WWW.IJRPR.COM

**International Journal of Research Publication and Reviews**

Journal homepage: www.ijrpr.com ISSN 2582-7421

# Green Tea *(Camellia sinensis)* extraction by different methods and it's antibacterial, antifungal activities : A Review

***Afroz Shaikh*[1]**

Postgraduate scholar[1],Department of Microbiology, KTHM College, Nashik, Maharashtra.

ABSTRACT:

Green tea extract contains several polyphenolic components with antioxidant properties, but the flavanol monomers known as catechins are the most active antioxidant compounds, with epigallocatechin-3-gallate and epicatechin-3-gallate being most effective(1).Catechins, which include epigallocatechin gallate (EGCG), epicatechin gallate, and gallocatechin gallate, are polyphenolic flavonoids found in green tea. EGCG is regarded to be the most pharmacologically active of the catechins(2),(3).Catechins constitute approximately 30-42% of the dry weight of brewed green tea, with EGCG being the most common type. Other polyphenols, such as quercetin, kaempferol, and myricetin, as well as alkaloids like caffeine and theobromine, are detected in lower doses in tea leaves. A typical brewed green tea beverage contains 240-320 mg of catechins, of which 60-65 percent is EGCG and 20-50 mg is caffeine(4).

## Introduction:

Green tea has now become a popular component in the growing market for nutraceuticals and functional foods due to a variety of health claims(1).Antioxidant, antibacterial, anti-collagenase, antimutagenic, and chemopreventive properties have shown to be beneficial in the treatment of chronic disorders such as periodontal disease. As per research, this kind of processing has the biggest impact on catechin concentration. Green tea also contains antihypertensive, cardiovascular risk-lowering, antibacterial, antiviral, and antifungal effects, according to several epidemiological research.In China, Japan, Korea, and Morocco, green tea is widely consumed. Since ancient times, it has been considered as a healthy beverage. This plant is used in traditional Chinese medicine to treat headaches, body pains, general pain, digestion, depression, as an energizer, and to help people live longer(5)

## Extraction:

1. **Ultrahigh pressure extraction (UPE):**
   The freshly crushed green tea leaves were sieved. Green tea leaves powder samples were weighed and blended with 200 mL of % (v/v) ethanol solvent before being placed in a sterile polypropylene bag. After removing all air from the bag, it was sealed and placed inside the ultrahigh pressure vessel. The mixture was filtered through a filter paper after being processed in an ultrahigh pressure apparatus for 5 minutes at ambient temperature (high pressure level: 400 MPa)(6),(7).The supernatants were pooled after centrifugation at 4000 g for 10 minutes. The supernatants were pooled after the extracts were centrifuged again under the same conditions. The resulting supernatants were mixed and concentrated in a rotary evaporator at 40°C under decreased pressure before being lyophilized. Green Tea Extract by UPE was created in this manner.(8)

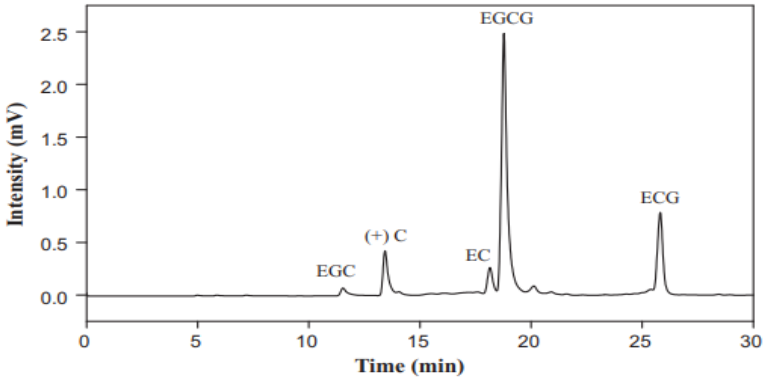


**Fig. 1.** The chromatogram of polyphenols components of GTE by UPE.

**2. Soxhlet extraction (SE)**

The sample was weighed exactly (10 g) and deposited in the Soxhlet extractor, along with 200 mL of a 50% (v/v) ethanol solution.The solvent was then refluxed for 6 hours in a water bath to allow it to boil constantly(9).The extracts were obtained by using UPE procedure.(8)

