IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 6 Ver. VI (Nov. - Dec.2016), PP 113-119
www.iosrjournals.org

Phytochemical, Antibacterial and Antioxidant Activity of Camellia Sinensis Methanolic and Aqueous Extracts

Noor S. Latteef
Institute of Genetic Engineering and Biotechnology for postgraduate studies / Baghdad University, Baghdad, Iraq.

Abstract: *This study evaluated the antioxidant and antibacterial activity of green tea by using the methanolic and aqueous extract. phytochemical analysis also detected. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl(DPPH) free radical. The methanolic crude extracts of green tea (Camellia sinensis) was the strongest, The EC50 of the methanolic extracts was 2.9 µg/ml and that of ascorbic acid was 2.9 µg/ml. Followed by BHT with value 4.0 µg/ml while The EC50 of the aqueous extracts was 6.5 µg/ml that showed less free radical scavenging activity with the DPPH method. The study reveals that the consumption of green tea has several beneficial effects by virtue of their antioxidant activity.Phytochemical analysis revealed the presence flavonoids, alkaloid, tannins and absence of glycosides, terpens and saponins. Camellia sinensis can inhibit the growth of Gram-positive and Gram-negative bacterial species with moderate potency. The study was carried out on E coli and Staphylococcus aureus, Pseudomonas aeriginosa and Bacillus subtilus and shows that the methanolic extract have better inhibitory effect than aqueous one in a concentration (40 mg/ml) against Escherichia coli and Staphylococcus aureus, the diameter of the inhibition zones was (15and 16 mm) respectively and show a lesser extent to Bacillus subtilus and Pseudomonas aeriginosa.*

Keywords: *Antibacterial, Antioxidant, Camellia sinensis, DPPH, HPLC*

I. Introduction

Great interests has been focused on the natural foods, medicinal plants and phytoconstituants due to their ability to scavenging freed radicals (antioxidant power) (1). Many medicinal plants contain large amounts of antioxidant such as polyphenols, vitamin C, vitamin E, selenium, lycopene and other carotenoids, which play important roles in adsorbing and neutralizing free radicals (2).

One of the advantages of tea is that it has high antioxidant activities due to the presence of polyphenols that enable it to scavenge free radicals. The term green tea refers to the product manufactured from fresh *Camellia sinensis* leaves in which significant oxidation of the major leaf polyphenols known as catechins. Green tea extract has strong antioxidant due to the presence of (+)catechin, (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG) and (-)-epigallocatechin-3-gallate (EGCG). The most significant compounds with antioxidant activity in *Camellia sinensis* leaf are catechins. Catechin is a compound which does not evaporate and it contained about 8-15% from the dry weight of plant (3). The use of catechins as natural antioxidants in oils, fats, animal feeds, animal products (4). The content of catechins decreases within the fermentation procedure employed to produce oolong and black tea, and although new phenolic compounds are formed from catechin condensation, the antioxidant activity decreases, indicating that not only the phenolic content responds this property, but the phenolic profile is very important.

Chinese green tea extracts showed strong inhibitory effect on major pathogens (5). Antimicrobial mode of action of plant extract might be related to their phenolic compounds present. Phenolic compounds are known to be synthesized by plants in response to microbial infection. It is therefore possible that they can act as effective antimicrobial substances against a wide array of microorganisms. However, the antimicrobial activity of plant extracts depend not only on phenolic compounds, but also the property is contributed by the presence of different secondary metabolite like hydroxyl groups on the active constituents. The biologically active constituents of plant extract are considered as antimicrobial agents, because of the ability of these substances to bind to bacterial adhesions and by doing so they disturb the availability of receptors on the surface (6).

The mechanism of active compounds via which they exerts stronger bactericidal effect is attributed to their effect on cellular membranes. Some reports indicated that active constituents might attack the cell wall and cell membrane, thereby destroying their permeability barrier and causing the release of intracellular constituents like ribose and sodium glutamate. Also they interfere with electron transport, nutrient uptake, protein and nucleic acid synthesis and enzyme activity leading to the inhibition of bacterial growth (7).

The aim of the present work was to evaluate the phytochemical composition of *Camellia sinensis* and to assess the antibacterial activities of *Camellia sinensis* extracts (aqueous and methanolic) using in vitro

antibacterial screening techniques, also investigation the antioxidant activities of these extracts using different techniques such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and FTIR (Fourier transform infrared).

