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Assessment of physical properties and antimicrobial activity of activated charcoal impregnated with silver nanoparticles against *Escherichia coli*

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Abstract

Activated charcoal possesses small pores that help in the increment of the surface area available for chemical reactions. In this study, coal samples were taken from Kalimati, Dharan, Eastern Nepal with the goal of determining the antibacterial activity of activated charcoal against *Escherichia coli*. This study also measured the moisture content, volatile substances, fixed carbon, and pH of coal using proximate analysis. This study was carried out by the activated carbon impregnation with silver nanoparticles by varying with the different $AgNO_3$ concentrations: a) 0.1 mol/liter (mol/L) b) 1 mol/L c) 1.5 mol/L one at a time. By the proximate analysis, it was found that the moisture content was 2.2%, the volatile matter was 15%, the fixed carbon was 53.8%, and the pH was 5.83. The antimicrobial activity was performed by agar well diffusion methods. With 25 mg nanoparticles the zone of inhibition against *E. coli* was found to be 7 mm, 8 mm and 10 mm respectively and with 50 mg nanoparticles the zone of inhibition against *E. coli* was found to be 9 mm, 9 mm and 12 mm with concentration 0.1 mol/L, 1 mol/L and 1.5 mol/L of $AgNO_3$ respectively. According to Pearson correlation ($r = 0.697$) and a simple linear regression ($R^2 = 0.798$), there was a positive relationship between the concentration of $AgNO_3$ and the zone of inhibition observed against *E. coli*. The highest zone of inhibition (ZOI) of activated charcoal impregnation against *E. coli* was 12 mm at 1.5 mol/L of $AgNO_3$ with 50 mg silver nanoparticles, which was comparatively less against seven standard antibiotics (13-29 mm ZOI).

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1. Introduction

Coal can be used as one of the widely distributed sources of fossil fuel all over the world. Coal and charcoal are closely related terms but at the same time are different in their own ways. Coal occurs naturally under the earth's crust for millions of years whereas charcoal is a mineral made by humans in a short period of time. When charcoal is heated with steam in absence of oxygen at approximately 100ºC, it is activated (Marsh & Rodriguez-Reinoso, 2006). These activities result in the increment of the surface area of charcoal and the formation of large volume pores, which makes activated charcoal the universal adsorbent that can bind with a variety of molecules (Chandy & Sharma, 1998). The surface area contains mostly microspores with pore diameters smaller than 2 nm (Romanos et al., 2012).

Activated charcoal is typically made from carbonaceous sources such as bamboo, coconut, husk, willow peat, wood, coir, lignite, coal, and petroleum pitch. Diarrhea, indigestion, flatulence, poisoning, and oral overdoses are all treated with various forms of activated charcoal tablets or capsules (Shreya et al.,

2022). Sometimes, activated carbon filtration is a successful method for treating water and organic farms can also use this method to produce wine and raise cattle (Shreya et al., 2022).

Since 1811, activated charcoal has been used for the various purposes by human beings for its high absorbance capacity such as industrial, medical, environmental, agricultural and so on (Petrov et al., 2000; Machnikowski et al., 2002; Olson, 2010; Zellner et al., 2019; Palandi et al., 2020). Activated charcoal has been reported to be used for the elimination of bacteria and bacterial toxins, both in vivo as well as in vitro (Watarai, 2005; Karnib et al., 2013; Krishnasamy, 2015). Moreover, it is also responsible for the removal of bacteria like Pseudomonas aeruginosa and *Escherichia coli* from fresh and potable water systems (Quinlivan et al., 2005). Attachment of microorganisms to activated carbon particles is due to strong Lifshitz van derWaals forces rather than electrostatic repulsion (Jucker et al., 1996). Additionally, the use of different types of nanoparticles in the activated charcoal had started due to their antimicrobial activity (Hsiao et al., 2006). Therefore, activated charcoal with larger surface area impregnated with several antibacterial nanoparticles acts as a combination of adsorbing and damaging bacterial cell membrane damage (Priester et al., 2009).

Globally nanoparticles have been used for their effective antibiotic properties. Silver has been used in the Europe against planktonic bacteria (Silvestry-Rodriguez et al., 2007) which has been very effective. Nanoparticles are created with special qualities that make them useful in biology and materials research. Silver nanoparticles typically have sizes of less than 100 nm and contain 20 to 15,000 silver atoms (Oves et al., 2018; Yin et al., 2020). Silver nanoparticles have extraordinary antibacterial action, even at low concentrations, due to a high surface-to-volume ratio (Oves et al., 2018). Additionally, they have minimal costs, little cytotoxicity, and little immunological reaction (Samuel et al., 2020). As a result, there are numerous potential biomedical uses for silver nanoparticles.

