

## Comparative Study of Antioxidant Properties and Cytotoxic effect of Three Different Plants of *Myrtaceae* Family

S.B.Nasrin Fathima<sup>1</sup>, Dr.R.Pandian<sup>2</sup> and Florida Tilton<sup>3</sup>  
nasrinfathima.sb@gmail.com<sup>1</sup>, drpandian50@gmail.com<sup>2</sup>, md@biozone.co.in<sup>3</sup>

**ABSTRACT**-The antioxidant activity of the ethyl acetate and methanol extracts of *Syzygium tamilnadensis*, *Syzygium densiflorum* and *Eugenia candolleana* was evaluated using various antioxidant tests such as H<sub>2</sub>O<sub>2</sub>, NO, SO, FRAP and DPPH scavenging activities. The cytotoxicity activity of the extracts was also determined against the breast cancer cell line MCF-7 using the MTT assay. The various antioxidant activities were compared to the standard ascorbic acid. Compared to the results of all the antioxidant assays the methanol extract of the plant *Syzygium tamilnadensis* exhibited the highest scavenging activities. The results of the MTT assay also provide evidence that the methanol extract of the plant *Syzygium tamilnadensis* has good cytotoxicity when compared to the other extracts.

**KEY WORDS**-*Syzygium tamilnadensis*, *Syzygium densiflorum*, *Eugenia candolleana*, Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>), Nitric oxide (NO), Superoxide (SO), Ferric reducing antioxidant power (FRAP). 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Cytotoxicity, MTT assay

### 1. INTRODUCTION

Free radicals are everywhere, in the air, our bodies, and the materials around us. They cause the deterioration of plastics, the fading of paint, the degradation of works of art, aging related illnesses, and can contribute to heart attacks, stroke and cancers. Free radicals may be either oxygen derived (ROS, reactive oxygen species) or nitrogen derived (RNS, reactive nitrogen species) (Evans *et al.*, 1999). The oxygen derived molecules are O<sup>-2</sup> [superoxide], HO [hydroxyl], HO<sub>2</sub> [hydroperoxyl], ROO [peroxyl], RO [alkoxyl] and H<sub>2</sub>O<sub>2</sub> oxygen as non-radical. Nitrogen derived oxidant species are mainly NO [nitric oxide], ONOO [peroxy nitrate], NO<sub>2</sub> [nitrogen dioxide] and N<sub>2</sub>O<sub>3</sub> [dinitrogen trioxide] respectively (Devasagayam *et al.*, 2003).

Free radicals are molecules with unpaired electrons, the presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron or accept an electron from other molecules, therefore behaving as oxidants or reductants (Cheeseman, K.H and Slater, T.F. 1993). Free radicals attack important macromolecules leading to cell damage and homeostatic disruption. Targets of free radicals include all kinds of molecules in the body. Among them, lipids, nucleic acids, and proteins are the major targets. Free radical formation occurs continuously in the cells as a consequence of both enzymatic and non enzymatic reactions. In their quest to find another electron, they are very reactive and cause damage to surrounding molecules. Cancer and atherosclerosis, two major causes of death, are salient "free radical" diseases. Cancer initiation and promotion is associated with chromosomal defects and oncogene activation. It is possible

that endogenous free radical reactions, like those initiated by ionizing radiation, may result in tumor formation. The highly significant correlation between consumption of fats and oils

and death rates from leukemia and malignant neoplasia of the breast, ovaries, and rectum among persons over 55 years may be a reflection of greater lipid peroxidation (Lea, A.J. 1996).

. Florida Tilton, Managing Director, Biozone Research Technologies Pvt Ltd, Chennai Tamil nadu, India

However, free radicals are also useful because they play an important role in our bodies and can be utilized to manufacture pharmaceuticals, custom-designed plastics and other innovative materials.

An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property (Halliwell B, 1995). Antioxidants are substances that may protect living cells against the effects of free radicals. A great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems. Recently there has been an upsurge of interest in therapeutic potential medicinal plants as antioxidants in reducing oxidative stress-induced tissue injury (Pourmorad *et al.*, 2006).

The antioxidants have been reported to have radical scavenging activity and inhibition of hydrolytic and oxidation to prevent oxidative damage caused by free radical. To prevent free radical damage, the body has a defense system of antioxidants which include extracellular and intracellular antioxidants. Enzymes like Superoxide dismutase [SOD], Catalase [CAT], glutathione peroxidase [GPO], glutathione reductase are intracellular antioxidants. Within biological systems, a number of small molecular weight antioxidants are capable of acting as repair or sacrificial antioxidants because they are chain breaking antioxidants which are extracellular antioxidants e.g., uric acid, bilirubin, transferrin, albumin, heptaglobin, tocopherol, ascorbic acid, glucose etc.

