ISSN: 0976-3031

Available Online at http://www.recentscientific.com

*International Journal of Recent Scientific Research*
*Vol. 7, Issue, 3, pp. 9451-9456, March, 2016*

*International Journal of Recent Scientific Research*

## RESEARCH ARTICLE

# ANTIOXIDANT AND ANTICANCER ACTIVITIES OF (+)-CATECHIN ISOLATED FROM *ANNONA RETICULATA LINN*

**Rayar A* and Manivannan R**

Department of Chemistry, Government Arts College (Autonomous), Kumbakonam, Tamilnadu, India 612 001

**ARTICLE INFO**

***Article History:***

Received 06th December, 2015
Received in revised form 14th January, 2016
Accepted 23rd February, 2016
Published online 28th March, 2016

***Keywords:***

Annona reticulata Linn; Anticancer; Antioxidant; Catechin; Human Breast cancer.

**ABSTRACT**

Biologically important polyphenol, (+)-catechin was isolated from *Annona reticulata Linn* fruit. The isolated (+)-Catechin was characterized by UV, IR, $^{1}$H- NMR $^{13}$C- NMR and evaluation of its antioxidant and anti-cancer activities.

High intake of fruits and vegetables has been correlated with a reduced risk of contracting different illnesses including cancer, diabetes, and cardiovascular diseases. This beneficial effect seems to be caused by phytochemicals with antioxidant properties as polyphenols, which have been proposed to be capable of controlling the oxidative stress caused by the overproduction of free radicals that can damage biomolecules such as carbohydrates, proteins, DNA and lipids. The (+) - Catechin showed significantly higher inhibition percentage at concentration 1000 µg/ml (62.42 ± 0.045) than MAR (45.55 ± 0.041) and positively correlated with total phenolic content.

Cancer is a class of diseases in which group of cells division beyond the normal limits Invasion and metastasis. The profile of cell growth after treated with (+)-Catechin and MAR are found that both (+)-Catechin and MAR showed a significant reduction in the number of viable cells at the concentration higher than 1000 treatment with (+)-Catechin (87.40±1.2**)** was more than MAR (66.56±0.2) against MCF-7 cell lines.



## INTRODUCTION

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Natural remedies from medicinal plants are found to be safe and effective. Many plant species have been used in folkloric medicine to treat various ailments. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries. Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties Zaman, *et al* (2014). In the last few years food consumption has acquired an importance based not only in nutrients which are essential for life, but also in other bioactive compounds that promote health. High intake of fruits and vegetables has been correlated with a reduced risk of contracting different illnesses including cancer, diabetes, and cardiovascular diseases. This beneficial effect seems to be caused by phytochemicals with antioxidant properties as polyphenols, which have been proposed to be capable of controlling the oxidative stress caused by the overproduction of free radicals that can damage biomolecules such as carbohydrates, proteins, DNA and lipids Estrogen and cancer website, 2006. Custard apple, *(Annona reticulata Linn*.) also known as Seetaphal in India, is a subtropical fruit belonging to the *Annonaceae* family. The fruit grows on a small deciduous tree and is known by different names worldwide. The fruit is heart-shaped or spherical and very aromatic to hard with a repulsive taste. The flavor of the fruit is sweet and pleasant. The skin of the fruit is thin and tough, mostly black and green in colour. The fruit is native to West Indies, Central America, Peru and Mexico. Custard apple contains high amounts of vitamin A and C, which is very beneficial for maintaining the skin, eyes and hair of the fetus Jamkhande. at al, (2013).This vitamin is also renowned for its innate anti-inflammatory and immune boosting properties. Include just one serving of this creamy fruit in your daily diet for a better resistance against infectious agents. It scavenges harmful free radicals in the body, preventing the onset of various illnesses and diseases. It is also high in potassium and magnesium that protects our heart from cardiac disease. Not

**Corresponding author:* **Rayar A**
Department of Chemistry, Government Arts College (Autonomous), Kumbakonam, Tamilnadu, India 612 001

only that, it also controls our blood pressure. It is a highly apparent plant in ayurvedic system of medicine for the treatment of various ailments. The plant is traditionally used for the treatment of epilepsy, dysentery, cardiac problems, worm infestation, constipation, haemorrhage, antibacterial infection, dysuria, fever, and ulcer Padam, at al (2013).

