

OXIDATIVE STRESS OF BIOACTIVE MOLECULES ANTIOXIDANT (EGCG) POLYPHENOLS EXTRACT OF GREEN TEA (*CAMELLIA SINENSIS* L.)

AMROUCHE Zoheir^{1,2,*}, El HADI Djamel¹, FAUCONNIER Marie-Laure², BENRIMA-GUENDOUZ Atika³ and BITAM Arezki⁴

1. Functionnal analysis of chemical process laboratory. chemistry department, University of Blida 1-Algeria

2. General and organic chemistry laboratory Agro-biotech Gembloux, University of Liege-Belgium.

3. Plant biotechnology research laboratory, Faculty of Science of the nature and the life, University of Blida 1-Algeria.

4. Department of food technology, National Higher school of Agronomy Algiers Algeria.

Reçu le 10/12/2017, Révisé le 05/06/2018, Accepté le 07/06/2018

Abstract

Description of the subject. Polyphenols are bioactive molecules exhibiting a lot of scientific attention due to their multiple activities antioxidants, therapeutic and biological.

Objective. Consists on extract polyphenols from green tea *Camellia sinensis* L. by ultrasound, and search some therapeutic aspect : antioxidant effects, antibacterial activity and slimming activity.

Methods. Ultrasonic extract polyphenols and identification of bioactive molecules with LC-MS. Mice and rabbit used *In-vivo* for toxicity test and slimming activity respectively. *In-vitro* DPPH test for antioxidant activity, nutrient agar for the antibacterial and antifungal activity.

Results. Analytical results of polyphenols extract allows us to identify the presence of bioactive molecules so-called Epigallocatechine gallate (EGCG) revealed by LC-MS (Up to 60,1 %). This bioactive molecules is not lethal and have powerful antioxidant activity. Therapeutic aspect of this molecules have a strong slimming activity and a very effective antibacterial activity but not antifungal activity.

Conclusion. The application of green tea polyphenols as a natural remedy is very efficient so it is a return to the traditional pharmacy, and used this antioxidant bioactive molecule in the pharmaceutical industry.

Keywords: *Camellia sinensis* ; Antioxidant ; Bioactive molecules ; Polyphenols (EGCG) ; slimming activity ; antibacterial activity.

STRESS OXYDATIF DES MOLÉCULES BIOACTIVE ANTIOXYDANTE DES POLYPHÉNOLS (EGCG) EXTRAIT DU THÉ VERT (*CAMELLIA SINENSIS* L.)

Résumé

Description du sujet. Les polyphénols sont des molécules bioactive attire l'attention des scientifiques en raison de leurs multiples activités antioxydants, thérapeutiques et biologiques.

Objectifs. Consiste à extraire des polyphénols des feuilles du thé vert *Camellia sinensis* L. par ultrason, et de recherché quelque aspect thérapeutique : effet antioxydant, activité antibactérienne et l'activité amincissante.

Méthodes. Extraction des polyphénols par ultrason et identification des molécules bioactive par LC-MS. *In-vivo* on réalise un test de toxicité sur souris, et l'activité amincissante sur lapin. *In-vitro* la recherche de l'activité antioxydant par test de DPPH, la recherche de l'activité antibactérienne et antifongique sur milieu gélosé.

Résultats. Les résultats analytiques de l'extrait polyphénolique ont identifié les molécules bioactive par LC-MS, dont l'Epigallocatechine gallate (EGCG) révélé de l'ordre de 60.1%. Ces molécules bioactive sont pas létale et possède une activité antioxydante très puissante. Leurs aspects thérapeutiques ont une forte activité amincissante, et une activité antibactérienne très efficace, mais pas d'activité antifongique.

Conclusion. L'application des polyphénols du thé vert comme un remède naturel est très efficace, qui permet un retour à la pharmacie traditionnelle d'utiliser les molécules bioactives dans l'industrie pharmaceutique.

Mots clés: *Camellia sinensis* ; Activité antioxydants ; Molécules bioactive ; Polyphénols(EGCG) ; Activité amincissante ; activité antibactérienne.

