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Studying the antimicrobial effect of green tea extract against common microbial pathogens

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Abstract
Consumption of healthy and organic food for a safe and happy life has been the priority of the current generation. In his context there are several age old foods and beverages that are re gaining their importance in the daily life. Once such very commonly used beverage of ages is green tea. Consumption of green tea which is a full load of antioxidants, on an empty stomach is known to be the one shot solution of several known ailments. Apart from its oral consumption use of green tea extracts in several products like cosmetics, tooth paste, bath soap etc has gained an immense popularity. Green tea is a type of beverage that is made from *Camellia sinensis* leaves. Many studies have shown that green tea extract can promote weight loss, blood sugar regulation, disease prevention and exercise recovery. Aim of the study is to test the antimicrobial activity of green tea against some common microbial pathogens. In this context the green tea leaf extraction was performed to obtain the active components in it which can be tested further. The solvents used for the extract preparation were water, ethanol and chloroform all in their pure form without any combinations. For testing inhibition of microbes some specific bacteria are needed in the work which wre also cultured directly from the source. The sample used for microbial extraction was skin and scalp source. This sample would allow the isolation of common skin and scalp contaminants. To obtain this bacterial culture spreading and streaking methods are used for the bacterial isolation. Bacteria isolated were later tested for their identity using microscopy and biochemical tests. Studying the inhibitory effect based on zone of inhibition by well diffusion is the most standard method which was selected in the current work. Obtaining a good inhibition of pathogens can reveal hopes for several other related research options which may include the use of green tea extracts in various cosmetics and heath drinks.

Keywords: Green tea, Beverages, Anti oxidants, Microbial pathogens, Anti microbial activity

Introduction
Camellia sinensis (L.) O. Kunze commonly known as green tea has been one of the wonderful health drinks in the present day [6]. This made the cultivation of the crop economically more valuable. Thus the crop is declared to be a commercial crop [4]. Apart from its nutritional and commercial value, it has a huge and a vital role in being a medicinal, antioxidant and anti microbial beverage [3]. Green tea is one of the aromatic and edible beverages that is prepared by hot and boiling water over the cured leaves of *Camellia sinensis* [2]. More than two third of the wold population is known to be consuming this tea according to the current statistics [5]. Green tea was exported from India to Japan for the first time during 17th century [1]. However its origin is owned from China [9]. It has been declared to be the 2nd most drink consumed worldwide after water [10]. Two major strains of *C. sinensis* are *Camellia sinensis sinensis* and *Camellia sinensis assamica* [7]. Among these two varieties the *sinensis* plant strain is originated from China and the second strain a*ssamica* being the natural inhabitant of Assam region in Northern India.

Major phytoconstituents present in the fresh leaves include 3-4% of alkaloids known as methylxanthines which may include caffeine, theobromine and theophylline. Phenolic acids are also present in the plant which include allergic acids and some characteristic amino acid such as theanine. Green tea also known to possess polyphenols like flavanols, flavodilols, flavonoids, and phenolic acid. Several studies based on animal models revealed that catechins of green tea provide a great protection against degenerative diseases. Further the anti proliferative activity of green tea extracts on hepatoma cells and a hypolipidemic activity in hepatoma-treated rats was well established by several research studies. Green tea was also known to be used as preventive agent against mammary cancer post-initiation [8].

Materials and Methods

Sample Collection and extract preparation

- Commercially available green tea powder was bought from the local distributor of Hyderabad, Telangana in its closed packaged form

Defatting of Tea powder

- The dry powdered content of the leaves which is very fine and coarse is collected into a glass-stoppered conical flask and mixed followed by maceration using required quantity of petroleum ether with a constant shaking which was than allowed to stand for over to night.
- Spot test on ordinary filter paper is used as a confirmation for the completion of defatting process.
- The solvent is than completely removed by filtration and air dry. This can be stored in a dry and closed glass container until further extraction process.

Extract reparation for study:

- A finely measured 8gms of previously prepared tea powder was macerated with 10 ml of ethanol, water and chloroform in separately labeled test tubes respectively and incubated for 24 hours.
- Filtered the above extracts using filter paper into separate labeled tubes.
- Filtered liquid is collected which appears to be coloured due to the presence of phyto chemicals from the leaves.

Obtaining the test bacteria for the study

According to the major focus of the study the effect of green tea extracts on skin and scalp pathogens has to be tested. Thus the initial step of the bacterial collection is to collect the samples from scalp and skin and isolate the inherent bacteria present.

Nutrient agar media being one of the basic and all purpose media for isolation of any unknown bacteria it has been prepared according to the standard composition and poured into respective sterilized plates for isolation of bacteria. All the plates were prepared under sterile conditions and in laminar air flow chamber to prevent the contamination by other microbes.

