International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Synthesis, Characterization and Biochemical Aspects of Bimetallic Nanoparticles

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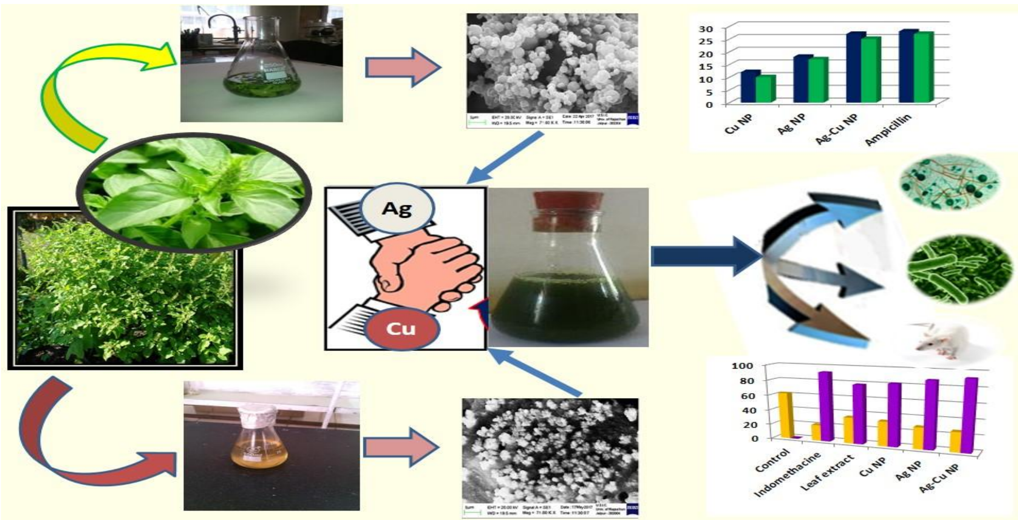
Abstract
The present study focuses on the preparation of copper-silver bimetallic hybrid nanoparticles by green method using leaf extract from Ocimum sanctum leaves. The resulting single pot green synthesized nanoparticles were characterized by analytical and spectral techniques. Here, UV-Visible spectroscopy is useful to examine the optical measurements of the prepared nanoparticles. The scanning electron microscope (SEM) and Transmission electron microscopy (TEM) images visibly envisaged the formation of spherical nanoparticles with an average size of 12-25 nm. The FTIR analysis was used to identify the capping and stabilizing capacity of the leaf extract. The antibacterial activities of the nanoparticles were revealed against some human pathogenic bacteria. It was found that these nanoparticles can inhibit the bacterial growth and hence can be used as antibacterial agents. The positive findings of anti-inflammatory activities of these compounds have been discussed.

Keywords: Bimetallic, Nanoparticles, scanning electron microscopy Transmission electron microscopy Antimicrobial activity, Anti inflammatory activity

Graphical Abstract



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Introduction

Development of nanoscience and nanotechnology needs a constant innovation and improvement in nanomaterials. In recent years, a new field of science focused on the creation, manipulation and use of materials at the nanometer scale has boomed up in the scientific world [1]. Nanoscience involves the synthesis of nanoparticles covering tremendous applications in many scientific areas such as chemistry, biology, medicine, material, physics, etc. Nanoparticles are of great interest due to their novel physicochemical, magnetic and optoelectronic properties that are governed by their size and shape [2, 3]. A large number of methods have been employed for the synthesis of nanoparticles in order to meet the increasing demand for various metallic and non-metallic nanoparticles.