The MTT Cell Proliferation Assay measures the cell proliferation rate conversely, when metabolic events lead to apoptosis or necrosis, the reduction occurs in cell viability. For each cell type the linear relationship between cell number and signal produced is established, thus allowing an accurate quantification of changes in the rate of cell proliferation.

The present study emphasises on the antioxidant potentials of the three medicinal plants *Syzygium tamilnadensis*, *Syzygium densiflorum* and *Eugenia candolleana*. The Hydrogen Peroxide scavenging activity, Nitric oxide radical inhibition and Superoxide scavenging activity of the three plants were determined. The free radical scavenging activity was performed using the

. S.B.NasrinFathima, Department of Plant Biology and Biotechnology, Presidency College, Chennai, Tamil nadu, India.

. Dr. R. Pandian, The Principal, Government Arts and Science College, Kovilpatti, Tuticorin district, Tamil nadu, India

1,1-diphenyl-2-picryl hydrazyl (DPPH) and Ferric reducing antioxidant power (FRAP).

The research was also extended to study the anticancer activity of the extracts using the MTT assay against the breast cancer cell line MCF-7 cell line.

## 2. MATERIALS AND METHODS

### 2.1 Plant Collection and Identification

The three medicinal plants *Syzygium tamilnadensis*, *Syzygium densiflorum* and *Eugenia candolleana*, were collected from the Wayanad district of Kerala. The leaves of the three medicinal plants were shade dried, powdered and subjected to sequential extraction using Hexane, Ethyl acetate, Methanol solvents respectively. Among all the three solvents used ethyl acetate and methanol showed the best results for phytochemical analysis and antioxidant assays were carried out for ethyl acetate and methanol extracts.

### 2.2. Hydrogen Peroxide scavenging assay

The scavenging ability of hydrogen peroxide for the three medicinal plant extracts was determined according to the method of (Ruch *et al.*, 1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically at the absorption of 230 nm (8500 IU, Bio-Crom GmbH, Zurich, Switzerland). Extracts (200–1000 µg) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). Phosphate buffer without hydrogen peroxide was determined at an absorbance of 230 nm after ten minute against a blank solution. The scavenging percentage of hydrogen peroxide for all the three extracts and standard was calculated using the following equation:

$$\% \text{ of Inhibition} = (A \text{ of control} - A \text{ of Test}) / A \text{ of control} * 100$$

### 2.3. Nitric oxide radical inhibition assay

Sodium nitroprusside in an aqueous solution which spontaneously generates nitric oxide, it interacts with oxygen to produce nitrite ions, which can be estimated by the use of GriessIllosvoy reaction (Garratt, 1964). In the present investigation, GriessIllosvoy reagent was modified using naphthylethylenediaminedihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and different concentration of extract (200–1000 µg/ml) or standard solution (0.5 ml) were incubated at 25 °C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylenediaminedihydrochloride (1%) was added, mixed and allowed to stand for 30 min. A pink colored chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against the blank. Vitamin C was used as positive control.

### 2.4. Superoxide scavenging activity

Superoxide scavenging activities of ethyl acetate and methanol extracts were determined by monitoring the competition of those with NBT for the superoxide anion generated by the PMS–NADH system (Liu, Ooi, and Chang, 1997). Superoxide radicals were generated in 1 ml of 20 mM Tris–HCl buffer pH 8.0 containing 0.05 mM nitrobluetetrazolium (NBT), 0.01 mM phenazinemethosulphate (PMS) and different concentration of extracts (200–1000 µg/ml) were pre incubated for 2 min. The reaction was initiated by the addition of 0.078 mM NADH. Blue chromogen, formed due to NBT reduction and was read at 560 nm. Results were expressed according to the percentage of inhibition in superoxide radicals. Vitamin C was used as a positive control.

### 2.5. Total Antioxidant activity- FRAP assay

Ferric reducing antioxidant power (FRAP) is a modified method of (Benzie and Strain, 1996) was adopted for the FRAP assay. The different concentration of standard or sample extract (200 - 1000µg) was mixed with 300 µl of ferric–TPTZ reagent [prepared by mixing 300 mM acetate buffer, Ph 3.6, 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl<sub>3</sub>·6H<sub>2</sub>O at a ratio of 10:1:1 (v/v/v)]. The mixture was incubated at 37 °C, and the absorbance readings were taken at 593 nm after 4 min. Results were expressed in mM Fe(II)/g dry mass using a calibration curve of a freshly prepared ferrous sulfate solution. They were calculated using:

$$\frac{[A \text{ (Sample Final)} - A \text{ (Sample Initial)}] / A \text{ (Std Final)} - A \text{ (Std initial)}}{(A - \text{Absorbance}; \text{Std - Standard})} \times 2$$

