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Antibacterial activity of some natural bioactive materials: Green tea and Bee honey against pathogenic strains

• **Siuli Shaw** • **Surabhi Pramanik**
• **Sonal Suman**

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Corresponding Author : Sonal Suman

Abstract : *Natural bioactive products available in the market like Green tea and Honey are consumed for their antioxidant properties, medicinal properties etc. This study was done to determine the antibacterial activity of green tea and honey against clinically isolated pathogenic strains Escherichia coli and Staphylococcus aureus and their inhibition at different time intervals using concentrations similar to the amount usually taken for daily consumption. The clinical strains were checked for their susceptibility and resistivity against antibiotics. The aqueous as well as ethanolic extract of green tea and honey dilution were prepared. The antibacterial activity was determined by using paper disc diffusion method. The aqueous extract was effective at 150mg/ml concentration but ethanolic extract showed maximum zone of inhibition at the same concentration. Similarly, honey exhibited maximum zone of inhibition at 100% dilution among the other dilutions taken under observation. The phytochemical analysis was also done which defined the presence of phenol, tannin, flavonoid and absence of steroids. Time kill assay was performed in which tubes were taken, each with final volume of tea extract and nutrient broth of 10ml, similar steps were followed for honey dilutions. Complete inhibition of growth was observed at 6 hours and 12 hours in case of ethanolic extract at 150mg/ml against E. coli and S. aureus, whereas in aqueous extract it was achieved at 12 hours and 24 hours at the same concentrations. 100% dilution of honey exhibited complete growth inhibition of E. coli and S. aureus at 12 hours.*

Key Words: *Bioactive products, antibacterial activity, phytochemical analysis, time kill assay.*

Siuli Shaw
B.Sc. III year, Industrial Microbiology (Hons.),
Session : 2012-2015, Patna Women's College,
Patna University, Patna, Bihar, India

Surabhi Pramanik
B.Sc. III year, Industrial Microbiology (Hons.),
Session : 2012-2015, Patna Women's College,
Patna University, Patna, Bihar, India

Sonal Suman
Guest Faculty, Deptt. of Industrial Microbiology,
Patna Women's College, Bailey Road,
Patna – 800 001, Bihar, India.
E-mail : sonal.micro89@gmail.com

Introduction :

Natural bioactive products like Green tea (*Camellia sinensis*) and Honey have been reported to have antioxidant and antimicrobial properties. Green tea is a non fermented tea. The tea is an infusion of leaves that has been consumed for centuries as a beverage and is valued for its medicinal properties. It has been reported to have

antimicrobial activities against various pathogenic bacteria, including Methicillin resistant-*Staphylococcus aureus* (MRSA) (Stapleton et al. 2004) and Multi Drug resistant-*Pseudomonas aeruginosa* (Shibata et al. 2005). The properties of green tea which inhibit bacterial growth are mainly related to their polyphenolic components including epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate against various Gram-positive and Gram-negative bacteria. The phytochemical screening of tea revealed the presence of alkaloids, saponins, tannins, catechins and polyphenols (Mbata et al., 2006). Honey is also a very popular product because of its beneficial effect on human health. The pure honey contains phenolic substances includes cinnamic acid derivatives, alkaloids, auterquinone glycosides, cardiac glycosides, flavonoids, antioxidants and reducing compounds.The antibacterial properties of honey include release of H_2O_2,the phytochemical compounds and high sugar content which serve osmolarity sufficient to inhibit bacterial growth (Dumronglert 1983). Many pathogenic bacteria are susceptible to honey and so it is considered effective compare to several well known antibiotics. Dabur honey is pure, natural and contains no additives or preservatives. In addition to being orally consumed it is also used to condition skin and heal wounds. Infectious diseases still represent an important cause of morbidity and mortality among humans; especially in developing countries (Ghaleb et al. 2011). In the recent years human pathogenic microorganisms have developed resistance in response to indiscriminate use of commercial antimicrobial drugs commonly employed in treatment of infectious diseases. Commercial antibiotics have become inefficient to cure many food borne diseases which are very common these days. Therefore, alternative antimicrobial agents are needed to be developed and employed to control multi-drug resistant bacteria. The present study was done to test the benefit of daily consumption of commercial green tea and honey on the basis of its capacity to control disease. The antibacterial activity was tested against clinical isolates of pathogenic strains of *E. coli* and *S. aureus* using paper disc diffusion method and time kill assay. Moreover phytochemical analysis of green tea was also done.

