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A Comparative Studies on Antimicrobial Activity and Antioxidant Activity of Different Extracts of Green Tea *Camellia sinensis (*L.) Kuntze Leaf from Southern Shan State (Myanmar)

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Abstract

Green tea has many well-known effects and benefits. It has anti-diabetic, hypocholesterolemic, anti-inflammatory, anti-carcinogenic, anti-cavity, thermogenic, probiotic, antimicrobial and antiviral properties. In this research work, Green Tea *Camellia sinensis* (L.) Kuntze leaf was collected from Panglong area, southern Shan state ,Myanmar. In the present research work, according to the preliminary phytochemical investigation, alkaloids, phenolic compounds, flavonoid, saponin, glycoside, tannins, reducing sugar, carbohydrate and ὰ-amino acid were found to be present in green tea. The yield percentage (%) of green tea different extracts of H_2O, MeOH, EtOH and pet ether were 40, 15, 10 and 8 respectively .The vitro antimicrobial activity of different extracts (EtOH , MeOH and H_2O) of green tea was screened by Agar well diffusion method. In the present work, the tested microorganisms were *Bacillus sublitis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus,Candida albicans and Escherichia coli.* The antimicrobial activity against six microorganisms with the inhibition zone diameter ranged between 15 mm – 20 mm. The Antioxidant activity were determined by DPPH assay method. IC_{50} value of MeOH, EtOH and H_2O extracts of green tea were 13.57 μgmL^{-1}, 7.10 μgmL^{-1} and 5.21 μgmL^{-1} respectively.

Keywords: Camelliasinensis; phytochemical; antioxidantactivity and antimicrobial; activity.

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1. Introduction

Tea, derived from *Camellia sinensis (L.),* is one of the most widely consumed beverages in the world. Green tea has many well-known effects and benefits. It has anti-diabetic, hypocholesterolemic, anti-inflammatory, anti-carcinogenic, anti-cavity, thermogenic, probiotic, antimicrobial and antiviral properties [2,3].Green tea's antimicrobial properties are effective against a variety of microbes, which include *Helicobacter pylori* (gastric malignancy), *Staphylococcus aureus* (MRSA), *Oral streptococci* (dental caries), *Mycobacterium tuberculosis* (tuberculosis), *Bacillus cereus* (food poisoning), *Escherichia coli 0157*(severe diarrhea and kidney failure), *Legionella pneumphila* (pneumonia), *Candida albicans* (candidiasis), and Chlamydia trachomatis (chlamydia) [7].Its antiviral properties are effective against HIV, influenza, Epstein-Barr, Herpes, Hepatitis B and C, and Human T-cell lymphotropic virus type 1 (HTLV-1; leads to adult T-cell leukemia) [7]. Green tea helps to prevent or treatcancer in people .Daily consumption of black tea (but not green tea) has been associated with a significant reduction in death from all cancers. Green tea has been associated with a lower risk of death from cardiovascular disease.. There is no conclusive evidence that green tea aids in weight loss. Tea can be categorized into three main type , depending on the level of oxidation , as green tea , oolong tea and black tea [5]. Green tea is an ever green plant that grows primarily in tropical and temperate regions of Asia which mainly include China, India, Srilanka and Japan .The strain produces green, white, black and oolong teas. Green tea is a small shrub that can expand up to 30 feet high, but is customarily trimmed to 2-5 feet when cultivated for its leaves. The leaves are naturally murky green and glossy with notched edges and are 2-5cm broad and 4-15cm long. The flowers are white and contain bright yellow stamps. The immature, light-green leaves are preferably harvested for tea production. Mature leaves are deeper green in color than the young leaves. Different leaf ages produce varying tea qualities as their chemical compositions are different. These basic types of tea have different quality characteristics, including appearance, aroma, taste, and color. The manufacturing process of tea is designed to either preclude, or permit tea polyphenolic compounds to be oxidized by naturally occurring polyphenol oxidase in the tea leaves. Green tea is produced by inactivating the heat-labile enzyme polyphenol oxidase in the fresh leaves by either applying heat or steam, which prevents the enzymatic oxidation of catechins, the most abundant flavonoid compounds present in green tea extracts [6]. The tea leaves are then rolled, dried and packaged. The medicinal effects of tea have a history dating back almost 5000 years. The chemical components of green tea chiefly include polyphenols, caffeine and amino acids. Tea also contains flavonoids, compounds reported to have antioxidant properties having many beneficial effects. Tea flavonoids reduce inflammation, have antimicrobial effects and prevent tooth decay. Consumption of tea may have diuretic effects due to the caffeine. The main green tea polyphenols are catechins; (+,-)catechin C, (-) - epicatechin EC, (+) - gallocatechin GC, (-) - epigallocatechin EGC, (-)- epicatechin gallate ECG. Antioxidant and antimicrobial activity of these compounds and crude extracts of tea are described. The review hightlights also the potentials of green tea, its health benefits in terms of antimutagenic and anticarcinogenic properties as well as protective agents against cardiovascular diseases. . Traditionally, green tea has been used for a variety of medicinal purposes, such as the prevention and treatment of a variety of cancers, mental alertness, weight loss, lowering cholesterol level, and UV protection. Studies have shown that catechins, the polyphenols phenol found in tealeaves, are effective as anti-infectious agents by affecting the infection process instead of specifically targeting the virus. This treatment strategy has the potential of reducing the prevalence of drug-resistant viruses

