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Antioxidant Activity of Honey-Derived Carbon Nanoparticles Incorporated Herbal Formulation Mediated Silver Nanocomposite against Wound Pathogens

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The main aim is to synthesize honey-mediated carbon nanoparticles incorporated with herbal-based silver nanocomposite against wound pathogens and evaluate their antioxidant activity.Carbon Nanoparticles combined with silver nanocomposites are synthesized from honey and their antioxidant activity is evaluated using DPPH assay, Hydrogen peroxide assay, FRAP, Nitric oxide assay, and ABTS assay.For honey-mediated carbon nanoparticles combined silver nanocomposites, the analysis of antioxidant activity shows that the highest value of DPPH obtained at the highest concentration of 50µg/ml is 89.76%, for hydrogen peroxide the value obtained at the same concentration is 86.7 %, the value estimated for FRAP is 88.66%, for nitric oxide assay the value is 86.59% and 89.65% is the value obtained for ABTS assay.In this current paper, honey-mediated carbon nanoparticles combined with silver nanocomposites show impeccable potential for antioxidant activity as determined from the values shown from different assays and it can be utilized for future biomedical applications.

Keywords: Anti-inflammatory, antioxidant, biomedical applications, green synthesis, silver-coated carbon nanocomposite.

1. Introduction

The era of nanotechnology came into existence in 1959 by merging both areas of engineering and science which in various nanoscale-based dimensions are being utilized in applications of materials, devices, systems, design, manufacture and structures. The multiple nanoparticles developed can be incorporated into various applications like therapeutic, preventative, and diagnostic properties. For instance, drugs embedded with nanoparticles under observation show that side effects are not noted, absorbed more effectively and bioavailability is better. Nanoparticles can also cross the brain membranes and can be used on various membranes as part of the drug carrier system [1]. The types of nanoparticles including both organic and inorganic particles are utilized in the field of nanomedicine for applications. Inorganic nanoparticles when compared to organic nanoparticles are found to be hydrophilic, non-toxic, highly stable, and biocompatible. These materials are used because of specific thermal, mechanical,chemical, optical, and electrical features. Nanoparticles usually take up the chemical properties of the material from where they are synthesized which in turn helps in further action of the applications [2].

Carbon nanoparticles are obtained from pure carbon thus they have impeccable features like good heat and electrical conductivity, and are hydrophobic because of their sp2 hybridization, excellent biocompatibility, low toxicity, high stability, and mechanical properties like strength, stiffness, and toughness. Multiple carbon-based materials like nanodiamonds, sp2 carbon nanomaterials(graphene quantum dots, carbon nanotubes, graphene, fullerene, and carbon nanohorns), and amorphous carbon nanoparticles(carbon dots, ultrafine carbon particles, carbon nanoparticles). The usage of carbon nanoparticles is applied in medicine, food, agriculture, electronics,pharmaceuticals, cosmetics, bioengineering, biosensors, anticancer drugs, imaging, and drug delivery-based vehicles. The most commonly studied CNP is graphene which is used in multiple applications [3]. Detrimental effects are not seen when nanoparticles are exposed just for a short period. CNPs don't show any adverse actions or health issues when short-term exposure is seen. However long-term exposure especially to the staff can be seen as risky. The risk of toxicity can be minimized by causing alterations in their functionalization which in turn could lead to a reduction in toxicity by causing modifications in the functions of CNPs [4].

Honey is a bioproduct obtained from bees containing vitamins, amino acids, glucose, minerals and fructose. Honey has been an element in folk medicine since ancient times and has been seen as an impeccable therapeutic agent. There are different types of honey in the world which differ based on flavor, sensory perception, color, and medical action. Honey has been known for its antimicrobial, anti-inflammatory, antioxidant, and antitumor, and can be used as a cardiovascular influencing agent and helps in the regulation of glycemic response. It can also be a healing agent and a wound dressing [5]. One feature of honey is its antioxidant application. Oxidative stress happens when there is an error in the chemical reaction based on the production of free radicals and the protective effect of the body leading to irregularities in the genetic structure and damage to the cells. The main antioxidants present in honey are chrysin, quercetin, hesperetin, and melanoidins [6].