* Auteur correspondant : AMROUCHE Zoheir, E-mail: zoheir.amrouche@yahoo.fr

INTRODUCTION

The oxidative stress correspond to a disturbance of intracellular oxidative status, induced either by an excessive production of free radicals, or by a decrease in antioxidant defense capacity. The effects of free radicals are the intensity proportionnel and the duration of their production: a transitory and moderated production of free radicals correspond to a cells defense mechanism, allow it to destroy cancerous cells or pathogenic microorganism. The free radicals are substances produced by cellular metabolism that can be toxic for biologic fabric and source of DNA, lipids, cell membrane and proteins [1].

Polyphenols are secondary métabolites of plants, brought by a food of vegetable origin and present a big structural diversity. We find them, in general, in all vascular plants, where they can be located in various organs : roots, stems, wood, leaves, flowers and fruit. The phenolic compounds represent the most studied phytochemical element and were widely exploited as model systems in various domains of the search on plants [2].

Green tea (*Camellia sinensis* L.) is the second most common beverage in the world next to water [3]. Green tea is now moving from a traditional beverage to a healthy drink, a source of pharmacologically active molecules, an important member of the antioxidant food group, and a functional food endowed with beneficial health properties [4]. Polyphenols green tea contains powerful antioxidants catechins [5], integrating epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) [6, 7].

Green tea polyphenols is a particularly rich source of polyhydroxy flavan-3-ol derivatives [8]. Polyphenols demonstrated them very beneficial antioxidant properties and which play an important role in the biology of the alive. The major catechin found in green tea *Camellia sinensis* L. Apparently, EGCG functions as a strong antioxidant, preventing oxidative damage in healthy cells. EGCG polyphenols may have therapeutic health effects for a variety of chronic pathological conditions including cancer, neurodegenerative diseases, and cardiovascular diseases [9, 10], obesity and diabetes [11, 12].

The tea is known for a long time as slimming food [13], this examination is realized to prove the efficiency of the polyphenols of the green tea in the way of loss of weight. Studies on the model animal were operated and showed their beneficial effects, which begin today to receive the scientific attention which they deserve. The aim of this work to take profit from these molecules to extract them, and to estimate in vivo the effect on the obesity by ultrasound polyphenols extracts and to realize a study of some therapeutic effect, the antioxidant effect, slimming activity and antimicrobial activity.

MATERIALS AND METHODS

1. The plant material

Chinese typical green tea *Camellia sinensis* of 1st choice commercialised in Europe, whole sheet is used and not broken leaves.

2. Ultrasonic extract polyphenols of green tea

Ultrasonic (CPX750, 750w, 20kHz, Julabo Cole Parmer ultrasonic processor, Germany) at 95°C, transmitted a 10000 watts energy (10s/10s) . Infusions were centrifuged at 5000g for 15 min at 4°C and filtered using 0.45µm filter membranes (Millipore, MA, USA). The upper solution was taken and lyophilized by CHRIST Gamma 2-16 LSCplus. The lyophilized powder was stored in darkness at 4 °C until used.

3. LC-MS analysis

LC-MS, using a Agilent 1100 series HPLC, Mass detection was performed on ESI esquire HCT (ion trap BRUKER daltonics Germany) coupled to the chromatographic system. Column EC/3 NUCLEODUR 100-3 C18 ec (MACHEREY-NAGEL Germany). The mobile phase consisted of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The separation was performed on a linear gradient of acetonitrile in water (5–95% B) during 40 min. Injection volumes for all samples were 20 µL. The mass spectra were obtained at a mass-to-charge ratio (m/z) scan range from 100-600. The following MS parameters were used for the analysis:

capillary voltage, 4500V; nebulizer pressure, 50 psig; drying gas flow rate, 10 L/min; drying temp 365°C. Samples were analyzed in positive mode. Components of green tea extracts were identified according to their *m/z* values, UV/Vis absorption spectra and retention times as compared to those found in the literature [8]. The identification of peaks is confirmed by spectre of mass. Quantities of phenolics were calculated from peak areas on chromatograms.

4. Antioxydant activity DPPH radical scavenging activity

The radical scavenging activity of the phenolic compounds of the green tea extract was estimated by measuring their capacities to trap the free radical DPPH (1, 1-diphényl-2-*pycridyl*-hydrazyl). Its violet color is transformed into yellow as its reduction [5]. A concentration of DPPH is 0.037 mg/ml, prepared from 9.25 mg solubilized in 250ml of absolute methanol. An aliquot of 100 µl of green tea extract with concentration of 5 mg/ml (chosen after preliminary tries) was added to 3 ml of DPPH solution in methanol. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark, and then the absorbance was measured at 517 nm by Thermo Electron Corporation. For each dilution of the extract, the DPPH scavenging activity was calculated as $100 \cdot (A_0 - A_1) / A_0$, where A_0 is the absorbance of the control without sample at 30 min, and A_1 is the absorbance of the sample at 30 min.

