Original Article

Structural Analysis and Evaluation of Antimicrobial Activity of *Camellia sinensis* Aqueous Extract

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ABSTRACT

Introduction: The study is focused to determine the properties (antimicrobial, antioxidant, anticancer and phytochemical) of the *Camellia sinensis.* In this study the extract of *Camellia sinensis* performed to assess the antimicrobial potential against skin associated microorganisms. Antioxidant activity performed in the plant *Camellia sinensis* play a protective major role in different types of cancer, heart diseases and stroke.

Objective: To identify the rich components present in green tea leaves aqueous extract by phytochemical methods and then it is useful as drug for various human effects and problems.

Methods: The tea leaves extract was subjected to phytochemical tests, Gas chromatography-mass spectroscopy (GC-MS), Fourier transform infrared spectroscopy (FTIR) analysis and antimicrobial test. Phytochemical analysis of Green tea leaves (*Camellia sinensis)* was performed and the result showed that the presence of phenolic compounds (alkaloids, flavonoids, steroids, triterpenoids and tannins). Antimicrobial property was performed with two different gram-positive and gram-negative bacteria.

Result: Laboratory studies shows that herbs like *Camellia sinensis* (green tea leaves) can be effectively used in curing bleeding, mental health, regulate body temperature, healing wounds, and to aid digestion. It also shows positive impact on types of cancer such as breast cancer, bladder cancer, ovarian cancer, skin cancer and so on.

Conclusion: *Camellia sinensis* are rich in flavonoids, caffeine and catechins. The tea leaves extract possesses an excellent antimicrobial, antioxidant property which can be used as an alternative drug of choice due to its lower side-effects to human.

Key Words: *Camellia Sinensis*, Leaves Extract, GC-MS, FTIR, Cancer

INTRODUCTION

Green tea leaves scientific name is *Camellia sinensis* which is evergreen species, originated from China. They are native to southeast and east India but nowadays tea leaves are growing both in tropic and subtropic regions. Height of tea shrub is 9 meters and it will grow up to 5 to 10 meters in length. They are dark in colour with pointed tips and serrated edges. These tea leaves contain alkaloid mainly caffeine, which is present in tea or coffee and it is a stimulant of the central nervous system.[1] Tea leaves are rich in antioxidants.[2] The antioxidant compound present in it has anti-inflammatory, antitumor, antibacterial, antiviral, antiangiogenic, antimutagenic, anticarcinogenic activities.[2,3] As it contains nutrients, it helps in the release of nutrients to the soil and it makes the environment a nutrient-rich one and makes the soil health-

ier.[4] Though the tea leaves are rich in tannic acid they are used as good fertilizer and helps in treating sunburns.[5] They help freshen our breath and after some processing, it is used to soothe our skin after some wounds which will reduce the inflammation and keep the skin healthier.[2] They can also be used for the removal of unpleasant smell and to reduce bug bites.[6]

Green tea leaves belong to kingdom Plantae. Its order is *Ericales*. It comes under the family called *Theaceae*. Its genus is *Camellia*. And its species is *C. sinensis*. It contains catechins, polyphenolic compounds which give protective effects on the cardiovascular system. It helps in reducing cholesterol levels and also improves the ratio of the good cholesterol (HDL) to bad cholesterol (LDL), as well as it helps in curing diabetes, weight loss, inflammatory bowel disease and

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iron overload. It may lower blood pressure and even reduce the risk of stroke and coronary heart diseases.[7] Tea leaves reduce the effects of cancer by inhibiting the growth of cancer cells.[3,7] And even protect brain cells from oxidative stress as it contains antioxidants in it. Even helps in lowering the blood sugar levels.[8] Also helpful in rheumatoid arthritis, even in infection and impaired immune functions.[9,10,11] They contain polyphenols which include flavonoids(major), phenolic acids and flavanols. Major flavonoid of green tea leaves is catechins.[12] Most significant active compound is Epigallocatechin Gallate (EGCG). EGCG is the most powerful antioxidant in it as well as it inhibits the growth of cancer cells without harming the healthy tissues.[13] Catechins are described by the presence of a benzopyran structure which has at least one aromatic ring in it due to antioxidant and anti-inflammatory properties.[14-17] EGCG acts as an immune modulator, effective in neurological problems and reduces the risk of chronic diseases.[17] Consumption of green tea increases the mineral density of bones and even strengthens the immune system action. It keeps us fresh and reduces our depression.[15] Lowers the diabetes risk and even burns the fat.[18] Green tea consumption prevents various types of cancer includes the mouth, stomach, small intestine, and so on. Cell lines have various advantages like easy to use and even cost-effective so only it is used. Cancer cells will be kept on dividing and even it is growing over time. Cancer cell lines are used in the research of studying the biology of cancer and even to test cancer treatments.

