ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 7, Issue 2, pp: (195-200), Month: April - June 2019, Available at: www.researchpublish.com

ANTICANCEROUS AND ANTIOXIDANT ACTIVITY OF *Camellia Sinensis*"

S. Valli*[1], Divya[2]

[1]Assistant Professor, [2] Research Scholar

PG & Research Department of Microbiology, Mohamed Sathak College of Arts and Science, Chennai, Tamil Nadu, India

Abstract: **Green tea is currently an area of intense scientific research because of its effective actions in anticancer therapy. It is one of the most widely consumed beverages in the world and is currently perceived as a healthy drink now gaining worldwide popularity as a drink that is important in preventive medicine.It is derived from non-fermented leaves of the *Camellia sinensis* plant.. The aim of this research was to determine the anticancerous and antioxidant properties of green tea The antioxidant activity is measured by (1,1-diphenyl-2-picrylhydrazyl) assay method and FRAP (ferric reducing antioxidant power) assay method. The DPPH activity of the *Camellia sinensis* extract was found to be 22.53% at the concentration of 1000µg/ml. The FRAP activity of the *Camellia sinensis* extract is found to be 3520µM at the concentration of the standard is 1000µM and then FRAP value of absorbic acid is 2. HT 29 cell line was obtained from National Centre for Cell Sciences, Pune (NCCS). The highest concentration1000µg/ml of *Camellia sinensis* extract shows the minimum cell viability of 28%, the concentration of 7.8µg/ml shows the maximum cell viability of 77.41% and 100% cell viability by cell control.**

***Keywords: Camellia sinensis*- Antitumour –MTT assay FRAP-DPPH assay.**

1. INTRODUCTION

Tea has been extensively studied for its wide range of health benefits, including anti-diabetic anti-oxidant,anti-cancer and anti-microbial capabilities [1,2] Other benefits vary from enhanced metabolism leading to weight loss and reduction of total cholesterol but raise High Density Lipid (HDL). The leaves of the plant, *Camellia sinensis*, are processed in different ways to produce four different types of tea: white, green, oolong, and black These specific treatments produce tea leaves with varying chemical compositions and potential health benefits. Green tea has a high concentration of polyphenols. Specifically, it contains a large amount of catechins- compounds believed to be responsible for tea's effect in our biological activities

Green tea epigalocatechin galate (EGCG) has been shown to exhibit a growth-suppressive effect on human pancreatic cancer cells; however, the exact molecular mechanism by which EGCG suppresses cell proliferation is unclear. The effect of EGCG on cell adhesion and proliferation was examined using pancreatic cancer cells. EGCG-treated pancreatic cancer cells AsPC-1 and BxPC-3 decrease cell adhesion *ability* on micro-pattern dots, accompanied by dephosphorylations of both focal adhesion kinase (FAK) and insulin-like growth factor 1 receptor (IGF-1R), whereas retained the activations of mitogen-activated protein kinase and mammalian target of rapamycin.

Green tea (*Camellia sinensis*) is rich in catechins, of which (−)-epigallocatechin-3-gallate (EGCG) is the most abundant. Studies in animal models of carcinogenesis have shown that green tea and EGCG can inhibit tumorigenesis during the initiation, promotion and progression stages. Many potential mechanisms have been proposed including both antioxidant and pro-oxidant effects, the catechins are chemical antioxidants which can quench free radical species and chelate transition metals, there is evidence that some of the effects of these compounds may be related to induction of oxidative stress. Such pro-oxidant effects appear to be responsible for the induction of apoptosis in tumor cells.

DPPH ASSAY:

DPPH (1,1-diphenyl-2-picrylhydrazyl) is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalisation also gives rise to the deep violet colour, characterised by an absorption band in ethanol solution centered at about 520 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Blois,1958) with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present).Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is

Z• + AH = ZH + A•

Where, ZH is the reduced form and A• is free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorised) by one molecule of the reductant.