5. Toxicity test

The study of the toxicity is made on 50 mice of 18-20g. The applied dose is 2500 mg of the lyophilized powder polyphenols extract by kg weighty physical of mouse, on intravenous way [14].

6. Slimming activity

The variations of the weight to rabbits, by administering them the lyophilized powder polyphenols of the green tea 3 times by day to a quantity of 130 mg of polyphenols extracts by force-feeding, during 87 days. The first 3 days, the rabbits consume a balanced and 100% energy diet, after a usual diet is taken back.

7. Antimicrobial activity

Ultrasonic *Camellia sinensis* L. extracts were assayed for their antibacterial activities against three human-pathogenic microbial, and two antifungal activity. The latter included two Gram (-) bacterial species, namely *Escherichia coli* ATCC 10536TM, *Pseudomonas aeruginosa* ATCC 15442TM and one Gram (+) bacterial *Bacillus subtilis*. Antifungal species, namely *Aspergillus niger* and *Candida albicans*.

The Antimicrobial activity of polyphenols extracts (bioactive molecules) was performed according to the disc diffusion method described by Nakayama *et al.* [15]. The extracts were dissolved in distilled water to a final concentration of 10 mg/mL. The microbial strains were cultured in a nutrient broth for 24 h. After that, a 200 µL culture suspension of each microbial strain (10^6 CFU "colony-forming unit" estimated by absorbance at 600 nm) was spread on a Henton Agar medium. Bores were made on the medium using a sterile borer and loaded with 50 µL of polyphenols extract at 10 mg/mL. Streptomycine Gentamicin, and Furane were used as a positive reference bacteria for *Bacillus subtilis*, *Pseudomonas aeruginosa*, & *Escherichia coli* respectively. Ketoconazole antibiotic was used as a positive reference fungal for *Aspergillus niger* and *Candida albicans*. All the Petri dishes were incubated at corresponding temperatures for 24 h. Antibacterial activity was determined by measuring the diameter of the inhibition zone in millimetres. All experiments were carried out in three replicates.

8. Statistical analysis

All experiments were performed in triplicate and results were expressed as mean $\pm$ standard error. All statistical analyses were performed on Minitab 17. A p-value less than 0.05 was considered as statistically significant.

RESULTS

1. Phenolic composition of the green tea extract

Identification by LC-MS of the biochemical composition of total polyphenol extract by ultrasound of green tea is characterized by four main peaks (Fig. 1).

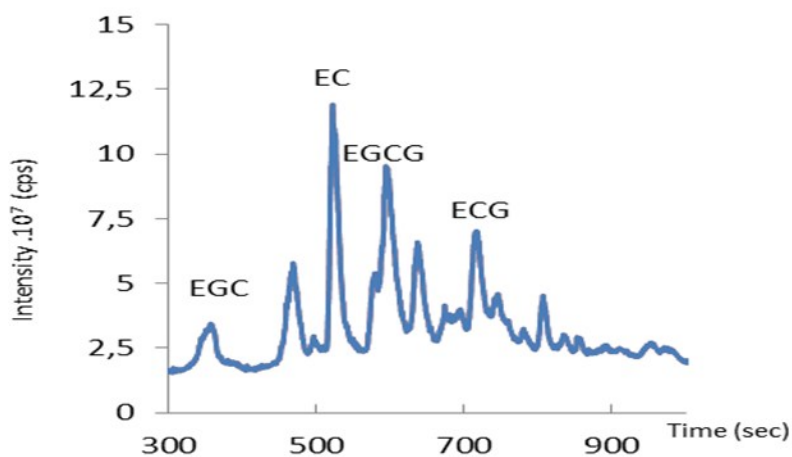


Figure 1 : LC-MS chromatograms.

