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Effect of extraction temperature on antimicrobial and antioxidant properties of Assam tea

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Abstract

Tea is traditionally prepared by boiling dry leaves in water. This study was taken to evaluate the effect of extraction temperature on antimicrobial and antioxidant properties of Assam tea (*Camellia sinensis* var. *assamica*). Dry tea leaves were extracted in water at 4 C, 37 C and by boiling at 100 C, and antimicrobial as well as antioxidant activities of the extracts were compared. Antibacterial activity was determined by agar cup diffusion assay against two important pathogens; *Staphylococcus aureus* and *Vibrio cholerae*. Antioxidant property was monitored by total reducing power (TRP) assay, ferric reducing antioxidant power (FRAP) assay and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay. Our results show that maximum antibiotic activities of the extracts are obtained by boiling tea leaves. We also show that boiling of tea leaves does not lead to loss of antioxidant properties.

Keywords: Tea, Staphylococcus aureus, Vibrio cholerae, Antimicrobial, Antioxidant, Agar cup diffusion assay.

Introduction

Tea is used as a beverage all over the world. It is popular because of its caffeine content which acts as mild stimulant. Various types of tea are available like green tea, black tea, red tea, oolong tea etc. It contains phytochemicals like alkaloids, saponins, tannins, catechin and other polyphenols which impart medicinal as well as pharmacological properties. Amount of these phytochemicals in the extracts often depends on the method of preparation [1]. Catechins are antioxidant and natural polyphenols present in high amount in tea leaves. Types of catechins present in tea are epicatechin (EC), epicatechin3gallate (ECG), epigallocatechin (EGC) and epigallocatechin3gallate (EGCg). ECG and EGC have antibacterial property and EC helps to prevent cancer [2-4]. Zaveri *et al* (2006) and Nagle *et al* (2006) [5,6] showed that in green tea EGCg is the major portion of total catechins which possess medicinal properties as anti-obesity, anti-inflammatory, anticarcinogen, antibacterial including antioxidant activity [7-11].

Tea extracts inhibit growth of various gastric and intestinal pathogens and used as prophylactic against typhoid [12]. It also inhibits the growth of pathogens causing dental infections like *Streptococcus mutans* [13-16]. Growth of *Staphylococcus aureus*, *S.epidermidis*, *Vibrio cholerae* 01 type, *V.cholerae* non O1 type, *V. parahaemolyticus*, *V. mimicus* were found to be inhibited by tea extracts [17]. Growth inhibition has been confirmed by observing the change in morphology of bacteria where polyphenols from green tea caused damage to the cell membranes by increasing outer and inner membrane permeability [18,19]. In addition to growth inhibition of *V.cholerae*, tea extracts are reported as potent inhibitor of cholera toxin *in-vitro* [20]. Tea extract has the ability to inhibit various types of viruses like rotavirus, enterovirus and influenza virus [21].

In addition to antibacterial activity polyphenols also exhibit antioxidant property. In green tea and black tea antioxidant property is imparted by the polyphenols and the theaflavins respectively [22,23]. According to Yang and Liu (2012) [24], green tea showed better antioxidant activity (4850.2 ± 60.7 μmol g^{1}, $P < 0.05$) than black tea and oolong tea. Antioxidant activity is reported to vary in different parts of the tea plants. Different antioxidant assays like ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] assay, FRAP (ferric reducing antioxidant power) and DPPH (2,2-diphenyl-1-picrylhydrazyl) assay showed that tea shoot has more antioxidant activity than leaves [25]. Process of tea preparation also affects the antioxidant content of tea. Baruah (2012) [26], reported that antioxidant activity is more in tea extract when prepared by microwave oven drying than when prepared by hot air oven.

Tea is prepared in different ways and variation in treatment temperature has been observed in most of the recipes. Assam tea is grown in north-eastern Himalayan region of India, and is famous all over the world for its flavour and liquor. In this study we compared the effect of temperature on antimicrobial and antioxidant activities of Assam tea.

Materials and Methods

Chemicals

Tryptone, sodium chloride, yeast extract, agar powder, dipotassium phosphate, potassium dihydrogen phosphate, potassium ferricyanide, trichloroacetic acid, ferric chloride, sodium acetate trihydrate, acetic acid, potassium persulfate, methanol, hydrochloric acid, glutaraldehyde, ethanol were obtained from Fisher Scientific. 2, 4, 6- tripyridyl-s-triazine, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), trolox and ascorbic acid were obtained from Sigma.