and the reliance on anti-viral drug therapies. This paper will explore the efficacy of green tea in preventing infections by the hepatitis B and C, influenza and humanimmuno deficiency virus.

2. Materials and methods

Collection of leaves

The green tea leaves *Camellia sinensis* (L.) Kuntze was chosen for the present research. The sample was collected from Panglong Township, Loilem District, Southern Shan State , Myanmar. The collected sample was identified in Department of Botany, University of Pang Long.

Chemicals

All chemicals used in this work were from British Drug House Chemical Ltd., Poole, England. All standard solutions and other diluted solutions throughout the experimental runs were prepared by using distilled water. In all the investigations the recommended methods and standard procedures involving both conventional and modern techniques were employed [8]. DPPH(2,2-diphenyl,1-picrythydrazyl) radical, gallic acid ,ascorbic acid and Folin –Ciocalteau reagent were obtained from Sigma-Aid-rich, USA . All other chemicals and reagents used were of analytical grate.

Preparation of leave extracts

The leave of green tea *Camellia sinensis* (L.) kuntze was chosen for the present research. The sample was collected from Pang Long area, Southern Shan State, Myanmar .The collected sample was cleaned by washing with water and then dried at room temperature. The dried sample was ground into parely fine powder by using an electric grinder. The powdered sample was labeled and stored separately in air tight plastic bottle to prevent moisture and other contaminations. The powdered material (15)g was extracted with 100ml of water ,ethanol, and methanol separately .The contents were kept as such in room temperature for 48h with constant stirring at regular intervals After the incubation period, the contents were filtered through Whatman No.1 filter paper. Then filtrate were vacuum dried using rotary evaporator and concentrates were store d at 4°C.The residues were redissolved with the appropriate solvents from which they were prepared and used for further studies.

Preliminary Phytochemical analysis

Qualitative phytochemical analyses were performed in filtrates of *Camellia sinensis* (L.) kuntze green tea leaf preliminary phytochemical test were carried out according to determine the presence of phytochemicals the alkaloid, α-amino acids, flavonoids, phenolic compounds, glycosides, and saponins as described by standard procedure.

Test organism

Screening of antimicrobial activity of various extracts such as 95% EtOH, MeOH and watery extract of green

tea *Camellia sinensis* (L.) kuntze. sample was investigated by Agar Well Diffusion Methods. In the present work, the test microorganisms were *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli.*

Preparation of inoculum

The microorganisms were inoculated into nutrient broth and rose Bengal broth for bioassay and incubated for 24 and 48 h at 37°C. The turbidity of the medium indicates the growth of organisms.