The main phenolic compounds in green tea are catechins (table 1), including (–) epigallocatechin-3-gallate (EGCG) 60.1 ± 0.17 %, (–) epigallocatechin (EGC) 12 ± 0.25 %, (–) epicatechin gallate (ECG) 13 ± 0.45 %, and (–) epicatechin (EC) 5 ± 0.2 %, with EGCG being the highest accounting for 60% to 65% of the entire catechin content [16]. EGCG is the major catechin in green tea and accounts for 50% to 80% [17].

Furthermore, chemical modification of an EGCG pharmacophore may modify relative therapeutic activities so that combinatorial supplementation may synergistically enhance beneficial health effects [18, 19].

Table 1: Proportion of Major Catechins polyphenols green tea

Compound	%
Epigallocatechin (EGC)	12 ± 0.25
Epigallocatechin-3-gallate (EGCG)	60.1 ± 0.17
Epicatechin (EC)	05 ± 0.2
Epicatechin-3-gallate (ECG)	13 ± 0.45
Other	09.89 ± 0.26

The results are expressed as averages of three independent measurements $\pm$ SD.

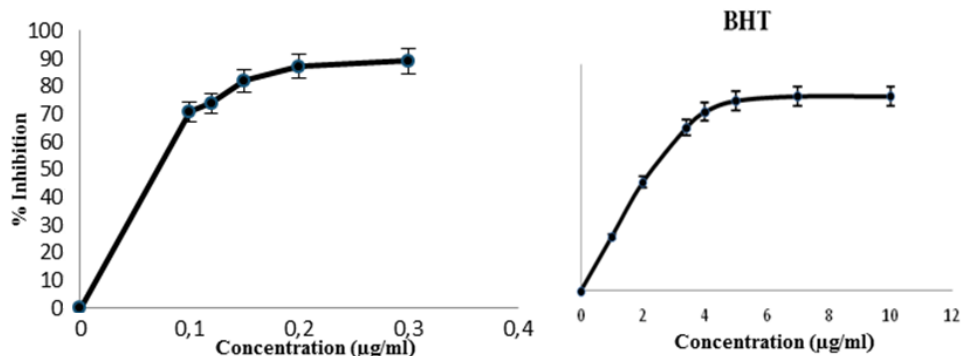


Figure 2 : Comparison antioxydant activity with BHT

2. Antioxydant activity DPPH radical scavenging activity

DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants. The method is based on the reduction of methanolic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form, DPPH-H, by the reaction. The total phenolic extract of *C.sinensis* showed a concentration-dependent antiradical activity by reducing the stable radical DPPH to the yellow coloured diphenylpicrylhydrazine derivative. The antioxidant activity ultrasound polyphenols extract of green tea present 90.22 % comparing our results with those of the standard antioxidant BHT 92% (Fig. 2). The liquor of green tea extract an antioxidant activity close to that of BHT.

However, scavenging activity of gallic acid, a known antioxidant used as positive control, was relatively high. Epigallocatechingallate (EGCG) antioxidant is the major chemical constituent of *C.sinensis*. Total phenolic extract showed potent DPPH free radical-scavenging activity. Natural polyphenols have chain-breaking antioxidant activities and are believed to prevent many degenerative diseases, including cancer and atherosclerosis [11, 12].

3. Toxicity.

The level of mortality is 15 % then, according to the standards of the European pharmacopoeia 2008, the applied dose (2500 mg / kg) is not toxic and thus she can be increased. If the mortality of the used animals is 50 % or more, it means that the administered dose is mortal. The level of mortality is equal at the level of survival (50 % mortality, 50 % survival), the administered dose is said toxic or more less toxic [14].

The showed that administration of 2500 mg/kg polyphenols intravenously is not lethal.

4. Slimming activity

The results of loss weight rabbits by consumption of 390 mg lyophilisate polyphenols extract of green tea was illustrated in the figure 3 decrease of physical weight mattering at rabbits 14.30 and 18.60%, the loss of the weight of which is $460 \pm 125.19\text{g}$ during 56 days, when the maximum weight loss of rabbit is 613g. this results are means of three different experiments; means, in the same line, with different small letters are significantly different ($p < 0.05$).

Shen et al. [20] estimated *in vivo* the efficiency of the form concentrated some green tea on the obesity on the animal model and proved that the green tea prevent significantly the weight gain. On the other hand the epidemiological data and the proofs obtained *in vitro* or *in vivo* animal studies suggest that the high consumption of polyphenols can reduce the risk of developing several diseases [21].