Plant materials

The tea leaves were provided by the Senior Manager, Chabua Tea Estate, Dibrugarh, Assam who is maintaining the plants in nursery through vegetative propagation. Further the leaves were identified through a Horticulturist, Archaeological Survey of India, Government of India, Agra, as described earlier (Mehrotra et al 2010)

Preparation of extracts

Dried tea leaves were taken and crushed in mortar and pestle. 10gm of powdered tea was taken in three different conical flasks and 40 ml of autoclaved distilled water was added in each flask and kept in 4 C, 37 C and 100 C for six hours. After temperature treatment these samples were filtered using Whatman filter paper no 1 and filtrates were lyophilized and stored for future use.

Inoculation of bacterial culture

Luria-Bertani broth 2 ml was taken in autoclaved test tubes, and isolated bacterial colonies were inoculated in the broth separately and kept overnight in the shaker at 37 C. *S.aureus* clinical isolates provided by NICED, Kolkata and *V.cholerae* clinical strains were isolated by authors of Assam medical college)
were inoculated in the broth separately and kept overnight in the shaker at 37 C.

Antibacterial activity of tea extracts

Antibacterial activity was checked by agar cup diffusion assay. The Luria-Bertani agar media was spread separately with 100µl of overnight grown bacterial cultures (*S.aureus* and *V.cholerae*) and wells were made in the plate using sterile cork borer. 100µl of 4 C, 37 C, 100 C treated tea leaf extracts were loaded in wells and autoclaved distilled water was included as negative control. The petriplate was incubated overnight at 37 C. Next day the zone of inhibition of the samples were measured. Each reading indicates diameter of zone of inhibition excluding the well diameter and experiment was done in triplicate.

Scanning electron microscopy

Bacterial strains were grown to optical density 0.5 at 600nm and treated overnight with sub-lethal doses of tea leaf extract. Next day bacterial cells were harvested and fixed in 2.5% glutaraldehyde and stored at 4 C. Before analyzing the samples were dehydrated with graded ethanol.

Antioxidant activity of tea extracts

TRP assay

Total reducing power of tea leaf, extracted in different temperature was measured to detect the antioxidant activity. 1ml of extract was taken and incubated at 50 C for twenty minutes along with 2.5ml of 0.2M phosphate buffer adjusted to pH 6.6 and 2.5ml of 1% potassium ferricyanide. After that 2.5ml of 10% trichloroacetic acid was added to it and centrifuged at 3000 rpm for ten minutes. 2.5ml of distilled water and 0.5ml of 0.1% ferric chloride was added to 2.5ml aliquot of upper layer. The absorbance was measured at 700nm and ascorbic acid was used as standard.

FRAP assay

The tea leaf extracts were checked for the presence of ferric reducing antioxidant power content (FRAP Assay). The stock solutions included 20mM $FeCl_3$.6H2O solution, 10mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40mM HCl and 300mM acetate buffer (3.1 g Sodium acetate trihydrate and 16ml acetic acid) pH 3.6. The fresh working solution (FRAP) was prepared by mixing 2.5ml TPTZ solution, 25ml acetate buffer and 2.5ml $FeCl_3$. 6H2O solution and heated at 37 C before use. 150 µl of plant extract solution was kept for thirty minutes in the dark along with 2850 µl of the FRAP solution. The absorbance of coloured product [ferrous tripyridyltriazine complex] was measured at 593nm. Trolox was used as standard compound for the quantification of the antioxidant power by FRAP assay.

ABTS assay

ABTS assay was performed to check antioxidant activity of tea leaf extract using trolox as standard. Stock solutions contained 7.4mM ABTS solution and 2.6mM potassium persulfate solution. The two stock solutions were mixed in equal quantities and allowed to react for twelve hours in dark at room temperature. To prepare working solution 1ml of ABTS solution was mixed with 60ml of methanol to obtain an absorbance of 1.10±0.02 units at 734nm. 150 µl of leaf extract was allowed to react with 2850 µl of the ABTS solution for two hours in dark and absorbance was recorded at 734nm.

