Green Synthesis, Characterization and Anti-Helmintic (Worms) Potency of Copper (II) Oxide and Zinc (II) Oxide Nanoparticles Derived From Azadirachta Indica (Neem) Bark

Gongden J. J. [1] • Musa S. [1] • Lohdip Y. N. [1] • Lungaka A. P. [2] • Chollom A. M. [1] • Idongesit N. A. [1]

[1] Department of Chemistry, University of Jos, Plateau State, Nigeria
[2] Department of Chemistry, Plateau State University Bokkos, Nigeria
gongdenjj@gmail.com

Abstract. Zinc (II) and copper (II) oxide nanoparticles (ZnO-Nps and CuO-Nps) were synthesized from Azadirachta indica (Neem) bark. The complexes were characterized, and their anti-helminths (worms) activities were studied. Direct synthesis was used for the synthesis and characterization using FTIR. UV-visible, XRD and SEM. In-vitro and In-vivo were used to test the biological activities (anti-helminthic). The UV-Visible absorption spectrum of zinc oxide nanoparticles shows the characteristic surface plasma resonance with a wavelength of approximately 200 – 440 nm and a peak maximum at 380 nm with attributes to the formation of nanoparticles. FT-IR Spectroscopy was used to identify the possible biomolecules responsible for reducing zinc nitrate ions and copper sulfate and capping the reduction of zinc nanoparticles. Copper nanoparticles synthesis used Azadirachta indica (Neem) bark extract and the result shows zinc oxide nanoparticles gave the IR bands 3220.4 cm-1, 2102.2 cm-1, 1990.4 cm-1, 1595.3 cm-1, 1401.5 cm-1, 1088.4 cm-1, 771.8 cm-1, 849.8 cm-1, 1028.7 cm-1,. The results observed from copper oxide nanoparticles are 3252.61 cm-1, 2918.62cm-1, 2850.90 cm-1, 2168.92 cm-1, 1636.30 cm-1, 1239.42 cm-1, 1083.80 cm-1, and 1035.91 cm-1. The bands which appeared at 3252.61cm-1 and 2918.62cm-1 corresponds to O-H stretching and symmetric C.H. stretching respectively. XRD shows sharp peaks of the nanoparticles, which indicates the formation of nanoparticles and crystallinity. SEM analysis also showed a magnified image of the nanoparticles, further confirming their formation. The nanoparticles were found to be potent in anti-helminthic activities at 200mg/ kg.

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1. Introduction

The field of nanotechnology is one of the most active areas of research in modern materials science. New applications of nanoparticles (N.P.s) and nanomaterials are emerging rapidly. Green synthesis provides advancement over chemical and physical methods as it is cost-effective, environment-friendly, and easily scaled up for large-scale synthesis. Furthermore, there is no need to use high pressure, energy, temperature, and toxic chemicals. Synthesis of N.P.s using biological entities has generated significant interest due to their unusual optical, chemical, photo-electron-chemical, and electronic properties (Mohanpuria et al., 2008).

The particles of size less than 100 nm in diameter were not uncommon on the planet earth as these were found in nature due to photochemical volcanic activity, combustion and food cooking, and vehicle exhausts. Nanoparticle synthesis has led to the introduction of nanotechnology in the last two decades which produced novel compounds that are applied in various fields. The pure metals formed in nanoparticles are applied in the field of diagnostics, antimicrobial agents, anti-worm activities, drug delivery, textiles (clothing), electronics, bio-sensing, food industry, paints, cosmetics, medical devices, and treatment of several acute and chronic diseases, such as malaria, hepatitis, and cancer.

Different metals are used in nanoparticle synthesis, but zinc oxide and copper oxide are preferred in this study because of their non-hazardous nature. Zinc nitrate is an inorganic compound with the formula ZnO. ZnO is a white powder that is insoluble in water and is widely used as an additive in numerous materials and products, including rubbers, plastics, ceramics, glass, cement, lubricants, paints, ointments, adhesives, sealants, pigments, foods, batteries, ferrites, fire retardants, and first-aid tapes (Hernandezbattez et al., 2008). Although it occurs naturally as the mineral zincite, most zinc oxide is produced synthetically (Marcel et al., 2006). ZnO is a wide-band gap semiconductor of the II-VI semiconductor groups. The

native doping of the semiconductor due to oxygen vacancies or zinc interstitials is n-type (Özgür et al., 2005).

