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Research Article

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Antibacterial activity of extracted and purified caffeine from used and unused tea powder

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Abstract

Tea powder contains different components like tannin, catechins, caffeine, flavonoids, polyphenol, polysaccharides and fluronine. But tea powder contains the high amount of caffine. Extraction of caffeine was done from used and unused tea powder with its qualitative and quantitative analysis. Its antimicrobial activity was performed and also it act as a plant bio fertilizer. For this purpose ethanol extraction was done for used and unused tea powder. The purification of extracted crude caffeine was done by using 100% ethanol. For TLC analysis, the sample mixtures were prepared by adding 0.001g of caffeine in 1ml of absolute ethanol. Further for HPLC analysis, the gradient solvent system was prepared. Antimicrobial activity was studied with different bacterial species. Amongst different bacteria *E. coli, B. megaterium, B. subtilis, S. typhi* showing more clear zone of inhibition than *B. amyloliquefaciens.*

Keywords: Tea powder, caffeine, extraction, antimicrobial activity, plant bio fertilizer.

Introduction

The caffeine is still facing many controversies and misconceptions like its intake could result in enhanced risks of caffeine addiction, cancer, miscarriages, breast diseases, osteoporosis and hypertension etc. Caffeine is one of the most thoroughly investigated ingredient in the human food. In 1958, the US Food and Drug Administration (FDA) designated Caffeine as "generally recognized as safe" (GRAS) for consumption (Aurora A. *et al* 2005).

Tea and coffee are the most popular beverages for centuries, primarily due to their aroma, pleasant taste and stimulant effects (Alan M. 2004). Caffeine can stimulate the nervous system and can cause relaxation of respiratory and cardiac muscles. Caffeine is well known to increase both the alertness level and attention span. Also caffeine is useful in plant growth and nourishment. Caffeine is a chemical stimulant which increase the biological processes such as ability to increase the photosynthesis and absorb water and nutrients from soil (M. Kranthi Kumar 2015).

Caffeine has antimicrobial and antioxidant activities. It has been reported immunologic, anti HIV, antioxidant, antibacterial and antifungal activities. It provides a dietary source of biologically active compounds considered to be beneficial to the human health (Ibrahim S *et al*. 2006).

Compost tea contains caffeine and this caffeine is useful in plant growth and nourishment. It is a chemical stimulant which increase the biological

processes such as ability to increase photosynthesis and absorb water and nutrients from soil. Caffeine contains nitrogen, hydrogen, oxygen and carbon. These components helps in many metabolic activities of the plant like nucleotide synthesis, respiration, cell division, cell growth, flowering and fruiting, etc.

Materials and Methods

Collection of Used tea powder

The used tea powder was collected from homes and hotels of Aurangabad city, Maharashtra, India. The obtained tea powder was near about 500g. This used tea powder was dried, labelled and stored in a plastic container for further use.

Extraction of caffeine from used tea powder

100 g of tea powder was weighed and dissolved in 1000 ml of distilled water. The tea mixture is boiled at 60^{o}C for 20-30 min on the hot plate. After boiling the mixture was cooled and filtered by using muslin cloth. Filtrate was collected in a conical flask, 810ml filtrate was obtained. To that filtrate 10.6g sodium sulphate was added. This was added to dissolve the tannin in water so that it can be removed in the separating process. The filtrate was then transferred in a separating funnel and 102.98 ml of CH_2Cl_2 mixed into it using hand gloves. CH_2Cl_2 was added to dissolve the other impurities. Bottom layer was collected in a beaker. The solution was yellowish. To make that solution clear some crystals of Na_2SO_4 were added and mixed gently. At the bottom the crystals of impurity were seen. The upper clear solution was transferred to another beaker. The solution was then boiled on a hot plate until all the liquid gets evaporated and we got the powder at the bottom (a glass rod was kept into the beaker while evaporation to avoid the overflow of solution). The yellowish powder at the bottom is the crude caffeine (eku_chem_lab 2015).

Purification of caffeine

The crude was dissolved in absolute ethanol and poured in a watch glass (wet of the watch glass was taken before use). This was place on a boiling water bath at 100^{o} C for the evaporation. Absolute ethanol is added to the watch glass until we get the white coloured powder. The extracted white powder i.e. caffeine was weighed in a weighing balance with the previously weighed watch glass. The same procedure was followed for extraction of caffeine from unused tea powder. The extract sample from used an unused tea powder was compared by weighing the amount (eku_chem_lab 2015).

