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Antimicrobial Activity of Antibiotic Doped Carbon Nanoparticles Extracted from Kitchen Soot against Pathogenic Gram-negative Bacteria

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Authors' contributions

This work was carried out in collaboration among all authors. Author Habiba designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IUD, MAB and MM managed the analyses of the study. Author ST managed the literature searches and supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

In this study carbon nanoparticles were extracted from kitchen soot and characterized by using UV/Visible spectroscopy. Amoxicyline and vibramycin were doped on the carbon nanoparticles by mixing the solutions of the aforementioned antibiotics and carbon nanoparticles and then evaporated to dryness at 50°C. The antibacterial potential of carbon nanoparticle, antibiotics and carbon nanoparticles doped antibiotics were evaluated using Agar tub dilution method against two bacterial strain i.e. *P. Aerogunis* and *Salmonella entrica*. The result indicated that the carbon nanoparticle doped antibiotics showed marked increase in the antibacterial potential with inhibition zone of 16.2 mm against *P. aerogunisa* and 12.5 mm against *Salmonela entrica* for Vibramycin Doped CNPs. The inhibition zone of Amoxicyline doped carbon nanoparticle is 25.0 mm against *P. aerogunisa* and 30.0 mm against *Salmonela entrica.*

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1. INTRODUCTION

1.1 Carbon-nanotubes (Buckytubes)

Carbon among other elements is considered to be the most abundant elements exists in the world [1-3]. Also, the interest in carbon based nano materials have grown rapidly in recent decades. For instance some of the popular developments in carbon based nanostructures are carbon nanotubes, nanofibers, nanoparticles and other carbonaceous materials [4-7]. Among other carbon, carbon nanotubes are standout as one of the most citedand popular creation of nanotechnology. Some of the varieties of carbon nanotubes are:single andmulti-walled, different open and closed spiral structure. The aforesaid typesare carbon allotropes based cylindrical nanostructure and has specific production costs and applications. Furthermore, nanotubes related to fullerene family and derived from their hollow structure and the walls are formed by single layer of carbon atoms known as graphene.

Due to the unique characteristic and properties (both physical and chemical), carbon nanoparticles (CNPs) have excellent scientific applications. CNPs not only result in a materials weight reduction and also gain impact strength, increase surface area, thermal stability, electrical conductivity, boast optical properties, dimensional stability and flame resistance [8]. The heat of conduction of CNPs are as efficient as most diamond and copper elements. Moreover, they can efficiently produce streams of electrons. Carbon Nanotubes, having long with thin carbon cylinders were discovered by Sumiolijima in 1991 [9]. Nanotubes consist of nanotubes within can act like a miniature spring which are seamless cylindrical layer (one or more) of graphene having closed or open ends. A perfect, CNTs structure have all carbon bonded to each other in hexagonal lattice except at the ends [10,11], whereas the desired properties degradation results by defects in mass produced heptagons, pentagons and other imperfections found in the sidewalls.

Furthermore, they are unique and large macromolecules because of their shape, size and significant physical properties. Alike a sheet of graphite where using straight carbon nanotubes may result in to branching, bending, plane buckling, closed ring and coils, etc. This is due to incorporation of pentagon–heptagon pairs on the straight nanotube walls in hexagonal network. In a regular orientation, a nucleation of pentagons and heptagons occurs along with nanotube body, results into coiled nanotubecarbon hexagonal lattice rolled into a cylinder.

These fascinating structures have generatedconsiderablepopularity recently, where extensive research has been devoted to understand their morphology. However, the physical properties until now are unclear and still undergoes further research. Nanotubes, encompasses broad range of structural, thermal and electronic properties that differs with different nanotube kinds (defined by its length, diameter and chirality).

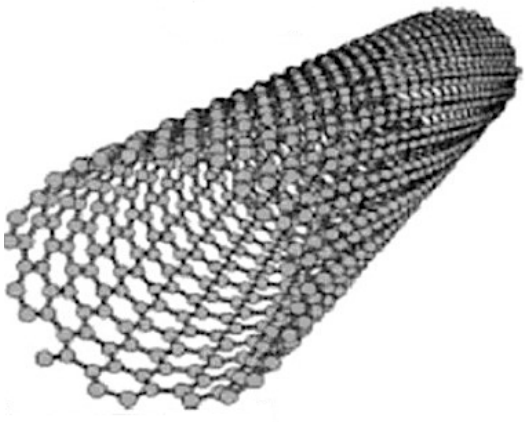

Fig. 1. Multiwall CNTs [12]

Carbon nanotubes (CNTs), could also called as another form of pure carbon, represented as a rolled hexagonal carbon networks capped by pentagonal carbon atoms Fig. 1. To fabricate carbon nanotubes, the most common method used is electric-arc discharge, which is an electrical breakdown of a gas produces a plasma discharge. This is similar to the prompt spark, which results when a current flowing through nonconductive material such as air. Due to vast application in different areas of science and technology, CNTsbecause of unique properties and potential are the focal point of intense research presently [3,5,13,14].