Nanomaterials have received much attention because their structure and properties vary from those of atoms and molecules concerning their bulk materials and thus have many potential applications. The size and shape of the nanoparticle depend on various parameters like pH, temperature, time of reaction, nature of plant extract, the relative concentrations of the extract and metals salts reacting as well as the rate of mixing of plant extract and metal salts. This research aims to synthesize and characterize zinc oxide and copper oxide nanoparticles using Azadirachta indica (Neem) bark extract and determine its possible anti-worm activities.

2. Materials and Methods

The following materials and equipment were used for the research:

Neem tree Bark; zinc nitrate; Copper sulfate; heating magnetic stirrer; pH meter; Genesys 10s UV-visible spectrophotometer; Perkin Elmer Frontier Fourier Transform Infrared Spectrophotometer; XRD; S.E.M.

2.1. Sample Collection and Preparation

For this study, Neem tree bark was collected from Kyarang in Tafawa Balewa Local Government Area of Bauchi State, Nigeria, in February 2019 during the dry season. The healthy bark of the neem tree (Azadirachta indica) was collected and washed thoroughly using running tap water before distilled water. After washing, the neem bark was air-dried on foil paper for seven days. Air drying was preferred over oven drying to prevent the loss of essential components. The dry bark of the neem was shredded and ground using mortar and pestle to a fine powder (nanosize). Exactly 50 grams of the powder was sucked into 800 ml of distilled water for 7 days. The supernatant liquid was decanted and filtered using Whatman filter paper no.1. It was collected in a 250 ml volumetric flask and then concentrated using a rotary machine for 5 hrs at 100 °C. This was further put in a water bath for 2hrs to dry up. The concentrates were stored for the synthesis of the nanoparticles (Adopted from Vimala et al., 2014).

2.2. Green Synthesis of Zinc Oxide Nanoparticles (ZnO-NPS)

Exactly 2 g of zinc nitrate was weighed and dissolved in 10 ml aqueous bark extract solution of Azadirachta indica (Neem). This was stirred continuously until the complete dissolution of the mixture. The solution was further kept under vigorous stirring at 80^0 C for 2 hours. It was then removed and allowed to cool at room temperature, and the supernatant was discarded. Next, the nanoparticles were centrifuged at 400 rpm for 15 min and washed thoroughly, and allowed to dry at 80^0 C for 4 hours.

2.3. Green Synthesis of Zinc Oxide Nanoparticles (ZnO-NPS)

Exactly 0.2g of copper oxide anhydrous was weighed and dissolved in 100 ml of distilled water. About 60 ml of copper oxide anhydrous was added to 5 ml of bark extract and stirred using an electric stirrer for 10 min at room temperature, change in color of the solution indicates the formation of copper oxide nanoparticles. Finally, the product was centrifuged at 300 rpm for 10 minutes and washed with distilled water; the nanoparticles were allowed to dry at room temperature (Caroling *et al*., 2015).

2.4. Characterization of Zinc (II) oxide and Copper (II) oxide Nanoparticles

The prepared nanoparticles were characterized by UV-Visible Spectroscopy, FTIR Spectroscopy, S.E.M. (Scanning Electron Microscopy), and XRD (X-Ray Diffraction).

2.5. Toxicology

A test for LD50 was carried out using Lorke's Method.

2.5. Anti-Worm Activity Experiment

Twenty (20) rats (three months old) were used to test for anti-worm activities. For in-vitro studies, Hymenolepis microstoma proved to be a helpful worm because of its prolonged survival in Phosphate Buffered Saline (PBS). The in-vitro trials for the anti-helminthic activity of the nanoparticles and albendazole were carried out using the motility assay of the adult and third larval stages.

2.7. Adult Motility Assay

Adult motility assay was conducted on mature *H. miscrotoma* worms following the method described by Sharma et al. (1971). The mature worms were collected from the lutestine of infected Rat freshly slaughtered in the approved house. The worms were picked manually and placed in Petri dishes. Ten activity-moving worms were exposed in triplicates to each of the following treatments in separate Petri dishes at room temperature (25ºC – 30ºC).

- Zinc oxide nanoparticles at 200 mg/ml
- Copper oxide nanoparticles at 200 mg/ml
- Albendazole 200 mg/ml (positive control)
- Phosphate buffer saline (negative control)

The dead worms were easily recognized by their straight flat appearance with no movement at the hail and tail regions of the body. The motility was recorded after 2, 4, 6, 8, and 12 hours intervals.

2.8. Larval Motility Assay

Adult female parasites of *H. Microstoma* was used. The eggs were cultured in sterilized rat faeces for 8 days at room temperature. The intestine larvae were harvested using standard Bearman techniques. The larval suspension was diluted in water. Approximately 10 larvae in 0.5 ml of

water were placed in a test tube and exposed in triplicates to each of the following treatments at 4ºC (Al-Qaraw et al., 2001). Observations on the mortality of the larva were made at 2, 4, 6, 8, and 12 hours intervals under the magnifying microscope.