MATERIALS AND METHODS

Plant material

Green tea leaves (*Camellia sinensis*) is collected from Ooty tea estate and brought to our laboratory. The leaves are torn into small pieces and kept in shadow dry for 48 hours. Then by using mixer the dried leaves are chopped down into a fine powder and it is stored in an airtight container.

Preparation of the leaf extract

Chopped tea powder of about 5g is weighed and kept in a beaker. Then the solvent is added for about 50mL of 100% ethanol into the sample powder. Then Ultrasonicator extraction was done for 20 mins with on pulse 9sec and off pulse 3sec. After the completion of extraction to remove ethanol the extract is kept at 50C for vapourisation. Then the sample is scrapped and kept in a sample container.

Qualitative phytochemical analysis

Phytochemical constituents were analysed by presence or absence by using the following procedures:

Test for proteins: Biuret's Test: The Tea extract of 1ml was added with 1ml of 4% NaOH and 1ml of 1% CuSO4. If the solution colour change to violet or pink colour it indicates the presence of proteins.

Test for Tannin's and Phenols: Ferric Chloride Test: The Tea extract of 1ml was added with 1ml of 5% Ferric Chloride. If the solution colour changes into blue-black or brown-green colour then it indicates the presence of tannins and phenols.

Test for Glycosides: Keller-Killani Test: The Tea extract of 1ml was added with 0.5ml of glacial acetic acid, 2 drops of 5% Ferric Chloride and 2 drops of concentrated Sulphuric acid. Then the disappearance of reddish-brown colour at the junction of two layers and bluish-green in upper layer indicates the presence of cardiac glycosides.

Test for Saponins: Foam Test: The Tea extract of 1ml was added with 20ml of water and shaken vigorously. If the persistent foam is observed then it indicates the presence of saponin.

Test for Flavonoids: Alkaline reagent Test: The Tea extract of 1ml was added with a few drops of NaOH. Then an intense yellow colour will appear and when a few drops of dilute acid is added if it becomes colourless then it indicates the presence of flavonoids.

Test for Triterpenoids: Salkowaski Test: The Tea extract of 1ml was added with 5 drops of concentrated Sulphuric acid. The appearance of a greenish-blue colour indicates the presence of triterpenoids.

Test for Phlobatannin's: The Tea extract of 1ml was boiled with 1% HCl. The occurrence of red precipitate deposition indicates the presence of phlorotannins.

Test for Alkaloids: Mayer's Test: The Tea extract of 1 ml was added with 1ml of Mayer's reagent (Potassium mercuric iodide). The occurrence of a white precipitate indicates the presence of alkaloids.

Test for Lipids: The Tea extract of 1ml was added with 0.5N alcoholic Potassium hydroxide and a drop of phenolphthalein. Then this mixture of the solution was heated on a water bath for about 1 hour and soap formation or neutralization of alkali indicates the presence of lipids.

Test for Steroids: The Tea extract of 1ml was added with 2ml of chloroform and concentrated Sulphuric acid. The upper layer turns into a red and Sulphuric acid layer showed yellow with green fluorescence and it indicates the presence of steroids.

Test for Terpenoids: The Tea extract of 1ml was added with 1ml of chloroform and after 5 mins 1ml of concentrated Sulphuric acid was added. Then greyish colour indicates the presence of terpenoids.