Materials and Methods :

Test Organisms : Pathogenic strains *Escherichia coli* and *Staphylococcus aureus* were obtained from "Gitanjali Patho Diagnostics", Vivekananda Marg; Boring Road, Patna. Source of *E. coli* was urine sample and source of *S. aureus* was pus sample. Strains were revived weekly in Nutrient Agar (NA) slants and stored at 4°C in refrigerator.

Reagents and Chemicals : All the chemicals were purchased from three different companies-Merck ltd, Nice ltd & Qualigens ltd. Extracts and dilutions were prepared in sterile distilled water. Antibiotics were purchased from medical stores.

Preparation of green tea extract : Lipton green tea was purchased from local market of Patna.

The aqueous and ehanolic extract of the green tea were processed according to the method of Nayak et al. (2011) and Yildrim et al. (2000).

Preparation of Honey Sample : Dabur honey was used. Different concentrations of Honey i.e. 20%, 40%, 60%, 80% & 100% was prepared with sterile distilled water (Chute et al. 2010).

Antibiotic Susceptibility Testing by Disc Diffusion Method : Standard antibiotic disc such as Streptomycin (10mg) and tablets i.e. Tetracycline (500mg), Erythromycin (250mg), Penicillin (200mg), Ciprofloxacin (500mg) were tested against pathogenic strains of *E .coli* and *S. aureus* by paper disc diffusion method (Aneja, 2013). Working solution of each antibiotic (30mg/ml) was prepared.

Antibacterial activity and MIC determination of Green tea and Honey : Green tea**:** The test was carried out on NA media by paper disc diffusion method. Three different concentrations of both aqueous and ethanolic green tea extract of 50mg, 100mg & 150mg respectively were prepared. Serial dilution up to 10^{-8} suspension was prepared. The zone of inhibition was observed and measured for different extracts (Kumar et al., 2012).

Honey**:** Various concentrations (20%, 40%, 60%, 80% & 100%) of honey were prepared by diluting it with sterile distilled water. Further test was carried out by disc diffusion method for both strains (Chute et al. 2010).

Phytochemical analysis of green tea : Analysis of green tea extract (phenol, tannin, flavonoid and steroid) was done following the method of Tariq and Reyaz (2012).

Test for Phenol : 0.5 ml of aqueous extract was taken in a test tube. Few drops of freshly prepared (0.5%) $FeCl_3$ solution was added in it. Appearance of dark green colour gave positive result for the presence of phenol.

Test for Tannin: 1 ml of aqueous extract was added to 5ml distilled water in a test tube. The tube was boiled for few mins and cooled down. 0.1% of freshly prepared $FeCl_3$ solution was added. Appearance of blue black colour showed the positive test for presence of tannin.

Test for Flavonoids (Ammonia Test): 1ml of aqueous extract and ammonia solution were added in the ratio (1:5) in a test tube. To this 3-5 ml of conc. H_2SO_4 was added. Appearance of yellow colour and its disappearance on standing showed positive result for the presence of flavonoid.

Test for Steroids: 2ml of acetic anhydride was added to 0.5 ml of extract. Further 2ml of conc. sulphuric acid was added slowly along the side wall of test tube. Change of colour from violet to blue or green showed positive result.

Time Kill assay of Green tea and Honey

Green tea: Different concentrations (50mg, 100mg, and 150mg/ml) were incorporated into nutrient broth to find their efficiency against isolated pathogenic strains. Bacterial cells at 10^4 cell/ml was inoculated to 10 ml of final volume and incubated at 37°C for 24hours. Time kill assay was determined by taking the absorbance at 600nm at intervals of 0, 2, 4, 6 and 24 hrs (Archana and Abraham 2011).

Honey: Different concentrations of honey (20%, 40%, 60%, 80% and 100%) were incorporated into the nutrient broth to find their efficiency against isolated pathogens. Bacterial cells at 10^4 cell/ml were inoculated to 10 ml of final volume and incubated at 37°C for 24hours. Time kill assay was determined by taking the absorbance at 420nm at intervals of 0, 2, 4, 6 and 24 hrs (Abdelmalek et al. 2012).

Results and Discussion :

Antibacterial susceptibility tests performed on microbial strains of *E. coli* and *S. aureus* obtained from "Gitanjali Patho Diagnostic". Ethanolic extracts of tea leaves at different concentration (50mg/ml,100mg/ml,150mg/ml) exhibited comparatively greater antibacterial effect against *E. coli* with zone of inhibition of 7mm,8mm and 12mm, respectively (Fig. 1c) , and in *S. aureus* with

zone of inhibition of 6mm,10mm and11.5mm, respectively, (Fig. 1d) than the aqueous extracts which showed zone of inhibition of 4mm, 5mm, 6mm against *E. coli* (Fig.1a) and 5mm, 5mm and 6mm against *S. aureus* (Fig.1b). Different dilutions of honey (60%, 80%, and 100%) exhibited inhibition zones of 5, 6, 6.5mm respectively, (Fig.1e) against *E. coli* and 5, 6, 6.25mm respectively against *S. aureus* (Fig.1f). However, there was no zone observed at lower concentration of 20% and 40%.