### 2.6. DPPH free radical scavenging assay

The ability of the extracts to annihilate the DPPH radical (1, 1-diphenil-2-picrylhydrazyl) was investigated by the method described by (Blois, 1958). Stock solution of leaf extracts was prepared to the concentration of 1mg/ml. 100µg of each extracts were added, at an equal volume, to methanolic solution of DPPH (0.1mM). The reaction mixture is incubated for 30min at room temperature; the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic Acid was used as standard controls. The annihilation activity of free radicals was calculated using:

$$\% \text{ of Inhibition} = (A \text{ of control} - A \text{ of Test}) / A \text{ of control} * 100$$

### 2.7. MTT assay for cell viability

The MTT assay (Mossman, 1983) is based on the ability of live cell but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product. Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at 37°C in humidified atmosphere with 5% CO<sub>2</sub>. The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2 X 10<sup>4</sup> cells/well and allowed to attach overnight at 37°C. The medium was then discarded and cells were incubated with different concentrations of the extracts (100, 200, 300µg) for 24 hours. After the incubation, medium was discarded and 100µl fresh medium was added with 10µl of MTT (5mg/ml). After 4 hours, the medium was discarded and 100µl of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570nm

in a microtitre plate reader. Cyclophosphamide was used as a positive control. Cell survival was calculated by the following formula:

$$\text{Viability \%} = (\text{Test OD} / \text{Control OD}) \times 100$$

$$\text{Cytotoxicity \%} = 100 - \text{Viability\%}$$

### 3. RESULTS AND DISCUSSION

#### 3.1. Hydrogen peroxide scavenging activity

Hydrogen peroxide is a weak oxidizing non reactive agent and has an ability to cross biological membranes. Because of the involvement of hydrogen peroxide in the generation of hydroxyl radicals, this property of hydrogen peroxide plays a prominent role in initiating cytotoxicity. Thus removing H<sub>2</sub>O<sub>2</sub> is very important for the protection of living systems (Van Wijk *et al.*, 2008). According to (Serhat, K *et al*) ethanol and water extracts were prepared from powdered *Crataegus monogyna* flowers, leaves and fruits. BHA and  $\alpha$  tocopherol were used as standard. 100  $\mu$ g of water and ethanol extracts of *C. monogyna* exhibited 15.44-30.13% scavenging activity on hydrogen peroxide. On the other hand, using the same amounts, BHA and  $\alpha$ - tocopherol exhibited 44.58% and 39.26% hydrogen peroxide scavenging activity. His work clearly explains that *C. monogyna* extracts had more total antioxidant activity than  $\alpha$ -tocopherol at the same concentration (100 $\mu$ g/mL). Water and ethanol extracts of the leaf *Crataegus monogyna* showed maximum H<sub>2</sub>O<sub>2</sub> scavenging activity compared to the flower and fruit of *Crataegus monogyna*.

According to the author (O.E. Ogunlana *et al.*, 2008) carried out his experiment with Crude Methanolic Stem Bark Extract of *Newbouldia Laevis* with different concentrations (0.0109, 1.0217, 0.0326, 0.0435 and 0.0543 mg/ml) for scavenging H<sub>2</sub>O<sub>2</sub> and BHA is a standard control, in which H<sub>2</sub>O<sub>2</sub> was found to be more effectively than BHA, at 0.0217mg/ml, but at 0.0435mg/ml, plant extract appeared to be about 37.5% more efficient than BHA at scavenging hydrogen peroxide.

The scavenging of H<sub>2</sub>O<sub>2</sub> by the extracts was increased in dose dependent manner. The tabulated (Table 1, Figure 1) results of our study indicates that the methanol extract of *Syzygium tamilnadensis* shows maximum activity of 36.02% in 1000  $\mu$ g /ml, whereas ascorbic acid at the same concentration exhibited 95.75% inhibition.

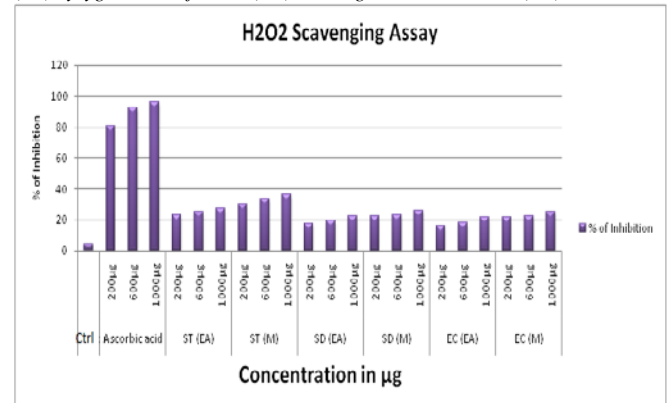
The ethyl acetate extract of *Syzygium tamilnadensis* shows an activity of 27.44%. The methanol and ethyl acetate extracts of *Syzygium densiflorum* revealed the inhibition of 22.43% and 25.64%. Similarly the methanol and ethyl acetate extracts of *Eugenia candolleana* showed an inhibition of 21.74% and 24.68%. For all the three plants, the inhibition was found to be at 1000  $\mu$ g /ml.