II. Materials and methods

2.1 Plant material:

Green tea was purchased from Baghdad markets. The leaves were washed under tap water, identification of the plant was carried out by Dr. Ali Al- Mosawy, Department of Biology, College of Science, University of Baghdad. The sample was powdered and used for the phytochemical study.

2.1.1 Extraction of Plant Material

Preparation of crude aqueous extract: Air dried leaves sample (50 gm) was soaked in 250 ml of water for 24 hr. at room temperature. The suspension was filtered through out filter of gauze to get rid of the large particles then filtered through a filter paper (Whatman no.1). The extracts was concentrated to near dryness under reduced pressure below 40 °C using rotary evaporator (8).

2.1.2 Preparation of methanol extract: A quantity of 50 g of plant powder was extracted with 250 ml of 95% methanol by soxhlet apparatus for 6 hrs at 40- 60 °C, and then evaporated by using a rotary evaporator at 40 °C. The extracts were diluted to 20 mg/ml with 10 % dimethyl sulfoxide (DMS) solution and stored in air tight glass bottles in a refrigerator till further use (9).

2.2 Detection of some active compounds of *Camellia sinensis*

Phytochemical Screening of Plant Extracts was tested to detect Flavonoids, Alkaloids, Tannins, Saponins, glycosides.

- Detection of tannins
  A quantity of (10) g of the plant powder was mixed with (50) ml DDH_2O using a magnetic stirrer. The mixture was left till boiling in a water bath for few minutes, and then filtered through a filter paper (Whatman no. 1). The filtrate was treated with a few drops of 1% lead-acetate solution. The presence of viscous precipitate was an indication of the presence of tannins (10).
- Detection of saponins
  A liquots of 5 ml plant extract was added to (1-3) ml of mercury chloride solution. A white precipitate was developed indicating the presence of saponins (11).
- Detection of flavonoids
  Ethanolic extracts of the plant material was partitioned with petrolium ether; the aqueos layer was mixed with the ammonia solution. The appearance of dark color was an indication for the presence of flavonoids (9).
- Detection of glycosides
  Equal amounts of water extract and Fehling's reagent was mixed in a test tube, then boiled in a water bath for 10 min. The formation of red precipitate indicated the presence of glycosides (10).
- Detection of alkaloids
  A quantity of 10 g of the powder plant material was added to 50 ml of 4% HCl in a steam bath, then 1 ml of the filtrate was treated with Mayer's reagent. The appearance of white precipitate was an evidence for the presence of alkaloids (11).

2.3 Microorganisms and media:

The bacterial isolates *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeriginosa* and *Bacillus subtilus* isolated from patients with food poisoning and wound infection, the bacteria were obtained, as clinical isolates, from Al–Yarmook Teaching Hospital, Baghdad, Iraq. Bacterial cultures were maintained on nutrient agar (NA). Subcultures were made monthly and stored at 4 °C until required for use.

2.3.1 **Culture preparation:** A loop full of 24 hr. surface growth on a Nutrient Agar of each bacterial isolate was transferred individually to 5ml of Brain heart infusion broth (pH 7.6) and incubated at 37°C for 24 hr. bacterial cells were collected by centrifugation at 3000 rpm for 15 min, washed twice and resuspended in 0.1% pepton water. Turbidity was adjusted to match that of as McFarland standard (10^8 CFU/ml). Then 1:10 dilution of the cell suspension was performed to give an inoculums concentration of 10^7(CFU/ml).

2.3.2 Antibacterial assay:

The activities of extracts were determined against bacterial isolates by using modified agar diffusion method. The stock solution was prepared by dissolving (5) g of plant extract residue with (50) ml sterile DDH2O. The extracts were prepared at different concentrations (0, 5, 10, 20 or 40) mg/ml.

The medium was mixed well and 20ml poured in Petri-dishes. The nutrient agar medium was inoculated with 0.1ml of ($1.5*10^5$ cfu/ml) bacterial target isolates of (E. coli, S. aureus *P. aeriginosa* and *B. subtilus*) by using sterile swabs. Five spaced holes 6mm in diameter were made in the agar of each plate with sterile cork borer. To identify the intrinsic extracts activity (water and methanolic extracts for: *Camellia sinensis*), one control well was filled with (100 µl) phosphate buffer saline. Equal volumes of different concentrations (0, 5, 10, 20 or 40) mg/ml of the extracts were dispensed into each well. Plates were then incubated at 37°C for 24 hrs, and zones of inhibition were measured using a ruler in millimeters (12).