Antimicrobial studies

The agar well diffusion method was employed for the determination of antimicrobial activity of extracts. Lawn culture of *E.coli, Candida albican, Bacillus puimilus, psrudomonus aeruginosa, Staphylococcus aureus* and *Bacillus subtils* were spread on nutrient agar and A niger & A flavus spread on rose bengal agar using sterile cotton swabs. The wells (6mm in diameter) were cut from the agar plates using a cork horer. 30µlof the extracts(7mg/ml)were poured into the well using a sterile micro pipette .The plates were incubated at 37±2°C for 24 hours for bacterial activity and 48 hours for fungal activity .The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

DPPH (2,2-Diphenyl-1-picryl-hydrazyl) radical scavenging activity

The ability of the extract to scavenge DPPH radical was determined according to the method described by [4].One ml of a 0.3 m M DPPH methanol solution was added to a solution of the extract or standard (250µg/ml,2.5ml) and allowed to read at room temperature for 30 min. The absorbance of the resulting mixture was measured at 518nm and converted to percentage antioxidant activity (AA%).Methanol (1.0 ml) plus extract solution (2.5ml) was used as a blank 1 ml of 0.3 mM DPPH plus methanol (2.5ml) was used as a negative control .Solution of gallic acid served as positive control.

3. Results and dissuasions

For the purpose of quality control ,assessment of purity and identification of any sample ,standardization is much essential .The standardization of a crude drug is an integral part for establishing its correct identity. Standardization including physicochemical evaluation is meant for identification, authentication and detection of adulteration and complication of quality control of crude drugs. The physical constant evaluation of the drug is an important parameter in detecting improper handling of drugs. The medical plants are rich in secondary metabolites which include alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, tannins and reducing sugar, α-Amino acid were found to be present. They are of great medicinal value and have been extensively used in drug and pharmaceutical industry. The extraction of green tea *camellia sinensis* (L.)Kuntze test was carried out according to the standard procedures. According to the experiment polor extract is more potent than the non polor extract the result obtained from these experiment are showed in Table (1) and figure (2). Medicinal value of plants depends on the bioactive phytocomponents in plants associated to antimicrobial activities. Hence ,there is a need to focus on the traditional medicines which can serve as novel

therapeutic agents. The increased frequency of resistance to commonly used antibiotic leads to the search for new effective and easily affordable drugs in the management of infections diseases. In the present study, Screening of antimicrobial activity of various crude extracts such as 95% EtOH, MeOH and watery extract of green tea Camellia sinensis (L.)Kuntze sample was investigated by Agar Well Diffusion Methods. In the present work, the tested microorganisms were *Bacillus sublitis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus,Candida albicans and Escherichia coli* .From this experiment, it was found that the antimicrobial activity against six microorganisms with the inhibition zone diameter ranged between 15 mm – 20 mm. In addition, watery extracts was more potent antimicrobial activity against six microorganisms. MeOH and EtOH extracts were found to exhibit antimicrobial activity 15mm-18mm against six microorganisms. The results of inhibition zone diameters are described in Table (2) and Figure (3). The antioxidant activities of 95% ethanol extract and watery extract were studied by DPPH method. This method is based on the reduction of colored of free radical DPPH in ethanolic solution by different concentrations of sample. The antioxidant activity was expressed as 50% oxidative inhibitory concentration (IC_{50}). The lower the IC_{50} values, the higher the antioxidant activity of the sample In this experiment, each sample was dissolved in ethanol to get 0.2 mg/mL. concentration and then it was diluted with ethanol to obtain 200, 100, 50, 2512.5, 6.25, 3.125, 1.56, 0.78,0.39µg/mL concentration. After mixing with DPPH solution, the absorbance of each solution was measured at 517nm. The IC_{50} values for each sample were determined by linear regressive excel program. By using DPPH free radical scavenging assay, watery extract was found to the most potent antioxidant activity than 95% ethanol extract and methanol extract. The results of antioxidant activity are shown in Table (3) and Figure (4,5).