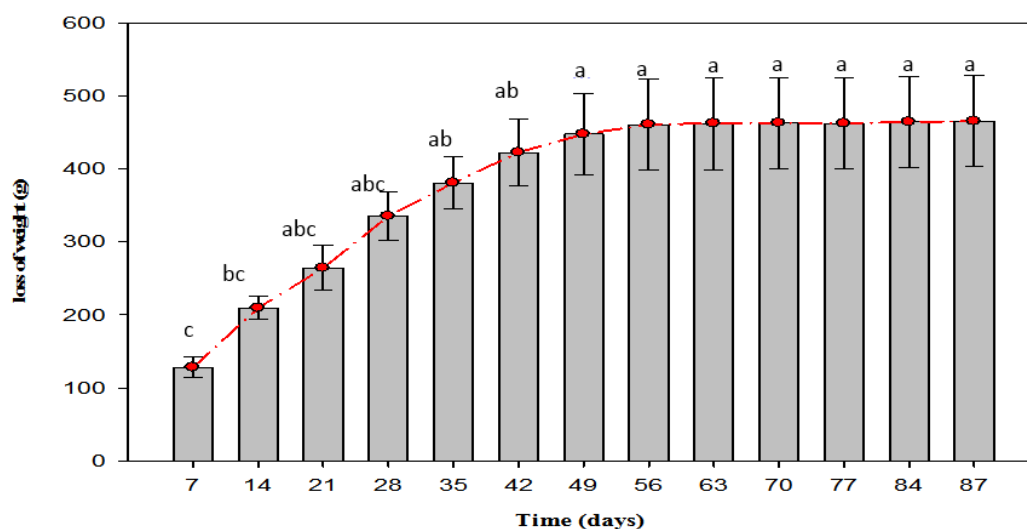


Figure 3 : The loss of the weight of rabbit according to time.

5. Antimicrobial activity

This study is made to show the efficiency of polyphenols extract and their protective effect against the bacterial attacks. The results obtained for Antibacterial and Antifungal are illustrated in the table 2. Polyphenols have, in one hand, an antibacterial

activity raised against diverse microorganisms: *Pseudomonas aeruginosa*, *Bacillus subtilis*, on the other hand the anti-microbial activity goes away at *Escherichia coli*. The antifungal activity revealed no zone of inhibition for *Candida albicans* and *Aspergillus niger*. Polyphenols have an antibacterial activity raised against diverse microorganisms [15].

Table 2: Values of the diameters of inhibition zones polyphenols extracts of *Camellia sinensis*

	Polyphenols extract BD	Reference antibiotic BD	
Bacterial			
Gram -			
<i>Escherichia coli</i>	0	Furane	24 ±0.1
<i>Pseudomonas aeruginosa</i>	18 ± 0.1	Gentamicin	13 ±0.1
Gram +			
<i>Bacillus subtilis</i>	06 ±0.1	Streptomycine	15±0.1
Fungal			
<i>Aspergillus niger</i>	0	Ketoconazole	13 ±0.1
<i>Candida albicans</i>	0	Ketoconazole	15±0.2

Values are means ± SD of three separate experiments done in triplicate. BD: bore diameter of inhibition (halo size) in (mm), polyphenols extract 50 µL/disc.

DISCUSSION

The present study addressed some therapeutic effect of the antioxidant effect, slimming activity and antimicrobial activity by using ultrasound polyphenols extracts from green tea and to estimate in vivo the effect on the obesity. The obesity is largely due to the westernization of the food. Experimental and epidemiological data suggest that a food rich in fat favors the development of the obesity and that there is a direct correlation between the lipid ration and the degree of obesity [22].

Green tea contains powerful antioxidants, catechins, which can destroy free radicals [5].

The analysis by LC-MS identified 4 polyphenolic components included in the obtained lyophilisate which EGCG is the most abundant. The major catechin found in green tea *Camellia sinensis* L., Apparently, EGCG functions as a strong antioxidant, preventing oxidative damage in healthy cells. Green tea polyphenols reduce considerably or prevent the increase of the physical weight to the male thin and obese subjects and the females. On the basis of the in-vivo effects, the components of the green tea such as the EGCG could be useful for the treatment of the obesity [23]. Any diseases are linked to obesity thus, it is important to indicate that the green tea is capable of inhibiting the biosynthesis of the cholesterol [24].