2.4 Fourier transform infrared (FTIR) assay: The Functional groups methanolic and aqueous extracts of *Camellia sinensis* (L.) were detected by using fourier transform infrared spectrophotometer (FTIR) and compared with standard value (13).

2.5 High-performance liquid chromatography (HPLC): The contents of catechins in the green tea extracts were analyzed according to the modified method described by (14) *Hu et al.* 2009. Prepared sample analyzed by HPLC (JASCO Co., Tokyo, Japan) using a XTerra RP18 column (3.5 µm, 4.6 × 150 mm, Waters, Milford, MA, USA) at 40 °C and multi-wavelength detector (MD-2010 Plus) was set at 210 nm. The mobile phase was composed of two solution A (0.2% orthophosphoric acid) and solution B (methanol) and eluted with a linear gradient elution of 0 min, 82% A; 15 min, 40% at a flow rate of 1.0 mL/min. Catechins contents were calculated by comparing with an external standard. The calibration curves were constructed by plotting concentrations *versus* peak area and showed good linearity, as shows in figure (6).

2.6 Evaluation of Antioxidant activity: DPPH assay: In order to obtain an indication of the antioxidant activity of *Camellia sinensis*, 5 ml of a freshly prepared 0.004 % of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol was mixed with 50 µl of different concentration of methanolic and aqueous extracts of *C. sinensis* (5 , 10 , 25 , 50 , 75 and 100 mg/ml) and the absorbance of each dilution, after 30 minutes, was measured at 517 nm. Butylated hydroxytoluene (BHT) and vitamin C was the antioxidant used as positive control (15). All tests were performed in triplicate and the methanol was used as blank solution. The percentage DPPH reduction (or DPPH radical scavenging capacity) was calculated as:

% Reduction = (Abs DPPH – Abs Dil.)/Abs DPPHx 100

Whereby: Abs DPPH = average absorption of the DPPH solution;

Abs Dil. = average absorption of the three absorption values of each dilution. With the obtained values, a graphic was made using Microsoft Excel. The EC50 of each extract (concentration of extract or compound at which 50% of DPPH is reduced) was taken from the graphic.

III. Results

3.1 Phytochemical Screening of Plant Extracts:

Different chemical reagents and solutions were used for detection of various active compounds found in the *Camellia sinensis*, in methanolic extract results obtained by chemical detection indicated the presence of flavonoids, tannins and alkaloid while Saponins, terpenes, Glycosides were not detected, but in aquouse extract result showed the presence of flavonoids, Saponins, tannins, alkaloid and absence of terpenes ,Glycosides, displayed in Table (1).

3.2 Antibacterial activity: *Camellia sinensis* methanolic extract exhibited antibacterial activity, against microorganisms at the concentrations (30 and 40 mg/ml). The diameter of the inhibition zones against *E. coli* was (12 and 15 mm) at (30 and 40 mg/ml) respectively. Whereas, decreased to (11 and 13 mm) against *S. aureus*, respectively, Results indicate that the inhibitory effects against *P. aeriginosa* at the concentrations (30 and 40 mg/ml) was (9 and 10 mm) inhibition zones diameter respectively, when (9 and 11 mm) was recorded in the same concentrations against *B. subtilus*.as shown in (table 2).

Results displayed in table (3) indicated that high concentrations of *Camellia sinensis* aqueous extract (30and 40 mg/ml) had inhibitory effects against *S. aureus* with (9 and 13 mm) inhibition zones diameter respectively, when (8 and 11 mm) was recorded in the same concentrations against *E. coli*. The aqueous extract exhibited antibacterial activity against *P. aeriginosa* at the concentrations (30 and 40 mg/ml) the diameter of the inhibition zones was (7 and 10 mm) at (30 and 40 mg/ml) respectively. It was nearly the same when compared to *B. subtilus*.