Carbon nanotubes after the decade of their discovery, has gone through much development with ongoing research. This points out CNTs application in numerous practical functions. The development also brought improvement in synthesis techniques, which help us to produced nanotube in a reasonably pure within evenquantities in grams. The rapid expansion of nanotechnology is support by in-depth studies of nanotubes structure-topology relations. Further strengthening comes from theoretical modeling which give experimentalist a new insights of possibilities. Due to right blend of properties such asstructural integrity, nanometer sized diameter, chemical stability and high electrical conductivity makes carbon anexcellent electron emitters. However, controlling nanotube diameter is considered as one of the most basic issues in developing nanotube growth methods. CNTs are cylindrical shells alike by rolling graphene sheets into a seamless cylinder. They exist as either multi-walled nanotubes (MWNTs) or single wall (SWNTs) nanotubes. The nanotubes individually are naturally aligned themselves in“ropes”which are held together by weak van derWaals forces (pi-stacking). Similar to graphite, chemical bonding of nanotubes is entirelyconsisting of sp^2 bonds. Compared to diamond (sp^3 hybridization), the three folded bonding (sp^2 hybridization) of curved graphene sheet is stronger asindicated by C-C bond length difference (0.154 and 0.142 nm for diamond and graphene,respectively). The tensile strength of SWNT is 20 times that of steel, and even higher for MWNT. Carbon nanotubes without any defectcould transfigure currently available high performance fibrous materials panel.

Infection caused by bacterial pathogen led the development of several diseases, which is a major challenge in healthcare system [15,16]. Till now antibacterial agents difficult to meet the challenges due to bacterial infection particularly on account of multidrug resistance among various bacterial pathogens. Recently discovered carbon nanomaterials has effective antimicrobial resistance due to its photo-activated properties [17]. It has been reported that antibacterial properties of nanomaterials depend on the composition, surface modification, type of microorganisms [18]. It has been further claimed that oxidative stress rather than photo-activation is the main reason behind the antibacterial properties of carbon nanomaterials. But, some current research studies confirmed that physical interaction of carbon material with bacteria is the main cause of bacterial cell wall damage [19,20].

In present research carbon nanoparticles were prepared from easily available kitchen soot and was doped with some antibiotics. Antibacterial activity of the dopedcarbon nanoparticles was evaluated against known bacterial strains.

2. MATERIALS AND METHODS

2.1 Methods of Collection

Carbon nanoparticles obtained in this study were extracted from soot (also recognize as hydrocarbon in general) of kitchen. For isolation of carbon nanoparticles, this method is one of the inexpensive, versatile, and reproducible. For burning, natural woods consist of trees branches and husk of coconut are utilized, whereas, uniform sized glass plates were hung over chimney hearth. The smoke originated from hearth sticks over the glass plate were collected and designated as day 1, day 2, and onwards. Glass plates were removed from smoke hood after one, two and up to seven days. The smoke particles that sticks on the glass plates were scratched off and collected. Similarly, the soot was also collected after the duration of six-month and marked as infinity sample. The scratched/ collected carbon nanoparticles were stored by wrapping in aluminum foil to avoid any sunlight exposure.

2.2 Purification

For purification, acetone-water with the ratio of 3: 1 was used for the dissolution of the crude kitchen soot. After centrifugation, the undisclosed residue was discarded. After that clear solution was collected and vacuum rotary evaporator was employed for evaporation of the solvent from this solution. Furthermore, powder form of fluorescing CNPs, was collected. The solubility of the nanoparticles was tested and was found to be

soluble in acetone, methanol, chloroform and DMSO.

2.3 Chemicals Used

1. Acetone
2. Soots (three types of soots from daraadamkhel, kohat and Karak)
3. Dimethyl sulfoxide, (DMSO)
4. Methanol (CH_3OH)
5. Distilled water(H_2O)

2.4 Medicines Used

1. Vibramycine
2. Amoxil

2.5 Experiment

First of all 600l acetone was taken in large beaker and then added 200 ml distilled water (3:1). Kitchen soot was putted in it and stirred for some time to made a miscible solution after that filtrate it twice to get a clear solutionas shown in Figs. 2-3. It was condensed on water bath at 50ºC at vacuum rotatory evaporator and then added methanol to made homogenous solution and got UV spectra of this solution.

