In Vitro Adsorption Studies of bacteria to Activated Charcoal Powder

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DECLARATION

I hereby declare that this is my original work and has never been submitted before for the award of a degree or any other accolade whatsoever in part or wholly.

NAME: …….
DATE: ……. SIGN: …….

RECOMMENDATION

I hereby recommend that this is this student's original work, having read and acknowledged it.

NAME: …….
DATE: ……....SIGN: ……...

DEDICATION

Special dedication goes to my mum, Phyllis Kabiti for her immense support financially and mentally to finish this project.

ACKNOWLEDGEMENT

I thank the almighty God for seeing me through this project by providing a peace of mind, family, friends, teachers and lab technicians to help through this project.

Abstract

Activated charcoal has been used before to adsorb different molecules including toxins and drug overdoses. Little research has been done on its ability to adsorb microorganisms. The aim of the study was to examine the adsorption characteristic of activated charcoal on gram positive and gram negative bacteria using *Staphylococcus aureus* and *Shigella spp*. respectively as representatives. The other objective was to find out the effectiveness of activated charcoal on adsorbing bacteria. The study showed that it was effective on gram negative bacteria more than the gram negative bacteria. In all, it was more effective with treatment of 10mg activated charcoal as the effect was dose-dependent. This shows that activated charcoal can be used to remove bacteria from the gastro-intestinal tract thus treating diarrheal diseases as previously thought.

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Adsorption Effect of Activated Charcoal on Gram Positive and Gram Negative Bacteria

Is activated charcoal effective against bacteria?

Activated charcoal is a black powdery or granular substance that is carbon in nature. It has been prepared by controlled burning of wood and other agricultural waste. The chunks produced are ground to increase the surface area. It is activated for purification purposes by removing the already adsorbed material using heat. This is locally done by heating it when placed in a pot until black smoke is produced and closed to preserve its integrity. This can also be modified by adding other nanoparticles to it so as to improve its adsorbing properties including silver.

Figure 1 shows a locally obtained activated charcoal that was bought at 100 Kenyan shillings at Mfangano Street. This can also be obtained in various shops with a different kind of packing.

Adsorption is the ability of a substance to bind a certain material and hold the material to its surface. Activated charcoal in this case forms certain bonds like hydrogen bonds with the toxins or any other substance and holds on to the substance that has been adsorbed. Other research shows that the interaction with the surface of the substance disrupts the cells and eventually kills the cells in the case of bacterial cells.

There are a number of benefits of charcoal that have been known over the years and been used traditionally for a long time especially for medicinal purposes. The following are the effects of activated charcoal that have been done research on.

- In the case of poisoning, when given within one hour of Ingestion, it is able to remove toxins from the body (Anon, 1999), (Kent, R.O., 2010). This has been shown to be effective both to children and adults (Bucaretchi, F., et al, 2005).
- Activated charcoal has also been shown to be effective against side effects of drugs (Chyka, P.A., et al, 2005).
- It is able to restore the normal flora eliminated by antibiotics (Spector, R., et al, 1986).

- It is also able to prevent diarrheal effects of some drugs (Spector, R., et al, 1986).
- It is also able to treat overdoses of certain drugs that have been researched on. (Cooper, G.M., et al, 2005).
- It can also adsorb the compound that courses the skin to itch which occurs in chronic kidney failure patients (Spector, R., et al, 1986).
- It is also effective in adsorbing high levels of bile flow in pregnancy that causes bile flow problems (Kaaja, R.J., et al, 1994).
- There is research showing that is it able to adsorb gas though it is not properly done. (Hall, R.G. Jr., et al, 1981).
- It is reported to lower cholesterol (Neuvonen, P.J., et al, 1989), (Park, G.D., et al, 1988).
- Activated charcoal has been shown to treat diarrhea when combined with other drugs (Ilomuana M.O., 2017).
- It can also help with indigestion problems. (Hall, R.G. Jr., et al, 1981).
- It has been shown to handle diarrhea in children (Sergio, G. –C., et al, 2008).
- Kidney diseases associated with proteins have been shown to reduce when a low protein diet combined with the ingestion of activated charcoal (Wang, Z., et al, (2012).
- Light therapy as a form of treatment for jaundice in newborn babies caused by high levels of bilirubin has been shown to be improved by activated charcoal (Spector, R., et al, 1986).
- It has been used to clean the environment (Przepiórski, J., 2006).
- Activated charcoal has been shown to speed up wound healing by adsorbing bacteria though not clear (Kerihuel, J.C., 2009), (Kerihuel, J.C., 2009).
- Charcoal hemoperfusion where blood is passed through a column containing charcoal which is able to adsorb toxic substances (Adrade, J.D., et al, 1999).
- It has been reported to adsorb alcohol (Spector, R., et al, 1986).