3.3 Determination of Antioxidant Activity DPPH

Green tea (*Camellia sinensis*) is a well-studied source of polyphenols antioxidants, being the catechins the most abundant among them (16). The free radical scavenging activity measured using the stable free radical 1; 1-diphenyl-2-picryl-hydrayl (DPPH) is one of the main tests used to explore the use of herb extracts as antioxidants (17). Among the extracts and standard tested for the in vitro antioxidant activity using the DPPH method, the methanolic extracts of green tea, (*Camellia sinensis*), showed highest antioxidant activity, with

EC50 values 2.9 µg/ml, The EC50 value for ascorbic acid was 2.9 µg/ml. The results indicate that the antioxidant activity of the methanolic extract of green tea was the same when compared to ascorbic acid. The EC50 value of BHT was 4.0 µg/ml While green tea aqueous extract were found to be less active than ascorbic acid and BHT since their EC50 values was 6.5 µg/ml, These findings showed that methanolic extract exhibited strong antioxidant and higher than aqueous extract. The antioxidant activity is presented in the Figure (1).

3.4 FT-IR Technique:

FTIR Technique is advantageous as a simple and rapid quantitative determining analytical tool for antioxidant activity assays in herbal medicine (18). *Camellia sinensis* leaves extract contain many Functional Groups with Standard groups as shown in table (4). FT-IR spectrum of methanolic and aqueous extracts is shown in Figures (2 and 3).

3.5 High-performance liquid chromatography (HPLC):

HPLC chromatography of methanolic and aqueous extracts of *Camellia sinensis* were analyzed by HPLC (Figure4 and 5). Peak compound identification in *Camellia sinensis* extracts was achieved by comparing HPLC retention times of standards and extracts. Result showed that presence of gallocatechin at retention time (4.170) in methanolic extract and presence of caffeine at retention time (4.900) in aqueous extract of *Camellia sinensis.*

IV. Discussion

Phytochemical analysis revealed the presence flavonoids, alkaloid, tannins and absence of glycosides, terpens and saponins in methanolic extract but in aquouse extract showed the presence of flavonoids, Saponins, tannins, alkaloid and absence of terpenes, Glycosides, This result was agree with lee *etal*., 2004 (19) who show that the constituents like tannins, reducing sugars and proteins present in the green tea extracts may be responsible for antioxidant activity. The phytochemical tests indicated the presence of alkaloids, tannins, and flavonoids in the crude methanolic extract

Camellia sinensis can inhibit the growth of Gram-positive and Gram-negative bacterial species with moderate potency. The study was carried out on *E coli* and *Staphylococcus aureus*, *Pseudomonas aeriginosa* and *Bacillus subtilus* and shows that the methanolic extract of *Camellia sinensis* has better inhibitory effect than aqueous one. This result was agreed with Yam *etal*. 1997 (20) who showed that methanolic extract of *Camellia sinensis* had greater antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. In a similar study, the liquid extracts of green tea showed inhibitory effects on some Gram-positive as well as Gram-negative bacteria (21). And agreed with Toda *etal.* 1990 (22) who reported that daily consumption of green tea can kill Gram positive *Staphylococcus aureus* and other harmful bacteria. Also it has been reported that the green tea contain catechin and polyphenols. These compounds have been found to possess antibacterial and antiviral action as well as anticarcinogenic and antimutagenic properties. It was proved that green tea has anticancer and anti hypercholesterole activities, it has also anti-bacterial activity that includes inhibition of Gram positive cocci, Gram negative. Antimicrobial effect of plant extracts depends on pH and solubility of the extract in the model systems (23). Plant extracts that are having low pH are more effective in inhibiting the microbial growth (24). Various studies showed significant suppressive effects of green tea against many microorganisms, for example *Salmonella typhimurium , Salmonella typhi, Shigella dysenteriae*, *Yersinia enterocolitica, E. coli*, *S. aureus*, *Vibrio cholerae*, *Campylobacter jejuni*, *Plesiomonasshigelloides*, *P. aeruginosa* and many other species of bacteria (25) (26).

Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl(DPPH) free radical. The methanolic crude extracts of green tea (*Camellia sinensis*) was the strongest but the aqueous extracts showed less free radical scavenging activity with the DPPH method. This result was agreed with (27) who said that the antioxidant activity of methanolic extract of green tea nearly the same when compared to ascorbic acid.

Result showed that presence of gallocatechin at retention time (4.170) in methanolic extract and presence of caffeine at retention time (4.900) in aqueous extract of *Camellia sinensis*.