3. **Microwave extraction (ME)**

A microwave extractor with a pressure control and a second time-base design (MSP-100D, Beijing Rayme Science & Technology Co., Ltd., Beijing, China). After weighing the sample (10 g), it was placed in a 300 mL quartz extraction tank with a reflux system. In 200 mL of 50 % ethanol, all microwave extractions were carried out at a certain microwave irradiation power (1000W) for a specific time (10 minutes).(10)The pots were allowed to cool to room temperature before being opened after extraction. The extracts were made using the UPE method.(8)

**4. Hot water-based extraction**

The extraction technique of hot water extraction is a traditional one. Dried leaves are simply placed in a boiling water bath at a temperature of 80–100 °C in this method. The extraction time might be extended from 1 to 6 hours depending on the substrate(11).The procedure has been thoroughly studied and compared to other extraction techniques, despite the fact that it is time demanding(12).The caffeine was collected as a byproduct of a 60 ℃ hot water extraction of dried tea leaves followed by decaffeination(13).Prior to the extraction, the enzymes were deactivated. Ethyl acetate was utilized as a solvent to extract the polyphenols from the decaffeinated extract. The phenolic contents of the extract was determined using the Folin-Ciocalteu reagent, and the various polyphenols found were resolved using HPLC analysis. 2.56% total caffeine output and 19.33% total polyphenols were reported, respectively.(14)

**Analysis of constituents of Green Tea Extract (GTE)**

Green tea extracts were tested for polyphenol, saccharide, and amino acid content(15),(16). Ferrous tartrate colorimetry was used to determine polyphenol levels. Using glucose as a standard, phenol–sulfuric acid techniques were used to measure saccharide concentration. The Lowry–Folin technique was used to determine the amino acid content.(8).

**5. Preparation of green tea aqueous extract and determination of its concentration**

In 150 ml of water, a powdered sample (16 g) of dried organic Darjeeling green tea leaves (bought from FabIndia Organics) was cooked for 30 minutes. After centrifugation at 5000 rpm for 10 minutes, the infusion was filtered, and the extract concentration was measured.(17),(18)

**HPLC analysis of catechins in green tea aqueous extract**

A 0.22 micrometer Millex polyethersulphone membrane was used to filter the produced green tea aqueous extract (Millipore, USA).

For liquid chromatography analysis of the catechins present in the sample, an Agilent 1200 Series HPLC system with a UV–visible detector was employed. At 35 0.5 C, a stainless steel column (250 mm 4.6 mm i.d.) was utilized, packed with Thermo Scientific Hypersil BDS C18, 5 micrometer particle diameter. The sample was put into the column in a volume of 10 microliters. The flow rate was kept constant at 1 ml/min, and UV absorbance was measured at 280 nm.

Phase A (acetonitrile/acetic acid/double distilled water-9/2/89 v/v/v) and Phase B (acetonitrile/acetic acid/double distilled water-80/2/18 v/v/v) made up a gradient mobile phase. The mobile phase A (100%) was begun and held for 10 minutes, then linearly increased to 60% mobile phase A, 32% mobile phase B over 15 minutes, and kept for 10 minutes. Before the second injection, the column was re-equilibrated with 100 percent mobile phase A for 10 minutes.(19),(20).

## Activities

**1. Antibacterial effects**

Bacteria were cultured in Muller-Hinton broth for 18 to 24 hours before being injected onto MH agar plates with or without 5 percent defibrinated horse blood using 0.1 ml of MH broth containing bacteria.Wells were then made in the agar and filled with 0.1 ml of tea extracts. The plates for facultative anaerobes were cultured aerobically at 37°C for 2 days in an incubator, and anaerobically at 37°C for 5 days in an anaerobic glove box for obligate anaerobes (LAB-Line Co., Melrose Park, IL). The box's environment consisted of 85 percent nitrogen, 10% carbon dioxide, and 5% hydrogen. The diameter of any microbial inhibitory zones that resulted was measured.(21)