The bark of custard apple contains astringent properties and tannins, which is utilized for making herbal supplements. These supplements help in the treatment of several types of cancer and tumors. The fruit contains compounds like acetogenin and alkaloids that reduce the risk of cancer and renal failure. It acts against cancer cells, without adversely affecting healthy cells. Antioxidants such as asimicin and bullatacin are also found to have anti-cancer and anti-helminthes properties. These antioxidants neutralize the effects of free radicals, preventing cancer. It also contains significant fiber, which protects the colon membrane by warding off toxic substance from the gut, reducing the risk of liver and colon risk. It also provides protection from breast cancer. Among the human diseases treated with medicinal plants is cancer, which is probably the most important genetic disease. Every year, millions of people are diagnosed with cancer, leading to death in a majority of the cases Eva, at al, 2006. Cancer (medical term: malignant neoplasm) is a class of diseases in which group of cells division beyond the normal limits Invasion and metastasis. Every year, millions of people are diagnosed with cancer, leading to death. Several chemo preventive agents are used to treat cancer, but they cause toxicity that prevents their usage. Chemotherapy, being a major treatment modality used for the control of advanced stages of malignancies and as a prophylactic against possible metastasis, exhibits severe toxicity on normal tissues. Other cancer-promoting genetic abnormalities may be randomly acquired through errors in DNA replication, or are inherited Raju. at al (2007). Polyphenols have been considered as conducive for cancer prevention. Such phytochemicals can block the action of carcinogens on target tissues thereby suppressing cancer development. Experiments were carried out using different human cancer cell lines and mice induced to develop breast cancer to test and verify the efficacy of the Custard apple fruit extract in inhibiting cancer cell proliferation. The first breast cancer cell line to be established was BT-20 in 1958 [3] and what still remains the most commonly used breast cancer cell line in the world, MCF-7 established in 1973 at the Michigan Cancer Foundation.

Antioxidants are substances that inhibit or delay the oxidation processes. Therefore, they are able to protect the human body, foods and drugs from oxidative damages. It acts as free radical scavengers, reducing agents, quenchers of singlet oxygen molecule and activators for antioxidative enzyme to suppress the damage induced by free radicals in biological system. Free radicals play an important role in cell's life and death. It is normally balanced by endogenous antioxidant system. Imbalances in redox status may develop cellular oxidative stress. If the endogenous antioxidants fail to overcome the reactive metabolites production, then exogenous antioxidants would be necessary to balance redox status. Dietary sources, including plants, herbs, spices, vitamins and herbal extracts, play an important role in this regard. No reports are available on the isolation of the phytoconstituent and pharmacology studies (anticancer&antioxidant) of the fruit, hence, the present study was carried out to investigate the same Rayar. *et al*,(2015).

## MATERIALS AND METHODS

### *Plant materials*

The custard apples were collected from Aduthurai ,Tamil Nadu state, India. They were identified and authenticated by Dr. R.Stephan, Assistant Professor in Botany,and voucher specimens (Department of Botany) and voucher specimens (GACBOT-180) were deposited at the Herbarium of the Department of Botany, Government Arts College, Ariyalur, Bharathidasan University, India.

### *General Experimental Procedures*

UV spectral analyse was recorded using UV- Visible Spectrophotometer Lambda 35 from Perkin Elmer, UV(EtOH) $\lambda_{max}$. IR spectrum was recorded with a Perkin Elmer RXI FT-IR spectrometer as a thin film on KBr plate. IR spectra were recorded on a Perkin Elmer spectrum on spectrometer using KBr disc method. Supporting evidence for the structure of the compound is provided by the $^{1}H$ (DMSO, 300 MHz) and $^{13}C$- NMR (100 MHz, DMSO) spectra were recorded on a Bruker AMX 300 NMR spectrometer.