2.9. In-vivo anthelmintic activity

A total of 20 Rats collected from the University of Jo's animal house were used for these experiments. The Rats were treated with albendazole 200mg/kg for 2 weeks to make sure they were all free from the parasite.

2.10. Infection of the Rat with Cyst of H. microstoma

The rats were injected with 60ml eggs collected during the postmortem examinations. This follows the guidelines of the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) for evaluating anthelmintic efficacy in rodents. Ten days after infection, fecal samples were collected from Rats twice a week for 2 weeks from the recta of individual Rats. The number of eggs per fecal sample was determined using the Bearman funnel method. A gram of feces was suspended in 10 ml of water to suspend the eggs, 50 ml of PBS was added, and the Baaman method was used to extract the eggs. A Rat with approximately 170 eggs/ml of sample was used for the studies.

2.11. Faecal Egg Count Reduction Test (F.E.C.R.T.)

The infected Rat was divided into four groups and exposed to the following treatments for two weeks.

- Zinc oxide nanoparticles of 200 mg/kg.
- Copper oxide nanoparticles of 200mg/kg.
- Albendazole 200 mg/kg (positive control).
- Negative control.

2.12. Determination of the Adult Worms Burden of H. microstoma

The treated rats were sacrificed, and the number of worms in parasites was taken. The worms were washed with water and sieved through a coffee-tea sieve. Recovered worms were then individually picked up, counted, and identified.

2.13. Determination of the Adult Worms Burden of H. microstoma

The treated rats were sacrificed, and the number of worms in parasites was taken. The worms were washed with water and sieved through a coffee-tea sieve. Recovered worms were then individually picked up, counted, and identified.

3. Statistical Analysis

Data collected from various experiments were subjected to analysis of variance (ANOVA) using the graph pad Pearson version 8.2. Significant levels were taken at $p<0.05$.

4. Results and Discussion

Table 1: Physical properties of Copper oxide and Zinc oxide nanoparticles

Compound	Color	Initial	Boiling point	Melting point
Neem extrac with Coppe oxide	Black Brown	36ºC	102ºC	144ºC
Neem extrac Zinc Oxide	White cloudy	32ºC	13ºC	152ºC

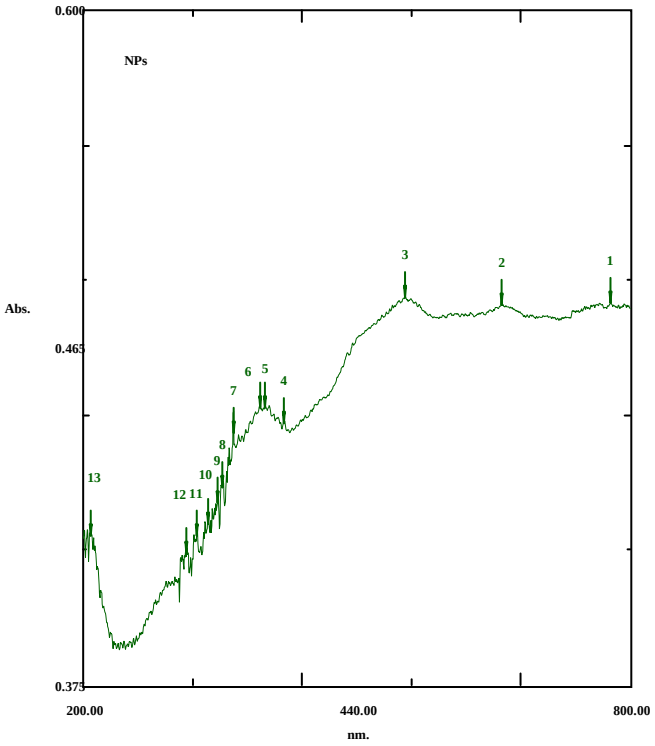

Figure 1. The UV-visible absorption spectrum of zinc oxide nanoparticles synthesized from Azadirachta indica (Neem) Bark extract.

4.1. UV-visible Absorption Spectrum of Zinc Oxide Nanoparticles

Figure 1 shows more information on zinc oxide nanoparticles. The reduction of zinc ions by aqueous extracts and the formation of ZnO-NPs were confirmed using U.V.–Visible spectroscopy. The UV-Visible absorption spectrum of zinc oxide nanoparticles shows the characteristic surface plasma resonance with a wavelength of approximately 200 – 440nm and a peak maximum at 380nm with attributes to the formation of nanoparticles.