The physical and chemical methods employed for nanoparticle synthesisinvolves an expensive procedure that often use toxic materials with potential hazards such as environmental toxicity, cytotoxicity, and carcinogenicity [7]. However all these factors can be possibly controlled by the use of biological mediated production. Copper displays substantial biochemical action either as an indispensable trace metal or as a component of innumerable exogenously administered compounds in humans. In its former role it is bound to ceruloplasmin, albumin, and other proteins, while in its latter it is bound to ligands of various types forming complexes that interact with biomolecules, mainly proteins and nucleic acids [4, 5]. Existing interest in Cu complexes springs from their probable use as antimicrobial, antiviral, anti-inflammatory, antitumor agents, enzyme inhibitors, or chemical nucleases.Heterobimetallic molecules embrace two different metals held in close proximity, either through a ligand scaffold or a direct metal-metal bond, which permits them to interact or act together. Interest in polymetallic coordination complexes endures to steer the development of novel synthetic strategies to access unique structural frameworks [6]. Herein, we have chosen for a cleaner, reliable, biologically compatible, benign, and environment-friendly green processes to synthesize bimetallic nanoparticles of copper-silver. These biosynthesized nanoparticles have been further evaluated for their antibacterial and anti-inflammatory activities.

Experimental

Preparation of leaf extract

The first step in the synthesis of nanoparticles involves the preparation of leaf extract from *Ocimum sanctum* leaves. The leaves (100g) were collected and washed thoroughly using the deionized water. These leaves were then cut into fine pieces and boiled with 100ml of deionized water for 20min in heating mantle at a temperature of about 80°C. The resulting product was allowed to cool, filtered and then stored in refrigerator for further experiments.

Synthesis of copper nanoparticles

The synthesis of copper nanoparticles embroils the preparation of 100ml of 1mM aqueous $CuSO_4.5H_2O$ solution in a 250ml Erlenmeyer flask. To this solution, 10ml of Ocimum sanctumleaf extract was added in a dropwise manner resulting into a change in color of the solution mixture from blue to light green. After complete addition of leaf extract, the mixture was kept for incubation for 24hrs, further causing the color change from light green to dark green. The Cu nanoparticles thus obtained were purified by repeated centrifugation at 12000rpm for 15min. followed by re-dispersion of the pellet in deionized water and dried in oven at 80°C.

Synthesis of silver nanoparticles

The silver nanoparticles were synthesized by preparing 100ml of 1mM $AgNO_3$ solution, to which 5mL of Ocimum sanctumleaf extract was added in a dropwise manner. The color of the resulting mixture changed from colorless to brown color clearly indicating the formation of silver nanoparticles. This resulting solution mixture was kept for incubation for 24hrs and further change in color to brownish black was observed. The silver nanoparticles thus attained were purified with the centrifugation of the solution carried out at 12000 rpm for 15min. followed by re-dispersion of the pellet in deionized water and dried in oven at 80°C.