GC-MS Analysis

The chemical composition of the floral sample was analysed through gas chromatography-mass spectrometry. The analysis was carried out using GC-MS Perkin Elmer model: Clarus 680 which is equipped with mass spectrometer Clarus 600 (EI) and analysed using Turbo Mass ver 5.4.2 software. A fused silica column which is packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30m × 0.25mm ID × 250μm df). To separate the components Helium was used as the carrier gas at a constant flow rate of 1mL/min. During the chromatographic run, the temperature of the injector was set at 260°C. The extracted sample of 1μL was injected into the equipment and the oven temperature were 60°C for about 2 minutes followed by 300°C at the rate of 10°C min-1; & 300°C for about 6 mins. The conditions of the mass detector were: transfer line temperature 230°C; ion source temperature 230°C and ionisation mode electron impact at 70 eV, a scan time 0.2sec and scan-interval of 0.1sec. The fragments from 40-600 Da. The spectrum of the components was compared with the database of known components stored in the GC-MS NIST (2008) library.

FTIR Analysis

Green tea leaves extract was analysed by FTIR method. The extracted sample was taken in the ratio of 1:9 and it was blended with KBr which was pelletized by KBr hydraulic press of pressure about 10 tons. FTIR was used to analyse the functional moieties in the pellet at the infrared range of 400-4000 cm^{-1}. The stretching at 3304.44 cm^{-1} was due to the C-H stretching of the alkaloids (Z, Z-6,13-Octadecadien-1-ol Acetate) present in the extract. The stretching at 2923.38 cm^{-1} and 2852.71 cm^{-1} was due to the aldehyde C-H stretching (6,11-Undecadiene,1-Acetoxy-3,7-Dimethyl) of the extract. The region from 1700 cm^{-1} to 1600 cm^{-1} was due to the C=O stretching, the region 1500 cm^{-1} to 1300 cm^{-1} was due to the C=C and acyl C=O stretching. From 1200 cm^{-1} to 1000 cm^{-1} indicate the alkoxy C-O stretching present in the extracted compound (1,9-Nonanediol, Dimethanesulfonate). The FTIR result conclude the presence of Z, Z-6,13-Octadecadien-1-ol Acetate, 6,11-Undecadiene,1-Acetoxy-3,7-Dimethyl, 6,8-Dodecadien-1-ol(6Z,8E) and 1,9-Nonanediol, Dimethanesulfonate.

Antibacterial assay

The antibacterial assay of *Camellia sinensis* was performed with five gram-positive bacteria and two gram-negative bacteria. Gram-positive bacteria such as *Bacillus subtilis, Streptococcus agalactiae, Listeria monocytogenes, Staphylococcus aureus, Streptococcus faecalis* and gram-negative bacteria such as *Escherichia coli, Klebsiella oxytoca, Klebsiella aerogenes* by the following agar well diffusion method. The following bacteria were inoculated in the sterile nutrient plates individually by swabbing technique. The wells are made up of diameter mm using the sterile agar well puncher.

Then 5 wells were punched. Then the Tea extract was diluted at different concentrations of (25%, 50%, 75%, 100%) using sterile distilled water. Then in the centre, well water and the other four wells, the extract with different concentrations was pipetted into it. Then the agar plates were placed in the incubator at ±37°C. After 24 hours of incubation the zone of inhibition was measured in mm.

Antioxidant activity

Antioxidant activity is nothing but the limitation of the oxidation of proteins, lipids, DNA or other molecules which occurs by inhibiting the propagation stage in the oxidation chains. Antioxidant activity of the extract is based on the scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. In this assay different concentrations of both standard and samples (ascorbic acid) were taken and their volume was adjusted to 100μl using methanol. By using methanol as a solvent, the DPPH solution of 0.01mM was prepared. From the solution prepared mix 3ml of 0.01 DPPH solution with samples of different concentrations. Prepare negative control by adding 100μl of methanol to the 3ml of solution. Kept the tube with mixtures to stand in a dark place at room temperature for 30 minutes. After 30 minutes of the process the shading of the tube with mixtures changes from violet to yellow which shows the presence of cancer-preventing agents. The above evaluation was estimated by its absorbance at 517nm. For both standard and sample, the IC50 esteem (inhibitory focus) was determined. The of hindrance was determined by utilizing the accompanying recipe

$$\% \text{ of hindrance} = [A0 - A1/A0] *100$$

In which A0 is the absorbance of the control (for example DPPH arrangement without test), A1 is the absorbance of the test or on the other hand standard (for example DPPH arrangement with test).