The activated charcoal obtained from Dharan, impregnated with silver nanoparticles may have a good potential to inhibit the bacterias. Thus, we have examined the antimicrobial activities of activated charcoal of Dharan area impregnated with silver particles. Moreover, physical properties of coal in various parameters have also been detected which included moisture content, ash content, volatile content, fixed carbon and pH.

2. Materials and Method

2.1. Collection of samples

The samples were collected from the suspected coal mine in the periphery of Kalimati, Dharan-4. Soil, dust and grasses from the coal seam were removed and packed in plastic bags. The collected sample was then brought to laboratory of Central Campus of Technology, Dharan, Sunsari, Nepal.

2.2. Activation of coal by the process of furnace

The samples were activated by keeping at the temperature of 510^{o}C for 5 hours. It helped to remove the organic matters from the coal as they are converted in the form of ash. Finally, surface area of coal was increased by changing coal into activated charcoal.

2.3. Collection of test organism and preparation of stock culture

The test organism (*Escherichia coli*) was collected from American Type Culture Collection (ATCC code: 25922; B.P Koirala Institute of Health and science, Dharan, Nepal). The given bacterial sample was inoculated in selective media. *E. coli* was cultured by using Eosin Methylene Blue agar medium. *E. coli* strain was subjected to pure culture technique and finally sub-culture of each strain was done for standard culture.

2.4. Activated carbon impregnation with silver nanoparticles (Ag-NPs)

One gram of activated carbon was weighed with the help of digital balance. The weighed activated charcoal was taken and added to 20 ml of different $AgNO_3$ concentrations (a) 0.1 mol/L (b) 1 mol/L (c) 1.5 mol/L one at a time in the volumetric flask. This impregnation of different concentrations sample was then filtered and washed with water to remove the loosely adsorbed $AgNO_3$. It was washed until no $AgNO_3$ was seen in the filtrate. After decantation, the powdered sample collected in the filtrate was air dried for 24 hours. 10 ml of 0.2 mol/L $NaBH_4$ was added in that powder sample and it was kept over 24 hours to reduce impregnated $AgNO_3$ to form Ag particles. The sample was again filtered and washed with water to reduce the excess $NaBH_4$ with the help of $KMnO_4$ solution. This filtrate was then air dried according to Bandyopadhyaya et al. (2008).

2.5. Plate assay method for the antimicrobial test from E. coli

We have taken two media (M-endo and MHA) and observed zone of inhibition separately. At first M-endo agar was prepared. The agar was fortified with 1.5 × 108 CFU/ml medium of *E. coli* which is equivalent to 0.5 McFarland. *E. coli* was poured in the M-Endo media and was dispensed in the petriplates. Sterilized 2 drops of sterilized water were dropped in that hole. Those plates were incubated at 37ºC for 24 hours, and then antimicrobial activities were tested. After that 50 mg of different concentration silver nanoparticles was added in holes at a time and the process was repeated again. cork borer was used to make seven mm diameter holes in the agar medium. 25 mg of different concentration silver nanoparticles was added in the holes at a time.

2.6. Antimicrobial assays

The antimicrobial activity was performed by agar well diffusion methods. First of all, MHA was prepared. Bacterial strains were then inoculated onto the MHA plate (108 cells/ml). The well of 3 mm diameter was bored in the inoculated plates. After that, Standard antibiotics with different concentrations [Ampicillin (10 µgm), Vancomycin (30 µgm), Streptomycin (10 µgm), Erythromycin (15 µgm), Cefotaxime (30 µgm), Nalidixic acid (30 µgm), Trimethoprim (30 µgm) and Tetracycline (5 µgm)] were added into seven mm labeled wells respectively. The plates were then allowed to stand for 15 minutes and then incubated at 37ºC for 24 hours. The zone of inhibition for each isolate was compared and analyzed. The zone of inhibition was compared against standard antibiotics and activated carbon impregnated with silver nanoparticles from our study. For this step, significant difference between the zone of inhibition observed in M-endo and MHA media was figured out and the data from both media were combined to compare with the standard antibiotics.

