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ISOLATION OF CAFFEINE FROM BEVERAGES AND EVALUATION OF ANTIMICROBIAL EFFECT ON VARIOUS HUMAN PATHOGENIC BACTERIAL STRAINS

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ABSTRACT

Caffeine is a natural alkaloid found in coffee beans, tea leaves, cola nuts, cocoa beans and other plants. In this study the caffeine was isolated from various products which were available in market and used for daily purpose in daily life like coffee and tea. Both tea and coffee had variable range of caffeine like Black Tea (49.3), Ice Tea (22.7), Coffee (drip) 8oz (139.6mg) and the instant coffee (74.8mg). Such products affect the human body in direct and indirect way as observed in earlier studies. For evaluating antimicrobial activity agar well diffusion and spetrophotometric method was used and it was observed that caffeine has its antibacterial activity and affect various microorganisms.

INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is a natural alkaloid found in tea leaves, coffee beans, cola nuts, cocoa beans and other plants. This caffeine is most commonly found in common beverages (coffee, tea, soft drinks), products containing cocoa or chocolate, and medications, including headache or pain remedies and over-the-counter stimulants [1,2,3,4]. Caffeine is most frequently and commonly ingested pharmacologically active substance in the world. It is found in common beverages (coffee, tea, soft drinks), in products containing cocoa or chocolate, and in medications [5]. Caffeine is most widely consumed as neuroactive drugs [6]. Caffeine has been reported as a protective substance on cellular damage [7,8] with beneficial antioxidant effects [9]; probably due to the main metabolites of caffeine, 1- methylxanthine and 1-methyluric acid, that are highly effective antioxidants and are able to prevent LDL oxidation in vitro[10]. Some components in coffee such as caffeine are reported to have antimicrobial activity reduces the growth rate of *Aspergillus versicolor, Penicillium citril1um* and *Penicillium urticeae* [11] and at certain concentrations inhibits the production of some mycotoxins such as aflatoxin produced by *Aspergillus parasiticus*[12]. The main objective of this study is to find out the quantity of caffeine in beverages like coffee and tea. One more is that we have to analyze its (caffeine) antimicrobial activity on various pathogenic strains.

MATERIALS AND METHODS

Sample collection

We have taken samples of beverages like coffee and tea from the various regions of Panipat and Sonipat (Haryana State India) market to estimate caffeine collected. For evaluating antimicrobial activity we have taken caffeine.

Table 1: Name of various collected samples of coffee and tea.

Coffee sample	Tea sample
Coffee (drip) 8oz	Black Tea
Coffee (espresso) 2oz	Green Tea
Coffee (instant) 8oz	Tea
	Ice Tea

Extraction of caffeine from coffee

Take 100mL of coffee extract in flask. Add 2g of sodium carbonate ($NaCO_3$) to the coffee solution. Mix it properly. Add 25mL of methylene chloride (CH_2Cl_2), and vigorously swirl the

mixture for 10 minutes. Filter this solution by using filter paper. After filtration methylene chloride solution of caffeine will remain on the filter paper then filtrate transferred to a flask. Add a scoop of anhydrous sodium sulfate (Na_2SO_4), in order to remove the last traces of water. After removal of water this solution was placed on warm hot plate and allowed the evaporation of methylene chloride. Only a fraction of a milliliter of liquid is left in flask. Allow the beaker to stand in the hood for a minute or two. The heat remaining in the glass will cause the last amount of methylene chloride to evaporate and produce a solid residue of crude caffeine. Weigh this and record this mass in the Table1.

Extraction of caffeine from tea

Take 5 tea bags add 100 mL of vigorously boiling water in flask. Set the beaker on the hot plate and boil gently for 10 min. stir the mixture carefully to avoid breaking any of the bags. After the solution has cooled, squeeze the tea bags to remove all the liquid. Filter this solution by filter paper. The filtered tea solution is mixed with 20ml of dichloromethane. Dry the combined dichloromethane solutions with anhydrous Sodium sulfite using filters papers. Wash the filter paper and add drying agent with about 1mL of CH_2Cl_2. Remove the CH_2Cl_2 by simple distillation until about 5 mL of liquid remains in the flask. Do not allow the flask to go dry or the caffeine may decompose. Place the round-bottom flask on a steam bath and evaporate the contents to dryness at low-medium heat. Weigh the flask to find the crude mass of caffeine.