Silver nanoparticles are known as silver-derived nanoparticles having a size range of 1–100 nm. Since ancient times, silver has been used as an antimicrobial agent all by itself or when combined with other elements. Silver has a flair to cause resistance to the growth of bacteria by the concept of blending into wound dressing and creams in the form of silver sulfadiazine and silver nitrate. Silver is also used for appliances like washing machines, and refrigerators, in food packaging and for industrial use. Thereby they are the essential element in applications of detection, targeted drug delivery, imaging, and diagnosis. With the evidence of antibacterial activity of silver, silver nanoparticles are developed in the field of nanomedicine for the study of antimicrobial activity [7]. Studies show that silver nanoparticles show more potent antioxidant activity than the leaf sample and, in most cases, the observation is compared with ascorbic acid. The increase in antioxidant activity of nanoparticles can be attributed to the process of adsorption occurring to the bioactive compounds over the nanoparticles. In addition to that feature, the antioxidant activity where the nanoparticle behaves as hydrogen donors, reducing agents, and singlet oxygen quenchers is mainly influenced by the presence of terpenoids, phenolic-based compounds, and flavonoids [8]. In this research paper, the green synthesis of carbon nanoparticles synthesized from honey is emphasized along with the blending of silver nanoparticles, and the antioxidant capacity of the nanoparticle extract is studied using Hydrogen Peroxide Assay, DPPH Assay, and FRAP, Nitric oxide, and ABTS assay.

2. Materials and Methods:

Synthesis of nanoparticles:

2 millimolar of silver nitrate is measured and added to 75ml of distilled water. 25 ml of prepared aqueous extract(ocimum gratissimum and Cissus quadrangularis) is measured and introduced into 75 ml of silver nitrate solution. The prepared solution was then kept at the orbital shaker. The optical density values were measured using a UV spectrometer after 3 hours. After 24 hours duration, the values of optical density were taken again using UV spectrometers. The color change observed indicated the synthesis of silver nanoparticles. The UV absorbent of the nanoparticles is taken between the range of 250- 650 nm.

Synthesis of Honey and PEG-Mediated FCNPs:

Honey is collected from the commercial store and PEG was utilized for the synthesis of FCNPs. 5g of honey & 1 g of PEG were mixed and kept in the microwave oven at 700 volts for 1 hour using a crucible. The residues settled in the crucible were crushed well and washed with a prepared ethanol solution (5:15) for the times. The supernatant collected was checked for its fluorescence. The pellet was collected and ground into fine powder followed by using an ultra-probe sonicator for 4 times at 5 min intervals. The FCNPs were kept in a hot air oven for drying and used for further studies.

Antioxidant Activity:

DPPH Assay:

The antioxidant property was analyzed using 1,1-Diphenyl-2-picrylhydrazyl assay. 0.03 g of DPPH powder was measured along with 10 ml of methanol, DPPH powder was then dissolved in the measured solution of methanol. 0.05 ml of stock solution was measured and dissolved into 49.5 ml of methanol. This solution is called a working standard solution. 1 ml of working standard solution was added to each 5 test tubes. Sample at various concentrationsss(10 µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, and 50 µg/ml) was added into the test tubes. Incubation was done for 10 minutes in a dark place. Color changes were observed.The readings were taken at 517 nm wavelength (9).

Hydrogen Peroxide Assay:

Hydrogen Peroxide Assay was used for the determination of antioxidant activity. 5 sterile test tubes were taken and 1 ml of hydrogen peroxide was added into each test tube. The prepared extract was added into the test tubes at different concentrations(10 µg/ml, 20 µg/ml,30 µg/ml,40 µg/ml and 50 µg/ml). The test tubes were kept under incubation in the dark for 10 minutes. The readings were taken by using a UV spectrometer at 530 nm.Ascorbic acid was used as standard(10).