2.7. Sample Preparation

The sample was prepared by crushing sample material. All the test and analysis were done using the prepared sample. The test methods for coal parameters were moisture content, ash content, volatile content, fixed carbon and pH.

2.7.1. Moisture content

1 gm of coal sample was taken into the clean and dry previously weighed porcelain empty crucible then introduced into the hot air oven at 105ºC. The sample was then dried until the weight was constant (Mishra, 2009).

The crucible was cooled in the desiccators and the final weight was taken from the loss in weight, the moisture content of the sample was calculated as reported by Mishra (2009);

$$Moisture\% = \frac{\text{Loss of weight}}{\text{Total weight of sample}} \times 100\%$$

2.7.2. Ash content

1 gm of prepared moisture-free coal sample was taken into a clean dry porcelain empty crucible and introduced into the furnace and the temperature was raised to 105ºC and the temperature was maintained constant for 30 minutes and then the temperature of the furnace was increased to 825ºC and dried until the constant weight. The crucible was cooled in the desiccators then the final weight was taken and from the weight of ash, the ash content of the sample was calculated as given by Mishra (2009);

$$Ash\ Content\% = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100$$

2.7.3. Volatile Matters

1 gm of prepared coal sample was taken into the clean dry previously weighed porcelain empty crucible and it was introduced into the muffle furnace by covering with the lid at 900ºC for exactly 7 minutes (Mishra, 2009).

2.7.4. Fixed Carbon

Fixed carbon was calculated by the following method proposed by Mishra (2009);

Fixed carbon % = 100 – Moisture Content % - Ash Content% - Volatile matter

2.7.5. pH

1 gm of coal was put into the conical flask. 100 ml of distilled water was added in the conical flask. Then the mixture was stirred by using magnetic stirrer. The sample was then for an hour and their pH meter by calibrating in the buffer 4 and 7.

2.8. Data analysis

Independent sample t-test was performed to observe the mean difference between the zone of inhibition against *E. coli* from standard antibiotics and samples of this study. For this test, all the samples from MHA and m-endo agar medium were combined and included. Therefore, mean difference between MHA and M-endo culture media were also observed where *E. coli* was cultured and zone of inhibition were observed. These

analyses were performed in SPSS version 16.0. Moreover, scatter diagram was made and a linear regression was performed to observe the relationship between zone of inhibition and concentration of $AgNO_3$ used. The test in study was repeated for 3 times and standard deviation (error bar) were presented in bar graphs. Bar graph with error bar were drawn in MS excel 2007. A Pearson correlation test was performed in R version 4.0.3 and normality of data was observed by Shapiro-Wilk test.

3. Results

3.1. Proximate analysis

Four parameters which were analyzed under this particular method as moisture content, ash content, volatile matter content and fixed carbon. The moisture content, ash content, volatile matter, and fixed carbon The moisture content, ash content, volatile matter, and fixed carbon of coal sample from Kalimati, Dharan were analyzed as 2.2%, 29%, 15%, and 53.8% respectively (Figure 1). The pH of coal collected from the Kalimati was found to be 5.8, which was slightly acidic.

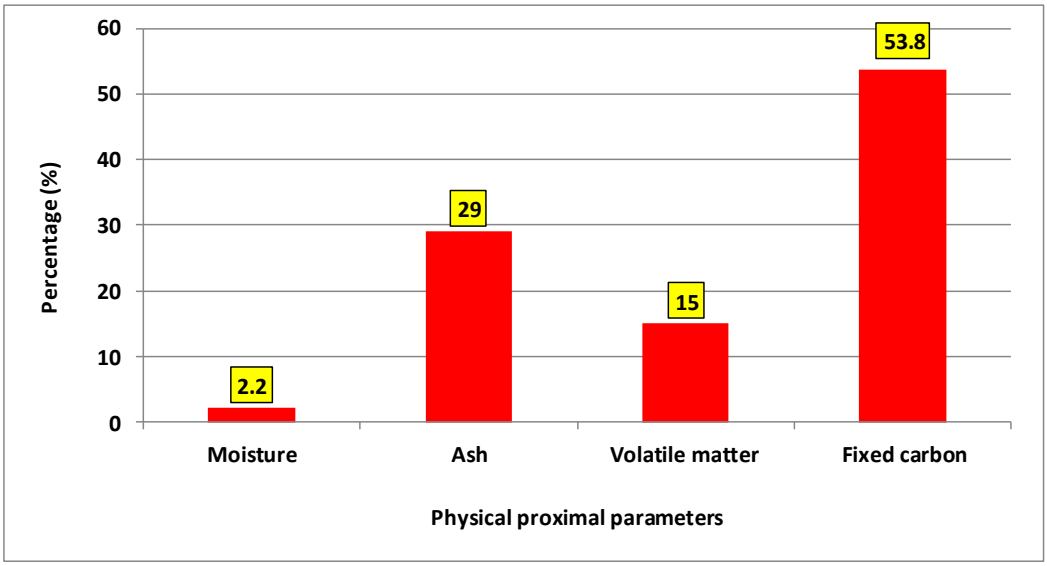

Figure 1: Bargraph representing the data of physical proximal parameters.