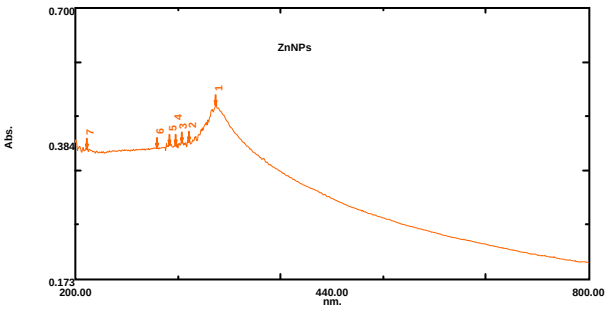

Figure 2. The UV-visible absorption spectrum of copper oxide nanoparticles synthesized from Azadirachta indica (Neem) bark extract

4.2. UV-visible Absorption Spectrum of Copper Oxide Nanoparticles

Figure 2 shows the UV-visible spectrum information on copper oxide nanoparticles by reduction with metal ion when exposed to Azadirachta indica (Neem) bark extract. The UV-visible absorption spectrum of copper oxide nanoparticles was scanned from 200 nm to 800 nm; it shows the characteristics of surface Plasmon resonance (S.P.R.) with absorbance at approximately 350 nm, which can be described as the formation of copper oxide nanoparticles.

4.3. FT-IR Absorption Spectra of Zinc Oxide Nanoparticles.

Figure 3 indicates the result observed from zinc oxide nanoparticles. The characteristic IR band 3220.4cm-1 corresponds to O-H bending. The band at 2102.2cm-1 may be due to -C=C– Stretching. The band at 1990.4 cm-1 could be attributed to C=C=C= Stretching. The IR. band observed at 1595.3cm-1 may be ascribed to C–C Stretching. The band at which 1401.5cm-1 is due to C-H bending, The band 1088.4cm-1 are due to C–N Stretching, The band 771.8cm-1 is due to C–Cl stretching, The band 849.8 cm-1 is due to C–Cl Stretching while The 1028.7 cm-1 band corresponds to C– N Stretching.

4.4. FT-IR Absorption Spectra of Copper Oxide Nanoparticles

Figure 4 describes the observed IR characteristics of copper oxide nanoparticles obtained. These included 3252.61cm^{-1}, 2918.62cm^{-1}, 2850.90cm^{-1}, 2168.92cm^{-1}, 1636.30cm^{-1}, 1239.42cm^{-1}, 1083.80cm^{-1}, and 1035.91cm^{-1}. The bands are similar to that of zinc oxide nanoparticles except for the bands at 1636.30cm^{-1} and 1239.42cm^{-1} which may be due to C=O and C-O stretching. Also, the bands observed at 2850.90cm^{-1} and 2168.92cm^{-1} could be attributed to CH_3 stretching and C≡C symmetric stretch. While the characteristic bands observed at 1083.80cm^{-1} and 1035.91 may be ascribed to symmetric C-O-C stretching and =C-O-C symmetric stretching.

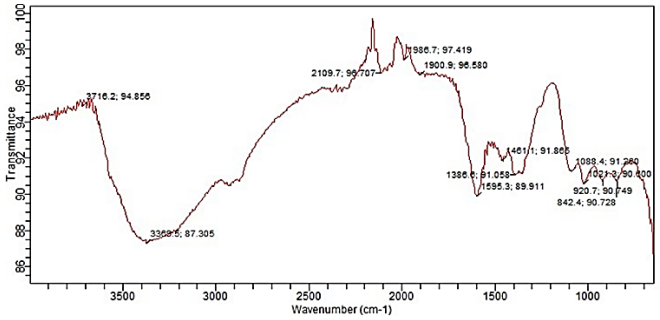

Figure 3. FT-IR absorption spectra of zinc oxide nanoparticles.

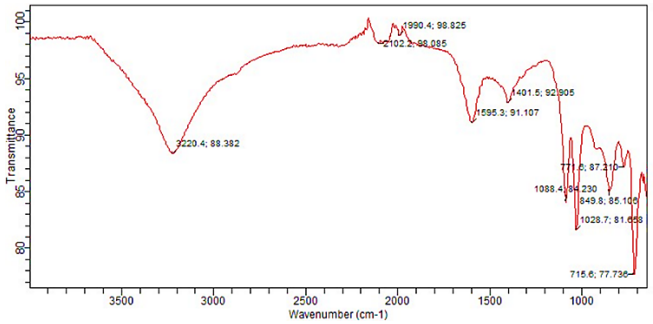

Figure 4. FT-IR absorption spectra of copper oxide nanoparticles.