**Table 1:** Hydrogen Peroxide scavenging activity of *Syzygium tamilnadensis* (ST) *Syzygium densiflorum* (SD) and *Eugenia candolleana* (EC)

Concentration ( $\mu$ g)	H <sub>2</sub> O <sub>2</sub> Scavenging Assay (ST)							
	Control	Ascorbic acid	ST		SD		EC	
			EA	M	EA	M	EA	M
200.00	3.87	80.30	22.74	30.13	17.63	22.42	16.32	21.55
600.00	3.87	92.19	25.08	33.55	19.54	23.07	18.25	22.32
1000.00	3.87	95.76	27.45	36.02	22.44	25.64	21.75	24.69

(EA-Ethyl acetate, M-Methanol)

**Figure 1:** Hydrogen Peroxide scavenging activity of *Syzygium tamilnadensis* (ST) *Syzygium densiflorum* (SD) and *Eugenia candolleana* (EC)



(Ctrl-Control)

#### 3.2. Nitric oxide scavenging activity

Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, and inhibition of platelet aggregation and regulation of cell mediated toxicity (Hagerman *et al.*, 1998). In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions (Nabavi *et al.*, 2008). According to (Nishaa.S, *et al.*, 2012) the ethanolic extract of *Maranta arundinacea* was treated with various concentrations ranging from (100-500  $\mu$ g/ml). The ethanol extract of *M.arundinacea* showed maximum activity of 62.96% at 500 $\mu$ g/ml for NO, whereas BHT which is used as a standard, also exhibited 87% inhibition at the same concentration. The IC<sub>50</sub> values were found to be 248.4 and 336.1 for BHT and the extract respectively the scavenging of NO by the extracts was increased in dose dependent manner.

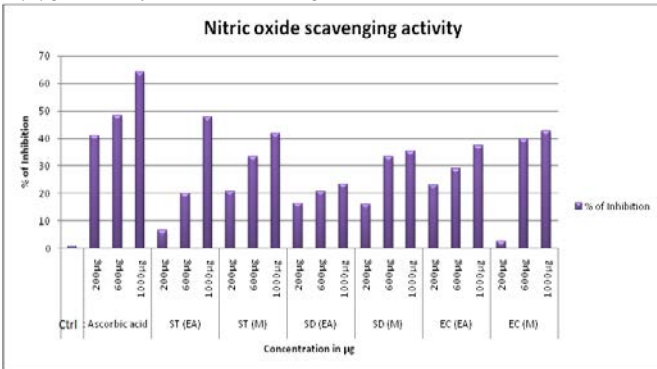
The tabulated results for (Table 2, Figure 2) the ethyl acetate extract of *Syzygium tamilnadensis* showed maximum activity of 47.74% 1000  $\mu$ g /ml, where as ascorbic acid at the same concentration exhibited 90.16% inhibition. The methanol extracts of *Syzygium tamilnadensis* showed an activity of 41.74%. The ethyl acetate and methanol extracts of *Syzygium densiflorum* exhibited an activity of 23.28% and 35.32%. The ethyl acetate and methanol extracts of *Eugenia candolleana* showed 37.19% and 42.62% activity, whereas at the same concentration ascorbic acid showed an activity of 64.10% at 1000  $\mu$ g /ml

**Table 2:** NO<sub>2</sub> Scavenging activity *Syzygium tamilnadensis* (ST), *Syzygium densiflorum* (SD) and *Eugenia candolleana* (EC).

Concentration (µg)	NO <sub>2</sub> Scavenging Assay (ST)							
	Control	Ascorbic acid	ST		SD		EC	
			EA	M	EA	M	EA	M
200.00	0.6853	40.901	6.508	20.73	16.31	9.951	23.11	2.65
600.00	0.6853	48.343	20.02	33.44	20.75	16.16	28.84	39.71
1000.00	0.6853	64.103	47.74	41.74	23.28	35.32	37.19	42.61

(EA-Ethyl acetate, M-Methanol)

**Figure 2:** NO<sub>2</sub> Scavenging activity of *Syzygium tamilnadensis* (ST), *Syzygium densiflorum* (SD) and *Eugenia candolleana* (EC)