3.2. Antimicrobial activity of activated carbon

With 25 mg nanoparticles the zone of inhibition against *E. coli* as found to be 8 mm, 9 mm and 10 mm respectively on M-endo medium (Figure 2 and 3). Similarly, with 50 mg nanoparticles the zone of inhibition against *E. coli* as found to be 9 mm, 10 mm and 11 mm with concentration 0.1 mol/L, 1 mol/L and 1.5 mol/L of $AgNO_3$ respectively (Figure 3).

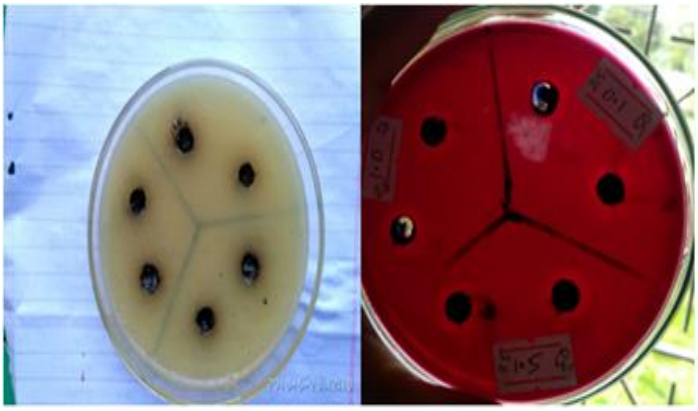

Figure 2: Zone of inhibition against *E. coli* in MHA media (left side) and M-endo media (right side) with 25 mg and 50 mg silver nanoparticles at three different (0.1 mol/L, 1 mol/L and 1.5 mol/L) $AgNO_3$ concentration.

Moreover, in MHA medium the inhibition zone with 25 mg nanoparticles at 0.1 mol/L, 1 mol/L and 1.5 mol/L of $AgNO_3$ was found to be 7 mm, 8 mm and 10 mm respectively (Figure 4). However, with 50 mg nanoparticles, the inhibition zone was found to be 9 mm, 9 mm and 12 mm at 0.1 mol/L, 1 mol/L and 1.5 mol/L of $AgNO_3$ respectively (Figure 2 and 4). On MHA media, numerically the zone of inhibition (Highest 12 mm at 1.5 mol/L $AgNO_3$) against *E. coli* was shown higher than in the comparison of M-endo agar medium (Highest 11 mm at 1.5 mol/L $AgNO_3$). However, statistically there was no significant difference (p value= 0.696; t value= -.405; degree of freedom= 8.260) between the two-culture media used for the *E. coli* where the zone of inhibition was observed at 95% confident interval (Table 1).