**TABLE 1. Antibacterial action of Japanese green tea extracts**

| | Refined Green* | | | Ordinary Green | | | Coarse Green | | | Roasted Green | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 20 | 5 | 10 | 20 | 5 | 10 | 20 | 5 | 10 | 20 |
| *S. mutans* | −† | − | − | − | ± | ± | − | − | − | − | − | − |
| *sanguis* | − | ± | + | − | ± | ± | − | − | − | − | − | − |
| *intermedius* | − | − | − | − | − | − | − | − | − | − | − | − |
| *Staph. aureus* | ++ | ++ | +++ | + | + | ++ | ± | + | + | − | ± | ± |
| *epidermidis* | + | ++ | ++ | ± | + | + | ± | + | ++ | − | ± | + |
| *P. mugnus* | − | − | − | − | − | − | − | − | − | − | − | − |
| *niger* | + | ++ | ++ | +++ | +++ | +++ | + | ++ | ++ | ++ | +++ | +++ |
| *Ps. micros* | − | − | − | − | − | − | − | − | − | − | − | − |
| *anaerovius* | − | − | − | − | − | − | − | − | − | − | − | − |
| *A. naeslundii* | − | − | − | − | − | − | − | − | − | − | − | − |
| *viscosus* | − | − | − | − | − | − | − | − | − | − | − | − |
| *israelii* | − | − | − | − | − | − | − | − | − | − | − | − |
| *L. casei* | − | − | − | − | − | − | − | − | − | − | − | − |
| *acidophillus* | − | − | − | − | − | − | − | − | − | − | − | − |
| *rogosae* | − | − | − | − | − | − | − | − | − | − | − | − |
| *Bifid. bifidum* | − | ± | + | − | − | − | − | − | − | − | − | − |
| *E. lentun* | ++ | +++ | +++ | +++ | +++ | +++ | ++ | ++ | ++ | ++ | +++ | +++ |
| *limosum* | ++ | ++ | +++ | ++ | ++ | +++ | ± | + | + | ++ | +++ | +++ |
| *Prop. acnes* | ++ | +++ | +++ | +++ | +++ | +++ | + | ++ | +++ | + | ++ | +++ |
| *V. parvula* | − | − | ± | − | − | − | − | − | − | − | − | − |
| *alcalescens* | ± | ± | ± | − | − | − | − | − | ± | − | − | ± |
| *F. nucleatum* | + | + | ++ | ± | ± | + | − | ± | ± | ± | ± | + |
| *B. ebdodontalis* | ± | ± | + | − | ± | ++ | − | − | − | − | − | − |
| *intermedius* | − | − | − | − | − | − | − | − | − | − | − | − |

* Concentration of tea (%).
† −, no inhibition; +++, diameter (D) > 20 mm; ++, 20 ≥ D > 16 mm; +, 16 ≥ 12 mm; ±, 12 ≥ D > 8 mm.

**2. Antibacterial susceptibility testing**

The Disc diffusion method was used to test antibacterial susceptibility. sterile Muller Hinton agar plates were used to test the antibacterial activity of tea leaf extracts. Muller Hinton agar was made and autoclaved for 15 minutes at 121°C. Under aseptic conditions, I poured the medium into sterile Petri plates. The media was then allowed to harden at ambient temperature before being stored at 4°C until needed. After solidification, 0.2 ml of inoculum suspension was inoculated with a micropipette and spread uniformly over the agar surface with a sterile glass spreader, then allowed to dry for 5 minutes. On sterile individual discs, different concentrations of tea leaf extracts (10, 20, 30 microliter) were inserted.The extract was allowed to diffuse for at least 5 minutes after the loaded discs were placed on the surface of the medium. The plates were held at 37°C for 24-48 hours to allow for incubation. Negative controls included methanol, ethanol, and distilled water. After 24-48 hours of incubation, plates were examined for the formation of zones of inhibition surrounding the discs. Antibacterial activity was determined by measuring the diameter of bacterial growth inhibition zones (in millimeters).(22)

Table 3. Zone of inhibition in (mm) for ethanolic extracts of *Camellia sinesis* against various bacteria isolated from environmental sources.