(Ctrl-Control)

### 3.3. Superoxide scavenging activity

Superoxide is generated *in vivo* by several oxidative enzymes, including xanthine oxidase. In the PMS-NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT (Ilhami Gulcin *et al.*, 2005). (O.E. Ogunlana *et al.*, 2008) carried out his experiment with Crude Methanolic Stem Bark Extract of *Newbouldia Laevis* use different concentrations (0.0109, 1.0217, 0.0326, 0.0435 and 0.0543 mg/ml) explains that even at the lowest of the concentrations used for Superoxide anion scavenging data shows (0.05mg/ml), plant extract scavenged 81.48% of superoxide anion generated in the system while BHA only showed 5.82% of same, a display of a scavenging efficiency of 1300% over BHA.

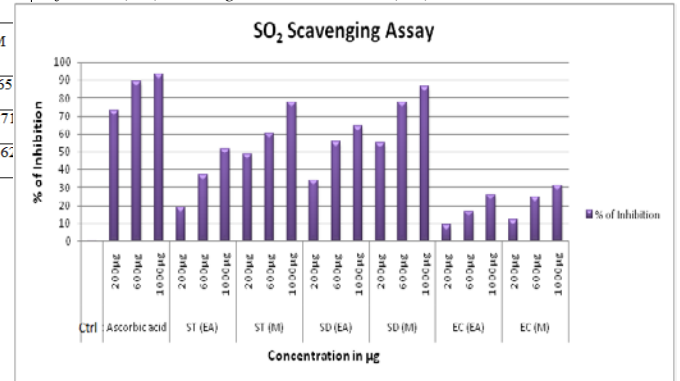
The maximum inhibition in our study was found to be 86.08% for methanolic and 63.80 % for ethyl acetate extracts of *Syzygium densiflorum* at 1000 µg /ml. The ethyl acetate and methanol extracts of *Syzygium tamilnadensis* exhibited an inhibition of 51.04% and 77.5%. Whereas the ethyl acetate and methanol extracts *Eugenia candolleana* has inhibition of 25.46% and 30.64% respectively (Table 3, Figure 3).

**Table 3:** SO<sub>2</sub> Scavenging activity of *Syzygium tamilnadensis* (ST), *Syzygium densiflorum* (SD) and *Eugenia candolleana* (EC).

Concentration (µg)	SO <sub>2</sub> Scavenging Assay (ST)							
	Control	Ascorbic acid	ST		SD		EC	
			EA	M	EA	M	EA	M
200.00	0.192	72.916	18.75	48.489	34.010	54.479	8.677	11.614
600.00	0.192	89.062	36.458	60.156	55.520	77.552	16.593	24.270
1000.00	0.192	93.229	51.041	77.5	63.802	86.083	25.468	30.640

(EA-Ethyl acetate, M-Methanol)

**Figure 3:** SO<sub>2</sub> Scavenging activity of *syzygium tamilnadensis* (ST), *Syzygium densiflorum* (SD) and *Eugenia candolleana* (EC)



(Ctrl-Control)

### D. Antioxidant Activity by FRAP

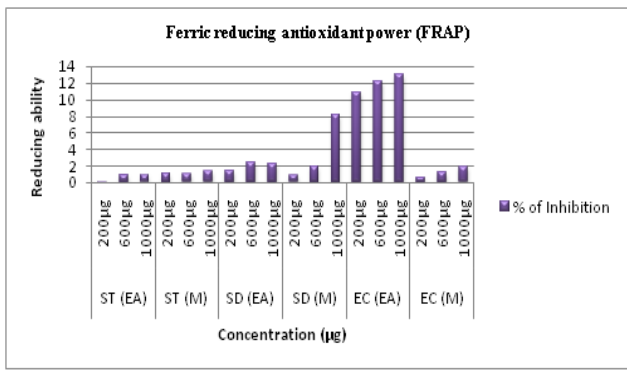
FRAP (Ferric Reducing Ability of Plasma) is one of the most rapid test and very useful for routine analysis. The antioxidative activity is estimated by measuring the increase in absorbance caused by the formation of ferrous ions from FRAP reagent containing TPTZ (2, 4, 6- tri (2 - pyridyl)-s-triazine) and FeCl<sub>3</sub>6H<sub>2</sub>O. The absorbance is measured spectrophotometrically at 593 nm (Netzel *et al.*, 2007). The higher the FRAP value the greater is the antioxidant activity. (Kandhasamy Sowndhararajan, *et al.*, 2012) determine phenolic content and antioxidant activities of chloroform, acetone, methanol and hot water extracts of leaves. The ferric reducing ability of the extracts revealed that all of them gave well FRAP activity (734.4–3257.8 mmol Fe (II)/g extract). Among the extracts, the highest activity was noted for methanol extract (3257.4 mmol Fe (II)/g extract). As well as the ethyl acetate extract of *Eugenia candolleana* and methanol extract of *Syzygium densiflorum* showed highest antioxidant activity with the percentage inhibition of 12.96% and 8.18% as shown in the table (Table 4, Figure 4). The ethyl acetate extract of *Syzygium densiflorum* showed 2.23% of antioxidant activity whereas the methanol extract of *Eugenia candolleana* showed 1.85% activity. The ethyl acetate and methanol extracts of *Syzygium tamilnadensis* possess 0.85% and 1.37% activity.