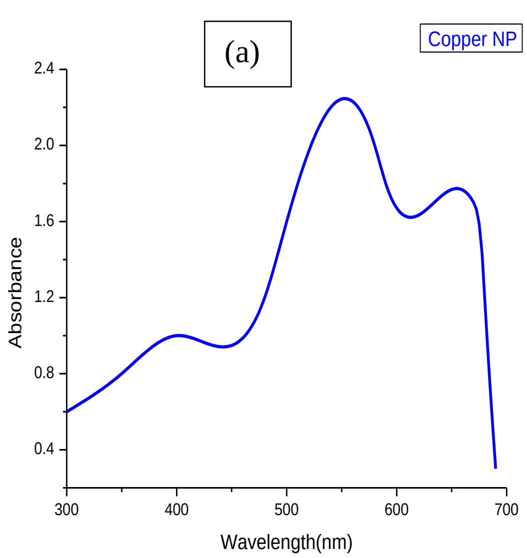
Synthesis of bimetallic nanoparticles

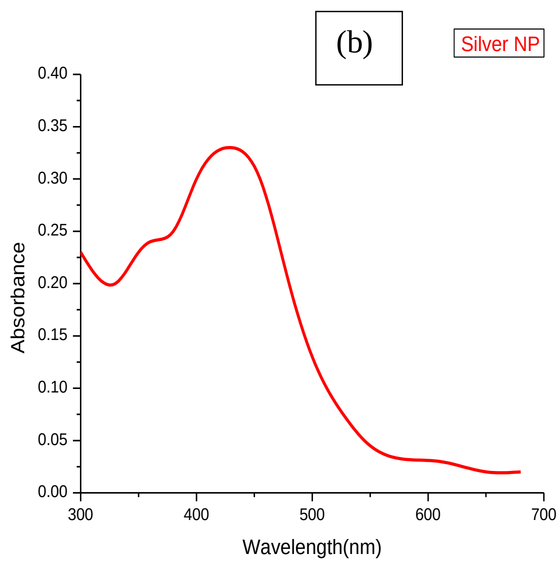
In the case of bimetallic nanoparticles synthesis, 20ml of leaf extract of Ocimum sanctumwas added to equal molar concentration mixture of 100ml $CuSO_4.5H_2O$ and 100ml of $AgNO_3$ solution in Erlenmeyer flask under constant stirring. The reaction mixture was incubated for 24hrs and a dark green color solution mixture was observed, clearly indicating the formation of nanoparticles. Then the solution was centrifuged at 15000rpm for 25min. followed by re-dispersion in deionized water to remove any unwanted biological materials. The suspension was then dried in oven and safely transferred to Eppendorf tubes.

Results and Discussion

Optical absorption studies

UV-Visible absorption spectrum is considered to be the most important study meant for the preliminary characterization of the synthesized nanoparticles (Fig.1). The spectrum of copper nanoparticles shows a band centered at 560nm and an addition absorption shoulder at 670nm which was assigned as the absorption characteristics for oxidation of copper [8]. The spectrum of Ag nanoparticles shows a band centered at 430nm [9]. In case of bimetallic nanoparticles an absorption band is obtained at 510nm indicating the Cu/Ag nanoparticles formation. The presence of a single absorption band in case of bimetallic nanoparticles clearly indicates that Ag-Cu NPs are in the alloy form [10] rather than core shell nanoparticles, which if present would have shown the existence of two separate absorption bands.





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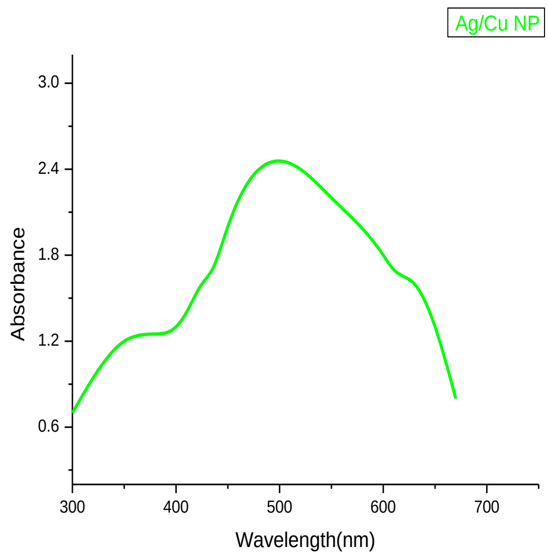


