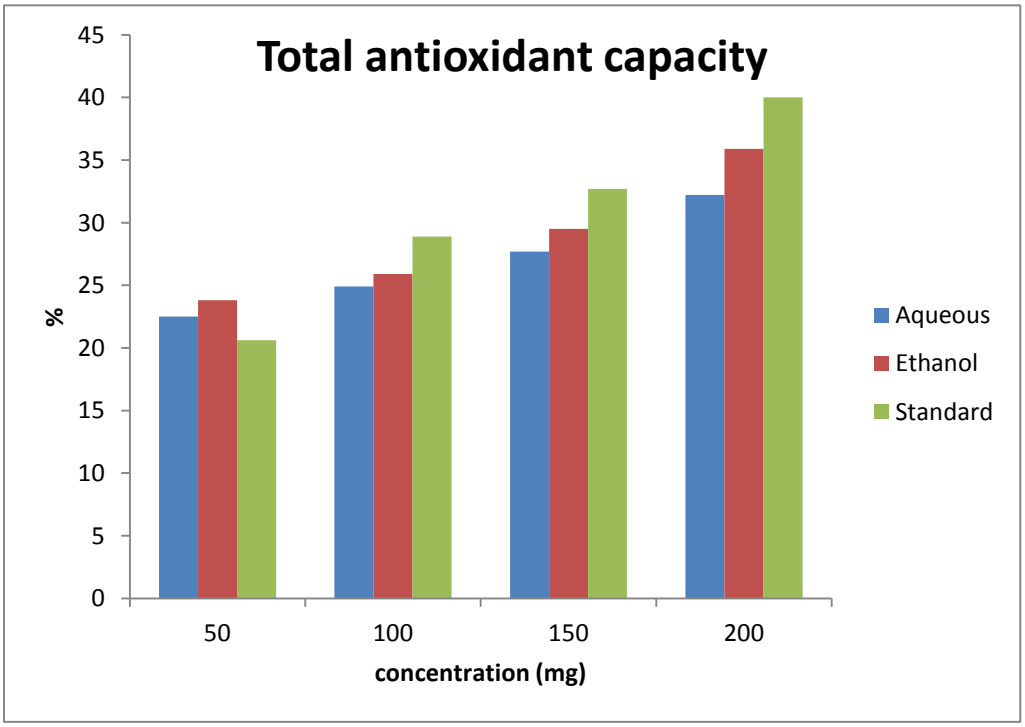

Figure 3

Figure 3 and table 4 indicates the total antioxidant activity of *Manilkara zapota* in comparison with standard ascorbic acid. It shows that the total antioxidant activity of *Manilkara zapota* which is increased in ethanolic extracts than aqueous extracts.

The antioxidant capacity of the fractions was measured spectrophotometrically through phosphor molybdenum method, based on the reduction of Mo (VI) to Mo (V) by the test sample and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 765 nm. The present study demonstrated that *Manilkara zapota* exhibited the highest antioxidant capacity for phospho-molybdate reduction. Recent studies have shown that many flavonoid and related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants. [13]

SUMMARY AND CONCLUSION

From the study it can be summarized that all the *Manilkara zapota* fruits are sources of various nutrients and can be helpful in fighting against inflammatory diseases. The following investigations were done in this study,

The Determination of Total antioxidant activity, DPPH scavenging activity and Hydrogen peroxide radical scavenging activity gives the *Manilkara zapota* fruit contains a high amount of antioxidant due to the presence of phyto constituents indicates the presence of Steroids, Tannins, flovonoids, Quinine, Terpenoids, saponins, Anthra quinines, carbohydrates and the absence of phenols, alkaloids, phlobatannins, in aqueous, ethanolic extracts. Glycosides, coumarins were absent in ethanolic extract.

It can be concluded that all the *Manilkara zapota* fruits contain different type of phytochemicals and higher activity of antioxidant. Most of the biologically active phytochemicals were present in the aqueous extract of *Manilkara zapota* fruit juice.