| Bacteria | Size of zones of inhibition (mm) | | | Control diameter (mm) |
|---|---|---|---|---|
| | 10 µl | 20 µl | 30 µl | |
| ***Staphylococcus aureus*** | 8 | 9 | 12 | - |
| ***Streptococcus*** | 9 | 10 | 13 | - |
| ***Pseudomonas aeruginosa*** | 7 | 9 | 10 | - |
| ***Bacillus*** | 10 | 11 | 12 | - |
| ***E.coli*** | 10 | 12 | 13 | - |
| ***Proteus*** | 8 | 9 | 11 | - |

**3. Antifungal activity of catechins**

Resting fungal cells were created to evaluate the effect of catechins on non-multiplying fungal cells. *C. albicans* was grown aerobically for 24 hours in brain heart infusion (BHI) broth (Difco Laboratories, Detroit, MI, USA). Harvested cells were washed three times in 0.15 M NaPB (pH 7.0), suspended in the same buffer to a final concentration of ~1×106 cfu/mL, and utilized in the test. Resting cells (~1×106 cfu/mL) in NaPB, pH 7.0, were given one milligrams per mL of each catechin, and the combination was incubated at 37°C with shaking. Over a long period of time, aliquots (0.1 mL) of the cell suspensions were collected.The samples were ten-fold diluted in 0.9 mL Tris–HCl buffer (0.05 M, pH 7.0) and inoculated onto Sabouraud agar plates. The cfu was calculated after the plates were cultivated aerobically at 37°C for 48 hours.(23)

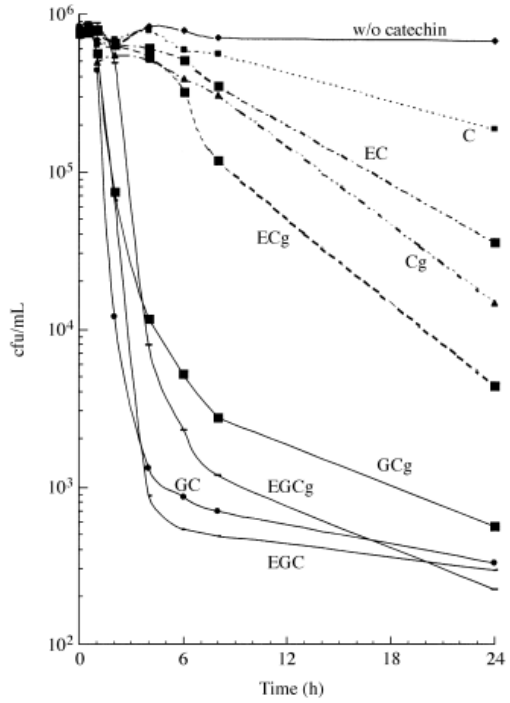


**Figure 1.** Antifungal effect of various catechins against *C. albicans* ATCC 90029 using resting cells with NaPB, pH 7.0.

4. **Antibacterial and antifungal activities of green tea polyphenols**
In two distinct conical flasks, a fungus *(Penicillium)* and bacterium *(Staphylococcus aureus)* were introduced. Using a flamed loop, contain 50 ml of SDA and NA (medium). Under aseptic circumstances, drops of fungus/water culture were combined with heated, melted, autoclaved PDA and put into different plates. The plates were covered and placed in the refrigerator to cool. The plates were inverted and left for a few minutes once the agar had partially solidified. Five wells were created in the center of the plate once it had cooled. A 6 mm cork borer or puncher that had been disinfected with alcohol and flame was used to create the wells.Using a microplate, ethanol and methanol extracted samples were dissolved in a solvent at a final concentration of 10 mg/1 mL and pipette into the various wells in a sterilized environment at varying volumes (0.1-0.2-0.4-0.6 ml). Chloramphenicol and Ketakonizol were used to inoculate control wells with bacteria and fungi, accordingly. The plates were marked, covered, inverted, and incubated for approximately 48 hours.The inhibitory zone was measured.(24)