Antibacterial activity

The antimicrobial activities of caffeine were tested by the Agar well Diffusion Method. caffeine is dissolved in sterile distilled water. In this well are made in agar having microbial cells in which caffeine (20µl) poured. As with the incubation period, the caffeine diffuses and creating a zone of inhibition around the well. Thus, the size of the zone of inhibition is a measured: the larger the clear area, the more effective the compound. The test organisms used in the study were *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aerogenosa, Bacilus Subtilis, Streptococcus agalactcia, Proteus vulgaris*. All the test cultures were obtained from the Microbiology laboratory where cultures were maintained at 4°C on nutrient agar. The purity of all the cultures was checked before use. The plates were allowed to solidify and used. Ten milliliter of sterile, Muller Hinton agar medium (seed agar) was seeded with organisms (200µl in 20 ml), in semi hot conditions and was poured uniformly on the base agar. The agar wells were

made in cultured plate. The plates were incubated at 37°C for 24 h and zone of inhibition was measured. We also performed another test in which each bacterial strain were taken and small amount of caffeine put in them. After every 1 hour optical density by spectrophotometer was measured at 610nm. Decrease in OD values was indicative of antimicrobial activity of cow urine.

RESULTS

As the extraction process started caffeine recovered from each sample. The caffeine content in tea ranges from 22 to 50 mg. Black Tea has the maximum caffeine (49.3) and Ice Tea has the minimum (22.7). The caffeine content in coffee ranges from 75 to 140 mg. The Coffee (drip) 8oz has maximum (139.6mg) caffeine and the instant coffee has least caffeine content (74.8mg). On the basis of extraction of caffeine from different samples of coffee and tea, the quantity of caffeine varied which was shown in table2 and table3 respectively.

Quantity of caffeine in coffee:

Table2: Quantity of caffeine in various coffee samples

Sample	Average Caffeine(in mg)
Coffee (drip) 8oz	139.6
Coffee (espresso) 2oz	88.6
Coffee (instant) 8oz	74.8

This table 2 shows present amount of caffeine in various coffee sample which we were taken. The amount also varies like in case of Coffee (drip) 8oz caffeine amount is 139.6mg and in case of Coffee (instant) 8oz is 74.8.

Quantity of caffeine in Tea

Table3: Quantity of caffeine in various tea samples

Sample (8oz)	Average Caffeine(in mg)
Black Tea	49.3
Green Tea	28.7
Tea	32.2
Ice Tea	22.7

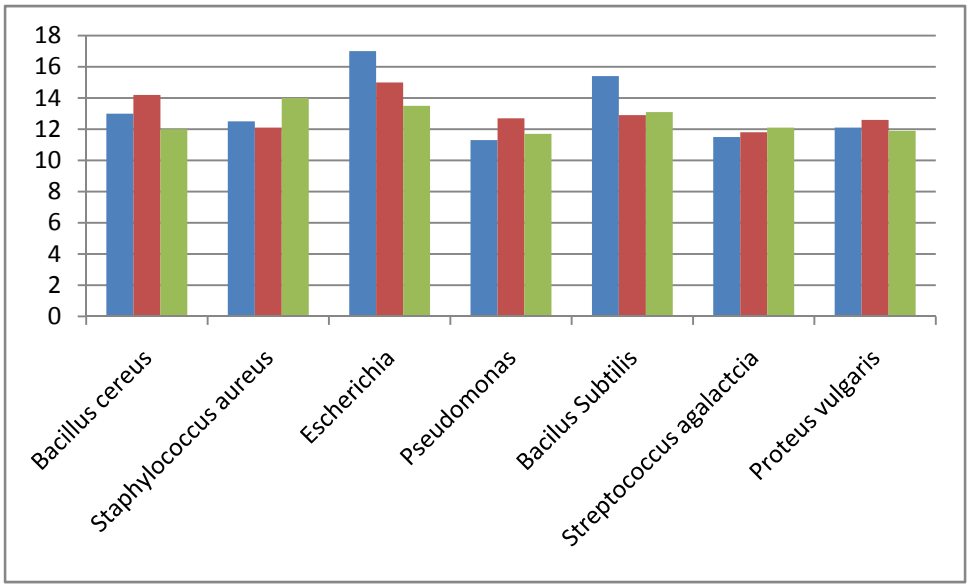
This table 3 shows present amount of caffeine in various tea sample which we were taken. The amount also varies like in case of black tea amount is 49.3mg and in case of ice tea is 22.7. This variable range showed that their effectiveness also varied with their intake.

In case of antimicrobial activity, the caffeine showed it own antimicrobial activity against various bacterial strains which were collected from microbiology lab as a stock culture. The antimicrobial effect of caffeine varies from one to another bacterial strain. Here this caffeine showed that its own antimicrobial effect against various bacterial strains.