(a) UV-Vis spectra of Cu-NPs

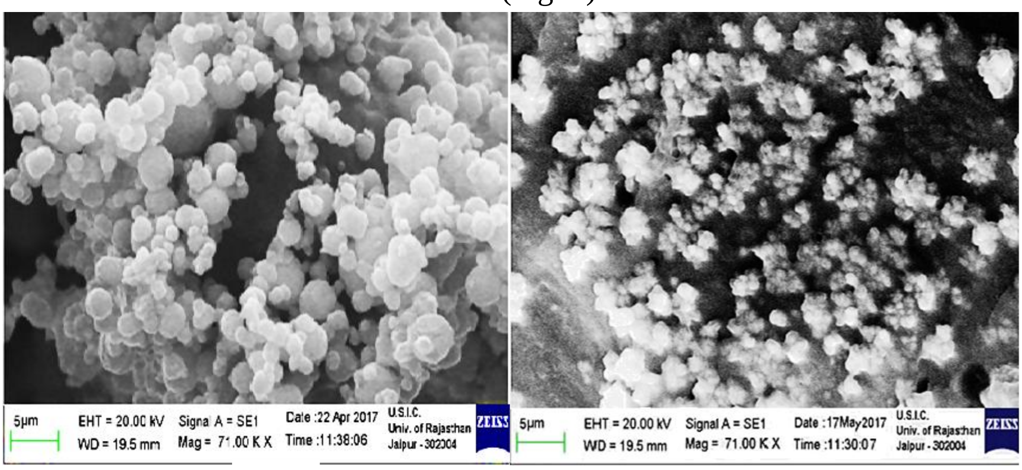
(b) UV-Vis spectra of Ag-NPs

(c) UV-Vis spectra of Bimetallic Ag-Cu NPs

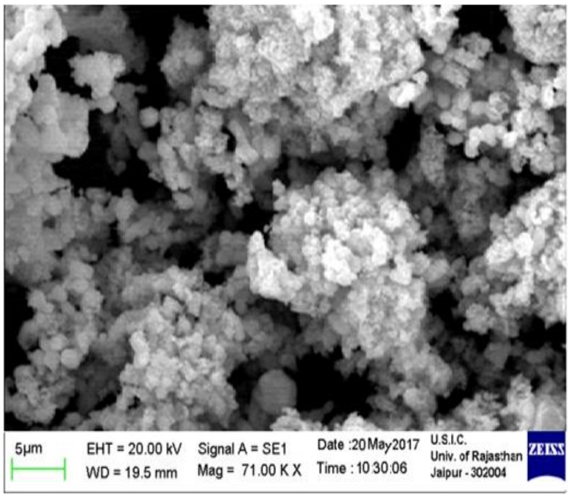
Figure1.UV-Visible specta ofthe monometallic and bimetallic copper nanoparticles synthesized using Ocimum sanctum leaf extract.

SEM analysis

The scanning electron microscope (SEM) image of the test sample surface is obtained by scanning it with a high energy beam of electrons in vacuum chamber. When the beam of electrons strikes the surface of the specimen and interacts with atoms of sample, signals in the form of secondary electrons and back scattered electrons are generated that contain information about sample's surface morphology [11]. The SEM images of the bio-synthesized copper and silver nanoparticles indicates a spherical morphology, while in case of bimetallic silver-copper nanoparticles clusters of spherical beadlike structure with non-uniform distribution is observed (Fig. 2).



(a) (b)



(c)

(a) SEM image of biosynthesized Cu-NPs.
(b) SEM image of biosynthesized Ag-NPs.
(c) SEM image of biosynthesized bimetallic Ag-Cu NPs.
Figure2.SEM images of the bio-syntheszied copper, silver and bimetallic coppernanoparticles.

TEM analysis

Transmission electron microscopic (TEM) analysis was carried out by preparing thin films of the sample on a carbon coated copper grid by just dropping a very small amount of the sample on the grid and removing the extra solution using a blotting paper and then the film on the TEM grid was allowed to dry before measurements [12]. TEM images clearly unveil the spherical shape of the nanoparticles as earlier confirmed by the SEM images. The images exhibits the size of nanoparticles which lies in the range of 12-25nm with bimetallic nanoparticles being the smallest in size as compared to the monometallic nanoparticles. Copper nanoparticles are largest in size followed by the silver nanoparticles. The smaller size of bimetallic nanoparticles is due to the reason that silver does not allow copper to undergo oxidation thereby resulting into decreased size.