The consumption of Sapodilla fruit benefits in reducing the level of infections. It helps in lessening viral diseases in addition to bacterial infections within the body. It also decreases inflammation caused by swelling and helps to ease pain. Chikoo or Sapodilla is an excellent source of minerals such as potassium, copper and iron. In addition to these minerals, Chikoo also consists of foliate and niacin acid. These vitamins and minerals help in making the body powerful and energetic.

REFERENCES

[1]. Rampriya R, Vasantha Devi. K. P, Thahira Banu. A, (2015), Nutrient Content, Phytonutrient Composition and Antioxidant Activity of Sapota Pulp Powder, international journal of scientific research Volume: 4 Issue: 9 Sept 2015.

[2]. Poornima. K, Cariappa M, Asha K, Kedilaya H.P and Nandini M, (2003), oxidant and antioxidant status in vegetarians and fish eaters. Indian journal of biochemistry, 18 (2) 197-205

[3]. Shanmugapriya K, Saravan P.S, Harshapayal, Peer Mohammed S, Williams Binnie, (2011), antioxidant activity, total phenolic and flavonoid contents of artocarpus heterophyllus and *Manilkara zapota* seeds and its reduction potential International Journal of Pharmacy and Pharmaceutical Sciences ISSN- 0975-1491 Vol 3, Suppl 5.

[4]. Chanda S. V and Nagani K. V, (2010), Antioxidant Capacity of *Manilkara zapota* L. Leaves Extracts Evaluated by Four *in vitro* Methods. Nature and Science 8 (10).

[5]. Markpercival DR, (1998)**,** Antioxidants. Clinical nutrition insights. NUT031 1/96 Rev. 10/98.

[6]. Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. Antioxidant properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of agricultural and food chemistry, 1992; 40: 945-948.

[7]. Trease G.E. And Evans W.C a Textbook of pharmacogency, 1 4th Ed. Bailliere Tindall Ltd, London, 2013; 832.

[8]. Prieto, P., M. Pineda and Aguilar, M. Spectrophotometric Quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem, 1999; 269: 337-341.

[9]. Klein SM, Cohen G, Cederaum AI. Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system. Biochemistry 1991; 20: 6006-6012.

[10]. Nabavi S. M, Ebrahimzadeh M. A, Nabavi S. F, Fazelian M, Eslami B, (2009) *Pharmacognosy Magazine*, 4,123-127.
[11]. Ebrahimzadeh M. A, Pourmorad F, Hafezi S; *Turk. Biol J*, (2008), 32, 43-49.
[12]. Dehpour A. A, Ebrahimzadeh M. A, Nabavi S. F, Nabavi S. M; *Grasas Aceites*, (2009), 60, 405-412.
[13]. Nunes PX, Silva SF, Guedes RJ, Almeida S: Biological oxidations and antioxidant activity of natural products, Phytochemicals as nutraceuticals - Global Approaches to Their Role in Nutrition and Health. 2012
[14]. Krishnaiah D, Sarbatly R, Nithyanandam RR: A review of the antioxidant potential of medicinal plant species. Food Bioprod Process. 2011, 89: 217-233.
[15]. Gulcin I, Berashvili D, Gepdiremen A (2005): Antiradical and antioxidant activity of total anthocyanins from Perilla pankinensis decne. J Ethnopharmacol. 2005, 101: 287-293. 10.1016/j.jep.2005.05.006.
[16]. Sahreen S, Khan MR, Khan RA, (2011): Phenolic compounds and antioxidant activities of Rumex hastatus D. Don. Leaves. J Med Plants Res. 2011, 5: 2755-2765.
[17]. Prale milind and preethi, (2015), chickoo: a wonderful gift from nature, Ayurveda pharm, received on 14. 4.15.
[18]. Mathangi T, prabhakaran p. Screening for antimicrobial activity of the leaf extracts of *Marsilea quadrifolia* against various bacterial pathogens. Herbal Tech Industry, 2012; 9 (8): 11-12.
[19]. Trease GE and Evans WC, *a textbook of Pharmacognecy*. 14th ed.Bailliere Tindall Ltd., London, 2013; 832.