**Table 4:** Ferric reducing antioxidant power of *Syzygium tamilnadensis* (ST), *Syzygium densiflorum* (SD) and *Eugenia candolleana* (EC)

Concentration (µg)	Ferric reducing antioxidant power (FRAP)					
	ST		SD		EC	
	EA	M	EA	M	EA	M
200.00	0.054	1.089	1.421	0.835	10.820	0.631
600.00	0.914	1.002	2.408	1.911	12.189	1.271
1000.00	0.858	1.378	2.238	8.189	12.968	1.855

(EA-Ethyl acetate, M-Methanol)

**Figure 4:** Ferric reducing antioxidant power of *Syzygium tamilnadensis* (ST), *Syzygium densiflorum* (SD) and *Eugenia candolleana* (EC)



**E. DPPH radical scavenging assay**

Antioxidants are considered vital nutraceuticals on account of many health benefits (Valko *et al.*, 2007). Scavenging of DPPH radical is the basis of the popular DPPH antioxidant assays (Alma *et al.*, 2003). According to the author (Rajamanikandan, S *et al.*, 2011) the DPPH radical scavenging activity of ethanolic extract of *Mollugo nudicaulis* was compared with BHT. It was observed that the plant extract had higher scavenging activity. At a concentration of 2.5 mg/ml, the scavenging activity of ethanolic extract of

*Mollugo nudicaulis* reached 74.96 %, while at the same concentration, the standard was 87 %.

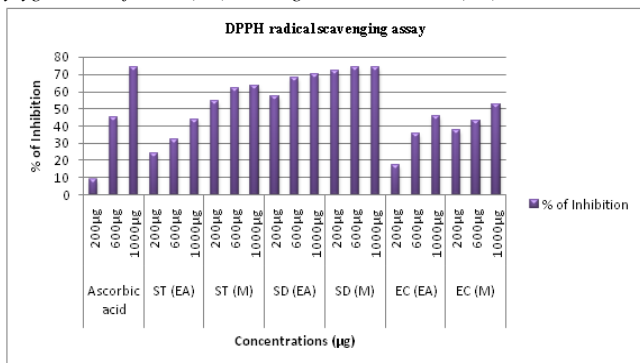
The maximum DPPH scavenging activity was exhibited by the methanol and ethyl acetate extracts of *Syzygium densiflorum* with the percentage of inhibition 74.24% and 69.75%. The methanol and ethyl acetate extracts of *Syzygium tamilnadensis* showed scavenging activity of 63.17% and 43.59%. The methanol and ethyl acetate extracts of *Eugenia candolleana* has 52.73% and 45.89% respectively (Table 5, Figure 5).

**Table-5:** DPPH radical scavenging assay of *Syzygium taminadensis* (ST) *Syzygium densiflorum* (SD) and *Eugenia candolleana* (EC).

Concentration (µg)	DPPH radical scavenging assay						
	Ascorbic acid	ST		SD		EC	
		EA	M	EA	M	EA	M
200.00	9.06788	24.3921	54.7872	56.9909	72.2644	17.6039	37.766
600.00	44.7822	31.9402	61.8288	67.6292	74.1135	35.7903	43.3637
1000.00	74.1641	43.5917	63.1712	69.7568	74.2401	45.8967	52.7356

(EA-Ethyl acetate, M-Methanol)

**Figure 5:** DPPH radical scavenging of *Syzygium tamilnadensis* (ST) *Syzygium densiflorum* (SD) and *Eugenia candolleana* (EC)



**F. Cytotoxicity Screening - MTT assay**

Percent cell viability was assessed using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay as described (Siddiqui *et al.*, 2008). The cytotoxicity of the *Eugenia candolleana*, *Syzygium densiflorum* and *Syzygium tamilnadensis* extracts on human breast cancer cell lines, MCF 7 were analysed. MTT assay as described is a technique of analyzing the cytotoxicity of substances based on the conversion of the yellow MTT reagent into purple insoluble formazon crystals, by the enzymatic action of mitochondrial dehydrogenase in live cells. Cells were treated with 100µg, 200µg and 300µg of the extracts. The results of the assay on the cancer cell lines showed dose dependent increase in cytotoxicity of the extracts on these cells, suggesting the anticancer activity of the extracts (Table 6, Figure 6). The methanol and ethyl acetate extracts of *Syzygium tamilnadensis* has the maximum cytotoxicity of 53.43% and 49.6%. The ethyl acetate and methanol extracts of *Eugenia candolleana* has cytotoxicity of 43.4% and 38.67%, whereas the ethyl acetate and methanol extracts of *Syzygium densiflorum* has cytotoxicity of 43.06% and 33.61% respectively.