Collection of samples from skin and scalp:

In order to isolate the inherent bacteria present in the skin and scalp of humans a normal cotton bud can be used, which needs to be rubbed on the surface of skin and scalp in replicas separately. The collected samples are allowed to sit in sterile distilled water for an incubation time of 10 minutes at room temperature under sterile environment. 0.1 ml of each sample is used to inoculate the above nutrient agar plates in replicas and incubated at 37^0C overnight to obtain visible colonies of bacteria.

Preparation of pure culture liquid broth

To test the inhibitory effect of tea extract the bacteria are required in liquid broth for which LB broth was prepared and sterilized in an autoclave. The cooled broth was than inoculated with the respective colony from the master plates which are than incubated overnight. A final thick broth indicating good growth of the bacteria can be further used for inhibitory assays.

Well diffusion method for inhibitory assay

Nutrient agar plates were prepared for testing the inhibitory role of leaf extracts against all the isolated bacteria. A total of 4 bacteria were isolated 2 each sfrom scalp and skin respectively. 0.5ml of the isolated bacterial broth was initially spread on the plates and kept for a minute. Later with the help of gel puncher 4 wells were punched in the centre of the media plates. 3 are used for 3 extracts and 1 is used as control which do not need any extract to be added. All the three wells were added with 0.5ml of 3 different extracts of the leaf. The plates were incubated in straight position without inversion in an incubator overnight.

Measurement of zone of inhibition

After an incubation period of overnight all the plates were collected and the zones if any around the central wells were measured with a help of a graduated scale. The results are recorded and compared for the final conclusion.

Identification of Bacteria

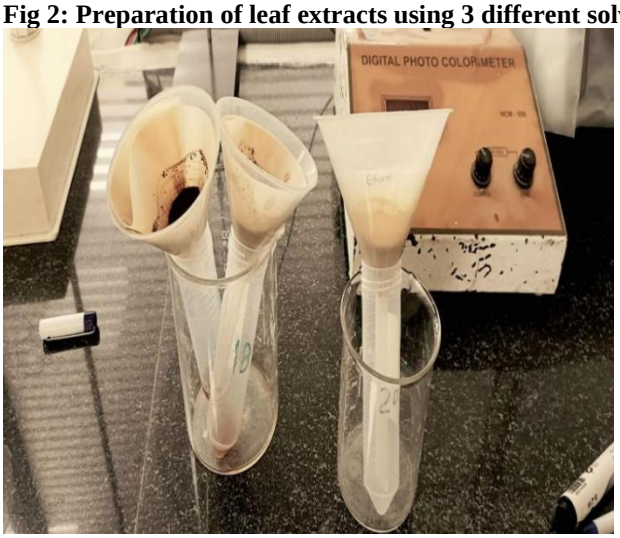
All the 6 bacterial species isolated were subjected for identification based on morphology and biochemical tests. For this 6 pure culture nutrient plates were prepared and used for further study. The first step in the identification of bacteria being the study of morphology and gram's nature, all the 6 cultures were subjected for Gram's staining using a freshly prepared smear of the cultures.

Gram's staining was performed according to the standard protocol and the slides were observed under microscope. Based on the Gram's nature and cell morphology the cells were later subjected for biochemical tests.

All the biochemical tests performed are in accordance with the standard Bergey's manual. Based on the biochemical characteristics and cell morphology the bacteria can be analyzed and identified.

Results and Discussion

Fig 2: Preparation of leaf extracts using 3 different solvents

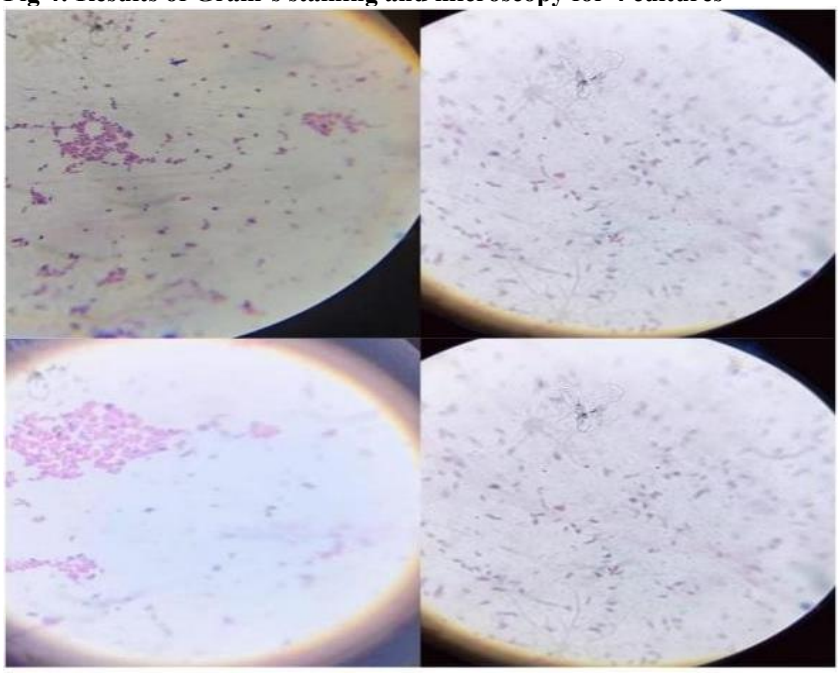


Inference: The above picture shows three different extracts being prepared and filtered using what-man filter paper.