Results and Discussion

Boiling of leaves leads to maximum extraction of antibacterial activity of the extracts

Tea extract, one of the most popular drinks worldwide, is loaded with beneficial effects such as antimicrobial and antioxidant properties. This study was taken to compare the antimicrobial and antioxidant properties of tea extracted at 4 C, 37 C and 100 C. Extractions in water at different temperatures were carried out for six hours as detailed in materials and method section. To compare the antimicrobial activity of the extract we chose the bacterial strains *V. cholera* and *S.aureus* that are reported to be susceptible to antibiotic effect of tea extract [27]. Standard agar-cup diffusion method [28] was used for antibacterial assay. All the extracts

showed higher antibacterial activity against *S. aureus* than to *V.cholerae*. Antimicrobial activity against both the strains was highest in 100 C followed by 4 C and 37 C (Fig 1). It was reported that, with the increase of extraction temperature, solubility of the phenolic compound, which is responsible for antibacterial activity, increases [29]. Thus tea leaf extract prepared at 100 C showed maximum growth inhibition activity as it contains highest amount of phenolic compound. On the contrary, extract prepared at 37 C showed least bioactivity amongst the extraction methods used. The possible reason of the loss of the antibiotic activity could be due to oxidation of phenolic compounds. Polyphenol oxidase enzyme causes oxidation of phenolic compound and remains active within 20 C to 70 C and it is inactive when temperature rises beyond 70 C or falls below 20 C [30,31]. This is also interesting to observe that in contrast to most synthetic antibiotics that get inactivated by boiling, antimicrobial activities of tea-extracts are quite heat-resistant. This observation is similar to earlier study made on plant-based antimicrobial activities [27] in which extracts were treated to 100 C for one hour and observed to retain its activity. The results suggest that boiling extract method is best for antimicrobial activities of the tea extracts. Scanning electron microscopy with tea extract (100 C) showed morphological changes in both *S. aureus* and *V. cholerae*. Deformation and surface ruffling of the bacterial cell were observed after treatment with tea extract prepared at 100 C (Fig 2).