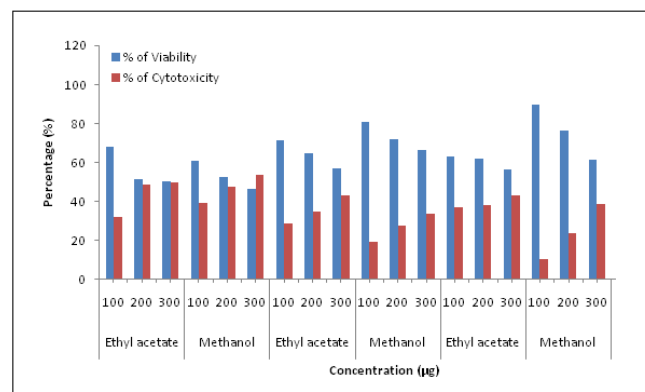
**Table-6:** Percentage of cell viability and cytotoxicity of compounds.

Test	Treatment (µg)											
	ST						SD					
	Ethyl acetate			Methanol			Ethyl acetate			Methanol		
	100	200	300	100	200	300	100	200	300	100	200	300
% of Viability	68.08	51.59	50.39	60.84	52.64	46.56	71.34	64.95	56.93	80.56	72.14	66.38
%of Cytotoxicity	31.91	48.40	49.60	39.15	47.35	53.43	28.65	35.04	43.06	19.43	27.85	33.61

Test	Treatment (µg)								
	EC						PC	C	
	Ethyl acetate			Methanol					
	100	200	300	100	200	300	73.34	100	
% of Viability	63.01	61.72	56.59	89.71	76.09	61.32	73.34	100	
% of Cytotoxicity	36.98	38.27	43.40	10.28	23.90	38.67	26.65	0	

(PC- Positive control - Cyclophosphamide, C- Control)

**Figure 6:** Effect of cell viability and cytotoxicity of extracts in MCF-7 Cancer cells



(PC- Positive control - Cyclophosphamide, C- Control)

**IV. CONCLUSION**

Cancer can be treated with antioxidant compounds derived from plant sources. They can be especially used in the treatment of breast cancer. To evaluate the antioxidant and cytotoxic potential the ethyl acetate and methanol extracts of

*Syzygium tamilnadensis*, *Syzygium densiflorum* and *Eugenia candolleana* were investigated for the antioxidant activity by Hydrogen peroxide scavenging, Nitric oxide scavenging, Superoxide scavenging, FRAP and DPPH. The cytotoxicity of the extracts against the breast cancer cell line MCF-7 was also performed. The results indicate that the methanol extract of *Syzygium tamilnadensis* has the highest Hydrogen peroxide scavenging potential whereas the Nitric oxide scavenging is maximum in the ethyl acetate extract of *Syzygium tamilnadensis*. The Superoxide scavenging is highly possessed by the Methanol extracts of *Syzygium densiflorum* and *Syzygium tamilnadensis*. The antioxidant activity by FRAP revealed that the ethyl acetate extract of *Eugenia candolleana* has good antioxidant activity whereas the methanol extract of *Syzygium densiflorum* showed good scavenging of the DPPH. The result of the antioxidant activity reveals that the extracts of *Syzygium tamilnadensis* have more antioxidant potentials when compared to *Eugenia candolleana* and *Syzygium densiflorum*. The MTT assay performed to predict the cytotoxicity activity of the extracts revealed that the methanol extract of *Syzygium tamilnadensis* has a potential cytotoxic effect.

The results of the MTT assay indicates that the antioxidant activity of the plant extracts have a greater impact on the anti cancer potential. The study reveals that the extract of the plant *Syzygium tamilnadensis* can be developed as antioxidant and anticancer agents.