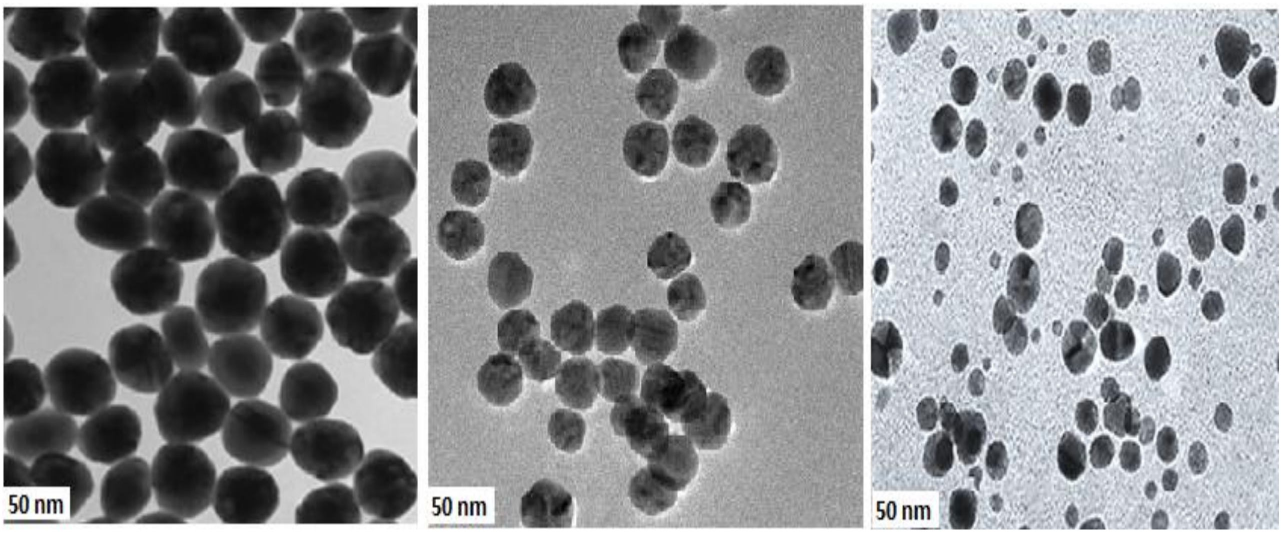
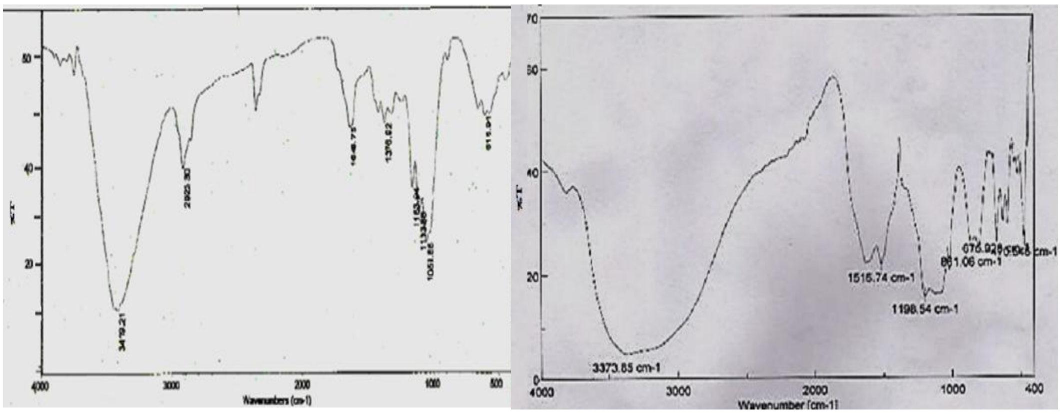


Figure3.TEM images of the bio-synthesized copper, silver and bimetallic coppernanoparticles.

FT-IR spectra

The FTIR analysis was used to identify the capping and stabilizing capacity of the leaf extract. The FTIR spectrum of monometallic nanoparticles exhibits a band in region of 3400-3412cm^{-1} which is assigned to the O-H stretching of H-bonded alcohols and phenols [13]. The band appearing in range of 2930-2935cm^{-1} is attributed to O-H stretching of carboxylic acids [14]. The band at 1620cm^{-1} corresponds to the N-H bending of primary amines while the bands at 1446 and 1524cm^{-1} are related to the C-C stretching of aromaticring structure [15]. A distinctive peak at 1362cm^{-1}corresponds to the C-N stretching of aromatic amine groupwhereas in the region of 1152-1286cm^{-1}are correspondingto the C-C stretching in carboxylic acids,alcohols, ethers and esters [16]. FTIR spectrum suggested that the nanoparticles are surrounded by different functional groups such as alcohols, ketones, aldehydes and carboxylic acids which are binding with the metal with to form a metallic nanoparticle (Fig. 4). The FTIR spectrum of bimetallic nanoparticles was similar to the FTIR spectrum of pure copper and silver nanoparticles.