FRAP ASSAY:

Total antioxidant activity is measured by ferric reducing antioxidant power assay [3] The FRAP assay, is presented as a novel method for assessing "antioxidant power." Ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine complex to form. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. Absorbance changes are linear over a wide concentration range with antioxidant mixtures, including plasma, and with solutions containing one antioxidant in purified form. There is no apparent interaction between antioxidants. The FRAP assay is inexpensive, reagents are simple to prepare, results are highly reproducible, and the procedure is straightforward and speedy. The FRAP assay offers a putative index of antioxidant, or reducing, potential of biological fluids within the technological reach of every laboratory and researcher interested in oxidative stress and its effects.

Owing to the increasing significance of green tea present work was carried out to characterize the anticancerous , antioxidant and antiradical performance of green tea extract using DPPH and FRAP assay

2. MATERIALS AND METHODS

PREPARATION OF EXTRACT FROM Camellia sinensis LEAF BY SOXHLET APPARATUS:

Camellia sinensis tea leaves were collected from Craigmore plantations from nilgiri tea estate, Ooty. 25g of *Camellia sinensis* extract sample was weighed and extracted with 300ml of the ethanol by continuous hot percolation with the help of soxhlet apparatus for 10hours of time. On completion the extracts were filtered and evaporated as per standard methods. the extract were filtered using Whatman filter paper, The filtrate was concentrated under reduced pressure in vacuum at 40°C for 25 min using a rotary evaporator. The concentration were stored in the refrigerator for further use.

ANTI-CANCER ACTIVITY *Camellia sinensis* LEAF EXTRACT:

Cell line and culture:

HT 29 cell line was obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in DMEM supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO2 at 37 °C.

***In Vitro* assay for anti cancer activity: (MTT assay) [4]**

Cells (1 × 105/well) were plated in 24-well plates and incubated in 370C with 5% CO2 condition. After the cell reaches the confluence, the various concentrations of the *Camellia sinensis* extract were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells .The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically. The % cell viability was calculated using the following formula:

% Cell viability = A570 of treated cells / A570 of control cells × 100

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ANTI-OXIDANT ASSAY OF *Camellia sinensis* LEAF EXTRACT:

DPPH ASSAY: [5] .

Aliquot 3.7 ml of absolute methanol in all test tubes and 3.8ml of absolute methanol was added to blank. 100µl of BHT were added to the tube marked as standard and 100µl of respective samples to all other tubes marked as tests. 200µl of DPPH reagent was added to all the test tubes including blank. Incubate all test tubes at room temperature in dark condition for 30 minutes. The absorbance of all samples was read at 517nm.

S.NO	REAGENTS	BLANK	STANDARD	TEST
1.	Methanol	3.8ml	3.7ml	3.7ml
2.	BHT	-	100µl	-
3.	Sample	-	-	100µl
4.	DPPH	200µl	200µl	200µl
Incubation at dark for 30 minutes				
O.D. at 517nm.				

CALCULATION:

$$\% \text{ Antioxidant activity} = \frac{\textbf{(Absorbance at blank) - (Absorbance at test)}}{\textbf{(Absorbance at blank)}} \times 100$$

FRAP ASSAY:

Total antioxidant activity is measured by ferric reducing antioxidant power assay[3] 100 µl of sample was added to the tube marked test and 3 ml of FRAP reagent was added to it. 3 ml of FRAP reagent was taken as a blank. Absorbance is measured at 0 minutes after vortexing at 593 nm. Samples are then placed at 37ºC in water bath and absorption is again measured after 4 minutes. Ascorbic acid was used as the standard.

FRAP value of sample (µM) = (Change in absorbance of sample from 0 to 4 minute / change in absorbance of standard from 0 to 4 minutes) x FRAP value of standard (1000 µM)

Note: FRAP value of ascorbic acid is 2.

3. RESULTS

EXTRACTION OF *Camellia sinensis* LEAF:

The ethanol extract of green tea sample were prepared, filtered and evaporated as per standard methods. Maximum percentage yield of 5% was observed in ethanolic extract of *Camellia sinensis* leaves 25g of fresh leaves used for extraction.