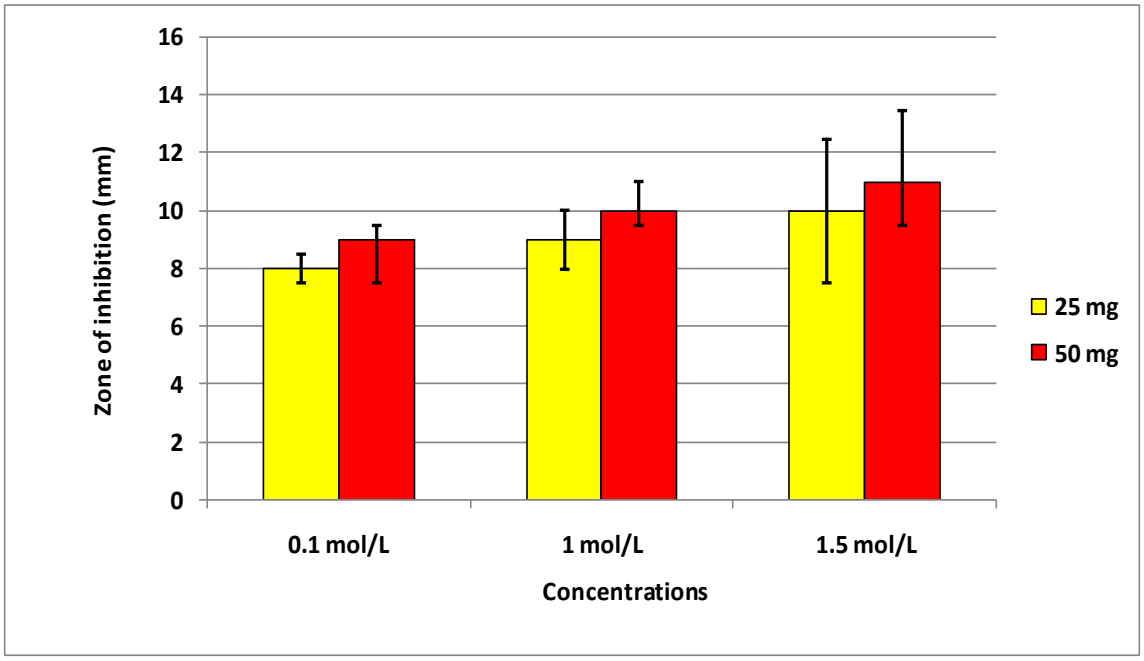

Figure 3: A bar graph showing zone of inhibitions (ZOI) against *E.coli* in M-endo agar medium representing mean value as ZOI and standard deviation on the top of each bar.

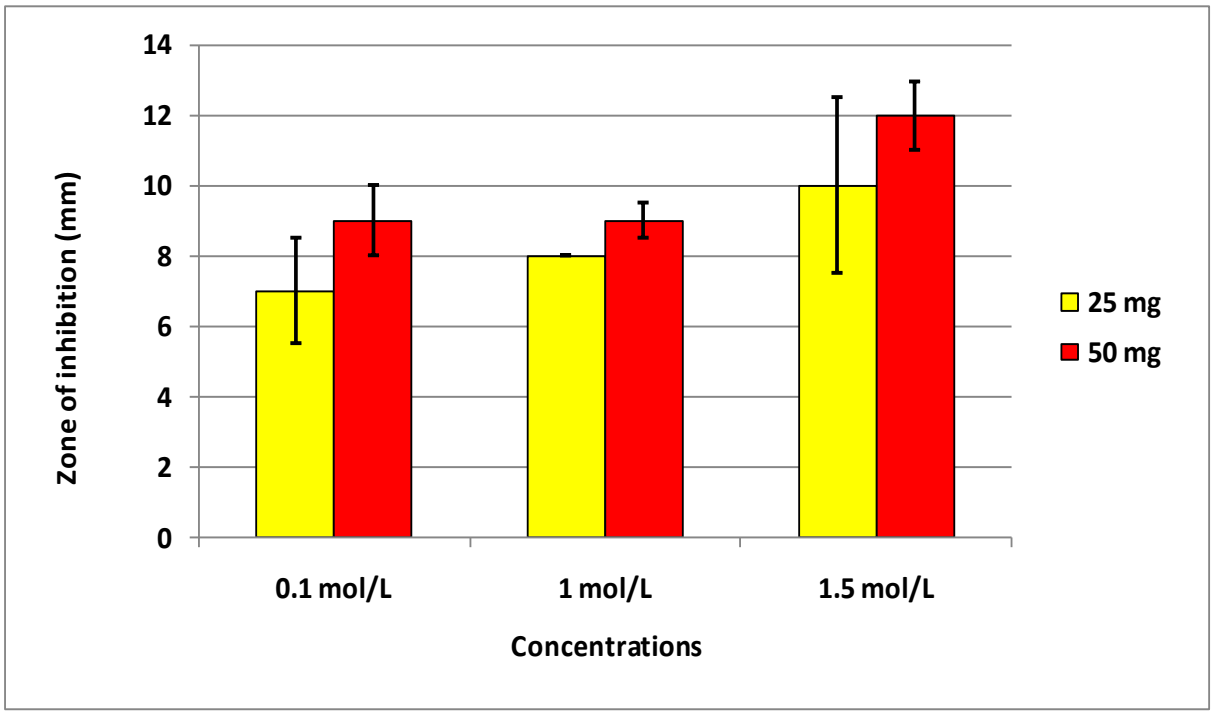

Figure 4: A bar graph showing zone of inhibitions (ZOI) against *E. coli* in MHA agar medium representing mean value as ZOI and standard deviation on the top of each bar.