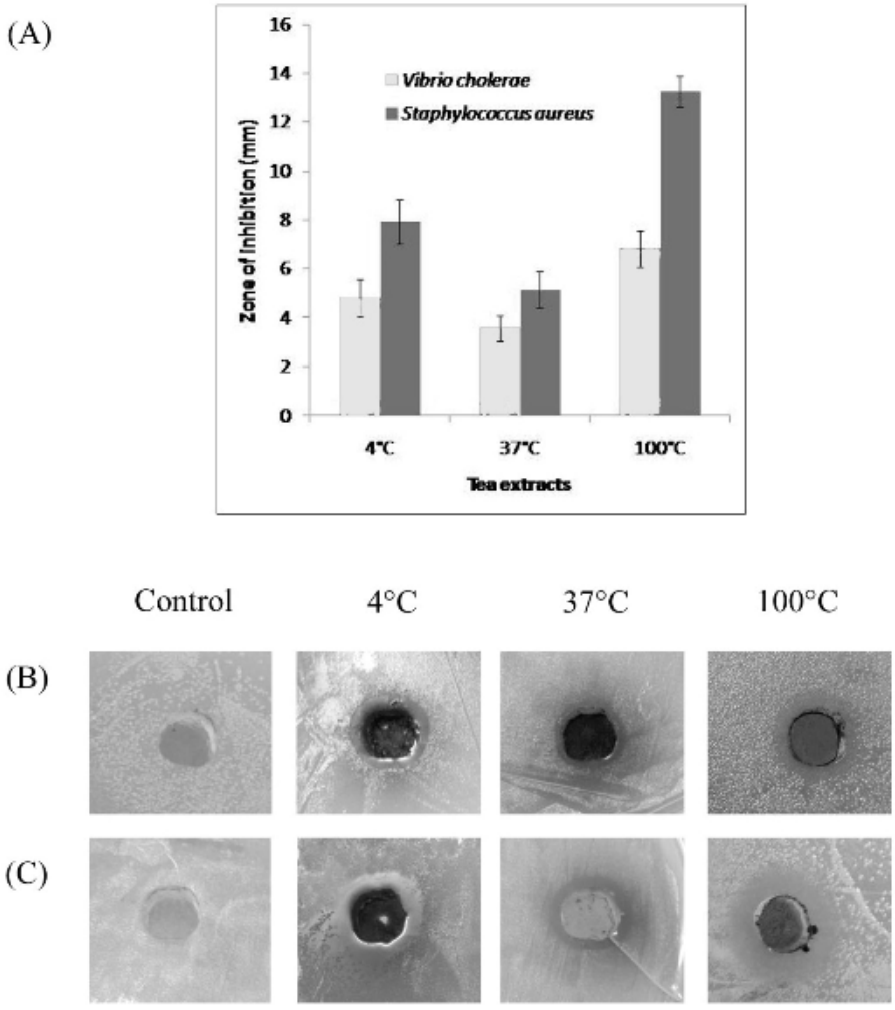


Fig 1: Antimicrobial activity of tea leaf extract (A) Zone of inhibition obtained by agar-cup assay. Each bar represents mean of standard deviation from triplicate readings. (B) and (C) shows representative inhibition assay with *V.cholerae* and *S.aureus* respectively.

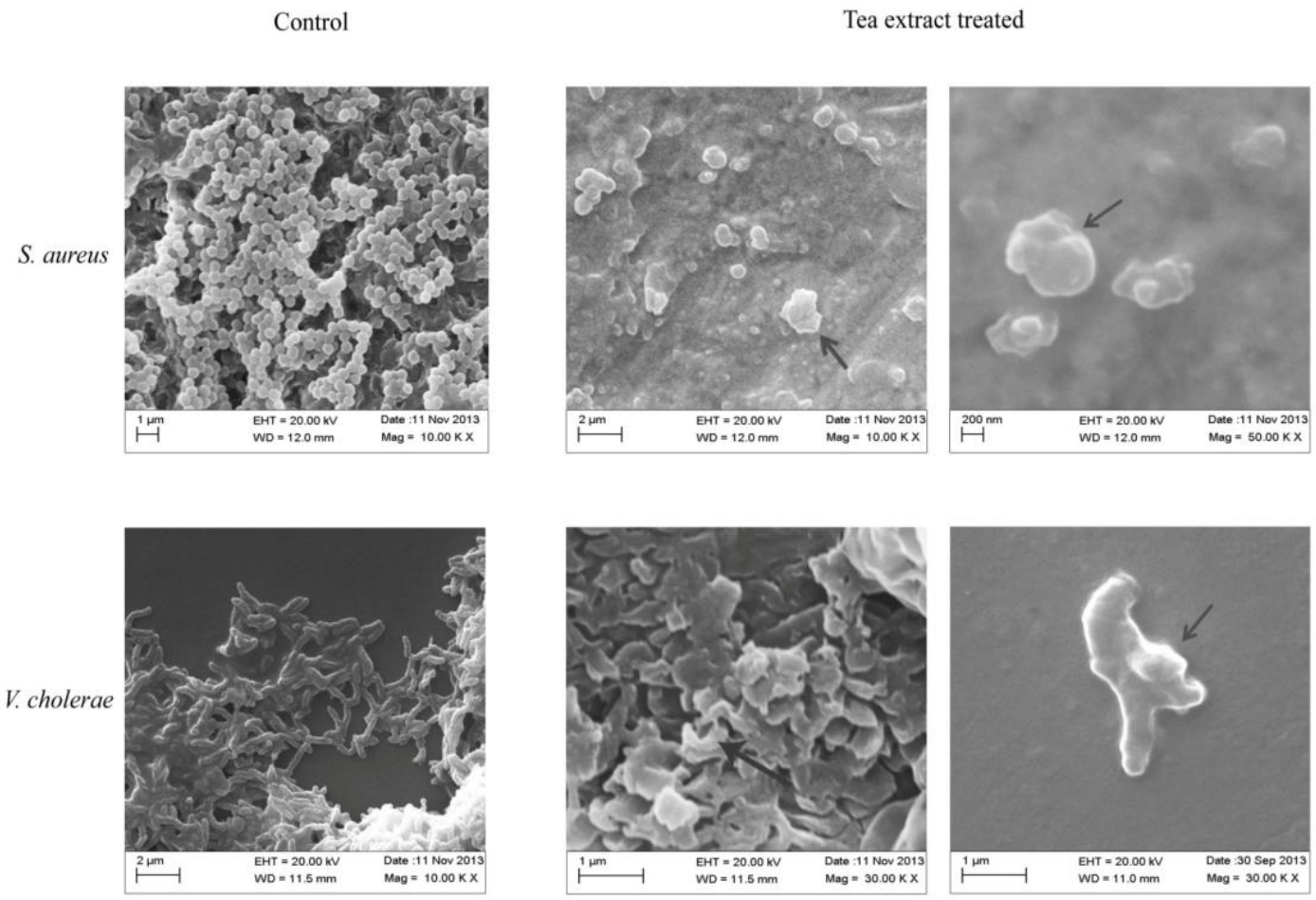
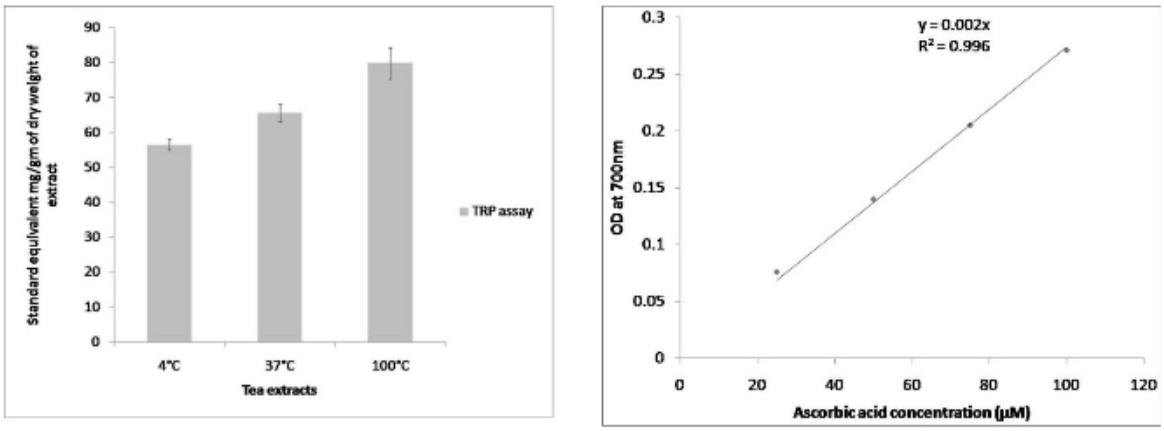