Conformation of caffeine

Sample was prepared as per given concentration for thin layer chromatography. Mobile phase was prepared by taking ethyl acetate: n-hexane:acetic acid in the ratio of 80:20:1 (P. A. Bansode *et al;* 2015). The TLC plate was taken and at 1cm from bottom 3 spots were marked distantly by using pencil and scale on that namely unused, standard and used (do not touch the TLC plate directly, use the forecep to handle it). The samples were applied on the spots by using a capillary tube and allowed to dry. The spotting was repeated for 3 times. The TLC plate was kept in mobile phase in a beaker. Allow the mobile phase to run 1/4th of the TLC plate. Mark the position of 1/4th run of mobile phase with scale and pencil. Place the TLC on a petri plate and allow it to dry in hot air oven for 5-10 min at 37^{o} C. After drying observe the spots under UV light. Rf values were calculated for standard and extracted samples.

HPLC for Caffeine

The extracted caffeine was confirmed by HPLC (Carmen Cabrera 2003). The samples were prepared as per above concentration (0.5mg/ml). The prepared sample mixtures were filtered by using Millipore membrane filter and stored in glass vials. Solvent system was prepared as shown in materials.

HPLC parameters were set as follow; Injection volume 20µl, column C18, Temperature 30^{0}C, flow rate 1ml/min, retention time 30min, wavelength 280nm and sample mixtures were allowed to run. The result was observed and recorded.

Disk diffusion method

For disk diffusion method the departmental known cultures viz. *E. coli*, *B. megaterium*, *B. subtilis*, *S. typhi*, *B. amyloliquefaciens*, *etc.* were revival in sterile nutrient broth and incubated at 37^{0}C for 24 h. The 250ml of nutrient agar was prepared Nutrient by using Peptone (2.5g), Nacl (1.25g), Yeast extract (1.25g), Agar (3.75g), Distilled Water (250ml). The concentration of caffeine was prepared as 0.005mg/ml in distilled sterile water. After solidifying the nutrient agar, the plates were spread with 20µl of bacterial

sample with sterile spreader. After 5min, the filter paper disks were dipped in the sample of caffeine and then put on the nutrient agar.5 disks were placed on each of the petri plate. Plates were kept in the refrigerator for half hour. Then the plates were incubated at 37^0C for 48hrs (University of Pittsburg).

Results and Discussion

Extraction of caffeine from used and unused tea powder

Extraction was done from 100gm of tea powder. In this amount of used tea powder we extracted 0.01g caffeine. Similar amount of unused tea powder was taken in which 0.02g caffeine was extracted. Purification of caffeine was done by using 100% ethanol as shown in fig. 1. (eku_chem_lab 2015).

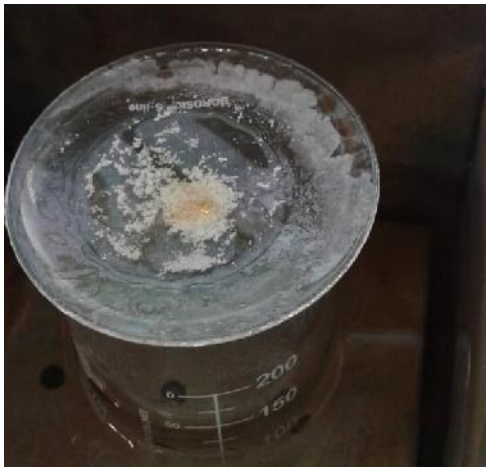


Fig. 1 Recrystallization of caffeine by absolute Ethanol

Conformation of extracted caffeine by TLC

Blue colour spots were observed on TLC plate after visualization under UV light. All the three Spots were migrated the same distance. Length of standard caffeine and length of the extracted sample was similar which confirms the sample is caffeine as shown in figure 2. (P. A. Bansode *et al.*, 2015).