Table 1: The mean difference of zone of inhibition between the two-culture media using t-test.

T	Df	P-value	Mean difference
-.405	8.260	.696	-.333

The present study revealed that increasing the concentration of $AgNO_3$, the diameter of zone of inhibition against *E. coli* also increases gradually. Larger zone of inhibition will demonstrate its higher antimicrobial activity. Statistically, it was proven that there was a positive relation between the concentration of $AgNO_3$ and the zone of inhibition observed against *E. coli* due to the antibacterial effect of activated charcoal impregnated with silver nanoparticles, according to Pearson correlation (r = 0.697) and a simple linear regression (R^2 = 0.798) (Figure 5).

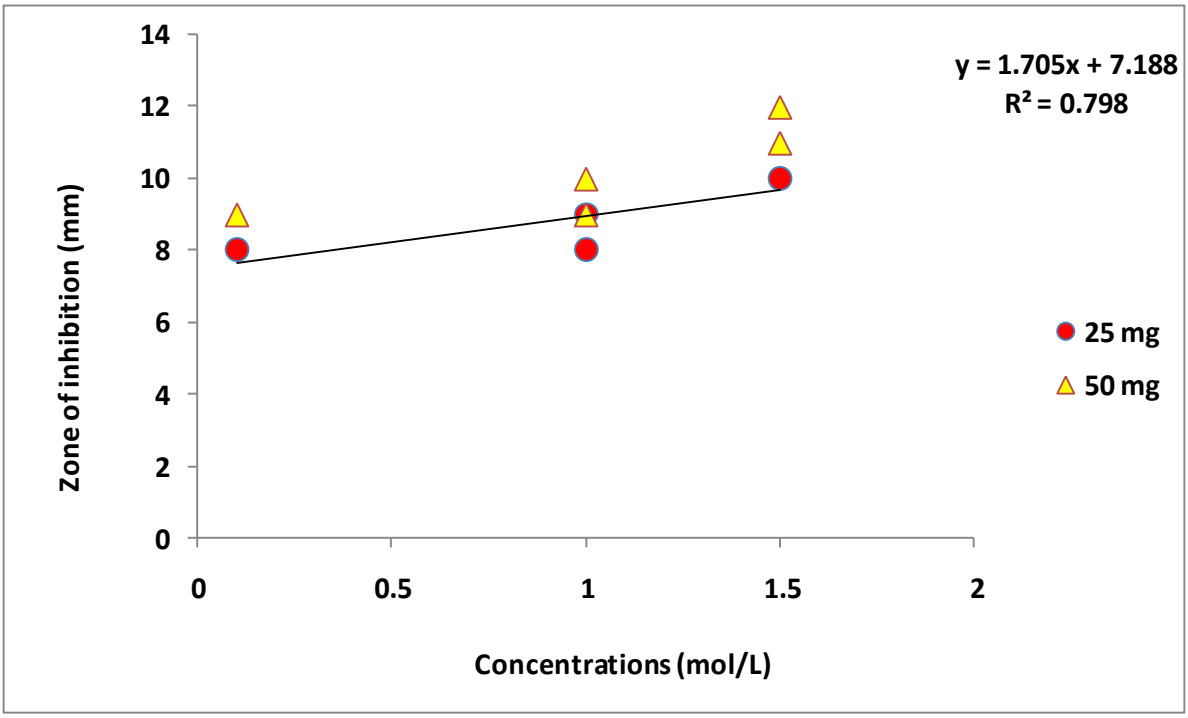

Figure 5: A simple linear regression showing relationship between zone of inhibition (mm) and concentration of reagent used.

3.3. Antibiotics susceptibility test and comparison

The test organism was tested against eight different standard antibiotics for valid comparison of antibacterial activity of activated charcoal and standard antibiotics. On testing eight different antibiotics i.e., Vancomycin (VA), Erythromycin (E), Tetracycline (TR), Cefotaxime (CTX), Ampicillin (AMP), Trimethoprim (TE), Naladixic acid (NA) and Streptomycin (S) against *E. coli*, it did not show inhibition zone against Vancomycin. However, it showed the highest inhibition zone (29 mm) against streptomycin (Figure 6). The highest zone of inhibition against *E. coli* from our sample and standard antibiotics was observed to be 12 mm and 29 mm respectively. From statistical analysis, it was proven that there was a significant difference (T value= 4.497; p value=.003; df= 6.445) between the zone of inhibition against *E. coli* observed from our study sample and standard antibiotics at 95% confident interval (Table 2).