### *Extraction and Isolation*

The custard apples were sliced using a home slicer and the slices obtained were shade-dried, pulverized and passed through 20 mesh sieve and stored in airtight container at room temperature About 2500 gm of dried, coarsely powdered plant material was extracted with methanol using Soxhlet apparatus. The solvent recovered by distillation, evaporated under vacuum to give semisolid mass (25% w/w) which further dried and used for isolation of phytochemicals. Gradient fractionation of acetone soluble part was performed by eluting the column with benzene:ethyl acetate:methanol (3:4:3) followed by methanol over silica gel (60-120 mesh), resulting from the first four fractions to give a compound. The separation of isolate was confirmed by spraying with ferric chloride ($R_f$ 0.74) Jennifer L. *et al*,(1999).

### *Identification of isolated compound*

The isolated compound (white powder) was identified as (+) – Catechin ($\mathbf{C_{15}H_{14}O_6}$) through UV ,FT -IR, $^{1}H$-NMR $^{13}C$-NMR spectroscopy and melting point. Finally recorded spectroscopic data and melting point of isolated compound was compared with previous Literatures to assign their identity.

### *Structural analysis of (+)-catechin*

***Melting point:*** A Reichart micro melting point apparatus was used for recording the melting points( 175-178$^{o}$C ). Care was taken to ensure that the heating was done steadily.

***UV spectra:*** UV spectra (λmax [EtOH]) showed maxima at 272nm and 222 nm

***IR spectra:*** IR $\upsilon_{max}$ (KBr) showed band at 2926-3091 (broad O-H), 1626(C=O), 1521, 1462, 1365, 1286(C-O-C), 1144(C-O), 1079, 1039, 968 and 865cm-[1].

***$^{13}$C- NMR*** (DMSO-$d_6$, 75 MHz): 156.40 (C7), 156.12 (C5), 155.30 (C3'), 144.79 (C4'), 130.54 (C8), 118.39 (C6), 115.03 (C6'), 114.46 (C9), 99.01 (C10), 95.05 (C5'),93.80 (C2'), 80.94 (C3), 66.26 (C2) and 27.80 (C4)

***$^{1}$H-NMR*** (DMSO-$d_6$, 300 MHz): d 9.20(s), 6.72 (1H, H6, 8, s), 6.58 (1H, H2',H5', s), 6.60 (1H, H6', s), 5.89 (s), 5.69 (1H, H5, H7, s), 4.89 (1H, H3', H4', s), 4.49 (1H, H3, s), 3.38 (1H, H3,s),3.38 (s), 2.67 (2H, H4, d) and 2.38 (s).

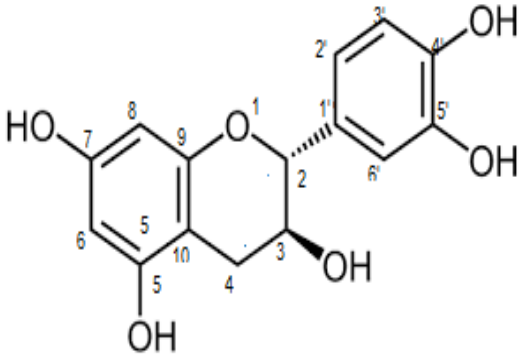


**Figure-1 Structure of *(+)-catechin***

***Evaluation of In Vitro Antioxidant Activity By DPPH Assay***

A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518nm and converted into percentage radical scavenging activity as follows.

$$Scavengingactivity(\%)=\frac{A_{518}\,\text{Control}-\,A_{518}\,\text{Sample}}{A_{518}\,\text{Control}}\times 100$$

Where $A_{518}$ control is the absorbance of DPPH radical with methanol; $A_{518}$ sample is the absorbance of DPPH radical with sample extract/ standard Mensor. *et al*, (2001).

**Table-2** DPPH radical scavenging activity of (+)- Catechin (CTN) and Methanolic extract of Annona reticulata Linn (EAR)

| S.No. | Concentration (µg/ml) | % of activity(Mean ±SEM) | | |
|---|---|---|---|---|
| | | Rutin | MAR | CTN |
| 1 | 125 | 20.65 ± 0.072 | 30.22 ± 0.046 | 35.87 ± 0.062 |
| 2 | 250 | 22.68 ± 0.054 | 33.24 ± 0.032 | 44.98 ± 0.026 |
| 3 | 500 | 52.22 ± 0.026 | 37.68 ± 0.025 | 54.56 ± 0.058 |
| 4 | 1000 | 68.84 ± 0.016 | 45.55 ± 0.041 | 62.42 ± 0.045 |
| 5 | **$IC_{50}$** | **480** | **1120** | **390** |