Fig 2: Scanning electron micrography after treatment of bacteria with tea leaf extract (extracted at 100°C). Magnification scale is indicated by bars.

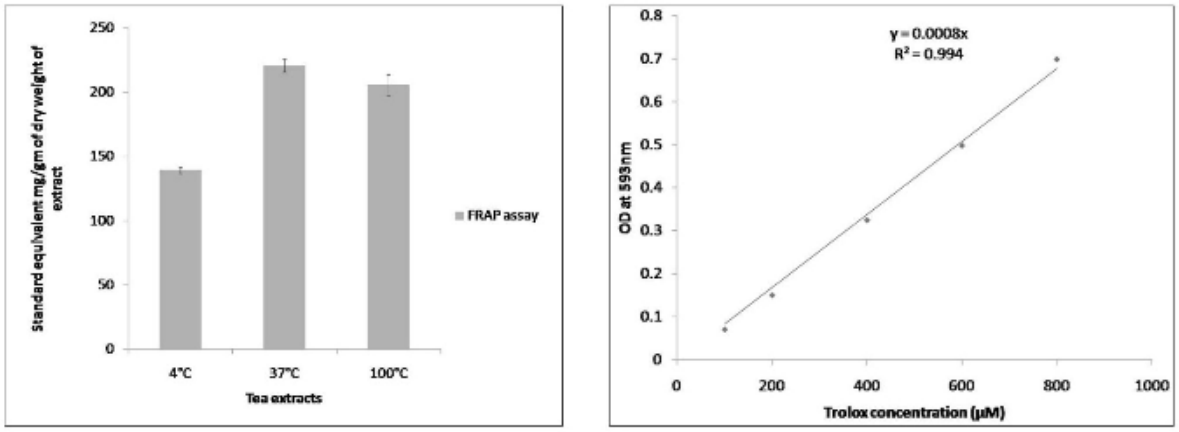
Boiling of leaves retains antioxidant activity of tea extracts