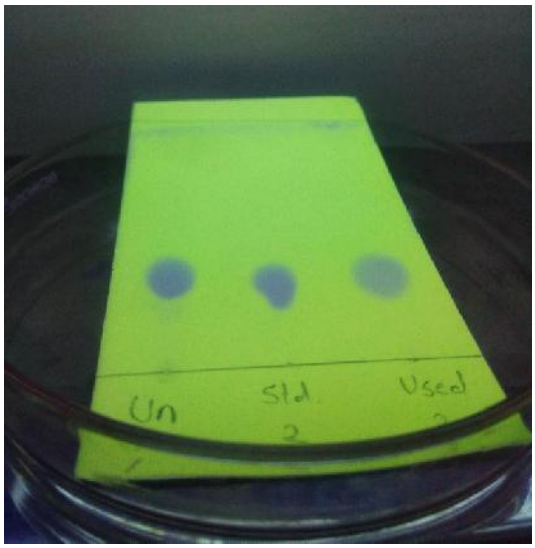
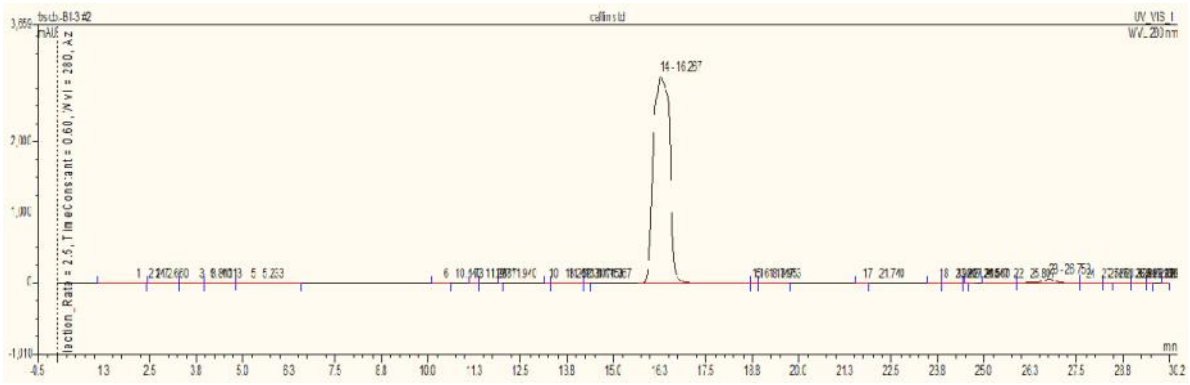


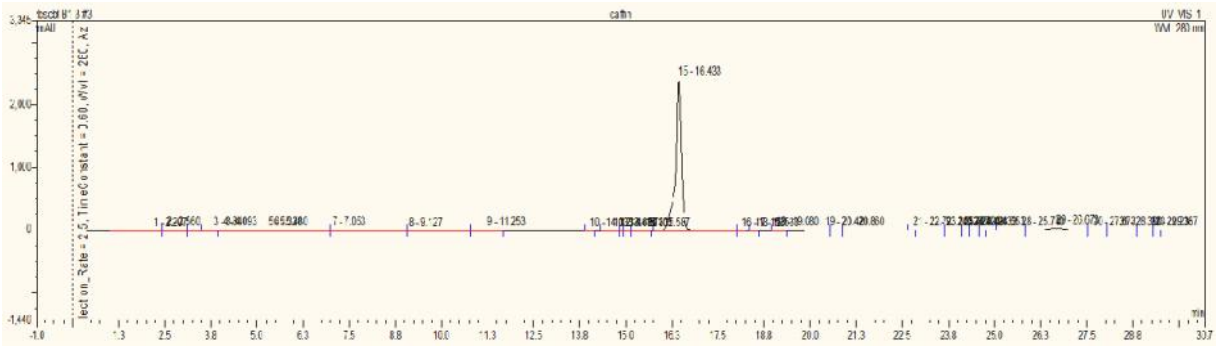
Fig.2 Visualization of spots under UV light

Conformation by High Performance Liquid Chromatography

By performing HPLC, determination of the relative peak area of extracted purified caffeine and standard caffeine. The retention time for peak of standard (as shown in Graph 1) as well as extracted caffeine (as shown in Graph 2) was 16.3 min. A single calibration curve was observed at 280nm which confirmed the presence of pure caffeine in the sample. From this study, our project reveals that similar result was obtained for standard caffeine (Carmen Cabrera, 2003).



Graph 1.HPLC analysis of standard caffeine



Graph 2. HPLC analysis of extracted caffeine

Disk diffusion assay of caffeine

A zone of clearance was observed against different bacterial species viz. *E.coli* (as shown Fig. 3), *B. megaterium (*as shown Fig. 4), *B.subtilis* (as shown Fig.5), *S. typhi* (as shown Fig. 6), *B. amyloliquefaciens* (as shown Fig.7). Extracted caffeine shows more zone of inhibition against these bacteria as compare to standard caffeine. This shows that caffeine has the antimicrobial activity against some bacteria (University of Pittsburg).