The spectra that were obtained are given below Fig. 4.

After spectra condensed the solution on water bath and then added acetone to make a solution and filter it, divided the solution in to two parts (4 ml, 2 ml). Standard solutions were taken in same ratio and mixed with nanoparticles and condensed it on water bath for antibacterial activity incubation of microorganism.

2.6 Standard Drug Solution

To make a standard solution methanol and pure drugs were taken as (1:1)

1. vibramycine (100 mg/100 ml)
2. Amoxicyline (100 mg/100 ml)

2.7 Culture Media Preparation for Incubation of Microorganism

1.9 g of nutrient broth(MHA) powder were dissolved in 50 ml water by using conical flask with a stoppered having cotton plug. The mixture in the conical flask was gently heated. The broth mixture was further sterilized in an autoclave at 15 Ibs and 121°C pressure and temperature, respectively for 15 minutes.Later the mixture was cooled in laminar hood which was thoroughly disinfected with absolute alcohol for 20 min. This was followed by UV irradiation.Wire loop sized stock microorganism was disinfected first beforetransferring to cold medium. After that cold medium was poured in petri dishes with equal agar around 2.5 mm thickness. To solidify the agar medium, dishes were cooled sufficiently. Miller Hinton Agar 2plates were lauded with bacteria (*P. aerogunisa and S. enterica*) utilizing sterile cotton swabs already dipped in nutrient broth culture of bacterial strains. The disc dipped in the carbon nanoparticles solution was applied in lauded plate. The dishes were covered and subsequently kept in incubator oven at 37°C for 24 hr for further test. The entire process was conducted in laminar hood having flame of spirit lamp in front. The zone diameter was measured from clear agar dish zone after inhibition as shown in Figs. 5-6. DMSO was used as control.

Fig. 2. Hot plate stirrer

Fig. 3. Filtration

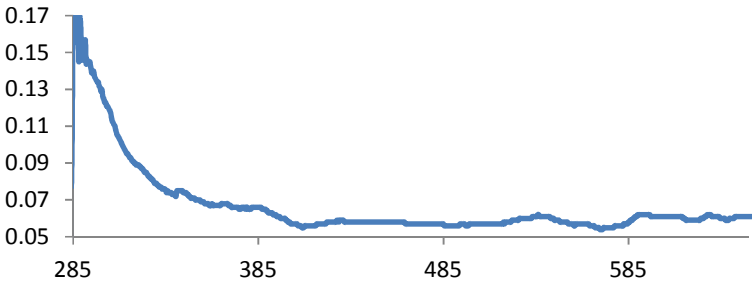

Fig. 4. UV spectra

3. RESULTS AND DISCUSSION

Carbon nanoparticles were extracted from kitchen soot by shaking finally divided soot with mixture of acetone and water (3:1) and then filtered. The filtrated was evaporated to dryness and then UV spectra of the carbon nanoparticles were recorded. The carbon nanoparticles were doped with vibramycin and amoxicyline by mixing the solution of 4mg of carbon nanoparticles and 2ml of the antibiotics and then evaporated to dryness. Pure carbon nanoparticles, pure antibiotics and antibiotic doped carbon nanoparticles were subjected to antibacterial activity against *P. aeruginosa* and *S. enterica* and the results were summarized in the Table 1. The results indicated that pure carbon nanoparticles did not show any antibacterial activity but vibramycin and amoxicyline doped carbon nanoparticles displayed enhanced antibacterial activity as compared to pure vibramycin and amoxicyline. The zone of inhibition of vibramycin doped carbon nanoparticles is 16.2 mm and 12.5 mm for *Pseudomonas aeruginosa* and *Salmonella enterica,* respectively while for pure vibramycin and then checked for antibacterial potential against *Pseudomonas aeruginosa* and *Salmonella enterica*. Further, results were compared with some already reported essential oils against known gram negative bacteria. One of the researchers recently studied the effect of Thyme essential oil on *E. coli* (a gram negative bacteria) and found maximum 31 mm zone of inhibition [21]. Antibacterial activity of *Eucalyptus* leaves extracts and its oils were found more active against gram negative strains than gram-positive one [22]. In an another piece of research gram negative bacterial strains showed less susceptible than gram positive bacteria especially against *P. aeruginosa* [23], which was certainly proofs superiority of our study against chosen gram negative bacteria.