4. Conclusion

The presence of phytochemicals such as alkaloids, phenolic compounds ,flavonoid, saponin, glycoside, tannins, reducing sugar, carbohydrate and ὰ-amino acid confirms the antiviral, antibacterial, antiparasitic and anti-inflammatory properties in green tea . This further revealed or provided some biochemical basis for the ethno pharmacological uses of the leaves. It can be deduced that green tea may be used as antioxidant in reducing of oxidative stress and some aged related orders. The fiber content was found to be the lowest amount in green tea. Green tea carry micronutrients that feed the immune system becomes stronger.

Table 1: Yield Percent of Green Tea extracts from Non Polar to Polar Solvent

No.	Solvent	Yield (%)
1	Petroleum ether	8
2	Methanol	10
3	Ethanol	15
4	Water	40

(a) (b)

(c)

Figure 1: (a) flower, (b) leave and (c) the whole plant of green tea in Panglong Tawnship

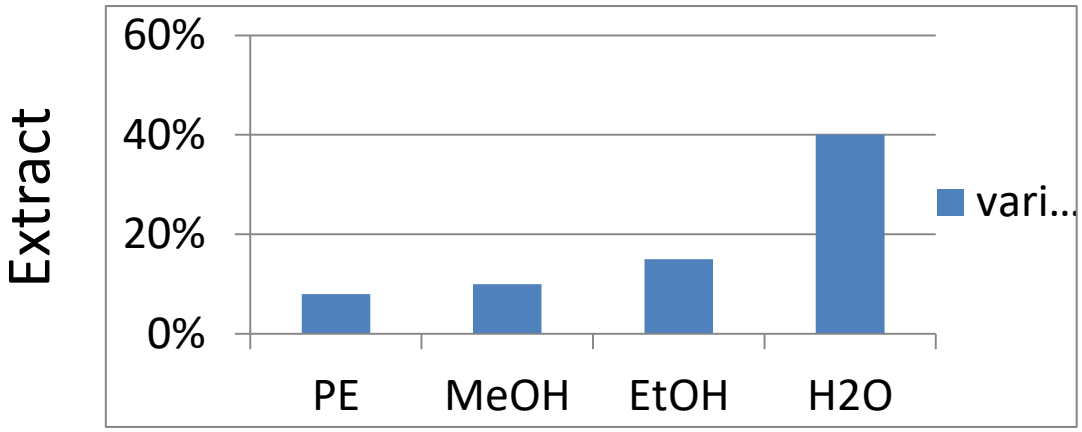

Figure 2: Bar graph of yield percent of green tea from non polar to polar solvent

Green tea has little caffeine and is one of the mildest of the teas , there are no adverse side effects. Pathogenic microorganisms are big threat for the human health as they show resistance to drugs. So, green tea is a pleasant, popular socially accepted and safe drink that is initially as medicine, later as beverage and now proven well as future potential of becoming an important pharmaceutical raw material. Therefore, watery extract of green tea can be used as medicinal purpose especially antimicrobial and antioxidant because green tea is more soluble in water than other solvent.

Table 2: Diameter of Inhibition Zone of Crude Extracts on Different Bacterial Strains

No	**Type of bacteria**	**Diameter of inhibition zone (mm)**		
		H_2O extract	**MeOH extract**	**EtOH extract**
1	*Bacillus Sublilis* (N.C.TC.8236)	20 (+++)	17 (++)	17 (++)
2	*Staphylo cocus aureus* (N.C.P.C.6371)	20 (+++)	15 (++)	14 (++)
3	*Pseudomonas aeruginosa* (6749)	20 (+++)	15 (++)	15 (++)
4	*Bacillus pumilus* (N.C.I.B.8982)	20 (+++)	15 (++)	15 (++)
5	*Candida albicans*	20 (+++)	15 (++)	15 (++)
6	*Escherichia Coli* (N.C.I.B.8134)	20 (+++)	14 (++)	14 (++)