ANTI- CANCER ACTIVITY OF *Camellia sinensis* LEAF EXTRACT:

The ethanol extracts of *Camellia sinensis* leaves extract were evaluated by using cell line culture HT 29 cell line. The highest concentration1000µg/ml of camellia sinensis extract shows the minimum cell viability of 28%, the concentration of 7.8µg/ml shows the maximum cell viability of 77.41% and 100% cell viability by cell control (Table1).

ANTI-OXIDANT ACTIVITY of *Camellia sinensis* LEAF EXTRACT:

DPPH assay:

The DPPH activity of the given green tea sample is found to be 22.53% at the concentration of 1000µg/ml was shown in table (2),

FRAP assay:

The FRAP activity of the given green tea sample is found to be 3520µM at the concentration of the standard is 1000µM and FRAP value of ascorbic acid is 2 was shown in table (3), and the calculation was given below.

4. DISCUSSION

Green tea is derived from non-fermented leaves of the *Camellia sinensis* plant. Green tea has been a favored drink, traditionally, in Asian countries. Because of studies that have shown the potential health benefits of green tea, it is now gaining worldwide popularity as a drink that is important in preventative medicine. Studies using green tea have shown it to have potential benefits, most notably in: cardiovascular disease, cancer, diabetes, obesity, oral health, bone health, and cognitive function[6].

Green tea is recognized as a cancer preventive beverage in leading countries Considering the significant cancer preventive activity of green tea catechins in humans in the present study the anti-proliferative effect of green tea extracts against Hep 2 cell lines were determined. In our study cell viability of hep2 cell lines decreased to 28% at the concentration of 1000µg/ml .*Camellia sinensis* extract at a concentration of 7.8µg/ml showed the maximum cell viability of 77.41% and 100% cell viability by cell control, this indicates that these plants contain potential bioactive compounds. The green tea polyphenols may inhibit cell growth through a variety of mechanisms. This indicates that these plants contain potential bioactive compounds, which if properly and extensively studied, could provide many chemically interesting and biologically active drug candidates, including some with potential antitumor and antiproliferative properties which have been found to exhibit anti-inflammatory, hepatoprotective, anti-ulcer anticancer and immunomodulatory properties[7]. Similarly the previous report on *Camellia sinensis* extracts showed antioxidant activity [8]

Several biological properties have been reported for green tea, including prevention of cancer, polyphenolic catechins were identified as active ingredients which deregulated a number of genes relating to cellular movement and cell morphology The anti-carcinogenic effects of green tea have been seen in many types of cancer, and the mechanisms may include inhibiting angiogenesis and cell growth, and inducing apoptosis in cancer cells [9]

Antioxidant effects come from the ability of green tea to limit the amount of free radicals by binding to reactive oxygen species (ROS).In the present study the antioxidant property of DPPH of the green tea extract is found to be 22.53% at the concentration of 1000µg/ml. The FRAP activity of the green tea sample is found to be 3250µM at the concentration of the standard is 1000µM.The basis for anticancer properties of green tea polyphenols *in vitro* and *in vivo* could due to their antioxidant or pro-oxidant properties Green tea polyphenols scavenge reactive oxygen species (ROS) by generating more stable phenolic radicals. A collection of assays have been developed and widely used to assess the radical scavenging/antioxidant activities of solutions, including green tea. One such assay is the ferric reducing/antioxidant power (FRAP) assay. A positive correlation exists between the phenolic content in green tea and the antioxidant activity measured by FRAP. In addition, green tea possesses more antioxidant capacity on average than Oolong and black teas (as measured by FRAP).

Antioxidants provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and concomitant lipid peroxidation, protein damage and DNA stand breaking [10]. Antioxidant compounds like Phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases

GC-MS analysis was done in the previous research to identify the components responsible for anticancerous and antioxidant activity. The compounds such as 1-Octadecene and 1-Heptadeceane possess anticancer, antioxidant and antimicrobial activity compounds such as squalene, tetracosane, ascorbic acid, and stigmasterol can be responsible for the antioxidant activities which are present in the ethanolic extract of *Camellia sinensis* leaves. Tetracosane with anticancer and antioxidant properties have been reported by [11]. Eicosane with antitumour and cytotoxic property was reported by [12] which was recovered from green tea leaves in the previous study.by the same authors

It is concluded from this research study that *Camellia sinensis* leaves showed good antioxidant activity which provides the protection to living organisms from the damage caused by the uncontrolled production of reactive oxygen species.All beneficial effects of tea have been attributed to the strong anti-oxidative activity due to phenolic compounds catechins which protect the body from damage caused by free radical-induced oxidative stress Natural antioxidants tend to be safer and also possess anti-viral, antiinflammatory, anti-cancer, antimutagenic, antitumor and hepatoprotective properties.