According to Panthee, S., 2008 the dosage of 1g/kg of activated charcoal was shown to be effective in adsorbing paracetamol overdose. The adsorption activity of activated charcoal was not affected significantly by changes in ph.

A lot of research has been done on the effectiveness on activated charcoal in treatment of drug overdose of medicine and poisoning by adsorbing the drug and toxins respectively. However, little research has been done on the effectiveness of activated charcoal adsorption on bacteria. This research was aimed examining the effectiveness of activated charcoal against gram positive and gram negative bacteria. The representatives used are *Staphylococcus aureus* for gram positive and S*higella spp.* for gram negative bacteria. The positive control for the experiment was *Escherichia coli* since it has been shown to be adsorbed by activated charcoal (Naka, K., et al, 2001).

Escherichia coli is known to be a normal flora in the gut but some strains produce an endotoxin that causes diarrhea that can be non-inflammatory or inflammatory are known as Enterotoxigenic *Escherichia coli*. Non-virulent strains may gain virulence when exposed to virulent Escherichia

coli which give their plasmid. It is a gram negative bacterium that is bacillus in nature (Baron, S., 1996).

The other gram negative bacterium used was Shigella spp. This genus has four sero-groups each with multiple serotypes thus the abbreviation spp. These include *S. dysenteriae, S .flexneri, S. boydii and S. sonnei.* It is non-motile, a facultative anaerobe and non-spore forming. It has a rod like structure. It causes Shigellosis which is characterized by abdominal pain, tenesmus, watery diarrhea and dysentery (Baron, S., 1996).

The only gram positive bacterium used was staphylococcus aureus which is commonly associated with boils. It is cocci in nature and has a diameter of 1μm but is commonly found in clumps. It causes a number of negative effects such as toxic shock syndrome, food poisoning, osteomyelitis, endocarditis, furunculosis, nocosomical infections (in surgical wounds) and abscesses in different parts of the body (Baron, S., 1996).

The aim of the project was to examine the effectiveness of the activated charcoal to bacteria with different morphology i.e. cocci and bacilli. The difference in activity of activated charcoal and non-activated charcoal was to be examined too but resources and time limited this objective.

With the increasing cases of antibiotic resistance, there is need for a medication that is less prone to resistance like an adsorbing substance.

An adsorbent is a substance that interacts with another and binds to it. Since activated charcoal has been shown to be able to adsorb various substances and has been used clinically before, it suits as the best remedy going forward to be used to treat bacterial infections.

Antibiotics have also been shown to interfere with the normal flora of the gut. This brings about superinfections that are hard to treat. Activated charcoal has two mainly recognized advantages over antibiotics. This includes its inability to pick up normal flora as shown in the Naka research and its ability to restore the normal flora of the gut that has been tampered with. The other advantages that come in handy especially with developing countries are its relatively lower cost of production thus cheap. It can be made at home as compared to antibiotics which need expertise to manufacture it. There is the resistance issue which has no supporting evidence yet but since it does not involve disruption of the bacterial metabolic system, there are lower chances of resistance.