Fig 3: Pure culture plates of bacteria obtained from scalp and skin samples

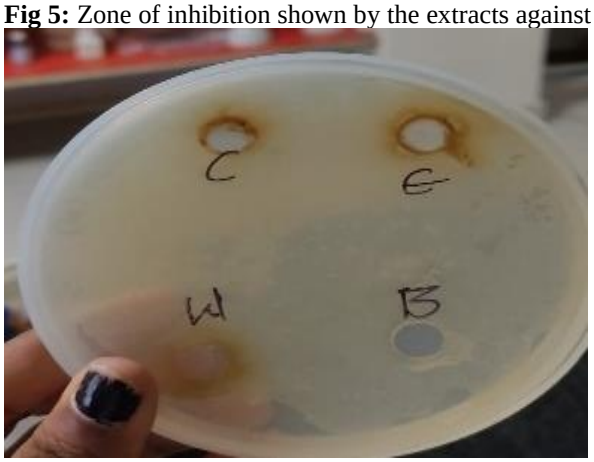
The above picture shows nutrient agar media plates containing the single streak of pure cultures obtained from 2 different sources

Fig 4: Results of Gram's staining and microscopy for 4 cultures



Inference: The above picture is the 100X image under microscope for all the 4 test bacteria. All of the images show gram positive bacterial species with variable morphology.

Fig 5: Zone of inhibition shown by the extracts against the test bacterial species



Inference: The above plate shows the zones that are very minute. Blank did not show any zone however the zones were obtained for water, chloroform and ethanol in the increasing order respectively. The image shown above corresponds to the test culture 1 of skin. Similar tests were performed for the other samples also and the results are noted furnished in the Table 1 below.

Table 1: Zone of inhibitions measured in centimeters obtained for all the test cultures against 3 extracts

Sample	Zone with water (cm)	Zone with Ethanol (cm)	Zone with Chloroform (cm)
Skin isolate 1 S1	0.2	0.5	0.6
Skin isolate 2 S2	0.4	0.3	0.8
Scalp isolate C1	0	0.3	0.5
Scalp isolate C2	0	0.2	0.3

Inference: From the above table the results are clearly readable and it can be observed that aqueous extracts of leaf were not showing a great inhibitory effect as it was zero for scalp isolates. Good degree of inhibition was seen by chloroform extract for all the 4 bacterial species. Ethanol showed a comparatively moderate inhibition.

Table 2: Biochemical tests for the identification of Bacteria

S.No	Sample	Gram's Nature	Identity based on biochemical tests
1	Skin isolate 1: S1	Gram +ve, Cocci	*Staphylococcus aureus*
2	Skin isolate 2: S2	Gram +ve Cocci	*Micrococcus*
3	Scalp isolate 1: C1	Gram +ve pleomorphic	*Corynebacterium sp*
4	Scalp isolate 2: C2	Gram +ve Bacilli	*Bacillus subtilis*

Inference: Based on the above test results the bacteria isolated from skin include *Staphylococcus aureus* and *Micrococcus* and the bacteria isolated from scalp sample were *Corynebacterium sp* and *Bacillus subtilis* . However all of them are aerobic and gram positive in nature.

Conclusion

There are two main objectives in the current work which include isolation and identification of common contaminant bacteria in skin and scalp and the second being testing the inhibitory effect green tea leaf extracts against the identified bacteria. All the bacteria were initially isolated from the respective samples onto the nutrient agar plates followed by their pure culturing in broth. The isolated bacteria were further identified based on Gram's staining and microscopic observation followed by biochemical characterization. The identified bacteria were tested against 3 different extracts of green tea which include ethanol extract, Chloroform extract and aqueous extract. Well diffusion method was used for the assay. The study revealed that aqueous extract is not much effective in inhibiting the growth of the study bacteria. However the extract using chloroform yielded a comparatively good inhibition than ethanol and aqueous extract. The highest inhibition was observed for the bacteria *Staphylococcus aureus* against chloroform extract yielding 0.8cms. All the bacteria isolated and identified include *Staphylococcus aureus, Micrococcus, Corynebacterium sp and Bacillus subtilis.* This work can be further extended to detect the application of these extracts in preparation of tooth paste, cosmetics, heath drinks etc and their role in the respective products.

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