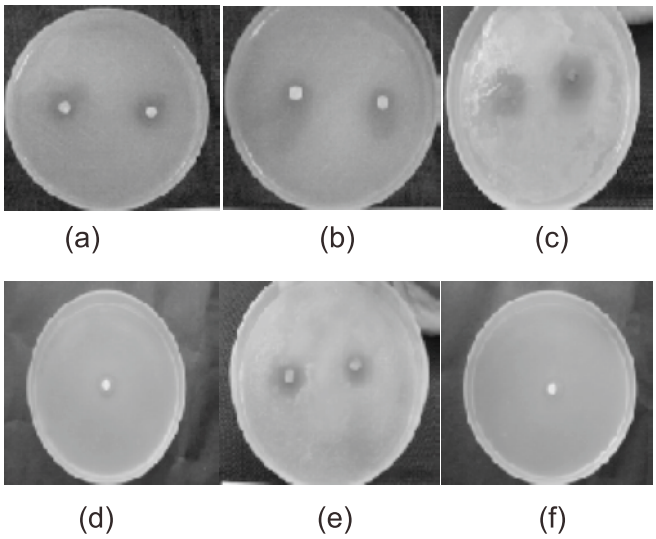

Fig.1. Antibacterial susceptibility test by disc diffusion method: (a) aqueous extract (150mg/ml) against *E. coli* (b) aqueous extract (150mg/ml) against *S. aureus.* (c) ethanolic extract (150mg/ml) against *E. coli* (d) ethanolic extract (150mg/ml) against *S. aureus.* e) honey (100%) against *E. coli* (f) honey (100%) against *S. aureus.*

As the growth was inhibited at lowest concentration therefore, 50mg/ml was considered as the MIC value among the others. Similar pattern of inhibitory zone was reported by Kumar et al (2012) in case of ethanolic extract against *S. aureus* and *E. coli*, respectively. According to Archana and Abraham (2011) the antimicrobial activity of methanolic extract of fresh green tea leaves, commercial leaves and dust tea exhibited highest zones compared to ethanolic and aqueous extract. Growth of pathogen was inhibited at 60% concentration of honey, so 60% concentration was determined as MIC value. The result of inhibitory zone of dabur honey was found in correlation with work of Chute et al (2010) against *E. coli* and *S. aureus* respectively.

Antibiotic susceptibility test showed more susceptibility towards Streptomycin with average zones of 12.5mm and 10mm. *S. aureus* was most susceptible to Tetracycline with average zone of 17mm. *E. coli* showed less susceptibility to other antibiotics used.

The study of time kill assay *E. coli* showed that the aqueous extracts (50mg/ml) were less inhibitory between 2-8 hours, however ethanolic extracts (50mg/ml) showed approx imately constant decrease in growth (Fig.2). At 100mg/ml concentration, growth of microbes was completely inhibited. However, ethanolic extracts completely inhibited the growth at 12 hrs but aqueous extracts inhibited completely at 24 hrs (Fig.3). Ethanolic extracts (150mg/ml) exhibited a rapid inhibitory effect within 6-12 hours than its corresponding aqueous extracts in which complete inhibition took place at 12 hours (Fig.4). The time kill assay of *S. aureus* showed that at 50 mg/ml concentration of aqueous and ethanolic extract growth were completely inhibited at 24 hours but the rate of inhibition was quite slow in both cases (Fig.5). At concentration of 100mg/ml, ethanolic extract resulted in low growth rate at 12 hours but in case of aqueous extract growth was inhibited at 24 hrs (Fig.6). Concentration of 150 mg/ml growth of ethanolic extract was completely inhibited at 12 hrs whereas in aqueous extract growth was completely inhibited at 24 hrs (Fig.7). This result was found in correlation with EL-Farmawi et al., (2014).