Activated charcoal has been shown to have health benefits to the human body and can be consumed in high doses without massive side effects (Brahmi, N., et al, 2006).This shows it can be taken without necessary diagnosis for medication to be applied. This reduces the time of the first sign been seen and the time of treatment. This project was geared towards giving a way of treating diarrheal diseases that are caused by a number bacteria such as *Escherichia coli* and *Staphylococcus aureus*, *Shigella spp*., *Salmonella typhi* and *Vibrio cholerae.*

Method

Bacteria used

Gram positive was *Staphylococcus aureus* while gram negative was S*higella spp.* The control used was *Escherichia coli* since it has been shown in a previous research to show activity of activated journal and was shown to be effective against it.

Design

The experiment used the above bacteria separately and examined the effect of activated charcoal on them after treating media containing the bacteria with the activated charcoal. This was done in the laboratories of Technical University of Kenya. It was done for a span of three weeks in the laboratory. A lot of repetition was done due to lack of instruments at the same period of time. There was a lot of contamination that needed repetition on the colony counts. The minute measurements were heard to use though it was done in 2X and 3X of the originally desired volume for easier manipulations. Higher concentrations of the activated charcoal obstructed the reading of the absorbance in the colorimeter.

Materials

A number of instruments were used to do various things. These include:

Centrifuge

- Heat sterilizer
- Autoclave
- Colorimeter
- Electronic balance
- Incubator.

The media used were;

- Agar type 1
- Mueller Hinton broth
- DCA agar
- MacConkey broth (purple)
- MacConkey agar (purple)

The other items used were;

- Cuvettes
- Portable Bunsen burner
- Cotton wool
- Aluminum foil
- Gloves
- 70% ethanol
- Conical flasks
- Boiling tubes
- Inoculating loop
- Centrifuge tubes
- Centrifuge tubes holder
- Spatula
- Distilled water
- Measuring cylinder

Procedure

Media preparation.

Media was prepared by measuring accurate amounts of media which added to distilled water with corresponding volume and autoclaved.

Bacterial culture.

Bacteria was isolated from stored and identified bacteria cultures and introduced to broth media in a sterile environment made possible by ethanol and open flame. *S. aureus* used Mueller Hinton to grow while the rest used MacConkey broth to grow. They were placed inside and incubator set at 37^0c and left overnight to grow.

Activated charcoal treatment.

Activated charcoal was purchased locally and weighed into different measurements of 1, 3, 5, and 10 mg. These were added into different centrifuge tubes containing 1ml of broth containing bacteria. This was done in triplicate for each bacterium. The result was shaken to mix and place in an incubator for one hour (Naka k., et al, 2001).

Screening using Number of colonies.

An inoculum was taken and streaked once across the agar in the different labeled petri dishes. These petri dishes were placed in 37ºc set incubator overnight and number of colonies produced counted the next day. Agar used for the gram negative bacteria was MacConkey while the one used on gram positive bacteria was DCA agar.

Screening using difference in absorbance.

Two ml of distilled water was added to each of the centrifuge tubes and placed in a centrifuge to sediment the activated charcoal at 3000rpm for 5 minutes. The supernatant was dispensed to cuvettes and the absorbance at 590nm was taken and recorded. For the 2X and 3X no dilution was done since the volume was enough for measuring absorbance.

Results

Bacterial culture

The first step of culturing bacteria in a broth medium is as shown in the figures below.

Figure 2 shows from the left MacConkey broth without bacteria which is the negative bacteria, followed by two boiling tubes with E. coli and a lighter purple color containing Shigella spp.

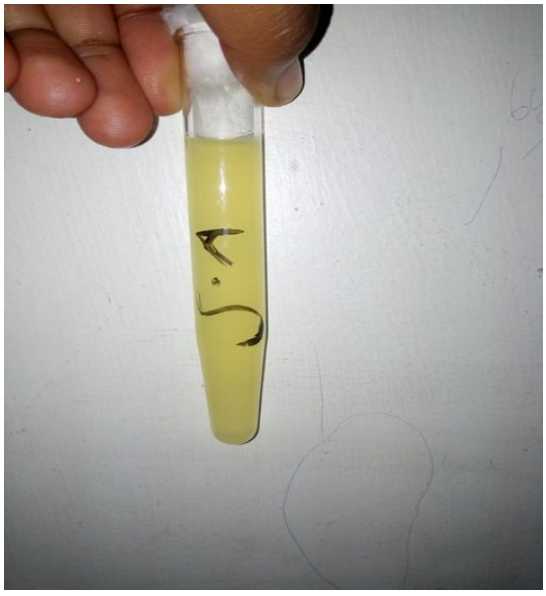


Figure 3 shows Staphylococcus aureus grown in Mueller Hinton broth manifested by its turbidity.