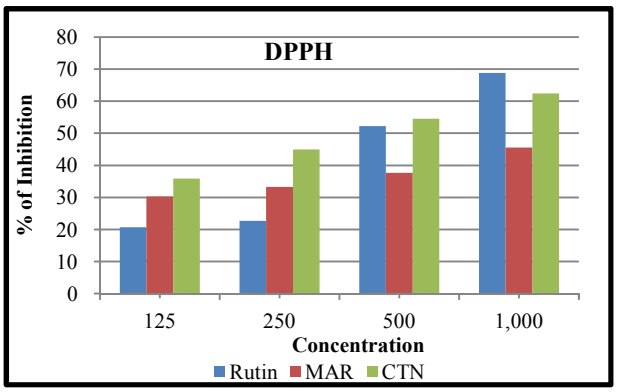


**Figure-2** DPPH radical scavenging activity of (+) - Catechin (CTN) and Methanolic extract of Annona reticulata Linn (EAR)

***Evaluation of In Vitro Anti-Cancer Activity By MTT Assay***

*In vitro* anti-cancer study of (+) –Catechin and the methanolic extract of Annona reticulata *Linn* fruit were assessed using 3-(4, 5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT) assay on Human Breast cancer (MCF-7) cell lines.

***MTT Assay Principle***

The MTT cell proliferation assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. The MTT assay is a colorimetric assay that measures the reduction of yellow MTT by metabolically active cells. The MTT enters the cells and passes into the mitochondria, where it is reduced to an insoluble, dark purple formazan product, which was then extracted using an organic solvent. The released formazan is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

***Cell Line and Culture Medium***

Human Breast cancer (MCF-7) cell lines was cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL) and amphotericin B (5 µg/mL) in atmosphere of 5% $CO_2$ at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 $cm^2$ culture flasks and all experiments were performed in 96 microtitre plates.

***Preparation of Test Solutions***

Plant extracts were diluted with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/mL concentration, which was sterilized by filtration and finally centrifuged. Serial dilutions (1000, 500, 250, 125, 62.5 µg / mL) were prepared.

***MTT Assay***

The monolayer cell culture was trypsinized and the cell count was adjusted to $1.0x10^5$ cells/mL using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 mL

of the diluted cell suspension (Approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µL of different concentrations of plant extracts were added. The plates were then incubated at 37°C for 3 days in 5% $CO_2$ atmosphere and microscopic examination was performed and observations were noted every 24 hours interval. After 72 hours, the drug solutions in the wells were discarded and 50 µL of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 hours at 37° C in 5% $CO_2$ atmosphere. The supernatant was removed and 100 µL of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader (ELx800, Bio-Tek) at a wavelength of 540 nm. The percentage growth inhibition (% GI) was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% ($CTC_{50}$) values is generated from the dose-response curves. The experiments were performed in triplicate Saravanan. *et al*, (2015).

$$\% \text{ GI} = 100 \quad \frac{\text{Mean optical density of individual test group}}{\text{Mean optical density of control group}} \text{ X } 100$$

**Table-2** *In vitro* anti-cancer efficacy test of (+)- Catechin (CTN) and Methanolic extract of Annona reticulata Linn (EAR) against Human Breast cancer (MCF-7) cell line

| Sample | Test conc (µg/mL) | % Cytotoxicity | $CTC_{50}$ (µg/mL) |
|---|---|---|---|
| MAR | 1000 | 66.56±0.2 | 782.67±5.8 |
| | 500 | 30.00±0.4 | |
| | 250 | 29.62±0.2 | |
| | 125 | 25.13±0.1 | |
| | 62.5 | 22.82±0.2 | |
| CTN | 1000 | 88.94±0.2 | 584.00±0.3 |
| | 500 | 79.20±0.3 | |
| | 250 | 26.04±0.2 | |
| | 125 | 22.69±0.3 | |
| | 62.5 | 20.00±0.4 | |