FRAP:

0.5 ml of ferric chloride was measured and the nanoparticles with different concentrations were taken in 5 test tubes. 2.5 ml of phosphate buffer was added to each test tube. Then again 2.5 ml of potassium ferricyanide was added to each test tube. It was kept for 30 minutes incubation. 2.5 ml of TCA was added to the test tubes. It was kept for 10 minutes incubation. The test tubes were incubated in a water bath for 10 minutes. After incubation,the samples were centrifuged and 2-5 ml of the centrifuged supernatant was taken. 2.5 ml of distilled water was added and kept for incubation for 10 minutes. Reading was taken at 700 nm (11).

ABTS Assay:

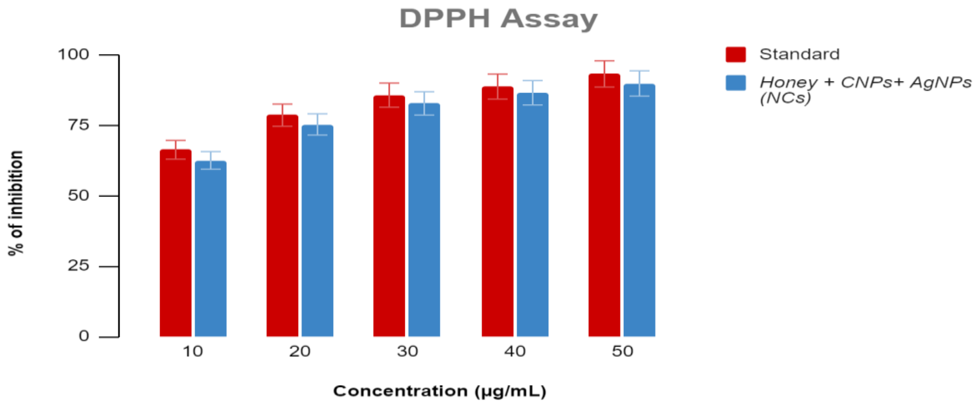
The formation of ABTS radical cation occurs by the reaction of 2.45 mM potassium persulfate(in distilled water) along with 7.0 mM ABTS(50% ethanol).The prepared reagent was refrigerated for 24 hours. The prepared reagent should be diluted with 50% ethanol before using until absorbance of 1.0 at 734 nm. 250 µl of ABTS was measured and 20 µl of carbon nanoparticles combined with silver nanoparticles based on nanocomposite taken at different concentrations (10, 20, 30, 40, and 50 µg/ml) were added together in a 96-well microplate. Incubation is done under darker conditions for 10 minutes. The readings were measured at 734 nm after incubation (12).

Nitric oxide Assay:

The estimation of Nitric oxide assay is done by GriessIllosvoy reaction. The modification of the GriessIllosvoy reaction occurs by the usage of naphthyl ethylenediamine dihydrochloride(0.1%w/v). 0.5 ml of phosphate buffer, 2 ml of sodium nitroprusside, andthe synthesized nanocomposite at different concentrations of 10, 20, 30, 40, and 50 µg/ml were taken in 5 test tubes and were kept for incubation at 25 C for 150 min. 0.5 ml of the reaction mixture after incubation, was added with 1 ml of sulfanilic acid reagent and was kept aside for 5 minutes to complete the process of diazotization. 1 ml of naphthyl ethylenediamine dihydrochloride was added and observed after 30 minutes at 25 C. Under diffused light, a pink-colored chromophore is observed. The absorbance of the mixture was measured at 540 nm. Rutin is used as a standard.