Absorbance results

The absorbance results were recorded in the table to see compare difference in absorbance in the first run.

Table 1 shows the absorbance taken before treatment with alcohol and absorbance taken after the treatment. It also shows the difference calculated

Bacteria	Without Charcoal	With Charcoal	Difference in Absorbance at 590nm
S. aureus	1.35	0.40	0.95
Shigella spp.	1.38	0.22	1.16
E.. coli	1.37	0.16	1.21

The work done in duplicate was statistically analyzed for the second run and the results are as shown in the graph projected below. T-test was used to analyze the data.

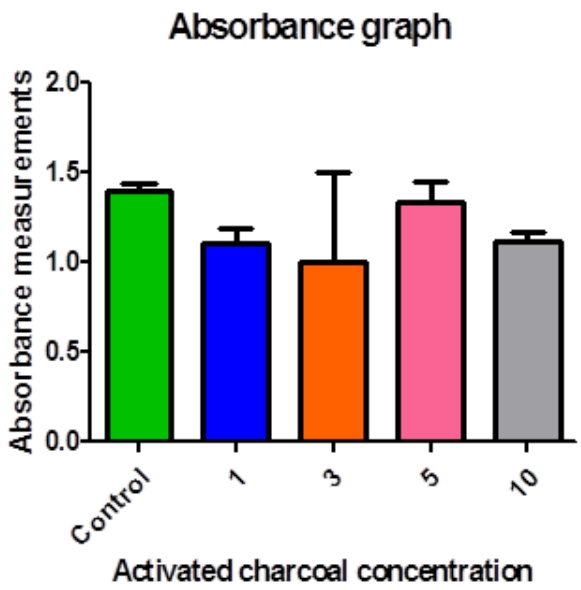


Figure 4 shows the statistical analysis of the absorbance data capture

Colony screening results

The cultured bacteria from the treated media produced results as shown below.