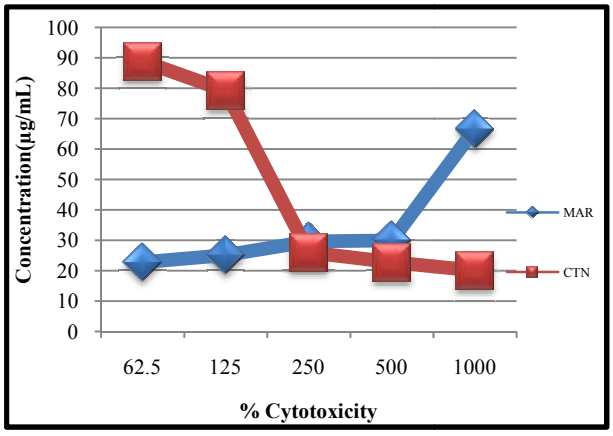


**Figure-3** *In vitro* anti-cancer efficacy test of (+)- Catechin (CTN) and Methanolic extract of Annona reticulata Linn (EAR) against Human Breast cancer (MCF-7) cell line

### *Calculation of 50% Inhibitory Concentration ($IC_{50}$)*

The concentration (mg/ml) of the fractions that was required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the fractions. Percentage of inhibition (%I) was calculated using the formula,

$$Scavengingactivity\,(\%) = \frac{A_{518}\,\text{Control} - A_{518}\,\text{Sample}}{A_{518}\,\text{Control}} \times 100$$

### *Statistical Analysis*

For *in-vitro* experiments values are represented by Mean ± SEM. The mean values are analyzed by one way analysis of varience (ANOVA) followed by Dunnett's Multiple comparisons test. The $p < 0.01$ was considered as statistically significant.

## RESULTS AND DISCUSSIONS

### *Structure of (+)-catechin*

Colomun chromatographic behaviour of the isolated (+)-catechin was in good agreement with the authentic (+)-catechin. The compound showed absorption bands at 220 and 277 nm in the ultraviolet. Anthocyanins give an absorption band at 275-280 nm regions. This ultraviolet behaviour indicated the possibility of the isolated compound belonging to the flavonoid group. In IR spectra of (+)-catechin, a very intensively broad band at 3091 $cm^{-1}$ and moderately intense band at 1365 $cm^{-1}$ were observed for the O-H bond vibrations which can be attributed to – OH

groups.The corresponding aromatic C=C vibrations was shown around 1521 $cm^{-1}$ as weakly intense band. The strcting and bending vibrations of methylene part were noticed by the intense band at 2926 $cm^{-1}$ and medium intensity band at 1365 $cm^{-1}$. The very weak band at 730 $cm^{-1}$ was attributed to the rocking movement of methylene part.The corresponding C-C vibrations was shown as weak intense band at 1039 $cm^{-1}$. Asymmetric band at 1626 $cm^{-1}$ observed may be due to C-O-C stretch for lactone and aromatic benzonoid ring. In the region 1521-1365 $cm^{-1}$ may be due aromatic C=C stretching. Other stretchings were comparable with IR spectra of authentic (+)-catechin. The $^{1}$H-NMR spectra of (+)-catechin was very clear and understandable. The observed signals in NMR spectra were in good agreement with the authentic (+)-catechin The $^{1}$H-NMR spectra of (+)-catechin showed that the signal at δ 9.20 due to aromatic phenolic groups.

The signals at δ 6.72,6.58,6.60,5.89 and 5.69 due to five different aromatic protons. (+)-Catechin molecule contains 1 secondary carbon, 7 tertiary carbons and 7 quaternary carbons. In the $^{13}$C-NMR spectra, signals appeared at δ 27.80, 66.26, 80.94, 93.80, 95.05,99.01 114.46, 115.03, 118.39 due to C4, C2, C3, C2́, C5́, C10, C9, C3', C6́ carbons respectively and others aromatic carbons showed peaks at δ 99.01, 130.54, 144.79, 155.30, 156.12 and 156.48. These data were also in good agreement with the authentic (+)-catechin Aher, *et al*, (2010).The data correlates the structure of the isolated compound to phenyl propanoid.