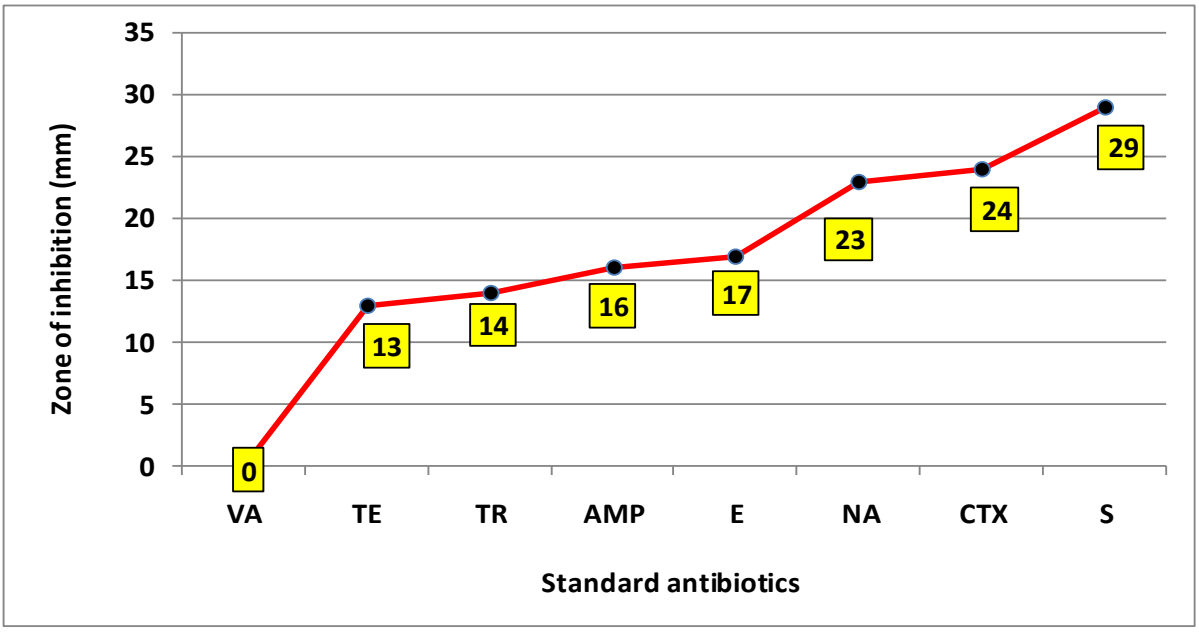

Figure 6: Antibacterial activity of standard antibiotics against *E. coli*.

Table 2: The mean difference of zone of inhibition between the study sample and standard antibiotics using t-test.

T	Df	P-value	Mean value
4.497	6.445	.003	10.329

4. Discussion

The present study has discussed the antimicrobial activity of activated charcoal against *Escherichia coli* and the physical properties of coal of Kalimati, Dharan under four parameters. The present study revealed that the increasing concentration of $AgNO_3$ gradually increased the diameter of zone of inhibition against *E. coli* with 25 mg and 50 mg nanoparticles. The zone of inhibition against *E. coli* was found to be 9 mm, 10 mm and 11 mm with concentration 0.1 mol/L, 1 mol/L and 1.5 mol/L of $AgNO_3$ at 50 mg nanoparticles. Inhibitory mechanism of silver nano-particles against *E. coli* is not fully known. However, some researchers have discussed that silver nano-particles attach to the thiol group of bacterial cells resulting to the cell inactivation (Yun et al., 2013). Moreover, a layer of lipopolysaccharides makes the exterior of gram negative bacteriaand those are composed of covalently linked lipids and polysaccharides. However, they lack strength and rigidity. Positively charged silver nanoparticles are attracted towards the negatively charged lipopolysaccharides which forms the basis of their attachmentdue to the electrostatic forces (Raffi et al., 2008). Therefore, when the silver nanoparticles enter the cell membrane it can hamper the bacteria by different ways. It has been reported that, it either destroys the cytoplasmic membrane or impede the cell wall synthesis (Sadeghi et al., 2010). It is also responsible for inhibition of specific enzyme synthesis and hold backof nucleic acid and protein synthesis which results in complete bacterial inhibition (Sadeghi et al., 2010). Hence, it is believed that silver particles bring some extra permeability in the cell membrane *E. coli*. This extra permeability affects the proper transport through the cell membrane which results in the incapability of proper material regulation and transport and finally contributing to cell death (Raffi et al., 2008). Moreover, some studies have also claimed that the destruction of bacterial cell is due to the attachment of silver nano-particles to sulphur and phosphorus of the DNA (Sadeghi et al., 2010). Krishnasamy (2015) have also reported the