Agar well- 10 mm , 10mm - 14mm (+) , 15 mm -19 mm (++) , 20mm above (+++)

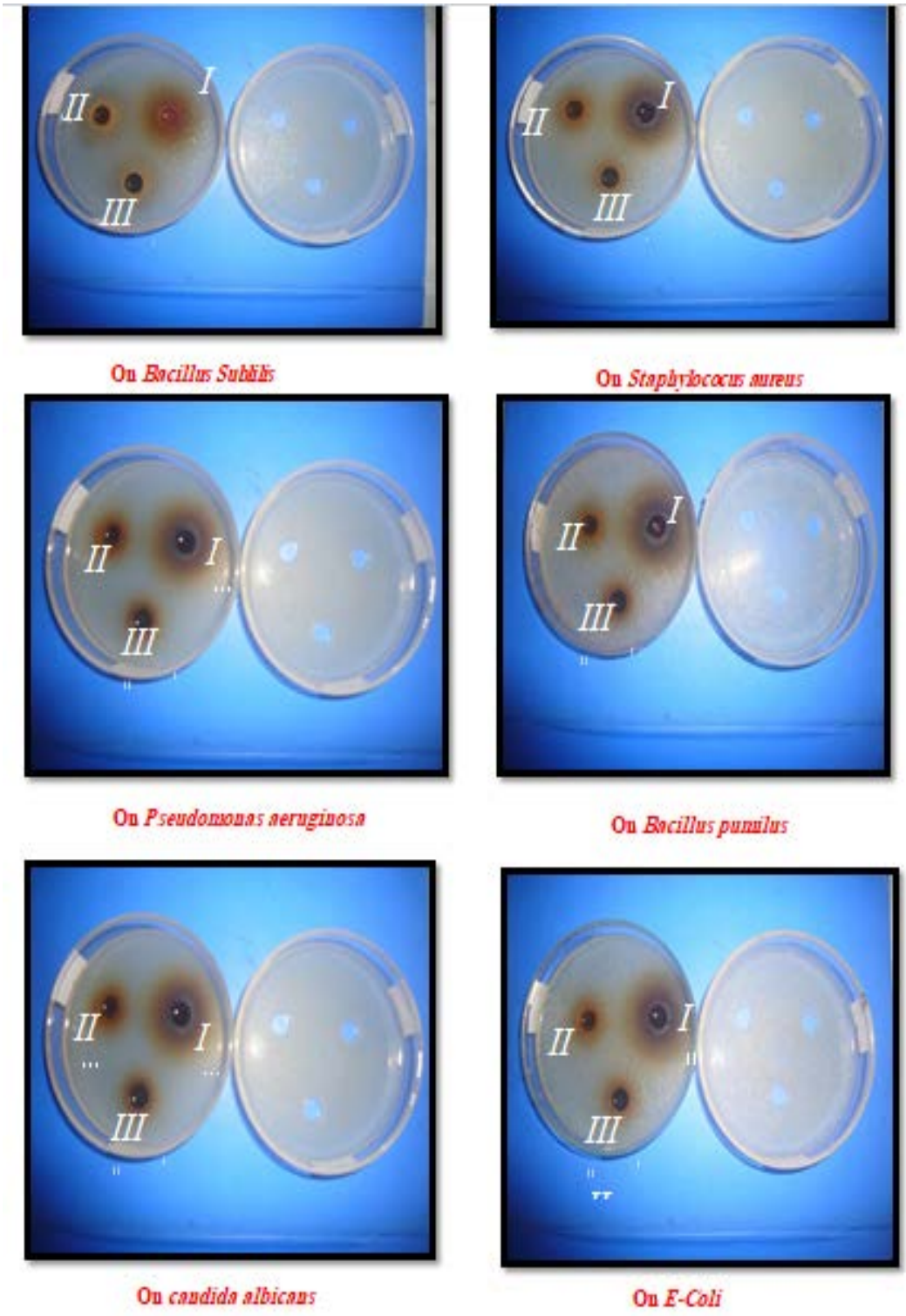

Figure 3: Antimicrobial activities of different extracts tested by agar well diffusion method