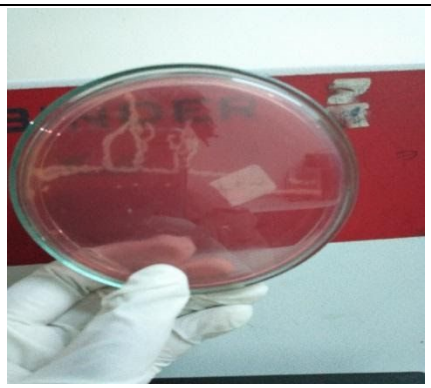
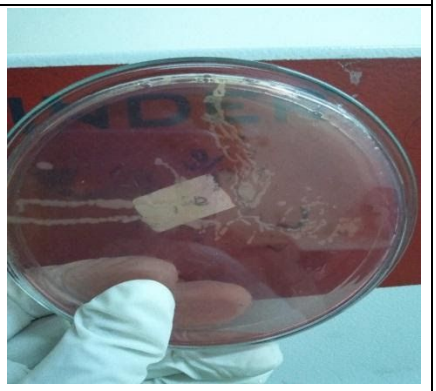
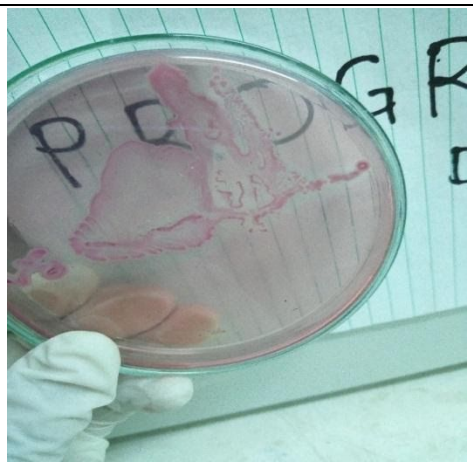
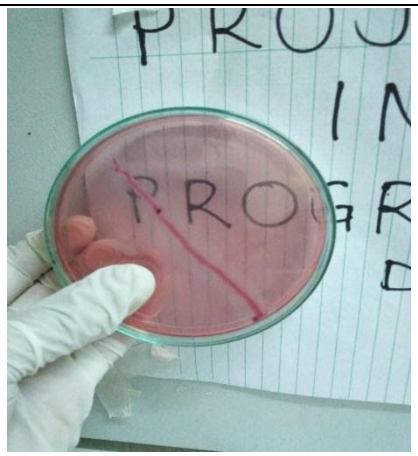
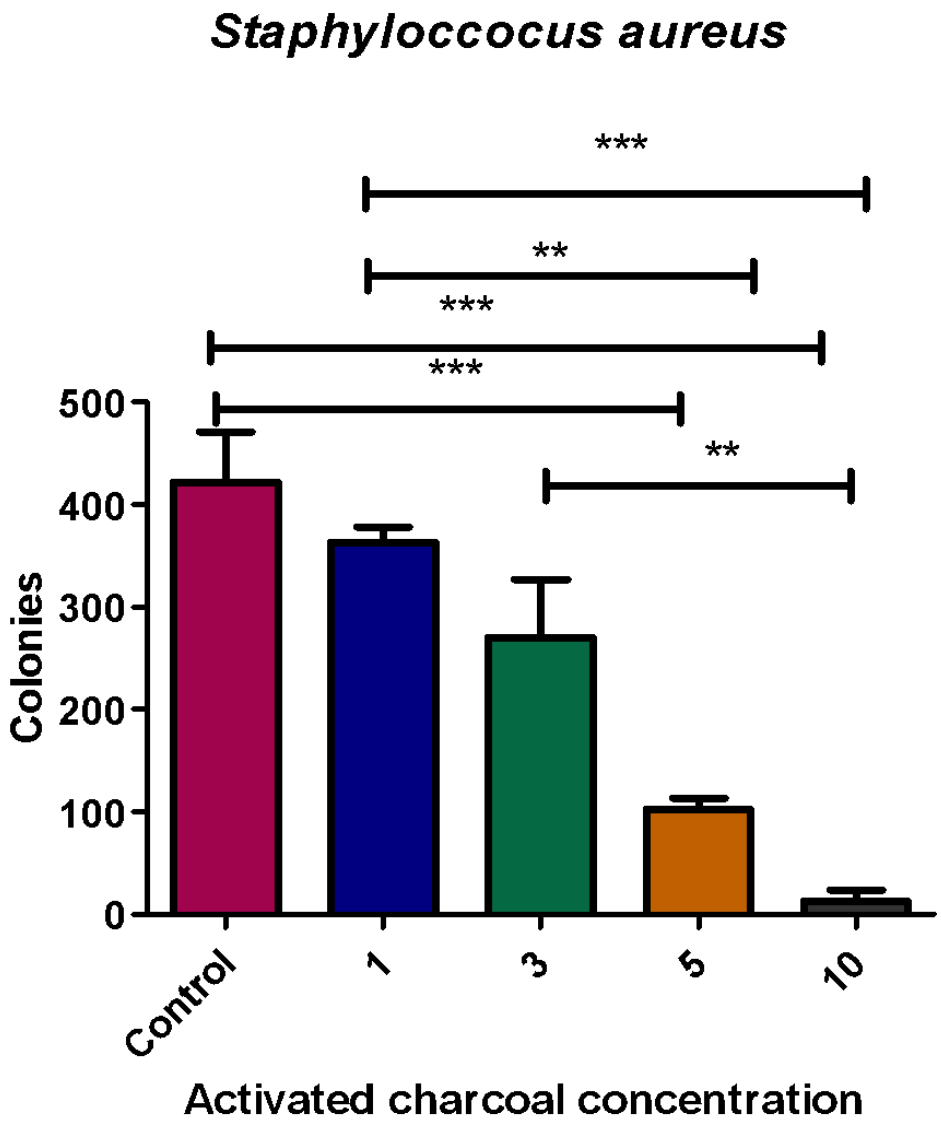
Bacteria	Concentration 1	Concentration 2
Shigella spp.		
Escherichia coli		