RESULT AND DISCUSSION

Laboratory studies show that herbs like *Camellia sinensis* (green tea leaves) can be effectively used in curing bleeding, mental health, regulate body temperature, healing wounds, and to aid digestion. It also shows the positive impact on types of cancer such as breast cancer, bladder cancer, ovarian cancer, skin cancer and so on. Research on *Camellia sinensis* shows that more amount of polyphenols can kill the cancerous cells and prevent them from their growth. People are taking tea for more health benefits such as to lose their weight, to avoid strokes, to reduce cholesterol, to cure type 2 diabetes, and for inflammatory skin diseases. It is considered as the world's number one health drink and it is rich in the number of antioxidants to prevent cell damage. Even though there are numerous benefits of *Camellia sinensis* in the pre-

sent world, future researches and studies need to define the actual magnitude of health benefits.

The phytoconstituents present in the organic extracts were determined qualitatively by HPTLC. The phytochemical studies showed the presence of compounds such as proteins, tannins, phenols, flavonoids, glycosides, lipids, triterpenoids, alkaloids, steroids in aqueous extract or ethanol extract whereas terpenoids, phlorotannin were absent in the ethanol or aqueous extract. The performed results were shown in table 1. The compounds present in *Camellia sinensis* which are identified by phytochemical studies have antimicrobial, anti-oxidant and anti-bacterial properties in which flavonoids have antioxidant properties. Similar, extraction from herbal leaves results in the antimicrobial activity and antioxidant activity.[19,20]

The components present in the extract which were obtained from the GC-MS results are shown in Table 2. The molecule 21.00 – bicyclo[4.1.0]heptane,7-pentyl- and 20.41-caffeine showed maximum peak followed by 27.77-1,9-nonanediol ,dimethanesulfonate showed highest peak , 20.09-2,7-octadiene-1,6,diol,2,6-dimethyl-,(z)-showed moderate peak and 19.59-z,z-6,13-octadecadien-1-ol acetate showed the lowest peak. Other than the molecule bicyclo [4.1.0] heptane,7-pentyl- and 6,8-Dodecadien-1-ol(6Z,8E) all other molecules contain oxygen group. The presence of oxygen may be the reason for the anti-oxidant properties.

To determine whether the floral extract possesses antibacterial activity the antimicrobial properties was been performed. Then to find the antibacterial properties disc diffusion assay was performed. The plates for different types of bacteria were prepared with respective agar. Then the plates were punched with a good puncher. After the wells are ready the samples were prepared in different concentrations like 20% 50% 75% and 100% concentrated samples. Then the different concentrated samples were transferred onto the wells of the plate and the zone of inhibition diameter around the wells reveals the antibacterial activity. The clear region around the wells shows that there is no bacterial growth in it and it is the zone of inhibition. Different types of bacterial strains were used to perform the antibacterial activity are *Bacillus subtilis, Streptococcus agalactiae, Listeria monocytogenes, Staphylococcus aureus, Streptococcus faecalis, Escherichia coli, Klebsiella oxytoca, Klebsiella aerogenes*. Among these strains, *Listeria monocytogenes* have shown the highest susceptibility in the floral extract. The bacterial species such as *Escherichia coli* have exhibited moderate susceptibility. The zone of inhibitions of bacterial species is shown (Figure 2).

The sample contains the high number of antioxidants which were indicated by DPPH results. The below Figure 1 is provided with the IC50 values for both the standard (ascorbic acid) and sample (floral extract). The graph reveals the standard has 400.6 μg/ml and the sample has 608 μg/ml.

Similarly, few researchers have performed the antimicrobial test on *E.coli,A. caliginosa*by disc method and the zone of incubation was 100 Au/mL.[20-21]

CONCLUSION

The Phytochemical analysis of the green tea leaves indicates the presence of flavonoids, alkaloids, triterpenoids. The antimicrobial activity of the extract shows an effective activity in Listeria monocytogenes of the zone of inhibition at different concentrations. The antioxidant compound in the green tea leaves extract can be used for the chemotherapeutic drug for cancer treatment.