I = watery extract , II = Ethanol extract , III = Methanol extract

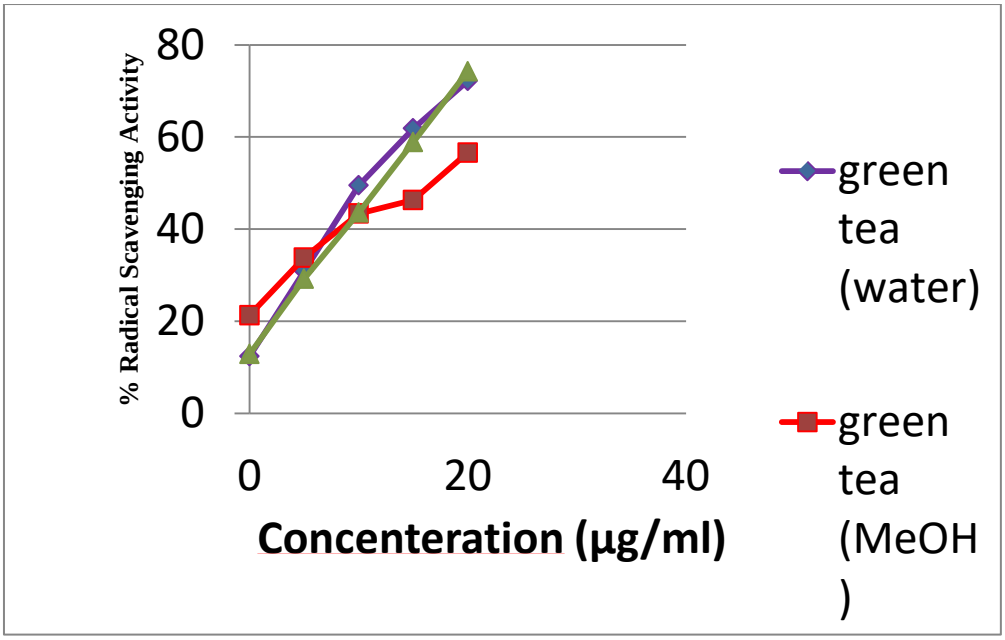

Figure 4: Radical scavenging activity of different concentrations of different extract of green tea

Table 3: Radical Scavenging Activity (IC_{50}) of Watery extract, MeOH extract and EtOH extract of Green Tea and Ascorbic Acid

Tested Sample	**% RSA (mean ± SD) In different concentration (µg/ml)**					**IC_{50} (µg/ml)**
	1.25	2.5	5	10	20	
Green tea (Water)	12.37 ± 1.46	29.21 ± 1.46	49.48 ± 1.46	61.86 ± 1.46	72.16 ± 1.46	5.21
Green tea (MeOH)	21.32 ± 3.12	33.82 ± 2.08	43.38 ± 1.04	46.32 ± 1.04	56.62 ± 3.12	13.57
Green tea (EtOH)	12.87 ± 1.40	29.21 ± 2.10	43.56 ± 2.80	58.91 ± 2.10	74.26 ± 0.00	7.10
Ascorbic acid	53.58 ±0.88	65.53 ±1.13	74.82 ±0.59	83.32 ±0.78	91.21 ±0.48	1.17