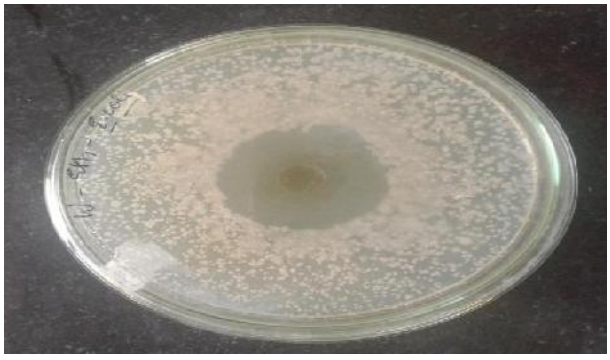


Fig.3 Zone of clearance against *E.coli*

Fig.4 Comparative Zone of clearance against *B.megaterium* with extracted and standard caffeine

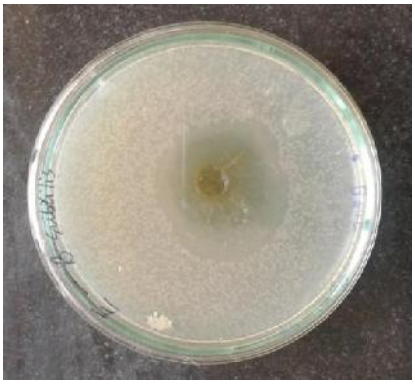


Fig.5 Zone of clearance against extracted *B.subtilis*

Fig.6 Comparative Zone of clearance against *S.typhi* with and standard caffeine

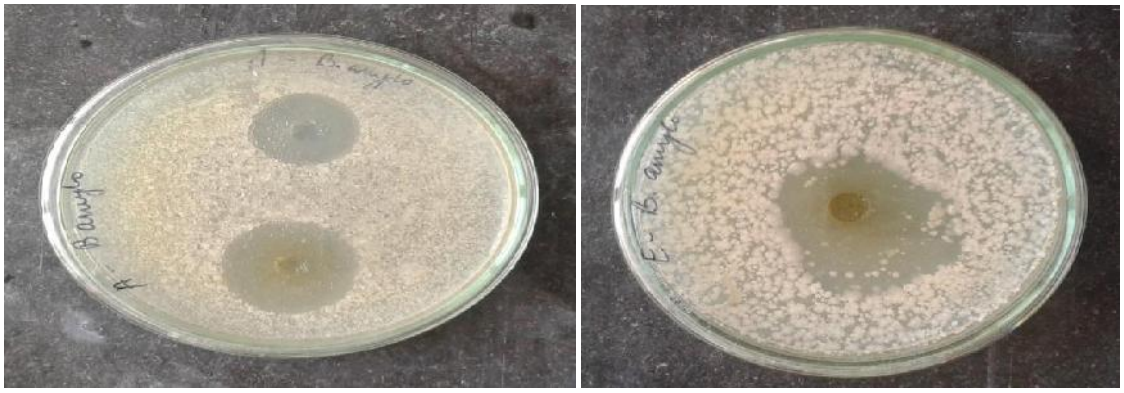


Fig.7 Comparative Zone of clearance against *B. amyloliquefaciens* with extracted and standard caffeine

Application of tea powder as a compost

Within less germination period, rapid growth of moong (*Vigna radiata*), wheat (*Triticum*) and bajra (*Pennisetum glaucum*) seedlings was observed by the application of compost used tea powder as a biofertilizer.

Following figure 8 shows the first day, seeds are sown in the soil contains compost tea powder.

Fig. 8 Seeds are spread in soil contains used tea powder

The following figure 9 shows germination of seeds after two days which indicates that use of compost tea powder can increase the germination of seeds in less period of time.

Fig. 9 Germination of seeds after two days

Every day watering the plants regularly and providing sufficient sunlight at window side in our laboratory were monitored. Day by day seedlings shows very rapid growth into plantlets within four days as shown in figure 10.

Fig, 10 Rapid growth of seedlings in plantlets

Conclusion

From the above study, we conclude that, unused tea powder has more amount of caffeine than used tea powder. It is present in almost double amount that was confirmed by extraction method after weighing. By performing TLC and HPLC, confirmation of extracted caffeine was done. Antimicrobial activity was shown by extracted caffeine against *E. coli, B. megaterium, B. subtilis, S. typhi, B. amyloliquefaciens, etc*. A rapid growth of seedlings to plantlet was observed in seven days, which was compared to soil when compost tea powder used as a biofertilizer.

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