## Discussion

1. The Japanese green teas utilized in this study are commonly consumed, and it has recently been shown that they have antibacterial properties against numerous bacteria found in the intestines (25,26,27). Various bacteria have been discovered from infected root canals, with the obligate anaerobes gaining popularity as provoking factors (10, 11). However, there have been few studies on Japanese green teas' antibacterial activity against the obligate anaerobe. If Japanese green teas are proved to have an antibacterial impact on bacteria found in infected root canals, they could be a potential medicament for use in the treatment of infected canals.(28,29),(21).
2. Methanolic extracts had the highest activity against *Staphylococcus*, *Streptococci*, and *Bacillus*, but were the least active against *Pseudomonas* and *Proteus*. Tea has been shown to have antibacterial properties against a variety of microorganisms. The presence of polyphenols in tea gives it antimicrobial properties. Catechins, a type of antioxidant polyphenol, play a key part in green tea's ability to prevent bacterial development. Epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), epicatechin (EC), and gallocatechin-3-gallate (GCG) are some of the most important catechins (30). Tea extracts have particularly specific antimicrobial properties.The concentration and kind of extracts determine the difference in their activity. These effects can vary depending on the bacterial species, and they might be growth inhibitory or stimulatory (31). Green tea leaf extracts examined in this study had variable levels of activity against microorganisms in the environment. Tea's active ingredient is thought to inhibit the growth and development of germs. The presence of catechins and polyphenols, which disrupt bacterial cell membranes, gives tea its strongest antibacterial effect. Green teas have been demonstrated to have stronger antibacterial activity than black teas. This discrepancy in results is most likely owing to the presence of varied active ingredient amounts in these tea varieties(32),(33).Green tea can kill Gram-positive *Staphylococcus aureus*, as well as many other dangerous bacteria, if consumed on a daily basis. Antibacterial, antiviral, anticarcinogenic, and antimutagenic activities are all present in tea components (34). The antibacterial action of plant extracts, on the other hand, is influenced by the presence of secondary metabolites such as hydroxyl groups on the active ingredients. Antimicrobials are physiologically active chemicals found in plant extracts(22).
3. In the case of fungi, Okubo et al.(35) found that 2.5 percent black tea extract completely found to inhibit the growth of *Trichophyton mentagrophytes* and *Trichophyton rubrum;* however, this extract did not inhibit the growth of *Candida albicans* or *Cryptococcus (Filobasidiella) neoformans* even at a 10 percent concentration. Antifungal activity has recently been reported in botanical(36,37), marine (38,39), and bacterial(40) natural compounds. The antifungal activity of catechins against *Candida albicans* was found to be pH dependent in the current investigation (Table 1). These data show that acidic circumstances decreased EGCg's antifungal activity. When the pH was dropped from 7.0 to 6.5, the MIC90 of EGCg increased by more than 10-fold, and then by several folds when the pH was reduced from 6.5 to 6.0.(23)

**Table 1.** $MIC_{90}$ and MFC of EGCg for *C. albicans* at various pHs

| | $MIC_{90}$ and MFC (mg/L) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ATCC 90028 | | ATCC 90029 | | ATCC 96901 | | ATCC 200955 | |
| pH | $MIC_{90}$ | MFC | $MIC_{90}$ | MFC | $MIC_{90}$ | MFC | $MIC_{90}$ | MFC |
| 6.0 | 2000 | 8000 | 2000 | 8000 | 2000 | 4000 | 2000 | 8000 |
| 6.5 | 1000–500 | 2000 | 1000–500 | 2000 | 500 | 2000 | 500 | 2000 |
| 7.0 | 250–62.5 | 2000 | 125–31.2 | 1000 | 15.6 | 250–125 | 62.5–31.2 | 1000–500 |