(a) (b)



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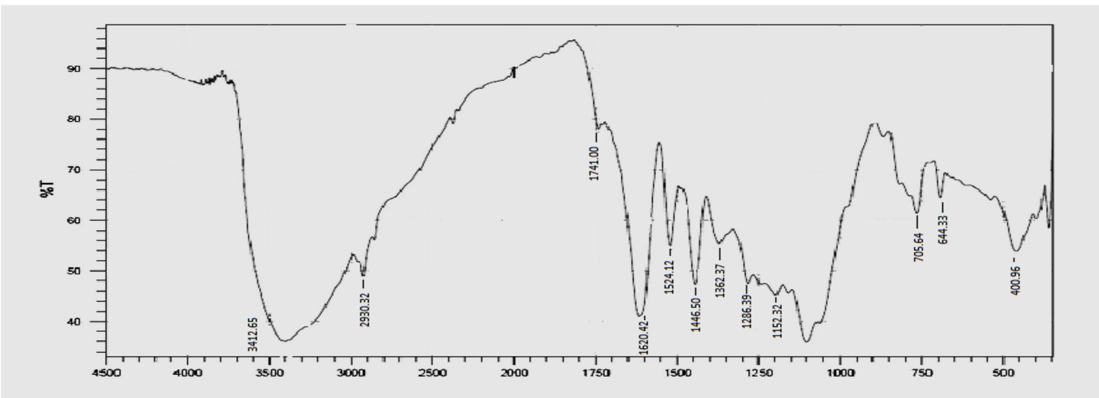

Figure 4.FTIR Spectra of (a)copper, (b) silver and (c) bimetallic copper-silver nanoparticles.

Biological Activities

Bacterial infections have been known to be the foremost reason responsible for the chronic infections and mortality. The most preferred method involved in the treatment of bacterial infections comprises the use of antibiotics owing to their cost-effectiveness and influential effects. However, the extensive use of antibiotics has led to the emanation of multidrug-resistant bacterial strains.The mode of action of NPs implicates a direct contact with the bacterial cell wall, without any need to penetrate the cell; thereby raising the hope of NPs being less prone to bacterial resistance. Therefore, currently attention has been engrossed on synthesis of nanoparticles with proficient antibacterial activity.

Antibacterial activity

The antibacterial activity of the monometallic copper and silver nanoparticles along with the bimetallic nanoparticles of copper with silver was evaluated against the pathogenic bacterial strains of *Escherichia coli* (-) (MTCC 729) and *Bacillus subtilis* (+) (MTCC 121) by the agar well diffusion method [17] (Fig. 5).

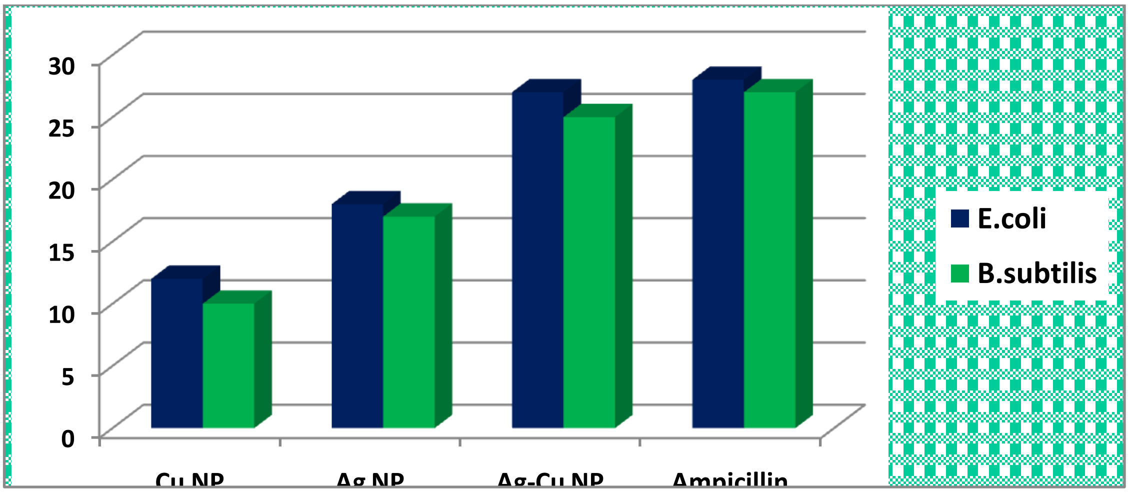

Figure 5.Antibacterial activity of the synthesized copper, silver and bimetallic copper nanoparticles against *Bacillus subtilis* and *Escherichia coli* by employing agar well diffusion method.