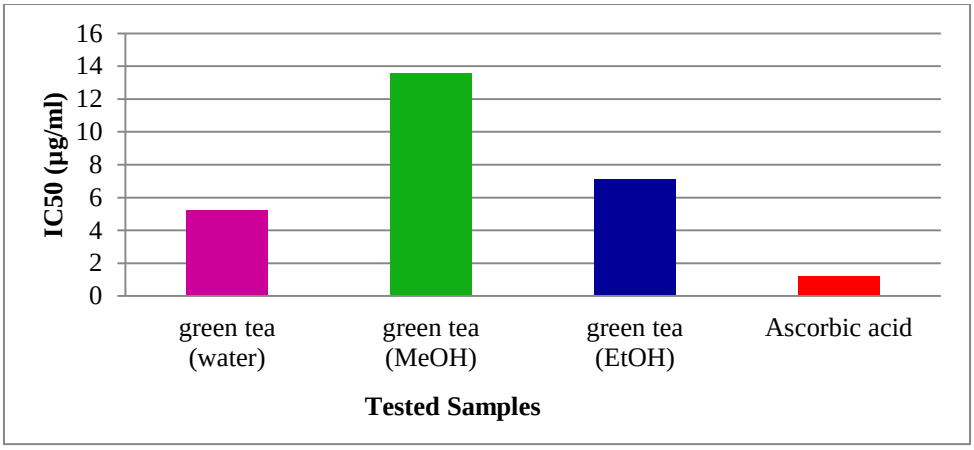

Figure 5: A bar graph of IC_{50} (µg/ml) of watery extract , MeOH extract and EtOH extract of green tea and ascorbic acid

Tea is the most popular drink after water. Increasing interest in its health benefits has led to the inclusion of green tea in the group of beverages with functional properties .Nowadays, green tea is considered one of the most promising dietary agents for the prevention and treatment of many diseases. The literature available suggests that aqueous extract of the green tea which mainly consists of catechins (EGCG, EGC, ECG and EC) possess antioxidant, antimutagenic, antidiabetic, anti-inflammatory, antibacterial and antiviral, and above all, cancer-preventive properties. Epidemiological studies suggest that consumption of green tea may have a protective effect against the development of several cancers. The tea also contains polyphenols which helps in decreasing the risk factor of specific type of cancers by inducing phase I and phase II metabolic enzymes that increase the formation and excretion of detoxified metabolites of carcinogens. Moreover the studies also reveal that regular green tea consumption has beneficial effects and it shows a significant rate of protection against the development of some oral diseases and against solar radiations. It also contributes to body weight control and to the rise of bone density as well as being able to stimulate the immune system. Most modern medicines used to treat cancer have serious side effects, high costs, and other associated risks. Green tea, on the other hand, is safe and widely available as a beverage and a nutritional supplement. Furthermore, growing scientific evidence suggests that green tea is effective in preventing many diseases associated with aging, including prostate and other cancers. Overall tea is an affordable beverage of natural origin compared to modern beverages such as soft drinks. It is yet promising area of research for future human studies.

References

[1]. AOAC, (2002).“Official Method of Analysis”, International Food Research Journal, **17**, 426–432.

[2]. Agarwal S, Maki KC, Rains TM: Antiobesity effect of green tea catechins: a mechanistic review. Journal of Nutritional Biochemistry 2010; 22: 1-7.

[3]. Ahmad I, Aqil F, Mehmood Z: Antioxidant and free radical scavenging properties of twelve

traditionally used Indian medicinal plants. Turkish Journal Biology 2006;30: 177-183.

[4]. Ashokkumar, R. and Ramaswamy, M. (2013). "Determination of DPPH Free Radical Scavenging Oxidation Effects of Methanolic Leaf Extracts of Some Indian Medicinal Plant Species", Journal of Chemical, Bological and Physical Sciences, **3** (2), 1273 – 1278

[5]. Call TG, Shanafelt TD: Phase 1 trial of daily oral polyphenone in patients with asymptomatic rai stage 0 to 2 chronic lympocytic leukemia. Jounal of Clinical Oncology 2009; 27: 3808-3814.

[6]. Glucin I: The antioxidant and radical scavenging activities of green tea and black pepper seed, International Journal of Food Science and Nutrition 2005;56: 491-499.

[7]. Tariq ,A.L.,Nirjantha, D and Reyaz, A.L,(2013),"Antimicrobial activity of Camellia sinensis leaves against gram positive and gram negative bacteria",World research journal of pharmaceutical research ,**1**,1-4

[8]. Vogel, A.I., (1978**),** Vogels Text Book of Practical Organic Chemistry, 4th Edition, 1984 Reprint LRS/Londons, England, 280