4. Using the agar gel diffusion method, the methanol and ethanolic extracts of polyphenols demonstrated considerable antibacterial and antifungal activity. In comparison to other extracts, the methanolic extract demonstrated significant antibacterial and antifungal activity against *Staphylococcus aureus* and *Penicillium sps*. The antibacterial susceptibility of polyphenolic methanolic and ethanolic extracts on *Staphylococcus aureus* shown in Figures 2 and 3 indicates that even low quantities of the extract were effective. The antifungal susceptibility of the methanolic and ethanolic extracts of the polyphenols on *Penicillium sps*. is shown in Figures 4, 5. When the concentration was 0.1 mg, neither extract had any antibacterial or antifungal activity. The extracts had different zones of inhibition (Table 2) that were comparable to the conventional medication.The maximum inhibitory concentration (MIC) of the methanol extract (0.4 ml) for antibacterial and antifungal activity was determined to be 26 mm and 15 mm, respectively, which was found to be similar to the inhibition levels of the standard chloramphenicol medication (43 mm).
The study found that polyphenol solvent extracts created significant zones of inhibition against *Staphylococcus aureus* and *Penicillium*. This verifies reports of its use as an anti-infective and reveals the presence of significant antibacterial and antifungal action.
Despite the fact that both the methanol and ethanol extracts of polyphenols had inhibitory effects on the test organisms, the methanol extract had a stronger inhibitory effect than the ethanol extract.(24)

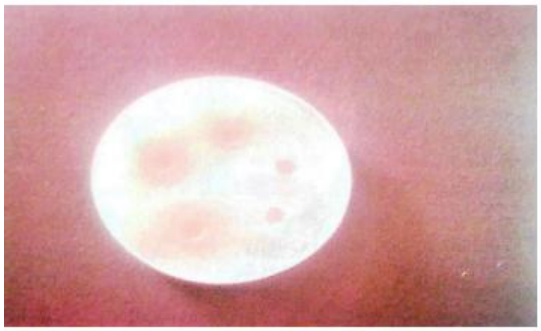

Figure 2: Antibacterial activity of Green Tea Polyphenols extracted with water.

Figure 5: Antifungal activity of Green Tea Polyphenols with Ethanol.

Figure 3: Antibacterial activity of Green Tea Polyphenols extracted with Ethanol.

Figure 4: Antifungal activity of Green Tea Polyphenols extracted with Water.

**Table 2: Antibacterial and antifungal activity of polyphenols.**

| Type of extract | Antibacterial activity | | Antifungal activity | |
|---|---|---|---|---|
| | Concentration of the extract (ml) | Zone of inhibition (mm) | Concentration of the extract (ml) | Zone of inhibition (mm) |
| Ethanol | 0.1 | 17.0 | 0.1 | - |
| | 0.2 | 18.0 | 0.2 | 17.0 |
| | 0.3 | 21.0 | 0.3 | 18.0 |
| | 0.4 | 32.0 | 0.4 | 20.0 |
| Methanol | 0.1 | 15.0 | 0.1 | - |
| | 0.2 | 24.0 | 0.2 | 18.0 |
| | 0.3 | 30.0 | 0.3 | 20.0 |
| | 0.4 | 26.0 | 0.4 | 15.0 |

**References**


1. Green tea extract: Chemistry, antioxidant properties and food applications – A review (https://doi.org/10.1016/j.jff.2013.08.011)
2. Green tea extract ingestion, fat oxidation, and glucose tolerance in healthy humans (https://academic.oup.com/ajcn/article-abstract/87/3/778/4633440)
3. Catechins from Green Tea (Camellia sinensis) Inhibit Bovine and Human Cartilage Proteoglycan and Type II Collagen Degradation In Vitro (https://www.koreascience.or.kr/article/JAKO201023064630588.view?orgId=anpor&hide=breadcrumb,journalinfo)
4. Recent Scientific Studies of a Traditional Chinese Medicine, Tea, on Prevention of Chronic Diseases (https://doi.org/10.4103/2225-4110.124326)
5. Green tea extract for periodontal health (https://dx.doi.org/10.4103%2F0972-124X.82258)
6. Annual trends and health risks of antibiotics and antibiotic resistance genes in a drinking water source in East China (https://doi.org/10.1016/j.scitotenv.2021.148152)
7. Separation of pollutants from aqueous solution using nanoclay and its nanocomposites: A review (https://doi.org/10.1016/j.chemosphere.2021.130961)
8. Comparison of in vitro antioxidant activities and bioactive components of green tea extracts by different extraction methods (https://doi.org/10.1016/j.ijpharm.2011.02.002)
9. Extraction of anthocyanins from grape skins assisted by high hydrostatic pressure (https://doi.org/10.1016/j.jfoodeng.2008.07.003)
10. https://www.researchgate.net/scientific-contributions/Yuko-Hamai-38488169
11. https://patents.google.com/patent/US6544581B1/en
12. https://doi.org/10.1007/s11947-015-1479-y