The method employed involves the usage of nutrient agar medium containing ingredients such as peptone, beef extract, NaCl, agar-agar and distilled water. The agar medium is then poured into the petriplates and was allowed to solidify. After solidification, the agar plates were swabbed with the bacterial strains using sterile L-shaped glass rod. The wells were then prepared using a cork borer (6mm) into each petri plate. The nanoparticles of about 50µl were added to the wells made in the culture medium plates. Ampicillin was used as a positive control for bacteria. Thereafter, the plates were placed in the refrigerator for about 10min. for diffusion and were later incubated at a temperature of 37°C for 24hrs. The antibacterial activity was evaluated by measuring the zone of inhibition. The antibacterial efficacy of the bimetallic nanoparticles of copper with silver in comparison to the pure copper and silver nanoparticles synthesized using the *Ocimum sanctum* leaf extractssplayed in Fig.5.

The zone of inhibition observed clearly indicates the bactericidal action of the synthesized nanoparticles. The antibacterial efficacy of the nanoparticles depends on the size of the nanoparticles. This is basically due to the fact thatsmaller is the size of nanoparticles more easily they get adsorbed on the surface of bacterial cells, thereby leading to disruption of the bacterial cell membrane. This disruption would lead to the leakage of the intracellular components, thus finally causing the death of the bacterial cells [18]. Amid the synthesized nanoparticles, the antibacterial activity of bimetallic nanoparticles is the greatest as compared to the monometallic copper and silver nanoparticles. This is due to the smaller size of bimetallic nanoparticles along with the presence of two metals which exhibits synergistic effect against the microbes. Hence, it is confirmed that the bimetallic nanoparticles of copper with silver are capable of proffering high antibacterial efficacy.

Anti-inflammatory activity

The aqueous leaf extract of *Ocimum sanctum* along with the synthesized monometallicand bimetallic nanoparticles was evaluated for their anti-inflammatory activity using the carrageenan induced hind paw edema model [18]. The procedure involves the use of 36 male albino rats (90-100 days old), divided into six groups comprising six animals in each group. Inflammation of the hind paw was induced by injecting freshly prepared 1% suspension of carrageenan in 0.9% saline into the sub planar surface of right hind paw of the rats. The negative control group was treated with 1ml of saline while the positive control group was treated with the standard Indomethacine. The remaining four groups were treated with the leaf extract and synthesized nanoparticles at the dose of 30mg kg^{-1} body weight respectively. Drug pretreatment was given 1h before the injection of carrageenan. After 4hrs of oral administration, the edema (%) and edema inhibition (%) were calculated using the following formula:

$$\text{edema (\%)} = (W_R - W_L \times 100)/W_L$$

$$\text{edema inhibition (\%)} = (M_C - M_T \times 100)/M_C$$

Where W_R = weight of right paw and W_L = weight of left paw, M_C = mean edema (%) in control group and M_T = mean edema (%) in drug treated group.

The carrageenan-induced hind paw edema model is recognized as an acute inflammatory model sensitive to the cyclooxygenase (COX) inhibitors and has been used to evaluate the effect of nonsteroidal anti-inflammatory agents (NSAID) [19]. The NSAID are found to inhibit the cyclooxygenase involved in the synthesis of prostaglandin (PG). The anti-inflammatory effect of the reference drug (Indomethacin), aqueous leaf extract, the synthesized monometallic (Cu-NPs, Ag-NPs) and bimetallic (Ag-Cu NPs) nanoparticles in carrageenan induced hind paw edema model in rats is displayed in Fig.6.