Antioxidant activities are usually monitored by several assays [32]. Three different antioxidant assays were done to include different kinds of antioxidant moieties present in tea extract, whose solubility varies with different reagents. One of the assay systems measures total reducing power (TRP) by monitoring the reduction of ferric ion (Fe3+) to ferrous ion with the help of ferric chloride [33]. Commercially available ascorbic acid served as a standard of our assay (Fig 3B). It was observed that tea extract prepared at 4 C, 37 C and 100 C had the ascorbic acid equivalent antioxidant capacity of 56.53 mg/gm, 65.55 mg/gm and 80 mg/gm of dry weight of extract respectively by TRP assay with reference to standard curve (y = 0.002 x, R^2 = 0.996). As shown in the Fig 3A, 100 C treated tea extract showed maximum reductive ability to transform Fe^{3+} to Fe^{2+}, followed by the extracts that were prepared at 4 C and 37 C. Thus, by boiling the antioxidant properties of tea leaves are not compromised. Another assay system measures ferric ion reducing antioxidant power (FRAP) by monitoring the maximum ferric reducing ability with the help of ferric chloride [33] and trolox was used as a standard (Fig 4B). It was observed that tea extract prepared at 4 C, 37 C and 100 C had the trolox equivalent antioxidant capacity of 139.42 mg/gm, 220.83 mg/gm and 205.83 mg/gm of dry weight of extract respectively by FRAP assay with reference to standard curve (y = 0.0008 x, R^2 = 0.994) (Fig 4A). In ABTS assay maximum inhibition of oxidation of ABTS was monitored by electron transfer radical scavenging [33]. It was observed that tea extract prepared at 4 C, 37 C and 100 C had the trolox equivalent antioxidant capacity of 636.26 mg/gm, 691.66 mg/gm and 518.09 mg/gm of dry weight of extract respectively with reference to standard trolox (y = 0.0007 x, R^2 = 0.996) (Fig 5A,5B). Thus, the antioxidant properties of the leaf-extracts are mostly retained in the boiling extract.



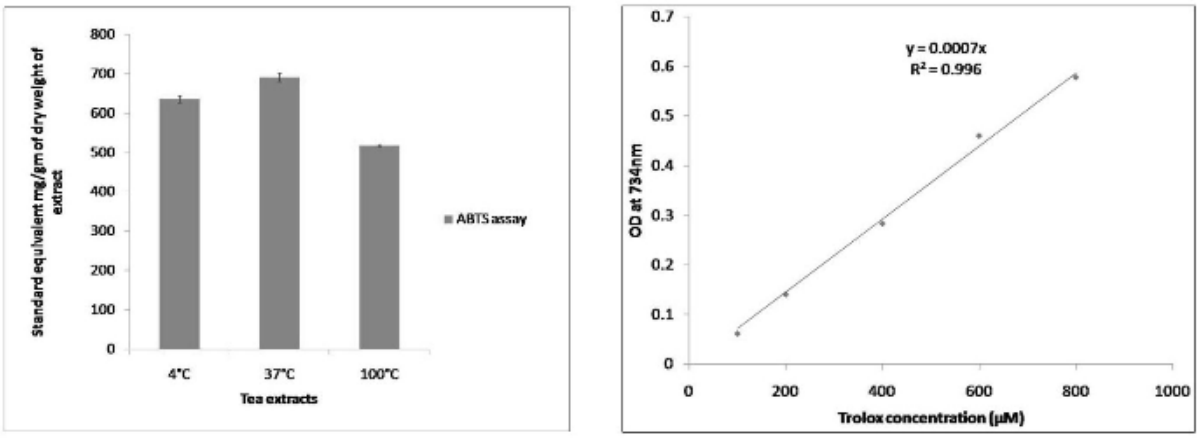
(A) (B)

Fig 3: Antioxidant activity of tea leaf extracted at different temperature. (A) TRP assay: Ascorbic acid equivalents in mg per gram of dry weight of tea extract; (B) Standard curve for TRP assay.



(A) (B)

Fig 4: Antioxidant activity of tea leaf extracted at different temperature. (A) FRAP assay: Trolox equivalents in mg per gram of dry weight of tea extract; (B) Standard curve for FRAP assay.