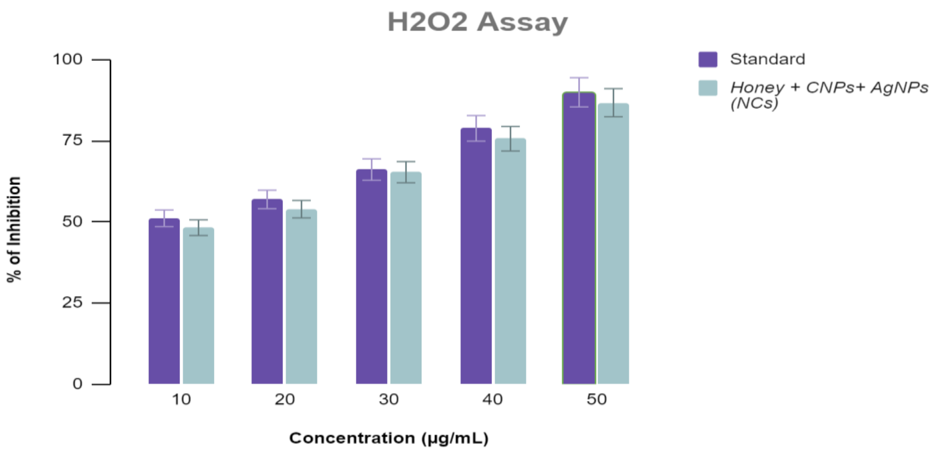
3. Results:

Figure 1: Graphical representation of percentages of antioxidant activity of the honey-mediated carbon nanoparticles incorporated silver nanoparticle-based nanocomposite and standard



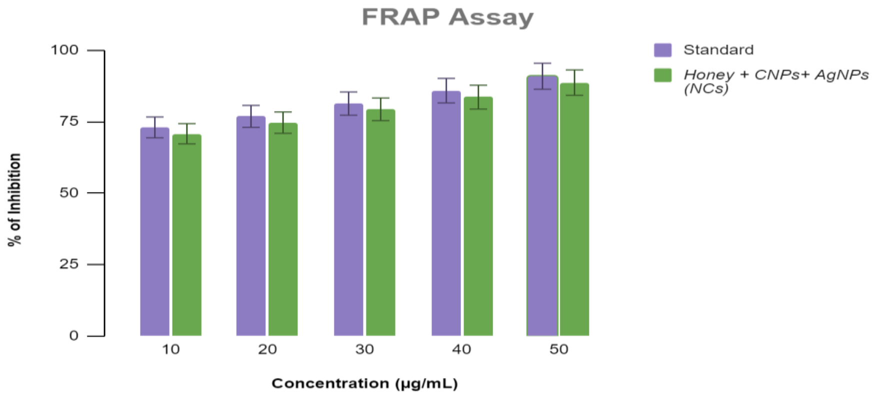
Honey-mediated carbon nanoparticles combined with silver nanoparticles-based nanocomposite when assessed for antioxidant activity using DPPH assay exhibit the values 62.48%, 75.24%, 82.69%, 88.47%, and 89.76%. The results show that the highest inhibition percentage is 89.76% reported at the highest level of concentration 50µg/mL and 93.15 is found for standard as shown in Figure 1.

Figure 2: Portraying the percentages of antioxidant activity of the honey-mediated carbon nanoparticles incorporated silver nanoparticle-based nanocomposite and standard using Hydrogen peroxide assay.