This result was agree with (28) how showed that showed that major compounds were identified and quantified from green tea with different plucking periods, this compound is(theanine at retention time(1.918), theobromine at (2.820), gallic acid at (3.283), (+)-gallocatechin at (4.172), caffeine at (4.998), (-)-epigallocatechin at (5.697), (+)-catechin at (6.188), (-)-epicatechin at (7.577), (-)-epigallocatechin gallate at (9.028), (+)-gallocatechin gallate at (10.388), (-)-epicatechin gallate at (10.319) and (+)-catechin gallate at (12.215) as shown in Fig. (6).

V. Tables and Figures

Table (1): Phytochemical analysis of the leaves extracts of *Camellia sinensis*

Phytochemical test	Mathanolic extract	aquouse extract
Tannins	+ve	+ve
Saponins	-ve	+ve
Flavonoids	+ve	+ve
Glycosides	-ve	-ve
Terpenes	-ve	-ve
Alkaloids	+ve	+ve

Table (2): Antibacterial activity of methanolic extract on some pathogenic bacteria

Zone diameter(mm)				**Bacterial Isolates**
Methanolic Extract cons.(mg/ml)				
40	**30**	**20**	**10**	
15	**12**	**10**	**7**	*Escherichia coli*
10	**9**	**7**	**-**	*Pseudomonas aeriginosa*
13	**11**	**9**	**8**	*Staphylococcus aureus*
11	**9**	**6**	**-**	*Bacillus subtilus*

(-) = no Inhibition

Table (3) : Antibacterial activity of aqueous extract on some pathogenic bacteria

Zone diameter(mm)				**Bacterial Isolates**
Aqueous Extract cons.(mg/ml)				
40	**30**	**20**	**10**	
11	**8**	**6**	**-**	*Escherichia coli*
10	**7**	**-**	**-**	*Pseudomonas aeriginosa*
13	**9**	**6**	**-**	*Staphylococcus aureus*
10	**7**	**-**	**-**	*Bacillus subtilus*

(-) = no Inhibition

Table (4): Frequencies of IR absorption of *Camellia sinensis* leaves extracts

I.R. Frequen cies of Aqueous extract	**I.R.Frequencies of Methanoli c extract**	**I.R Frequencies Standard Groups (cm-1)**	**The Functional Group**
3356.14	**3379.29**	**3200-3600**	**Phenolic– OH group stretching**
2935.66	**2927.94**	**2850-3000**	**C-H Stretch**
1608.63	**1627.92**	**1400-1600**	**Aromatic C=C**
1365.60	**1365.60**	**1345-1385**	**N-O Stretch**
1049.28	**1238.30**	**1000-1300**	**Aliphatic C–O**

Figure (1): The reduction percentage of DPPH using methanolic and aqueous extracts of *Camellia sinensis*