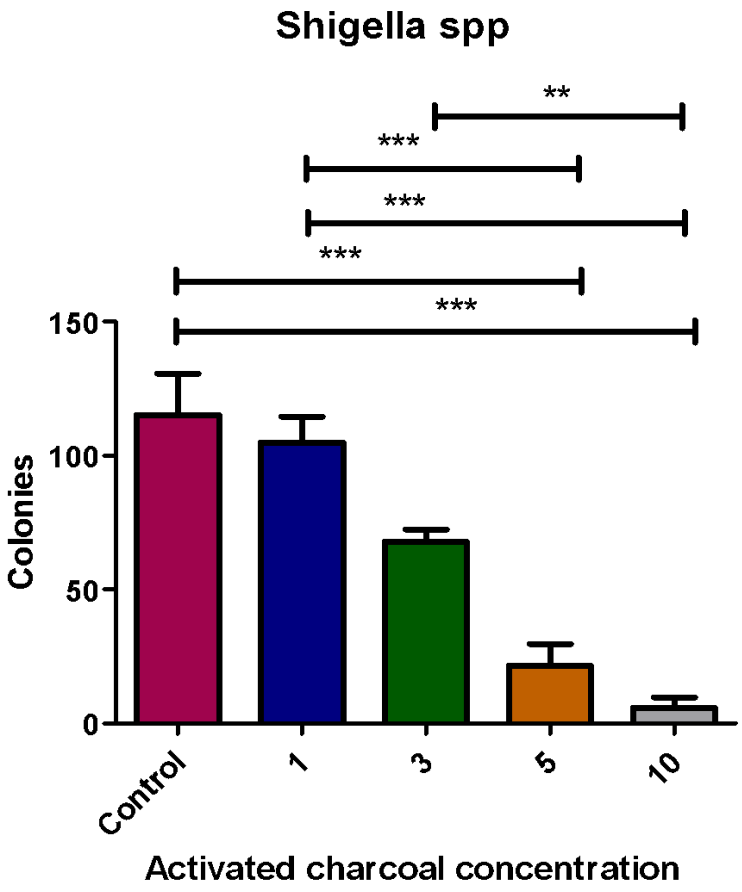
Table 2 shows bacteria cultures of different concentrations for the ones used on MacConkey media.

Statistical analysis

The colony count results are as shown in the graph below after statistical analysis. One way ANOVA was used to analyze the data.



The gram negative bacteria showed to have higher significant difference as compared to the gram negative one.



The graph shows the activity of activated charcoal on Escherichia coli which was the positive control. The significant difference of activity was much greater as compared to the other test organisms.