13. https://www.researchgate.net/profile/Chiranjit-Bhattacharjee/publication/265103214_Extraction_of_Polyphenols_from_Dried_Tea_Leaves/links/5594e09708ae99aa62c5b68c/Extraction-of-Polyphenols-from-Dried-Tea-Leaves.pdf
14. https://doi.org/10.1007/s11947-015-1479-y
15. https://doi.org/10.1007/s00217-006-0490-y
16. https://doi.org/10.1016/S0022-2275(20)39803-5
17. https://www.researchgate.net/profile/Julita-Regula/publication/51376524_Use_of_tea_extracts_Camelia_sinensis_in_jelly_candies_as_polyphenols_sources_in_human_diet/links/5d246d76299bf1547ca51ce6/Use-of-tea-extracts-Camelia-sinensis-in-jelly-candies-as-polyphenols-sources-in-human-diet.pdf
18. https://doi.org/10.1016/j.foodchem.2012.04.143
19. https://www.sciencedirect.com/science/article/pii/S0039914002000309
20. https://doi.org/10.1016/j.foodchem.2012.04.143
21. https://doi.org/10.1016/s0099-2399(06)81743-7
22. https://www.researchgate.net/publication/330703139_Antibacterial_activity_of_green_tea_Camellia_sinensis_extracts_against_various_bacteria_isolated_from_environmental_sources
23. https://doi.org/10.1093/jac/dkh046
24. https://www.researchgate.net/publication/352095649_Antibacterial_Antifungal_and_Anticorrosion_Properties_of_Green_Tea_Polyphenols_Extracted_Using_Different_Solvents
25. https://pubmed.ncbi.nlm.nih.gov/7251263/
26. https://www.researchgate.net/publication/230007691_The_Bactericidal_Activity_of_Tea_and_Coffee
27. https://pubmed.ncbi.nlm.nih.gov/2677434/
28. https://pubmed.ncbi.nlm.nih.gov/6109270/
29. https://www.sciencedirect.com/science/article/pii/S0099239987800882
30. https://pubmed.ncbi.nlm.nih.gov/11178724/
31. https://pubmed.ncbi.nlm.nih.gov/16106094/
32. https://books.google.co.in/books?id=K2O1DwAAQBAJ&pg=PA168&lpg=PA168&dq=wang,+z.y.,+m.t.+huang,+r.+chang,+w.+ma+and+ferraro+t.+1992.++inhibitory+effect+of+green+tea+on+the+growth+of+established+skin++papillomas+in+mice.+cancer+res.+52:+6657-6665&source=bl&ots=7LKJaCgrvz&sig=ACfU3U1gydkqEaHKiwt2mcnlHIVkLVQk3A&hl=en
33. https://books.google.co.in/books?id=di4v4jP9fawC&pg=PA293&lpg=PA293&dq=wang,+z.y.,+m.t.+huang,+r.+chang,+w.+ma+and+ferraro+t.+1992.++inhibitory+effect+of+green+tea+on+the+growth+of+established+skin++papillomas+in+mice.+cancer+res.+52:+6657-6665&source=bl&ots=GHoR2Wymyw&sig=ACfU3U3Z14DO_rbb06LpZT33TiylOV_Y-A&hl=en
34. https://pubmed.ncbi.nlm.nih.gov/2019547/
35. https://pubmed.ncbi.nlm.nih.gov/2062004/
36. https://academic.oup.com/jac/article-abstract/51/5/1223/784261
37. https://www.researchgate.net/publication/10711205_Antifungal_Antiprotozoal_Cytotoxic_and_Piscicidal_Properties_of_Justicidin_B_and_a_New_Arylnaphthalide_Lignan_from_Phyllanthus_piscatorum
38. https://pubmed.ncbi.nlm.nih.gov/12781505/
39. https://pubmed.ncbi.nlm.nih.gov/12385701/
40. https://pubmed.ncbi.nlm.nih.gov/12514051/