(A) (B)

Fig 5: Antioxidant activity of tea leaf extracted at different temperature. (A) ABTS assay: Trolox equivalents in mg per gram of dry weight of tea extract; (B) Standard curve for ABTS assay.

Conclusion

We conclude from the study that boiling extraction of tea leaves contains maximum antibiotic activity. Antimicobial as well as antioxidant properties of tea extracts are retained even after boiling at 100 C for six hours. Our experiments validate the traditional method of tea preparation, which is extraction of dry tea leaves in boiling water.

Authors' Contributions

Angana Payne Mandal and Mohd Hamza Hanfi have perfomed all above mentioned experiments and prepared the manuscipt. Dr. Lahari Saikia and Dr. Subhajyoti Deka have helped in analysis and interpretation of data. The whole conception of the manuscript have been given by Dr. Shoma Paul Nandi and she had designed all the experiments.

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References

[1]. Chen CC, Shi LL. Effect of extraction temperature and time on polyphenol contents and composition and sensory quality of oolong tea infusion. Food Sci Taiwan. 1996; 23: 285–298.

[2]. Saha A, Kuzuhara T, Echigo N. New role of ( )-epicatechin in enhancing the induction of growth inhibition and apoptosis in human lung cancer cells by curcumin. Cancer Prevention Research. 2010; 3: 953-962.

[3]. Hamilton-Miller JMT, Clifford MN, Clifford J, Schubert R, Spiro ME. In chemical and biological properties of tea infusions. Frankfurt am Main. 1997; 63-75.

[4]. Zhen YS. Tea bioactivity and therapeutic potential. Taylor and Francis. 2002; 176: 121-225.

[5]. Zaveri NT. Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. Life Sci. 2006; 78: 2073-80.

[6]. Nagle DG, Ferreira D, Zhou YD. Epigallocatechin-3-gallate (EGCG): chemical and biomedical perspectives. Phytochemistry. 2006; 67: 1849-55.

[7]. Katiyar SK, Elmets CA. Green tea polyphenolic antioxidants and skin photoprotection (Review). Int J Oncol. 2001; 18: 1307-13.

[8]. Khan N, Afaq F, Saleem M, Ahmad N, Mukhtar H. Targeting multiple signaling pathways by green tea polyphenol (-)-epigallocatechin-3-gallate. Cancer Res. 2006; 66: 2500-5.

[9]. Yang CS, Sang S, Lambert JD, Hou Z, Ju J, Lu G. Possible mechanisms of the cancer-preventive activities of green tea. Mol Nutr Food Res. 2006; 50: 170-5.

[10]. Hamilton-Miller JMT. Mechanism of synergy between epigallocatechin gallate and β-lactams against methicillin-resistant *Staphylococcus aureus.* Antimicrob Agents Chemother. 1995; 45: 1737–1742.

[11]. Wolfram S, Wang Y, Thielecke F. Anti-obesity effects of green tea: from bedside to bench. Mol Nutr Food Res. 2006; 50: 176-87.

[12]. Mandal S, DebMandal M, Pal NK, Saha K. Inhibitory and killing activities of black tea (*Camellia sinensis*) extract against *Salmonella enterica* serovar Typhi and *Vibrio cholerae* O1 biotype El Tor serotype Ogawa isolates. Jundishapur J Microbiol. 2010; 4: 115-121.

[13]. Das DN. Studies on the antibiotic activity of tea. J Ind Chem. 1962; 39: 849-854.

[14]. Ryu E. Prophylactic effect of tea on pathogenic microorganism infection to human and animals. Int J Zoonos. 1980; 7: 164-170.

[15]. Ryu E, Blenden DC, Wendall D. The inhibition of growth of selected bacteria by incorporating powdered tea in the medium. Int J Zoonos. 1982; 9: 73-76.

[16]. Scalbert A. Antimicrobial properties of tannins. Phytochemistry. 1991; 30: 3875-3883.

[17]. Toda M, Okubo S, Ohnishi R, Shimamura T, Nihon SZ. Antibacterial and bactericidal activities of Japanese green tea. Nihon Saikingaku Zasshi. 1989; 44: 669-72.