ACKNOWLEDGEMENT

REFERENCES

1. Nagashima, Goto T, Yoshida Y and Kiso M. Simultaneous analysis of individual catechin and caffeine in green tea. J Chromatogr Anal 1996;749(1-2):295–299.
2. Chopade VV, Phatak AA, Upaganlawar AB and Tankar AA. Green tea (Camellia sinensis): Chemistry, Traditional, Medicinal uses and its Pharmacological activities- a review. Pharma Rev 2008; 2(3):157-162.
3. Zaveri NT. Green tea and its polyphenolic catechins: Medicinal uses in cancer and noncancer applications. Life Sci 2006;78(18):2073-2080.
4. Intawongse M and Dean JR. Use of the physiologically-based extraction test to assess the oral bioaccessibility of metals in vegetable plants grown in contaminated soil. Env Pollut 2007;152(1): 60-72.
5. Sedaghathoor S, Torkashvand AM, Hashemabadi D and Kaviani B. Yield and quality response of tea plant to fertilizers. African J Agri Res 2009;4(6):568-570.
6. Utama-Ang N, Kopermsub P, Thakeow P and Samakradhamrongthai R. Encapsulation of Michelia Champaca L. Extractand Its Application in Instant Tea. Int J Food Eng 2017;3(1):48-55.7. Kavitha R, Kamalakannan P, Deepa T, Elamathi R, Sridhar S and Suresh Kumar J. In vitro antimicrobial activity and phytochemical analysis of Indian medicinal plant Couroupita guianensis Aubl. J Chem Pharm Res 2011;3(6):115-121.
7. Nammi, Boini KM, Lodagala SD, and Behara RBS. The juice of fresh leaves of Catharanthus roseus Linn. Reduces blood glucose in normal and alloxan diabetic rabbits. BMC Complemen Altern Med 2003;3(4):1-4.

8. Sinija VR and Mishra HN. Green tea: Health benefits. J Nut Env Med 2009;17(4):232-242.
9. Schneider C and Segre T. Green Tea: Potential Health Benefits. Am Fam Phy 2009;79 (7):591-594.
10. Abdel-Tawwab M, Ahmad MH and Sakr SFM. Use of Green Tea, Camellia sinensis L., in Practical Diet for Growth and Protection of Nile Tilapia, Oreochromis Niloticus (L.), against Aeromonas hydrophila Infection. J World Aqu Soc 2010;41: 203-213.
11. Kim YB, Kim YC, Lee LS and Kim SH. Quantitative analysis of major constituents in green tea with different plucking periods and their antioxidant activity. Molecules 2014;19 (7):9173–9186.
12. Juneja LR, Kapoor MP, Okubo T and Rao TP. Green tea polyphenols: nutraceuticals of modern life. Boca Raton: CRC 2016;348.
13. Anand J et. al. Green Tea Enhances Nutritional and Antioxidant Potential of Pearl Millet Based Cookies: A Healthy Approach. Int J Curr Res Rev 2020;12(18):48-54.
14. Benelli R, Morini M, Albini A, Garbisa S, Dona M, Dell'Aica I, Calabrese F et al. Neutrophil restraint by green tea: inhibition of inflammation, associated angiogenesis, and pulmonary fibrosis. J Immunol 2003;170(8):4335–4341.
15. Youdim MBH, Mandel S, Amit T and Weinreb O. Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. J Nutr Biochem 2004;15(9):506–516.
16. Yang CS, Hong J and Annu. Prevention of chronic diseases by tea: Possible mechanisms and human relevance. Rev Nutr 2013;17:161-181.
17. Whelan AM, Killian L, Jurgens TM, Doucette S, Kirk S and Foy E. Green tea for weight loss and weight maintenance in overweight or obese adults. Cochrane Database Syst Rev 2012;12.
18. Walsh DA and Pearson CI. Angiogenesis in the pathogenesis of inflammatory joint and lung diseases. Arthr Res 2001;3(3):147-153.
19. Ščerbová J, Kandričáková A, Hamarová Ľ and Lauková A. Relation to enterocins and herbal extracts of fecal hemolytic Escherichia coli from domestic ducks detected with MALDI-TOF mass spectrometry. Poultry Sci 2019;98(11):5925-5931.
20. Dkhil MA, Zreiq R, Hafiz TA, Mubaraki MA, Sulaiman S, Algahtani F, Abdel-Gabera R, Al-Shaebi EM, SalehAl-Quraishy, et al. Anthelmintic and antimicrobial activity of Indigofera oblongifolia leaf extracts. Saudi J Bio Sci 2020;27(2):594-598.