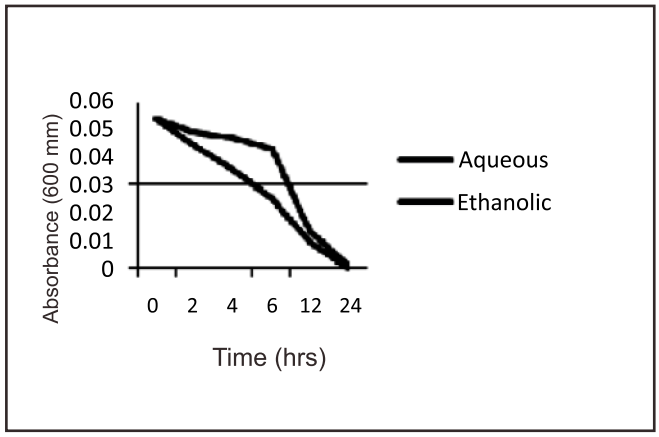

Fig.2. Time kill curve of green tea extract (50mg/ml) against *E. coli*.

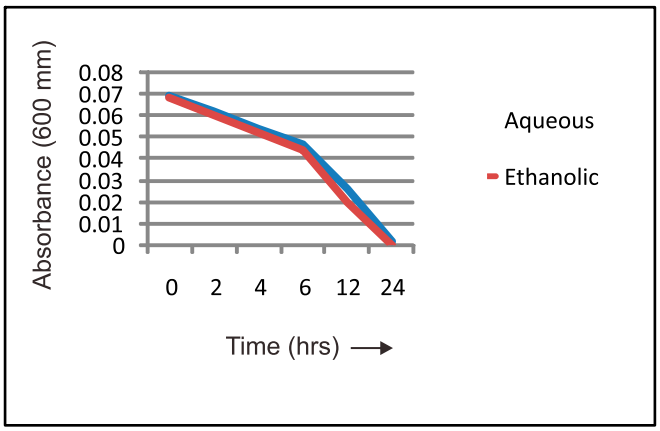

Fig.5. Time kill curve of green tea extract (50mg/ml) against *S. aureus*

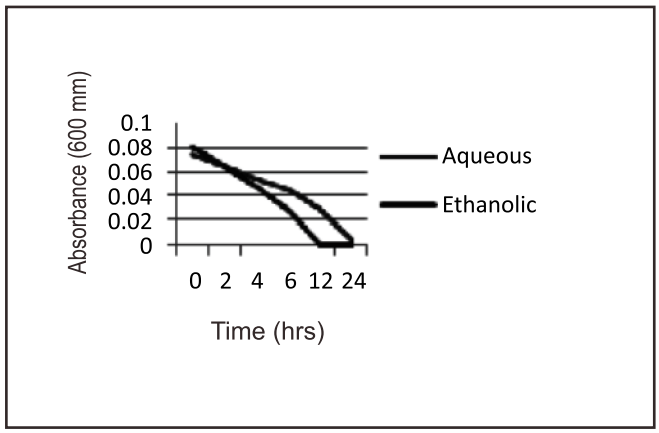

Fig.3. Time kill curve of green tea extract (100mg/ml) against *E. Coli*.

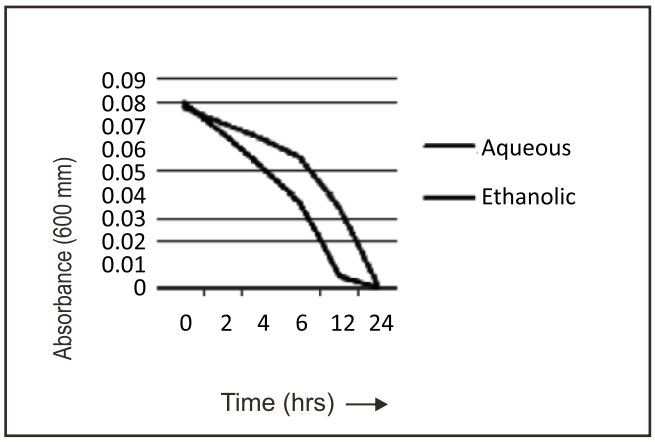

Fig.6. Time kill curve of green tea extract (100mg/ml) against *S. aureus*.

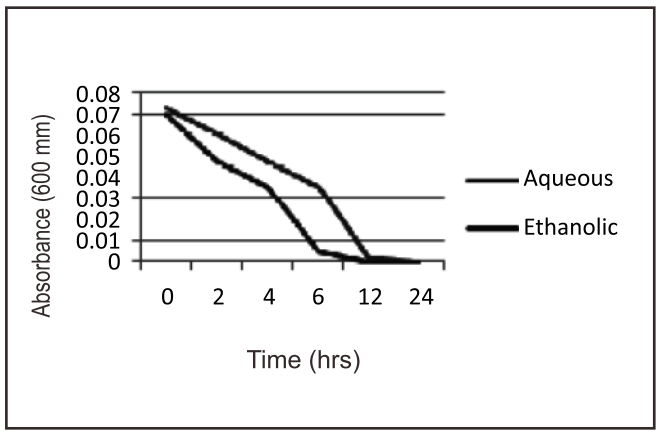

Fig.4. Time kill curve of green tea extract (150mg/ml) against *E. coli*.