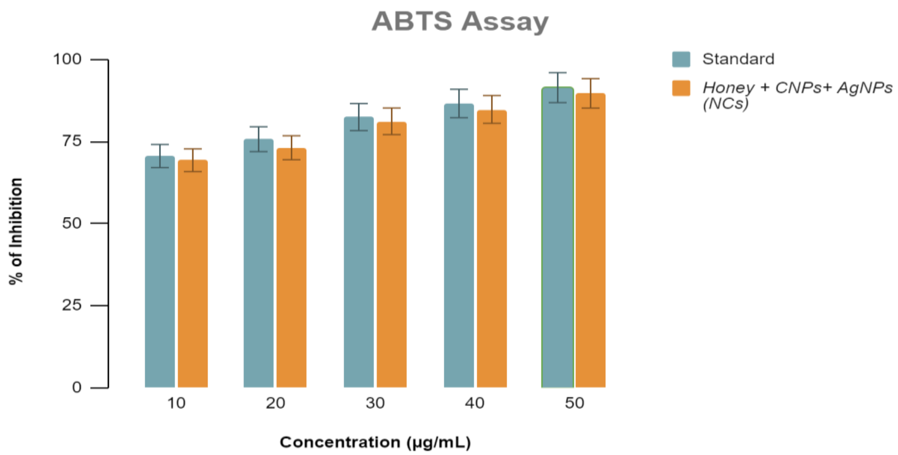
Honey synthesized carbon nanoparticles blended with silver nanoparticles based nanocomposite when evaluated for antioxidant activity using hydrogen peroxide assaydisplays the following values at 48.2%, 53.9%, 65.3%, 75.6% and 86.7%. The results show at the highest concentration of 50µg/ml, the highest inhibition percentage is 86.7 % and 89.9% is found for standard, which is shown in Figure 2.

Figure 3: Percentages of antioxidant activity of the honey-mediated carbon nanoparticles incorporated silver nanoparticle-based nanocomposite and standard using FRAP assay.



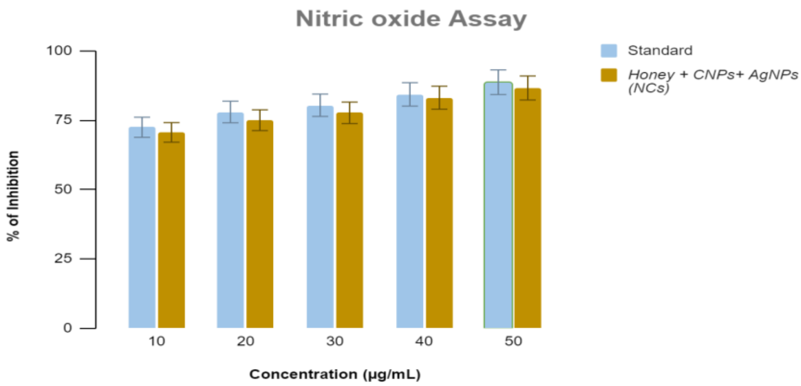
The results for the green synthesized nanocomposite when analyzed for antioxidant activity using FRAP assay reveals the following values at different concentrations. The values of inhibitory percentage are 70.75%, 74.64%, 79.31%, 83.57% and 88.66%. AT the highest concentration of 50µg/ml, the highest value of inhibition reported at 88.66%.The highest value obtained for standard is 90.89% as represented in Figure 3.

Figure 4: Graphical presentation of percentages of antioxidant activity of the honey mediated carbon and silver nanoparticle based nanocomposite and standard using ABTS assay.



The values obtained from the analysis of antioxidant properties of the nanocomposite using the ABTS assay are 69.28%, 73.08%, 81.14%, 84.74 and 89.65% at different levels of concentrations. The highest value reported is 89.65% at the concentration of 50µg/ml and for standard, the value at the same concentration is 91.39% as shown in Figure 4.

Figure 5: Portraying percentages of antioxidant activity of the green synthesized nanocomposite and standard using Nitric oxide assay.



In Figure 5, the values obtained from the antioxidant study of carbon and silver nanoparticles incorporated in nanocomposite at different concentrations are 70.58%, 74.97%, 77.63%, 83.08%, and 86.59%. The highest value obtained out of all concentrations is 86.59% at the level of 50µg/ml. The highest value for a standard is 88.67%.