Table 1: The Phytochemical constituents of *Camellia sinensis*

PHYTOCHEMICAL CONSTITUENTS	PRESENCE
Proteins	+
Tannins and Phenols	+
Glycosides	+
Saponins	+
Flavonoids	+
Triterpenoids	+
Phlobatannin	-
Alkaloids	+
Lipids	+
Steroids	+
Terpenoids	-

(+present, - absent)

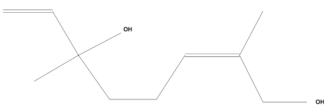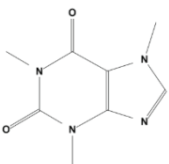**Table 2: GC-MS analysis result for major phytocomponents in Camellia sinensis extract**

S.No	RT	NAME OF THE COMPOUND	MOLECULAR FORMULA	MOLECULAR WEIGHT	PEAK AREA %	STRUCTURE
1.	19.590	Z, Z-6,13-Octadecadien-1-ol Acetate	$C_{20}H_{36}O_2$	308		
2.	20.105	2,7-Octadiene-1,6-Diol,2,6-Dimethyl-, (Z)-	$C_{10}H_{18}O_2$	170	5.002	
3.	20.406	Caffeine	$C_8H_{10}O_2N_4$	194	28.384	

Table 2: *(Continued)*

S.No	RT	NAME OF THE COMPOUND	MOLECULAR FORMULA	MOLECULAR WEIGHT	PEAK AREA %	STRUCTURE
4.	21.001	Bicyclo [4.1.0] Heptane,7-Pentyl-	$C_{12}H_{22}$	166	61.629	
5.	22.281	6,8-Dodecadien-1-ol(6Z,8E)	$C_{12}H_{22}$	166	1.242	
6.	26.513	6,11-Undecadiene,1-Acetoxy-3,7-Dimethyl-	$C_{14}H_{24}O$	208	1.456	
7.	27.479	1,9-Nonanediol, Dimethanesulfonate	$C_{11}H_{24}O_6S_2$	316	2.287	

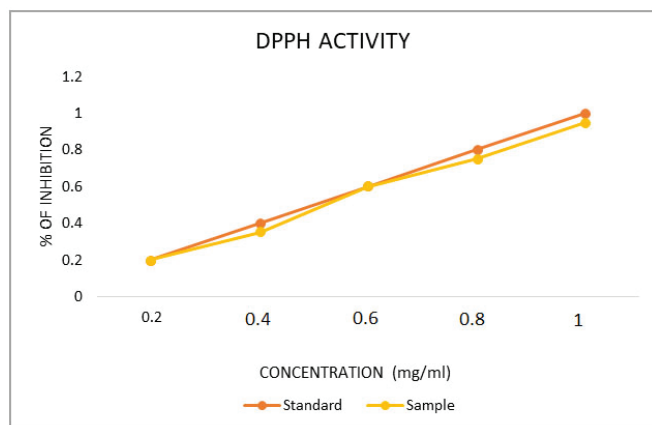**Figure 1:** The below graph is provided with the IC50 values for both the standard (ascorbic acid) and sample (floral extract).