[18]. Cui Y, Oh YJ, Lim J, Youn M, Lee I, Pak HK, Park W, Jo W, Park S. AFM study of the differential inhibitory effects of the green tea polyphenol (-)-epigallocatechin-3-gallate (EGCG) against Gram-positive and Gram-negative bacteria. Food Microbiol. 2012; 29: 80-7.

[19]. Yi SM, Zhu JL, Fu LL, Li JR. Tea polyphenols inhibit Pseudomonas aeruginosa through damage to the cell membrane. International journal of food microbiology. 2010; 144: 111-7.

[20]. Toda M, Okubo S, Ikigai H, Suzuki T, Suzuki Y, Hara Y, Shimamura T. The protective activity of tea catechins against experimental infection by *Vibrio cholerae* O1. Microbiol Immunol. 1992; 36: 999-1001.

[21]. Mukoyama A, Ushijima H, Nishimura S, Koike H, Toda M, Hara Y, Shimamura T. Inhibition of rotavirus and enterovirus infections by tea extracts. Jpn J Med Sci Biol. 1991; 44: 181-186.

[22]. Henning SM, Yantao N, Nicolas HL, Gail DT, Rosario RM, Hejing W, Vay LWG, David H. Bioavailability and antioxidant activity of tea flavanols after consumption of green tea, black tea, or a green tea extract supplement. The American journal of clinical nutrition. 2004; 80: 1558–64.

[23]. Yashin A, Yashin Y, Nemzer B. Determination of antioxidant activity in tea extracts, and their total antioxidant content. American Journal of Biomedical Sciences. 2011; 3: 322-335.

[24]. Un Y, Rui HL. The phenolic profiles and antioxidant activity in different types of tea. International journal of food science and technology. 2012; 48: 163-171.

[25]. Nor Qhairul Izzreen MN, Mohd Fadzelly AB. Phytochemicals and antioxidant properties of different parts of *Camellia sinensis* leaves from Sabah tea plantation in Sabah, Malaysia. International Food Research Journal. 2013; 20: 307-312.

[26]. Baruah S, Saikia D, Hazarika M. The role of drying system in antioxidant activity of tea leaves. Two and a Bud. 2012; 59: 119-121.

[27]. Mehrotra S, Srivastava AK, Nandi SP. Comparative antimicrobial activities of Neem, Amla, Aloe, Assam Tea and Clove extracts against *Vibrio cholerae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Journal of Medicinal Plants Research. 2010; 4: 2393-2398.

[28]. Mehrotra V, Mehrotra S, Kirar V, Shyam R, Misra K, Srivastava AK, Nandi SP. Antioxidant and antimicrobial activities of aqueous extract of *Withania somnifera* against methicillin-resistant *Staphylococcus aureus*. J Microbiol Biotech Res. 2011; 1: 40-45.

[29]. Chew KK, Khoo MZ, Ng SY, Thoo YY, Wan Aida WM, Ho CW. Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Orthosiphon stamineus* extracts. International Food Research Journal. 2011; 18: 1427-1435.

[30]. Subramanian N, Venkatesh, P, Ganguli S, Sinkar VP. Role of polyphenol oxidase and peroxidase in the generation of black tea theaflavins. Agric Food Chem. 1999; 47: 2571–2578.

[31]. Bello AB, Sule MS. Optimum temperature and thermal stability of crude polyphenol oxidase from some common fruits. Nigerian Journal of Basic and Applied Science. 2012; 20: 27-31.

[32]. Mehrotra, S, Jamwal R, Shyam R, Meena DK, Mishra K, Patra R, De R, Mukhopadhyay A, Srivastava AK, Nandi SP. Anti-*Helicobacter pylori* and antioxidant properties of *Emblica officinalis* pulp extract: A potential source for therapeutic use against gastric ulcer. Journal of Medicinal Plants Research. 2011; 5: 2577-2583.

[33]. Kuate D, Etoundi BCO, Soukontoua YB, Ngondi JL, Oben, JE. Comparative study of the antioxidant, free radical scavenging activity and human LDL oxidation inhibition of three extracts from seeds of a cameroonian spice, Xylopia parviflora (A. Rich.) Benth. (Annonaceae). International Journal of Biomedical and Pharmaceutical Sciences. 2011; 5: 18-30.