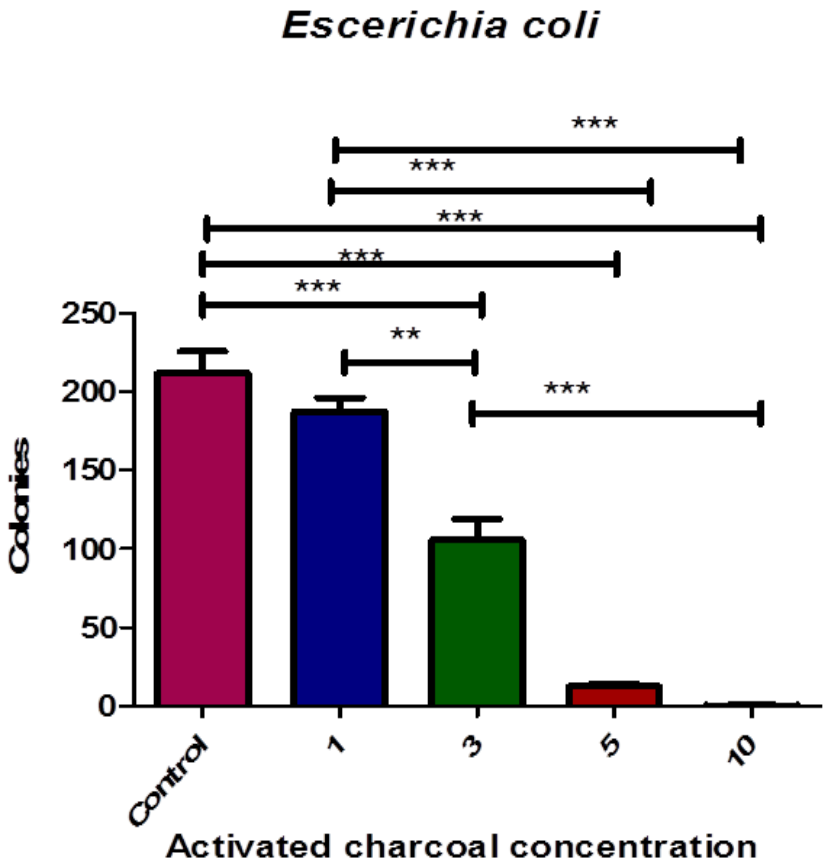
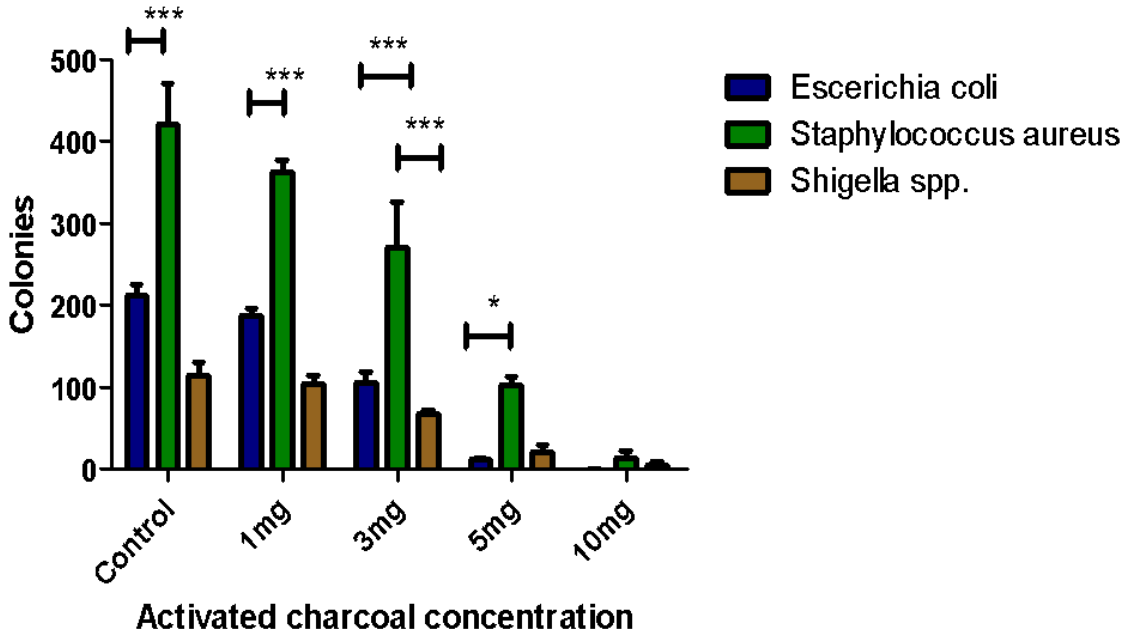


Figure 7 shows colony count results for Escherichia coli. The level of statistical significant difference is as shown by the asterisks. This was done by one way ANOVA with the confidence interval of 95%.