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APPENDIX- A

List of Tables:

TABLE 1: ANTI-CANCER EFFECT OF *Camellia sinensis* EXTRACT ON HT 29 CELL LINE

S.NO	CONCENTRATION (µg/ml)	DILUTIONS	ABSORBANCE (O.D)	CELL VIABILITY (%)
1	1000	-	0.625	28.57
2	500	1:1	0.782	35.75
3	250	1:2	0.960	43.89
4	125	1:4	1.113	**50.89**
5	62.5	1:8	1.264	57.79
6	31.2	1:16	1.421	64.97
7	15.6	1:32	1.541	70.46
8	7.8	1:64	1.693	77.41
9	Cell control	-	2.187	100

(-) = **NIL**

ISSN 2348-1218 (print)
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TABLE 2: ANTI-OXIDANT ACTIVITY OF *Camellia sinensis* EXTRACT USING DPPH ASSAY METHOD.

S.NO	SAMPLE	CONCENTRATION (µg/ml)	O.D	DPPH ACTIVITY (%)
1.	*Camellia sinensis*	1000	1.451	22.53

Blank O.D: 1.873

TABLE 3: ANTI-OXIDANT ACTIVITY OF *Camellia sinensis* EXTRACT USING FRAP ASSAY METHOD

S.NO	NAME OF THE SAMPLE	FRAP (µM)
1.	*Camellia sinensis*	3520

Difference in absorbance for Sample from 0 to 4 min = 0.704

Difference in absorbance for Ascorbic acid (standard) from 0 to 4 min = 0.2

List of Figures:

FIGURE 1: ANTICANCER EFFECT OF *Camellia sinensis* EXTRACT ON HT 29 CELL LINE

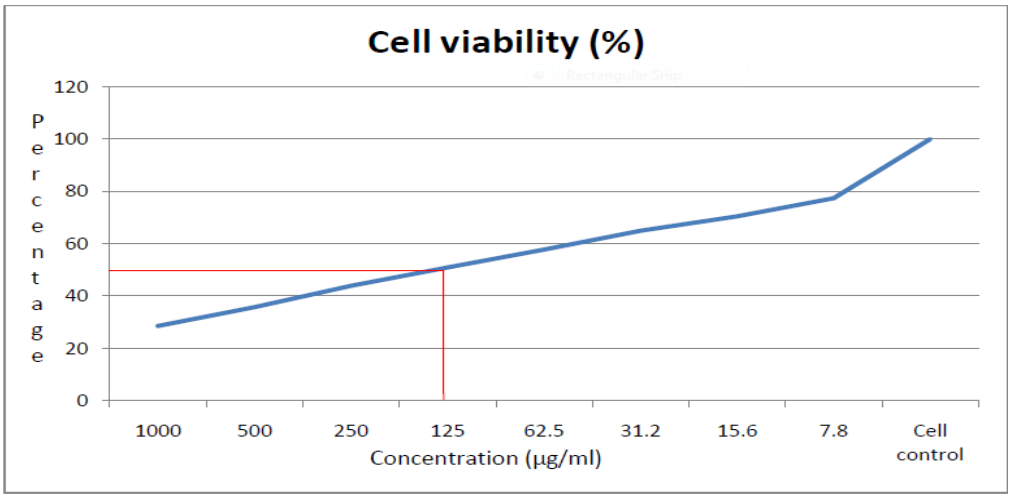

PLATE: 1 ANTICANCER EFFECT OF *Camellia sinensis* EXTRACT ON HT 29 CELL LINE