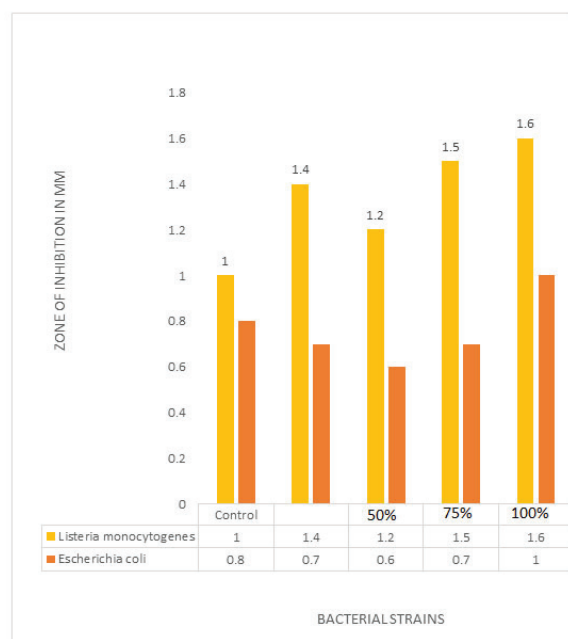|	Control		50%	75%	100%
Listeria monocytogenes	1	1.4	1.2	1.5	1.6
Escherichia coli	0.8	0.7	0.6	0.7	1

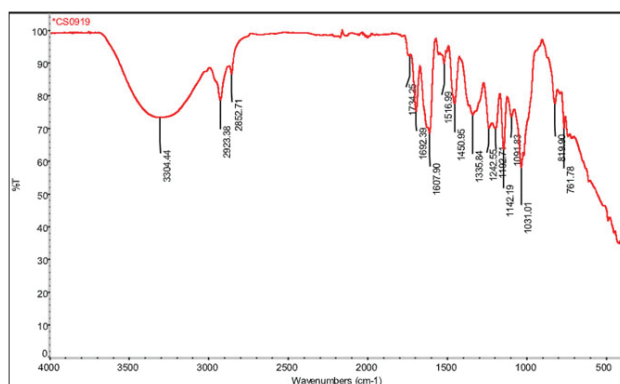**Figure 2:** The zone of inhibitions of bacterial species are shown in the below graph.

Figure 3: The structural compounds present in the extract which were predicted by using FTIR analysis is shown in the graph.
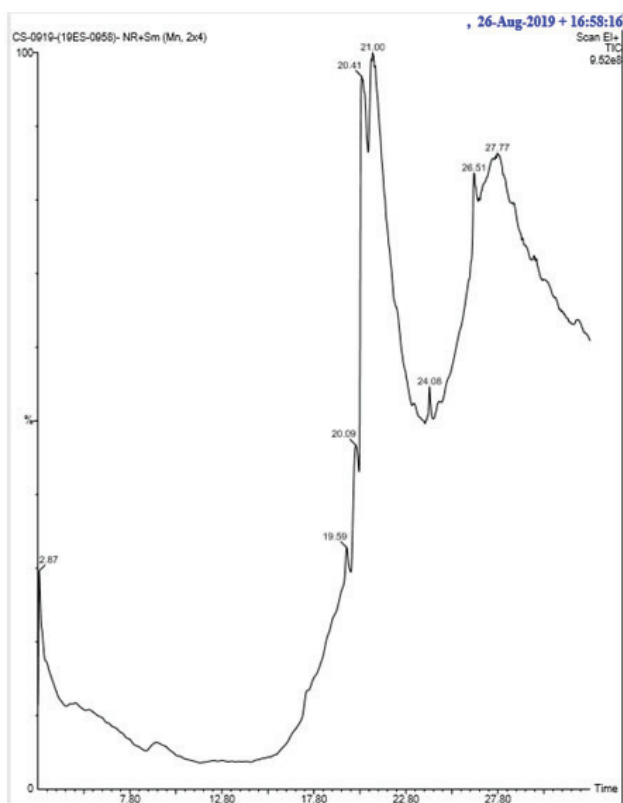

Figure 4: The chromatogram of compounds present in *Camellia sinensis.*

GRAPHICAL ABSTRACT

CAFFEINE

Bicyclo [4.1.0]

Heptane,7-

Pentyl-

Z, Z-6,13-

Octadecadien-1-

ol Acetate

6,11-

Undecadiene,1-

Acetoxy-3,7-

Dimethyl-

❖ GC- MS ANALYSIS
❖ FTIR ANALYSIS
❖ ANTIMICROBIAL
   PROPERTY
❖ ANTIOXIDANT
   PROPERTY