***Cell Growth Profile in MTT Assay***

MTT assay is a rapid and high accuracy colorimetric approach that widely used to determine cell growth and cell cytotoxicity, particularly in the development of new drug. It measures cell membrane integrity by determining mitochondrial activity through enzymatic reaction on the reduction of MTT to formazan. The MTT assay results for 24 hrs and 72 hrs incubation of Catechin and MAR at the concentrations 1000,500,250,125 and 62.5% showed increased Breast cancer (MCF-7) cell viability as the concentration got diluted. In the present study, the treatment with the Catechin suppressed the cell viability up to 62.5% at 250 µg/ml concentrations when compared to the untreated cells. The profile of cell growth after treated with (+)-Catechin and MAR are presented in Table-2 and Figure -3 respectively.

From the Table-2, it was found that both (+)-Catechin and MAR showed a significant reduction in the number of viable cells at the concentration higher than 1000 treatment with (+)-Catechin (87.40±1.2**)** was more than MAR (66.56±0.2) against MCF-7 cell lines. Therefore, the $CTC_{50}$ value were 584.00±0.3 and 782.67±5.8 µg/ml. The killing activity was specific toward tumor cells, as the compounds had no effect on primary cultures of healthy human cells. Cell death caused by the (+)-Catechin was via apoptosis. Varieties of chemical compounds have been reported to protect against chemical carcinogenesis and thus, are considered to be cancer chemo preventive agents. Among these, (+)-Catechin is promising physiochemical agent that have attracted interest due to its cancer chemo preventive activity in multistage carcinogenesis. In Figure-2 explained (+)-Catechin is more inhibited while increase the concentration than MAR Rayar. *et al*, (2015).

***DPPH radical scavenging activity***

The DPPH radical scavenging assay is an easy rapid and sensitive method for the antioxidant screening of plant extracts**.** A number of methods are available for the determination of free radical scavenging activity but the assay employing the stable 2, 2-diphenyl-1-picryl-hydrazylradical (DPPH) has received the maximum attention owing to its ease of use and its convenience. Most of the extracts and Polyphenols showed excellent free radical scavenging activity Wu. *et al*, (2013). Literature reports are evident that the reducing power of bioactive compound is associated with antioxidant activity. Thus a relation is evidenced between reducing power and the antioxidant effect. In the present study (+) - Catechin showed significantly higher inhibition percentage at concentration 1000 µg/ml (62.42 ± 0.045) than MAR (45.55 ± 0.041) and positively correlated with total phenolic content (Table-2 and Figure-3). Results of this study suggest that (+) - Catechin is capable of donating hydrogen to a free radical to scavenge the potential damage than MAR. The antioxidant activity remained practically unchanged (at the concentration 250 µg/mL), giving evidence that the fruit antioxidant potential depends on both, quantity and composition of phenolic compounds Frank. (2014). Based on phenolic composition, the custard apple may constitute a good source of antioxidant compounds.

## CONCLUSION

In conclusion this work describes for the first time both *in-vitro* antioxidant and *in vitro* anti-cancer activity of the methanolic extract and isolated constituent of Catechin from Pouteria sapota. The isolated Catechin was characterized by UV, IR, $^{1}$H- NMR and $^{13}$C- NMR. Large number of herbal species have been used traditionally or as folk medicines against cancer. Many of them have been studied scientifically and proved to be beneficial anti-cancer agents. (+) Catechin and MAR showed excellent free radical scavenging activity. Literature reports are evident that the reducing power of bioactive compounds is associated with antioxidant activity. Thus a relation is evidenced between reducing power and the antioxidant effect Boni. *et al*, (2014). People who eat fruits and vegetables have a lower risk of heart disease and some neurological diseases and there is evidence that some types of vegetables, and fruits in general, protect against some cancers .The antioxidant supplementation is widely used in attempts to prevent the development of cancer, it has been proposed that antioxidants may, paradoxically, interfere with cancer treatments. This was thought to occur since the environment of cancer cells causes high levels of oxidative stress, making these cells more susceptible to the further oxidative stress induced by treatments Yang. *et al*, (*2004*).

***Conflict of Interests***

The authors declare that they have no conflict of interests.

**Acknowledgements**

Authors are thankful to Assistant Professor Dr. S. Srrinivasan, Department of Chemistry, D.D.E. Annamalai University, Chidambaram, India for collecting spectroscopic data .