4. Discussion:

In the present research paper, emphasis is laid on the antioxidant activity of honey-mediated carbon nanoparticles combined with silver nanocomposites. The various values obtained from the different assays for the determination of antioxidant activity are compared with the findings of other related scientific papers. Using the DPPH assay, the studies show that the highest inhibition percentage obtained is 89.76% reported at the highest level of concentration 50 µg/mL. In comparison to other papers, CQDs synthesized from red cabbage increase with the increase in concentration level and it is observed that maximum activity is reported at 0.6 µg/mL for 61%(13). From carbon quantum dots derived from coconut husk, it is observed the antioxidant property increases with even minimal changes to the concentration levels thereby at the highest concentration of 0.30 µg/mL, 80 or 81% is reported (14). Compared to the MWCNTs synthesized, the plant extract of B. vulgaris records the highest antioxidant value of 70% whereas the value of MWCNTs is only 19% (15).

For honey-derived carbon and silver nanoparticle-based nanocomposite, the highest inhibition percentage value assessed on using hydrogen peroxide assay at the highest concentration of 50 µg/ml is 86.7 %.A nanocomposite of graphene oxide and vanadium oxide was synthesized using the Co-precipitation method along with pure graphene oxide. Various types of nanocomposites at different ratios were synthesized and analyzed for antioxidant activity using hydrogen peroxide assay. At the highest concentration level of 350 µg/ml, 53% is reported for GO 1:4, the value obtained for V2O5/GO1:3 is 60%, and 75% is seen for Ascorbic acid as standard(16). The synthesized graphene quantum dots/Zinc oxide is reported to have good scavenging activity thereby the values obtained are 68.20 % and 62.58 % for ascorbic acid and GQDs/ZnO at 100 µg/mL(17). Using the one-pot hydrothermal method, nanocomposites, where zinc oxide nanorods are incorporated on reduced graphene oxide nanocomposite, are synthesized. Using the hydrogen peroxide assay, the following value obtained for the nanocomposite was 62.1% at 1000 µg/mL where the standard used is gallic acid(18).

For the comparative study of ABTS values, the highest value obtained at the highest level of concentration(50 µg/ml)is 89.65%. For NPS-cooped carbon quantum dots, 70 or 71% is reported at the highest concentration of 0.03 µg/mL (19). An increase in the antioxidant activity is seen with the increase in concentration using ABTS assay, in comparison with both turmeric-based sulfur carbon dots and carbon dots. It is estimated that at the highest concentration of 200 µg/mL, sulfur-based carbon dots show 80% of free radical scavenging activity, and 90% of activity is seen from carbon dots (20). For the synthesized selenium nitrogen-doped carbon quantum dots and its standard Trolox, the concentration was increased from 0.2 to 0.5 mg/mL, and an increase in the scavenging activity was assessed from 3 to 93% for selenium-nitrogen-doped carbon quantum dots. Under the same conditions, no changes were observed much for Trolox (21).The highest value obtained on analyzing honey-mediated carbon and silver nanoparticle-based nanocomposite using nitric oxide assay is 86.59% at 50µg/ml. For the assessment of the antioxidant activity of zinc oxide nanorods decorated with reduced graphene oxide using Nitric oxide, the high value obtained is 42.7% inhibition at 1000 µg/mL. For the plant-based graphene oxide nanoparticles, the nitric oxide assay shows that for naringenin-based graphene oxide, graphene oxide, and naringenin extract, the values obtained are 24.2 ± 1.4%, 16.5 ± 2.61%, and 30.1 ± 0.9% (22).

5. Conclusion:

In this paper, a concise design of the antioxidant activity of honey-mediated carbon and silver nanoparticle-based nanocomposite is explained. The following characterizations performed on the synthesized nanoparticles are FT-IR and XRD. The various values obtained on performing multiple assays indicate that the nanocomposite can be used as a potential antioxidant. In the future,the antioxidant value can be studied and improved by incorporating carbon nanoparticles with another plant-based extract.

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