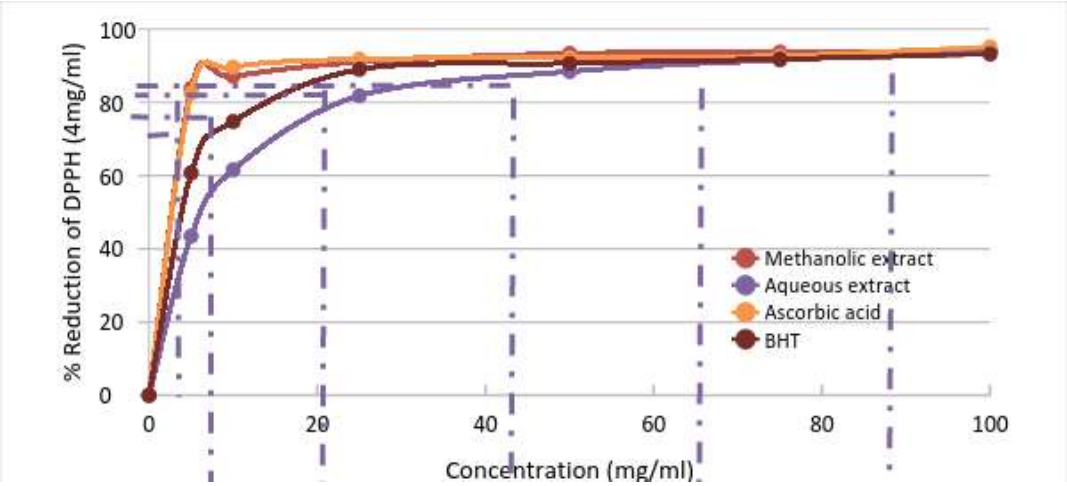


Figure (2): The infrared spectrum of methanolic extract

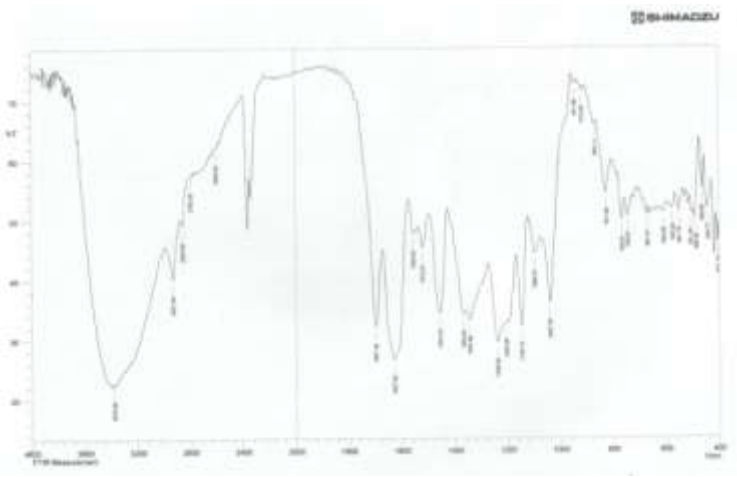


Figure (3): The infrared spectrum of aqueous extract

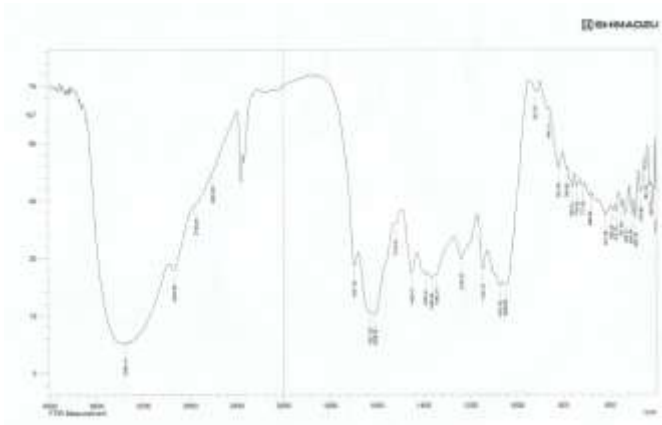


Figure (4): HPLC quantification of the methanolic extract

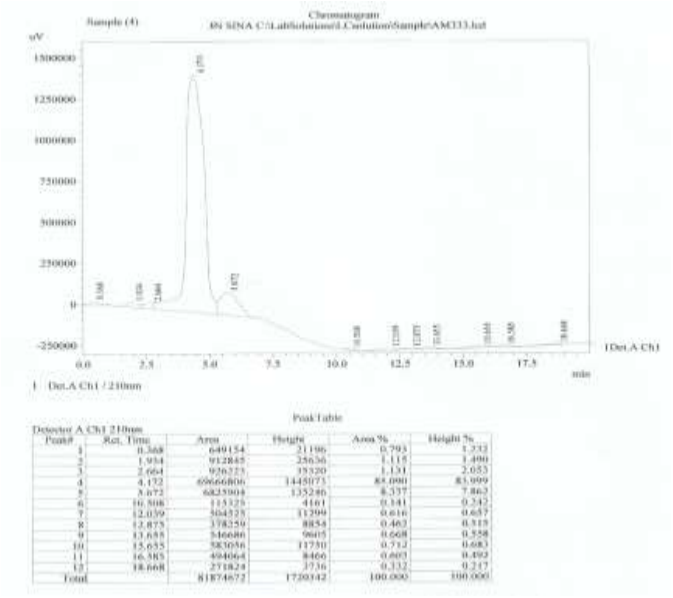


Figure (5): HPLC quantification of the aqueous extract

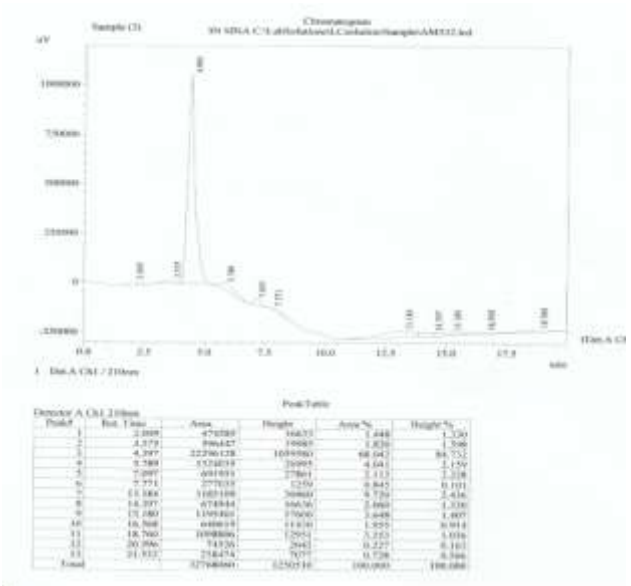
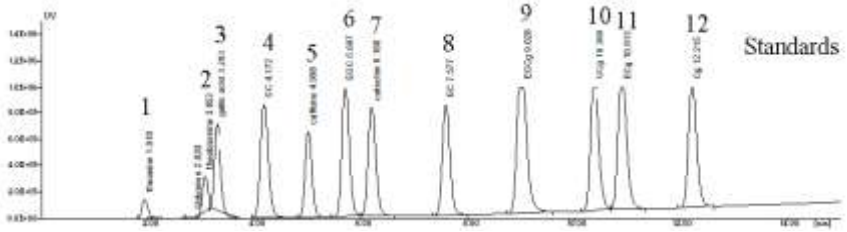


Figure (6): HPLC chromatograms of standard solution with different plucking periods.



VI. Conclusion

The results of the present study showed that the green tea (*Camellia sinensis*) leaves extracts could be used as natural antioxidant. The results demonstrated that green tea has antibacterial activity against many of pathogenic bacteria. In addition to the methanolic extracts showed antibacterial and antioxidant activity more than aqueous extract. Therefore, further studies are recommended for the isolation and purification the phytochemicals from methanolic extract which have great antibacterial and antioxidant activity.