## References

1. Aher, A.N,. Pal, S.C,. Yadav, S.K., Patil, U.K. and Bhattacharya, S.(2010). Isolation and Characterization of Phytoconstituents from *Casuarina equisetifolia* (Casuarinaceae). *Asian Journal of Chemistry,* 22(5): 3429-3434.
2. Estrogen and cancer website, **2006**; www.womenshealth.com, www.amazon.com.
3. Eva, J.M., Ange,l G.L., Laura, P., Ignacio, A., Antonia, C and Federico G.(2006). A New Extract of the Plant Calendula Officinalis Produces a dual *In-Vitro* Effect: Cytotoxic Anti-Tumor Activity and Lymphocyte Activation. *BMC Cancer*, 6(1): 119.
4. Frank N. (2014). *In- vitro* Anti-oxidant Activity and Free Radical Scavenging Potential of roots of Malawian*Trichodesma zeylanicumm* (burm. f.). *Asian Journal* of *Biomedical* and *Pharmaceutical Sciences,* 3(20): 21-25.
5. Jamkhande, P.G and Wattamwar, A.S. (2015). Annona reticulata Linn. (Bullock's heart): Plant profile, phytochemistry and pharmacological properties. *Journal of Traditional and Complementary Mediciens,* 5(3):144-152.

6. Jennifer L. Donovan, Devanand L. Luthria, Phil Stremple, Andrew L. Waterhouse. (1999). Analysis of (1)-catechin, (2)-epicatechin and their 39- and 49-*O*-methylated analogs A comparison of sensitive methods. Journal of Chromatography B, 726 277–283.
7. Mensor, L.L., Meneze, F.S., Leitao, G.G., Reis, A.S., Dos santor, J.C., Coube, C.S and Leitao, S.G.(2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy Research, 15: 127-130.
8. Padam, P., Acharya., Giri, R., Genwali and Meena Rajbhandari . (2013).Isolation of catechin from *Acacia catechu* Willdenow Estimation of Total Flavonoid content in *Camellia Sinensis* Kuntze and *Camellia Sinensis* Kuntze Var. *Assamica* collected from different Geographical region and their Antioxidant activities. *Scienticfic World,* 11(11):32-36.
9. Raju, J and Bird, R.P. (2007). Diosgenin, a naturally occurring furostanol saponin suppresses 3-hydroxy-3-methylglutaryl CoA reductase expression and induces apoptosis in HCT-116 human colon carcinoma cells, 255 (2): 194-204.
10. Rayar, A and Manivannan, R.(2015). *In Vitro* Cytotoxicity Activity of Phytochemicals Isolated from *Coriandrum sativum Linn. IOSR Pharmacy and Biological Science, 10(3) :38-49.*
11. Rayar, A and Manivannan, R. (2015).Evaluation of In Vitro Antioxidant Potential of Ethanolic Extract and 4-*Tert*-Butylcyclohexyl acetate isolated from *Decalepis Hamiltonii* Wight and Arn seed. *World Journal of Pharmacy and Pharmaceutical Science*, 4(10):1649-1662.
12. Saravanan, S., Hairul Islam, V.I., Gabriel Paulraj, M and Ignacimuthu, S. ( 2011). Protective effect of Enicostemma axillere on mutagenicity of Salmonella typhimurium by increasing free radical scavenging abilities. *Pharmaceutical Biology, 50(6):698-706.*
13. Wu, T, McCallum, J.L., Wang, S., Liu, R., Zhu, H and Tsao, R.(2013).Evaluation of antioxidant activities and chemical characterization of staghorn sumac fruit (*Rhus hirta* L.).Food Chemistry, 138:1333-1340.
14. Yang, J., Mani, S.A., Donaher, J.L., Ramaswamy, S., Itzkson, R.A., Come, C., Savagner, P., Gitelman, I., Richardson, A., Weinberg, R.A. (2004). Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell, 117, 927-939.
15. Zaman, K and Pathak, K .(2013). Pharmacognostical and Phytochemical Studies on the Leaf and Stem Bark of Annona reticulata Linn. *International Journal of Research in Pharmaceutical and Biomedical Sciences,* (5):1-7.


*******

**How to cite this article:**

Rayar A and Manivannan R.2016, Antioxidant and Anticancer Activities of (+)-Catechin Isolsted From *Annona Reticulata Linn*. *Int J Recent Sci Res.* 7(3), pp. 9451-9456.