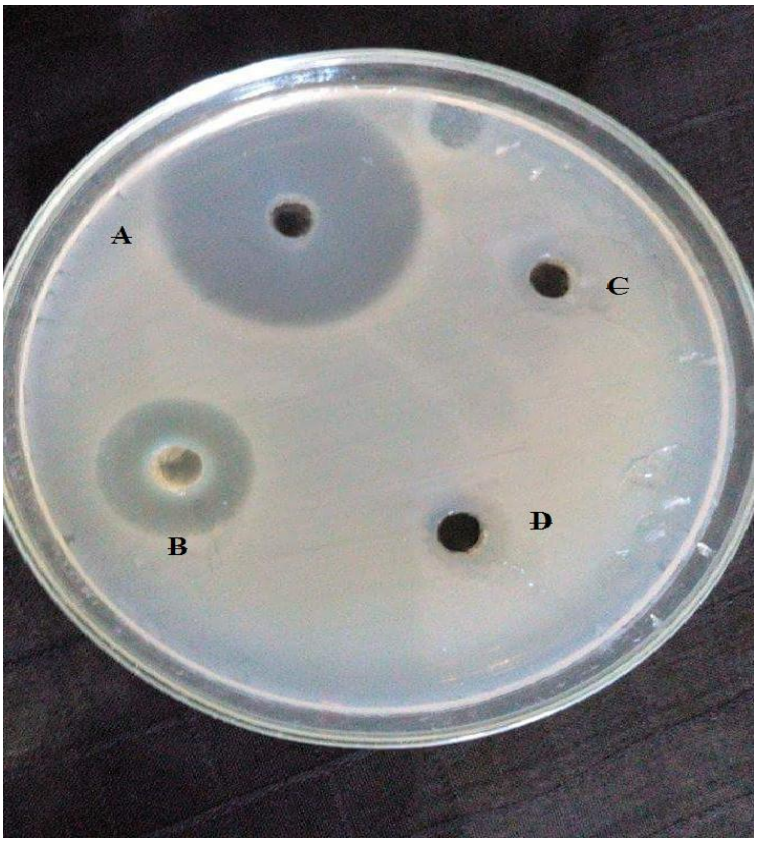

Fig. 5. Zone of inhibition in mm of Amoxycyline (A= 32 mm), Carbon naonaprticles with Amoxycylin (B = 30 mm), Vibriomycin (C= 19 mm) and Carbon nanoparticles with Vibriomycin (D = 12.5 mm) aginst*Salmonella enterica*

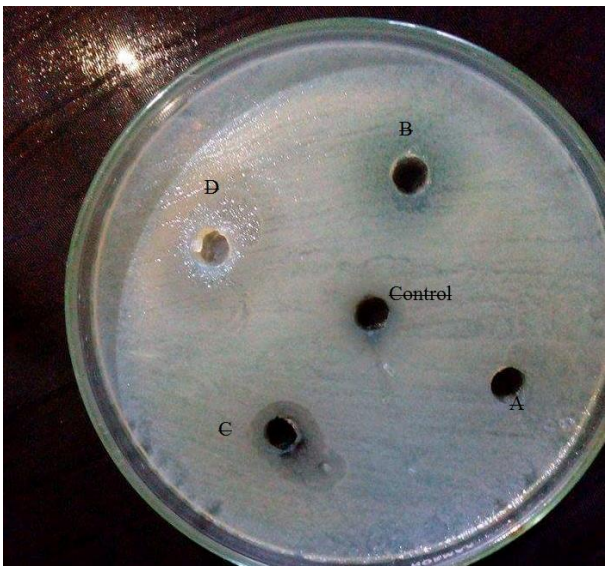

Fig. 6. Zone of inhibition in mm of vibriomycin (A= 12 mm), carbon naonaprticles with vibriomycin (B = 16.2 mm), amoxycyline (C= 15 mm) and carbon nanoparticles with amoxycyline (D = 25 mm) aginst *Pseudomonas aeruginosa*

Table 1. Vibromyxin and amoxicyline in different bacteria

S. No	Bacteria	Vibramycin		Amoxicyline	
		Pure	Vibramycindoped Carbon nanoparticles	Pure	Amoxicylinedoped Carbon nanoparticles
1	*Pseudomonas aeruginosa*	12 mm	16.2 mm	15 mm	25.0 mm
2	*Salmonella enterica*	19 mm	12.5 mm	32 mm	30.0 mm

4. CONCLUSION

In the current work isolation of carbon nanoparticles from soot of kitchen was conducted. It was further characterized by UV/visible spectroscopy. The isolated CNPs were further used and tested against *P. aeruginosa* and *S. enterica*. The antibacterial properties of carbon nanoparticles collected from kitchen soot were studied and found to be effective against *P. aeruginosa* and *S. enterica*. Future study was also planned to expand our research with more such carbon nanoparticles with many bacterial strains.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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