According to the works Sundaram et al. [25] the treatment orally everyday life of extract of green tea (physical 300mg/kg weighty) in rats diabetics during 30 days pulled a significant reduction of the levels of glucose in the plasma, the glycosyle heamoglobin (HbA1c) and the increase of the rate of insulin and some haemoglobin. Green tea extract has been approved by the US Food and Drug Administration as the first botanical drug [26]. Yi et al. [27] showed that the polyphenols of the green tea have a big antibacterial action to *Pseudomonas aeruginosa*. So, they have a big inhibitive efficiency, a power very powerful modifier of morphology and structure reaching the protein composition membranaire [28]. Sakanaka et al. [29] proved a strong activity against other germs such as *Bacillus stearothermophilus* and *Clostridium thermoaceticum*.

CONCLUSION

Certain diseases are linked to obesity, polyphenols reduce considerably or prevent the increase of the weight. Polyphenols antioxidants are capable of neutralizing the greater production of the free radicals. The DPPH test revealed that the extract of the green tea presents a powerful antioxidant activity. Polyphenols reduce considerably or prevent the increase of the physical weight to the thin and obese subjects male and female. On the basis of the in-vivo effects, the components of the green tea such as the EGCG could be useful for the treatment of the obesity.

REFERENCES

- [1]. Lobo V., Patil A., Phatak A. and Chandra N. (2010). Free radicals, antioxidants and functional foods : Impact on human health. *Pharmacogn Rev.* 4(8) : 118–126.
- [2]. Boudet A. (2007). Evolution and current status of research in phenolic compounds. *Phytochemistry.* 68 : 2722 – 2735.
- [3]. Wei H., Zhang X., Zhao J.F., Wang Z.Y., Bickers D. and Lebwohl M. (1999). Scavenging of hydrogen peroxide and inhibition of ultraviolet light-induced oxidative DNA damage by aqueous extracts from green and black teas. *Free Radicals Biology and Medicine.* 26 : 1427–1435.
- [4]. Adity B. (2016). Interaction of tea polyphenols with serum albumins: A fluorescence spectroscopic analysis. *Journal of Luminescence.* 169: 220–226.
- [5]. Sang S., Cheng X., Stark R.E., Rosen R.T., Yang C.S. and Ho C.T. (2002). Chemical studies on antioxidant mechanism of tea catechins: analysis of radical reaction products of catechin and epicatechin with 2, 2-diphenyl-1-picrylhydrazyl. *Bioorg. Med. Chem.* 10 : 2233– 2237.
- [6]. Meydani M. and Hasan S.T. (2010). Dietary polyphenols and obesity. *Nutrients,* 2: 737–751.
- [7]. Sano M., Tabata M., Suzuki M., Degawa M., Miyase T. and Maeda-Yamamoto M. (2001). Simultaneous determination of twelve tea catechins by high-performance liquid chromatography with electrochemical detection. *Analyst.* 126 : 816–820.
- [8]. Lin L.Z., Chen P. and Harnly J.M. (2008). New phenolic components and chromatographic profiles of green and fermented teas. *Journal of Agricultural and Food Chemistry* 56(17) :8130–8140.
- [9]. Tomas-Barberan F.A. and Andres-Lacueva C. (2012). Polyphenols and health: current state and progress. *J. Agric. Food Chem.* 60 : 8773–8775.
- [10]. Kishimoto Y., Tani M. and Kondo K. (2013). Pleiotropic preventive effects of dietary polyphenols in cardiovascular diseases, *Eur. J. Clin. Nutr.* 67 : 532–535.
- [11]. Iso H., Date C., Wakai K., Fukui M. and Tamakoshi A. (2006). The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. *Annals of Internal Medicine,* 144(8) : 554–562.
- [12]. Wu C.H., Lu F.H., Chang C.S., Chang T.C., Wang R.H. and Chang C.J. (2003). Relationship among habitual tea consumption, percent body fat, and body fat distribution. *Obesity Research,* 11(9) : 1088–1095.
- [13]. Krook M. and Hagerman A. (2012). Stability of polyphenols epigallocatechin gallate and pentagalloyl glucose in a simulated digestive system». *Food Research International.* 49 : 112 – 116.
- [14]. Hsu Y., Tsai C., Chen W., Huang C. and Yen C. (2011). A subacute toxicity evaluation of green tea (*Camellia sinensis*) extract in mice. *Food and Chemical Toxicology.* 49 : 2624 – 2630.
- [15]. Nakayama M., Shimatani K., Ozawa T., Shigemune N., Tsugukuni T., Tomiyama D., Kurahachi M., Nonaka A. and Miyamoto T. (2013). A study of the antibacterial mechanism of catechins: Isolation and identification of *Escherichia coli* cell surface proteins that interact with epigallocatechin gallate. *Food Control,* 33 : 433 - 439.
- [16]. Yang C.S. and Hong J. (2013). Prevention of chronic diseases by tea: possible mechanisms and human relevance. *Annu Rev Nutr.* 33: 161–242.
- [17]. Khan N., Afaq F., Saleem M., Ahmad N. and Mukhtar H. (2006). Targeting multiple signaling pathways by green tea polyphenol (-)-epigallocatechin-3-gallate. *Cancer Res.* 66 : 2500–2505.
- [18]. Bose M., Hao X., Ju J., Husain A., Park S., Lambert J.D. & Yang C.S. (2007). Inhibition of tumorigenesis in ApcMin/pmice by a combination of (-)-epigallocatechin-3- gallate and fish oil. *J. Agric. Food Chem.* 55: 7695–7700.
- [19]. Giunta B., Hou H., Zhu Y., Salemi J., Ruscin A., Shytle R.D and Tan J. (2010). Fish oil enhances anti-amyloidogenic properties of green tea EGCG in Tg2576 mice, *Neurosci. Lett.* 471: 134–138.
- [20]. Shen C., Smith B., Lo D., Chyu M., Dunn D., Chen C. and Kwun I. (2012). Dietary polyphenols and mechanisms of osteoarthritis». *Journal of Nutritional Biochemistry.* Vol.23 : 1367 – 1377.
- [21]. Oak M., El Bedoui J. & Schini-Kerth V. (2005). Antiangiogenic properties of natural polyphenols from red wine and green tea. *Journal of Nutritional Biochemistry.* 16 : – 8.
- [22]. Francis D.K., Vanden Broeck J. and Younger N. (2009). Fast food and sweetened beverage consumption association with overweight and high waist circumference in adolescents. *Public Health Nutr.* 12(8): 106- 1114.
- [23]. Bogdanski P., Suliburska J., Szulinska M., Stepień M., Pupek-Musialik D. and Jablecka A. (2012). Green tea extract reduces blood pressure, inflammatory biomarkers, and oxidative stress and improves parameters associated with insulin resistance in obese, hypertensive patients. *Nutrition Research.* 32 : 421 – 427.