Table4: Inhibition zone of microbes against caffeine (in mm)

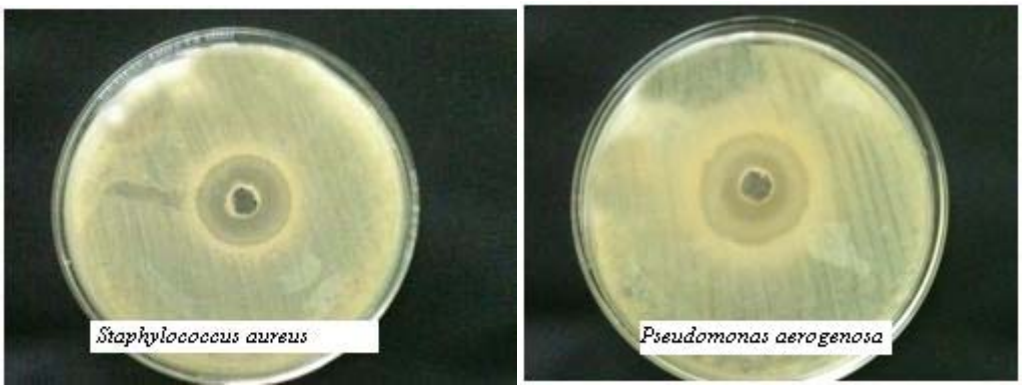
Samples	*Bacillus cereus*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aerogenosa*	*Bacilus Subtilis*	*Streptococcus agalactcia*	*Proteus vulgaris*
Caffeine	13	12.5	17	11.3	15.4	11.5	12.1
	14.2	12.1	15	12.7	12.9	11.8	12.6
	12	14	13.5	11.7	13.1	12.1	11.9

This table 4 showed the zone of inhibition when prepared caffeine dilution is poured and diffused in to agar. There is most effective method i.e. Agar well diffusion method was used. The zone of inhibition clarified that caffeine had its own antimicrobial activity (as per shown in image 1, 2 of *Staphylococcus aureus and Pseudomonas aerogenosa*) and the range is also vary from strain to strain as per shown in table 4.



Zone of Inhibition (in mm)

Graph 1: this graph showed that the zone of inhibition of each bacterial strain varies and from this it was cleared that caffeine had most of it effect against *Staphylococcus aureus* and *Proteus vulgaris.*



Staphylococcus aureus *Pseudomonas aerogenosa*

Figure 1: antimicrobial activity of caffeine

Table 5: Representing the optical density value of 5 samples in which caffeine was inoculated with cultures and the O.D was taken after every 1 hr. The declines in the O.D values indicate that the caffeine is showing its antimicrobial activity.

Incubation hours	***Bacillus cereus***	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aerogenosa***	***Bacilus Subtilis***	***Streptococcus agalactcia***	***Proteus vulgaris***
1	1.135	0.982	0.701	1.234	1.071	1.301	0.983
2	1.121	0.911	0.672	1.191	0.991	1.254	0.911
3	1.054	0.814	0.61	1.117	0.916	1.128	0.852
4	0.912	0.756	0.581	1.074	0.872	1.045	0.795
5	0.851	0.661	0.524	0.991	0.801	0.914	0.719
6	0.791	0.611	0.498	0.901	0.755	0.875	0.695
7	0.712	0.562	0.445	0.874	0.691	0.817	0.614

DISCUSSION

From above result we have calculated the quantity of caffeine varies in every product either it is of tea or coffee like Black Tea (49.3), Ice Tea (22.7), Coffee (drip) 8oz (139.6mg) and the instant coffee (74.8mg). Agar well diffusion method and spetrophotometric method displayed its antimicrobial activity. It was found that caffeine showed most antibacterial effect on *various bacterial strains.*

The above all evaluations mentioned that the effect of caffeine is very harmful if overdosed. Mostly reproductive-aged women and children are at high risk [5]. This caffeine induces antimicrobial effect on human body as earlier research had shown this. Caffeine induces apoptosis in the central nervous system [13]. The coffee induces effects on heart rate, maze reaction time, and subjective alertness [14]. The antioxidant ability of caffeine has been reported with its pro-oxidant effects derived from its action mechanism such as the systemic release of catecholamines [15].

It was observed that Caffeine influences a variety of conditions in a women's body that may be due to its demonstrated effect on the body's sex hormones. One of the actions of caffeine is that it causes increases in estrones, or female hormones, while it decreases available testosterone[16]. Polymorphism in the metabolic enzyme cytochrome P-450 is associated with risk of myocardial infarction in caffeine users [17]. Caffeine can decrease insulin sensitivity in healthy humans, result as elevated plasma epinephrine levels [18]. Caffeine-containing beverage consumption has been reported to be associated with reduced bone mass and increased fracture risk [19].

CONCLUSION

This study showed that the caffeine concentration varies in various tea and coffee sample. This caffeine induces its effect on bacterial strains as well as on human body. Their effectiveness also varies. Thus its consumption should be decreased.

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