References

[1]. Kukic, J,.Petrovic J and Niketic. antioxidant activity of tour endemic stachy a taxa. *Bio, pharma. Bus. 29*, 2006, 725-729.
[2]. Astley, S.B. Dictary antioxidants. Past, present and future. Trends. *Food. Sci. Technol. 14*, 2003, 93-98.
[3]. Farhoosh, R., Golmovahhed, G. A. and Khodaparast, M. H. H. Antioxidant activity of various extracts of old tea leaves and black tea wastes (*Camellia sinensis* L.). *Food Chemistry. 100*, 2007, 231 – 236.
[4]. Yilmaz, Y. Novel uses of catechins in foods. *Trends in Food Science & Technology. 17*, 2006, 64–71.
[5]. Si, W., Gong, J., Tsao, R., Kalab, M., Yang, R. and Yin, Y. Bioassay-guided purification and identification of antimicrobial components in Chinese green tea extract. *Journal of Chromatography A.* 1125, 2006, 204-210.
[6]. Branter, A., Males, Z. &Antolic, A. Antimicrobial activity of paliurus spina-christi Mill.(christis thorn). *Journal Ethnopharmacol . 52*, 1996, 119-122.
[7]. Zhang,Y.M., White, S.W.&Rock, C.O. Inhibiting bacterial fatty acid synthesis. *Journal Biol. Ghem*. 281, 2006, 17541-17544.
[8]. Mingarro, D. M., Acero, N., Linares, F., Pozuelo, J. M., Mera, A. C. and Peres C. Biological activitiesfrom Catalen bignonoides walt. (Bignonieceae). *Journal Ethnopharmacol 87*, 2003, 163-167.
[9]. Harbone, J. B. Phytochemical methods. Aguide to modern techniques of plant analysis. Chapman and hall: London. 1973.
[10]. Shihata, I. M. A Pharmacological study of Aangalis arvensis, M. D. Vet. Thesis, Cairo University, Egypt 1951.
[11]. Stahl, E. Thin-Layer-Chromatography, Laboratory Hand book, 2nd ed. Translated by Ashworth, M. R. F. Spring. Verag, Berlin, Heidelberg, New York, U.S.A. 1969.
[12]. Nathan, C. In vitro activity of Sch 21420, derivative of gentamicin. B compared to that of amikacin antimicrob. Agents Chemother. 1978; 14: pp 786-787.
[13]. Ilverstein, R. M.; Bassler, G. C.; and Morrill, T. C. Spectrometric Identification of Organic Compounds. 4th ed. New York: John Wiley and Sons 1981; QD272.S6 S55.
[14]. Hu, B.; Wang, L.; Zhou, B.; Zhang, X.; Sun, Y.; Ye, H.; Zhao, L.; Hu, Q.; Wang, G.; Zeng, X. Efficient procedure for isolating methylated catechins from green tea and effective simultaneous analysis of tea catechins, three purine alkaloids, and gallic acid in tea by high-performance liquid chromatography with diode array detection. *Journal Chromatogr.* 1216, 2009, 3223–3231.
[15]. Huang, D., Ou, B. and Prior, R. L. The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry. 53*, 2005, 1841–1856.
[16]. Yokosawa, T.; Nakagawa, T.; Kitani, K. Antioxidative activity of green tea polyphenol in cholesterol-fed rats. *J. Agric. Food Chem. 50*, 2002, 3549–3552.
[17]. Parejo, I.; Viladomat, F.; Bastida, J.; Rosas-Romero, A.; Saavedra, G.; Murcia, M. A.; Jiménez, M.; Codina, C. Investigation of Bolivian plant extracts for their radical scavenging activity and antioxidant activity. *Life Sci.* 73, 2003, 1667–1681.
[18]. Andrei A. Bunaciu, Hassan Y. Aboul-Enein & Serban Fleschin. FTIR Spectrophotometric Methods Used for Antioxidant Activity Assay in Medicinal Plants. *Applied Spectroscopy Reviews.* 47(4), 2012, 245-255.
[19]. Lee J, Koo N, Min DB. Reactive oxygen species, aging and antioxidative neutraceuticals. *Comp Rev Food Sci Food Safety*. 3, 2004, 21-33.
[20]. Yam T.S., Shah S. and Hamilton-Miller J.M.T. FEMS *Microbiology Letters*. 152, 1997, 169-174.
[21]. Gadang, V.P., Hettiarachchy, N.S., Johnson, M.G. and Owens, C.M. Evaluation of antibacterial activity of whey protein isolate coating incorporated with nisin, grape seed extract, malic acid, and EDTA on a turkey frankfurter system. *Journal of Food Science. 73*, 2008, 389-394.
[22]. Toda M, Okubo S. and Shimamura T. Antibacterial and bactericidal activities of Japanese green tea. Jpn. *Journal Bacteriol*. 00(0), 1990, 009-072.
[23]. Hao, Y.Y., Brackett, R.E. andDoyle, M.P.Inhibition of Listeria monocytogenes and Aeromonas hydrophila by plant extracts in refrigerated cooked beef, *Journal of Food Protection. 61*, 1998, 307-312.
[24]. Conner, D.E. and Beuchat, L.R .Effects of essential oils from plants on growth of food spoilage yeasts. *Journal of Food Science*. 49, 1984, 429-434.
[25]. Shetty M., Subbannayya K. and Shivananda P.G. Antibacterial activity of tea *Camellia sinensis* and coffee Coffeearabica with special reference to *Salmonella typhimurium. Journal Commun. Dis.*, 20, 1990, 107-152.
[26]. Yam T.S., Hamilton-Miller J.M. and Shah S. The effect of a component of tea *Camellia sinensis* on methicillin resistance. PBP2' synthesis and beta-lactamase production in Staphylococcus aureus. *Journal Antimicrob. Chemother*. 02, 1990, 211-210.
[27]. Nooman A. KHALAF, Ashok K. SHAKYA, Atif AL-OTHMAN, Zaha EL-AGBAR, Husni FARAH. Antioxidant Activity of Some Common Plants. *Turk J Biol. 32*, 2008, 51-55.
[28]. Lan-Sook Lee, Sang-Hee Kim, Young-Boong Kim and Young-Chan Kim. Quantitative Analysis of Major Constituents in Green Tea with Different Plucking Periods and Their Antioxidant Activity, *Molecules* 19, 2014, 9173-9186.