antimicrobial property by the activated charcoal against *E. coli*, *B. subtilis*, *S. aureus* and *K. pneumonia*. However, the activated carbon in their study was prepared from *Tribulus terrestris* which had maximum antibiotic effect against these organisms. Karnib et al. (2013) have also reported the antimicrobial ability of silver impregnated activated charcoal and silica sand against water borne *E. coli* BL21 under Shake flask and plate assay technique.

Despite of activated carbon and silver nanoparticles, further studies have reported the antibacterial activity of carbon nanotubes against *E. coli*. It has been reported that size of carbon nanotubes is an important factor to determine its antimicrobial activity (Kang et al., 2008). They prepared two types of nanotubes depending upon its wall i.e., single-walled carbon nanotubes (SWNTs) and multi-walled carbon nanotubes (MWNTs). They finalized that SWNTs were much effective and toxic to *E. coli* than MWNTs. Similarly, Graphene oxide was shown inhibitory effect on the growth of *E. coli*. Azimi et al. (2014) have reported the cell membrane damage of *E. coli* when sharp edges of grapheme sheets interact with bacteria resulting RNA efflux through damaged cell membrane.

Moisture is an important parameter determining coal quality. The low moisture containing coal is found to have higher calorific value (Aina et al., 2009). As this study analyzed that moisture contained of tested coal is of 2.2%. Ash is impurity that will not burn. The content of ash in the coal sample of Kalimati was found to be 29%; which is very high ash content. In combustion, high ash content is an indicator of reduced heat resulting from the given quantity of coal. Higher ash content can create problem in handling and disposing of larger amounts of ash residues produced during the combustion. Thus, it results in high coke rate (Sahni et al., 2006). In this work, the volatile matter of coal sample was estimated as 15% which is low volatile. Volatile matter of coal is directly related to coal rank. It is also described in previous studies that as the rank increases, the volatile content decreases (Mackowsky et al., 1997). Volatile matter and the carbon rank are found to have opposite relation with each other. Both the above factors are used to determine the quality of coal in the U.S classification system [ASTM method D388-12-2013]. The fixed carbon content was found to be 53.8%. It is used for the estimation of quantity of coke that will yield from the sample of coal by removing volatile matters from the original sample. According to Sahni et al. (2006), fixed carbon determines the coal rank. Higher is the amount of fixed carbon the greater is the burning efficiency which indicates the high rank of coal and vice versa. pH of the Kalimati was found to be 5.8% which is slightly acidic. Jha et al. (2015) reported that generally low rank coal is more in acidic groups. According to Singh and Singh (2006), acidic pH is directly proportional to the sulphur content. According to Baruah et al. (2006), acidic coals also negatively impact the water environment, plant life, soil environment due to its acidity.

5. Conclusions

The result of proximate analysis showed that the moisture content of the coal from Kalimati is low which indicates the good quality of coal. It has high volatile matters contained which helps to ignite and burns quickly and ash content was very high which causes decreases in fixed carbon. Experimental and available data indicate theusefulness of using activated charcoal impregnated with silver against *E. coli*. The activated charcoal prepared from Dharan has good antibacterial properties and good zone of inhibition against *E. coli*. However, it could not compete the antibacterial activity performed by standard antibiotics against *E. coli*. The zone of inhibition produced by standard antibiotics was very high compared to the ZOI reported in the present study. However, it can be used as an alternative to Tetracycline and Trimethoprim, as the ZOI of these two antibiotics and activated carbon impregnated with AgNO3 was almost similar.

Author's Contribution

Conception, data acquisition, and drafting were done by RK, SC, and SR. Experimental work was performed by RK and SC. Writing and preparation of the manuscript were performed by SC and RK. Statistical analysis was done by SD and SC. Critical revision of the manuscript was done by SR and SC. Final approval of the manuscript was done by all the authors.

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Competing interests

The authors declare no competing interests.

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Ethical Approval and Consent

Not applicable

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