## V. REFERENCES

- [1] Alma, M.H, Mavi, Yildirim, A, Digrak, M, and Hirata, T. (2003). Screening chemical composition and *in vitro* Antioxidant and Antimicrobial activities of the essential oils from *Origanum syriacum* L. growing in Turkey. *Biological and Pharmaceutical Bulletin*, (26): 1725–1729.
- [2] Benzie, I.E.F, and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Anal Biochem*. (239):70–76.
- [3] Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical, *Nature*, (29): 1199-1200.
- [4] Cheeseman, K.H, and Slater, T.F. (1993). An introduction to free radicals chemistry. *Br Med Bull*.(49): 481–93.
- [5] Devasagayam, T.P.A, and Kesavan, P.C. (2003). Radio protective and Antioxidant action of caffeine: mechanistic considerations, *Indj exp boil*. (41): 267 – 269,
- [6] Evans P, and Halliwall B, (1999). “Free radicals and hearing” *Ann N Y Acad Sci*, 884- 19.
- [7] Garratt, D. C. (1964). The quantitative analysis of drugs, Japan: Chapman and Hall Ltd., (3): 456–458.
- [8] Hagerman, A.E, Riedl, K.M, Jones, G.A, Sovik, K.N, Ritchard, N.T, and Hartzfeld, P.W.(1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. *J Agric and Food Chem*. (46): 1887-1892.
- [9] Halliwell, B. (1994). Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet*. (344): 721-724.
- [10] Ilhami Gulcin, Haci Ahmet Alici, and Mehmet Cesur. (2005) Determination of *in vitro* Antioxidant and Radical Scavenging activities of Propofol. *Chem Pharm Bull*. (53): 281 – 85.
- [11] Kandhasamy Sowndhararajan and Sun Chul Kang, (2012). Free radical scavenging activity from different extracts of leaves of *Bauhinia vahlii* Wight & Arn. *Saudi Journal of Biological Sciences*. (20): 319–325.
- [12] Lea, A.J. (1966). Dietary factors associated with death rates from certain neoplasms in man. *Lancet* (2) :332–3.
- [13] Liu, F, Ooi, V. E. C., and Chang, S. T. (1997). Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sciences*. (60): 763–771.
- [14] Nabavi, S.M, Ebrahimzadeh, M.A, Nabavi, S.F, Hamidinia, A, and Bekhradnia, A.R. (2008). Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica* Mey. *Pharmacologyonline*. (2): 560-567.
- [15] Netzel, M, and Netzel, G. (2007). Native Australian fruits-A novel source of antioxidants for food. *Innovative Food Science and Emerging Technologies*. (8) : 339 – 346.
- [16] Ogunlana, O.E, Olubanke Ogunlana, and Farombi, O.E (2008). Assessment of the Scavenging Activity of Crude Methanolic Stem Bark Extract of *Newbouldia Laevis* on Selected Free Radicals *Advances in Natural and Applied Sciences*, 2(3): 249-254.
- [17] Pourmorad, F, Hosseinimehr, S.J, and Shahabimajd, N. (2006). Antioxidant activity, phenols, flavanoid contents of selected Iranian medicinal plants. *S. Afr. J. Biotechnol*. (5): 1142-1145.
- [18] Rajamanikandan, S , Sindhu, T , Durgapriya, D , Sophia, D , Ragavendran, P and Gopalakrishnan, V.K. (2011). Radical Scavenging and Antioxidant Activity of Ethanolic Extract of *Mollugo nudicaulis* by *Invitro* Assays. *Indian Journal of Pharmaceutical Education and Research*, 4(45):310-316.
- [19] Ruch, R.J, Cheng, S.J, and Klaunig, J.F. (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechin isolated from Chinese green tea. *Carcinogenesis* (10): 1003–1008.
- [20] Serhat Keser, Sait Celik, Semra Turkoglu, Ökkes Yilmaz and Ismail Turkoglu. (2012). Hydrogen Peroxide Radical Scavenging and Total Antioxidant Activity of Hawthorn. *Chemistry Journal* (02):9-12

[21] Siddiqui, M.A, Singh, G, and Kashyap, M.P. (2008). Influence of cytotoxic doses of 4-hydroxynonenal on selected neurotransmitter receptors in PC-12 cells. *Toxicol. In Vitro.*(22): 1681-88.

[22] Valko, M., Leibfritz, D, Moncol, J, Cronin, M, Mazur, M, and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology.* (39): 44-84.

[23] Van Wijk, R, Van Wijk, E.P, Wiegant, F.A, and Ives, J.(2008) Free radicals and low-level photon emission in human pathogenesis: State of the art. *Indian J Exp Biol.* (46) :273-309.

[24] Young, I.S, Woodside, J.V. (2001). Antioxidants in health and disease. *J. Clin. Pathol.* (54): 176-186.

[25] Zhang, H, Jiang, L, Ye, S, Ye, Y, and Ren, F. (2010). Systematic evaluation of antioxidant capacities of the ethanolic extract of different tissues of jujube (*Ziziphus jujuba* Mill.) from China. *Food Chem Toxicol.* (48): 1461-1465.

IJSER