After carrageenan administration, paw edema in rats reached at its peak value at 3hr. Maximum per cent inhibition of edema exhibited by leaf extract of Ocimum sanctum, Cu-NPs, Ag-NPs and Ag-Cu NPs at 30mg kg^{-1} body weight concentration are 78.3%, 81.7% , 88% and 92.10%, respectively, and the effect is comparable to that of the standard drug Indomethacin 92.18% .

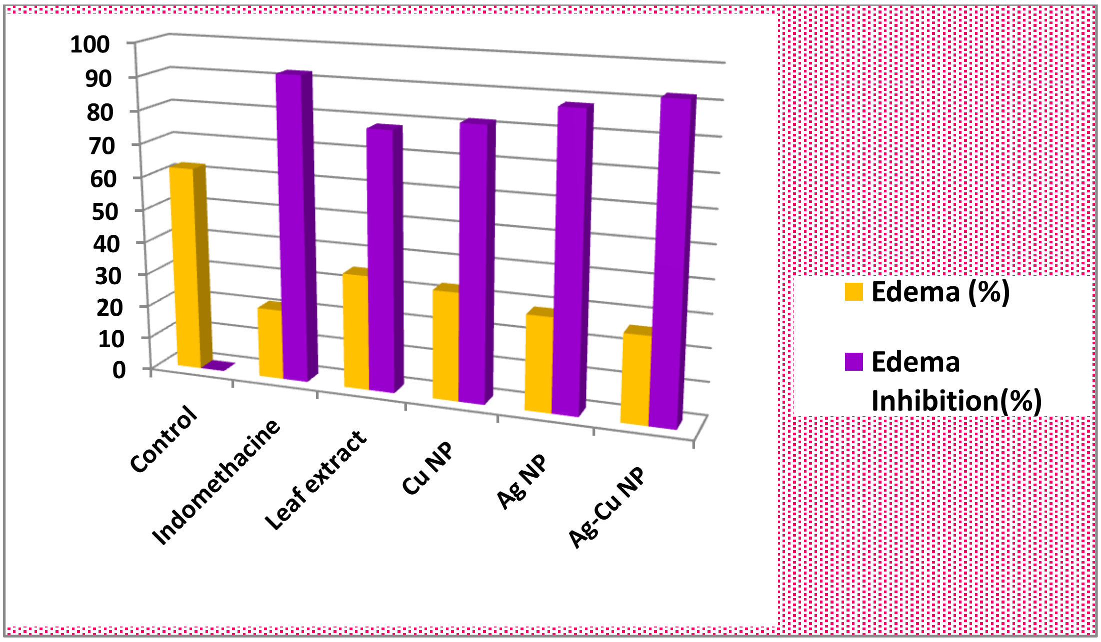

Figure 6. Acute anti-inflammatory activity of the leaf extract (Ocimum sanctum) and of the synthesized nanoparticles in male albino rats.

It is believed that the inhibitory effect of the leaf extract on the carrageenan induced inflammation could be due to the inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. In case of bimetallic nanoparticles, the anti-inflammatory activity is found to be the greatest; the reason being the synergistic effect of the two metals (Cu and Ag) which together consolidate to give an extraordinary and enhanced anti-inflammatory effect.

Conclusions

In the present study, we report the synthesis Cu-Ag bimetallic nanoparticles using the non-toxic and eco-friendly aqueous leaf extract of leaf extract of Ocimum sanctum. Here, Ocimum sanctum extract act as a good stabilizing and reducing agents. All the spectral and morphological characterizations of prepared bimetallic nanoparticles showed an excellent physiochemical property, less size and good crystallinity. Due to these features, the synthesized bimetallic nanoparticles mediated in biological applications. Antibacterial evaluation reveal that the bimetallic nanoparticles of copper with silver are capable of proffering high antibacterial efficacy. Anti-inflammatory activity is found to be the greatest in bimetallic nanoparticles; the reason being the synergistic effect of the two metals (Cu and Ag) which together consolidate to give an extraordinary and enhanced anti-inflammatory effect. As a result, the current research emphasizes biochemistry as a viable nanotechnology technique. The findings could aid in the development of novel, eco-friendly antibacterial and anti-inflammatory agents.

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