- [24]. **Lu C. and Hwang L. (2008).** Polyphenol contents of Pu-Erh teas and their abilities to inhibit cholesterol biosynthesis in Hep G2 cell line. *Food Chemistry*, 111 : 67 – 71.
- [25]. **Sundaram R., Naresh R., Shanthi P. and Sachdanandam P. (2013).** Modulatory effect of green tea extract on hepatic key enzymes of glucose metabolism in streptozotocin and high fat diet induced diabetic rats. *Phytomedicine*. 20(7):577-584.
- [26]. **Kumazoe M., Yamashita M., Nakamura Y., Takamatsu K., Bae J., Yamashita S., Yamada S., Onda H., Nojiri T., Kangawa K. and Tachibana H. (2017).** Green Tea Polyphenol EGCG Upregulates Tollip Expression by Suppressing Elf-1 Expression. *J Immunol*, 199(9):3261-3269.
- [27]. **Yi S., Zhu J., Fu L. and Li J. (2010).** Tea polyphenols inhibit *Pseudomonas aeruginosa* through damage to the cell membrane. *International Journal of Food Microbiology*, 144 : 111 - 117.
- [28]. **Cui Y., Oh Y., Lim J., Youn M., Lee I., Pak H., Park W., Jo W. and Park S. (2012).** AFM study of the differential inhibitory effects of the green tea polyphenol epigallocatechin-3-gallate (EGCG) against Gram-positive and Gram-negative bacteria. *Food Microbiology*. 29 : 80 - 87.
- [29]. **Sakanaka S., Juneja L. and Taniguchi M. (2000).** Antimicrobial Effects of Green Tea Polyphenols on Thermophilic Spore-Forming Bacteria. *Journal of Bioscience and Bioengineering* 90 (1) : 81 – 85.