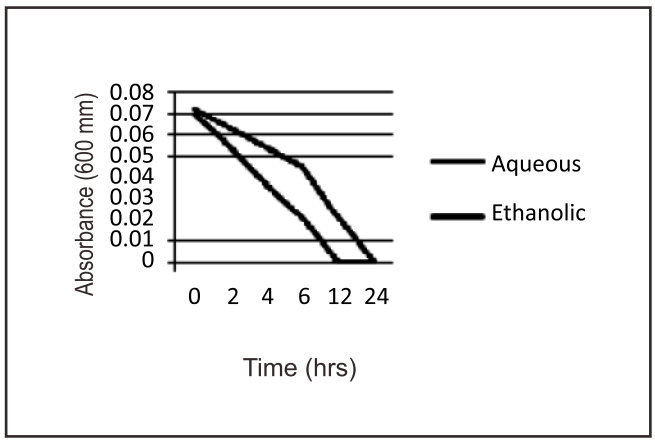

Fig.7. Time kill curve of green tea extract (150 mg/ml) against *S. aureus*.

Phytochemical analysis of green tea exhibited the presence of phenol, tannin and flavonoid and absence of steroids.This finding is in agreement with the findings of Tariq and Reyaz, (2012). In case of honey, similar assay was carried out at dilutions (20%,40%,60%,80% and 100%) for *S. aureus* and *E. coli*.

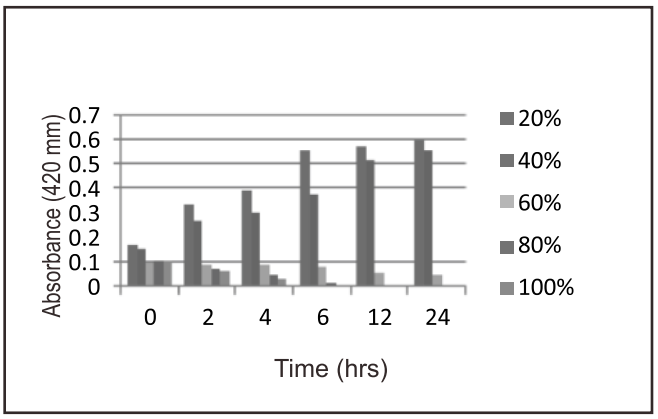

Fig.8: Time kill curve of Honey at different dilutions

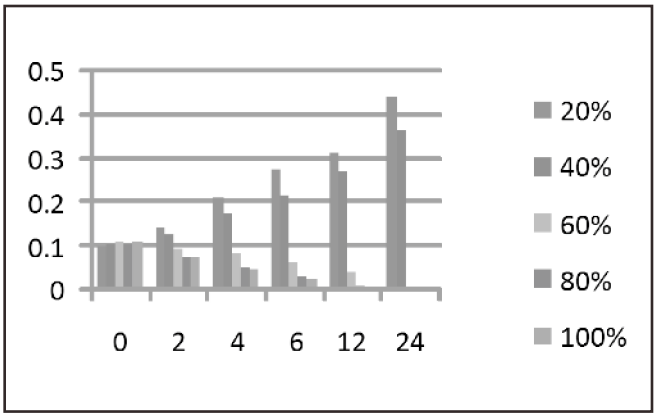

Fig.9: Time kill curve of Honey at different dilutions against against *S. aureus. E. coli*

Complete inhibition of growth was observed within 6-12 hours at dilution 80% and 100% for *S.aureus* (Fig.8) and constant rate of inhibition was found at dilution 100% for *E. coli* (Fig.9).This result was found to be in correlation with the work of EL-Farmawi et al (2014) in which it was seen that methanolic extract of green tea exhibited good and rapid bactericidal effect within 2- 4hrs for MRSA.

Conclusion :

From the above results it can be concluded that Commercial Lipton green tea and Dabur honey possess the antibacterial property in addition to their other beneficial attributes. This may be due to the presence of polyphenolic components including epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate in green tea and release of H_2O_2 as well as osmotic effect of the high sugar content in Honey. Hence consumption of green tea regularly can inhibit the intestinal pathogenic bacteria and its consumption along with honey instead of sugar is a better option.

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