To compare the effect the activity of activated charcoal on the various bacteria worked on, a comparative graph was made to compare their activity at each concentration of activated charcoal. This produced the following graph after two way ANOVA statistical analysis.

Comparison graph of activity of activated charcoal on different bacteria



Discussions

When the bacteria grow in a clear broth, it becomes turbid which can be seen physically. The Mueller Hinton having the staphylococcus aureus became turbid. MacConkey broth on the other side being purple in color changes to yellow for *E. coli* due to production of acid but *Shigella spp*. does not cause change in color because it is not lactose fermenting.

At 590nm wavelength a colorimeter was used to quantify the rabidity of the broth. The more the turbidity, the more the absorbance of the pale yellow color, the less the absorbance measure the less the pale yellow color to adsorb. As shown in the figure, there was physical evidence showing change in color of the broth. This shows reduction of bacteria from the broth after being treated with activated charcoal.

This reduction of bacteria was confirmed by use of inoculum from the treated broth and inoculating in agar media. This was in done in triplicate and the colonies were counted just to confirm the reduction of the bacteria. There was difficulty in counting the colonies due to its small size especially in the colorless colonies formed for the staphylococcus aureus. The more the concentration of the activated charcoal, the more effective it was against the bacteria.

This shows adsorption of the bacteria, both the gram negative and the gram positive. The gram positive showed less effect of the activated charcoal on it. This might be probably due to its high concentration of the bacteria grown compared to the rest. This can also be explained by structural difference. *Staphylococcus aureus* being cocci in nature and stacked together to form clumps, this makes it cumbersome to bind to the activated charcoal (Baron, S., 1996).

The activated charcoal compound has a tendency to adsorb more non-polar compounds as compared to polar compound. The charge on the outer side of a bacterium determines the efficiency of activated charcoal adsorption on it. This explains the difference in activity of activated charcoal on the gram positive bacteria (Hays, H.C.W., et al, 2005).

The other possible explanation apart from the high concentration of staphylococci aureus would be the adsorption time. Clinically it has been shown, that activated charcoal causes constipation. This is due to the adsorption of water too from the gut. It is needed to take a lot of water to elute the charcoal with adsorbed material from the gut. It has also been shown that re-adsorptions may occur when left for long. The *Staphylococcus aureus* having being treated first before the rest of the test microorganisms might have started the process of re-adsorption from the pores of the activated charcoal (George, N., et al, 2010).

The pore size also determines the activity of the activated charcoal. As much as there wasn't enough reagents and time to accomplish the research on the comparison of activated charcoal activity and non-activated charcoal activity, research on papers has shown the activated charcoal is more effective on bacteria. As explained before, there are two forms of charcoal, the granular one and the powdered one (George, N., et al, 2010). The activated charcoal available on market is powdered and the non-activated charcoal is more of powdered. The bacterium being small needs to be trapped by the small size charcoal too. The surface area of the activated charcoal is larger than the home-made conventional one. The activated charcoal is more purified. This is

demonstrated by the petri dishes cultures shown in figure 2. There was no other bacterium with a different color confirming its purity.

The statistical method used for analysis was limited to one-way ANOVA due to multiple entities to compare. The grouped data used T- test for analysis for the combined data. The combined graph showed that the interaction between the activated charcoal and bacteria was significant.

The absorbance graph showed that there was significant difference as much as it could be seen in the graph. This was the same even when the confidence level was reduced to 90%.

Conclusion

Activated charcoal is effective against bacteria but dose dependent. The research shows that activated charcoal was less effective against the staphylococcus aureus which was gram positive as compare to *Shigella spp.*

Recommendation

In-vivo studies should be done on the same in references to the bacteria done and others that cause diarrhea and other gut related diseases. More research should be done in the learning the mechanisms of adsorption of the activated charcoal and devise more ways to activate it and modification for better working.

Purity tests should be done on the activated charcoal to ensure no introduction of foreign bacteria to the

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