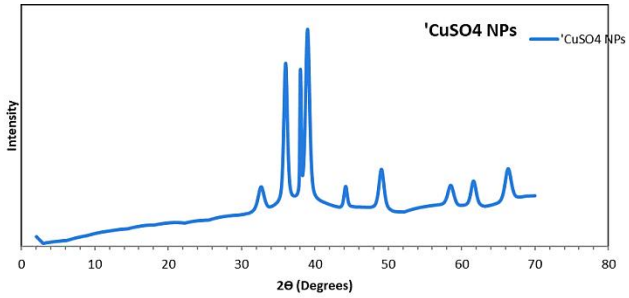

Figure 5. XRD analysis of Copper oxide nanoparticle

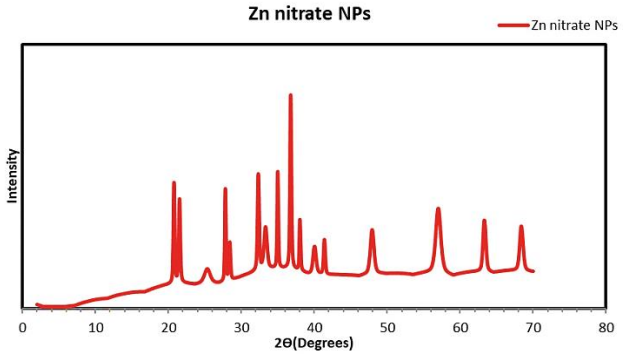

Figure 6. XRD Analysis of zinc oxide nanoparticle

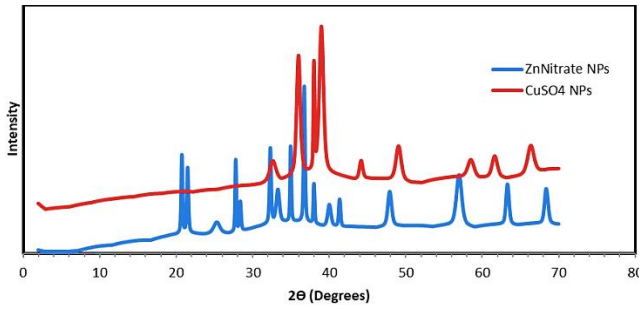

Figure 7. XRD Analysis of Both Zinc oxide and Copper oxide nanoparticles

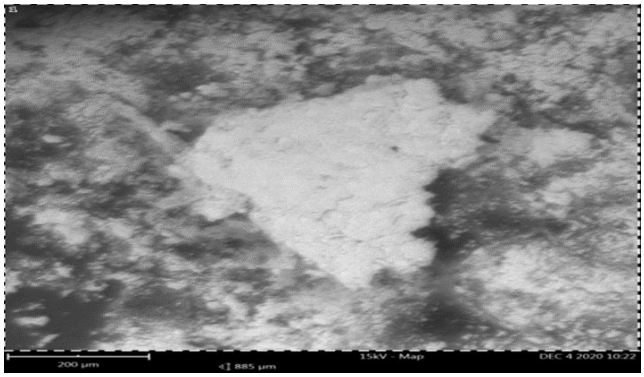

Plate 1. S.E.M. analysis of Copper Oxide Nanoparticles

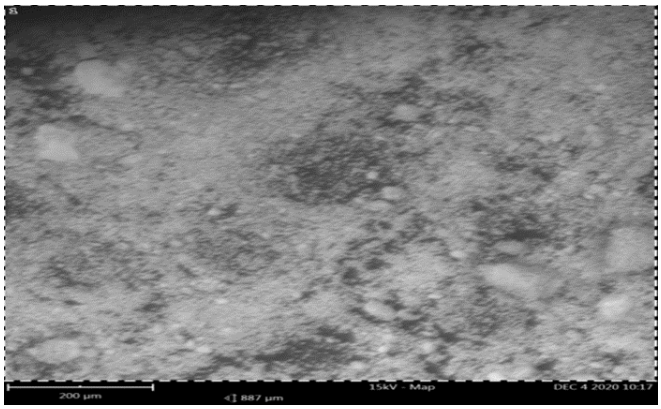

Plate 2. S.E.M. analysis of Zinc Oxide Nanoparticles

Table 2: Phytochemical Screening

Constituents	Extract	Copper oxide nanoparticles	Zinc oxide nanoparticles
Alkaloids	+	++	-
Saponin	+	-	-
Tannins	+++	-	-
Flavonoids	++	-	-
Carbohydrate	+	-	+
Steroids	++	-	-
Troponins	-	-	-
Anthroponins	-	-	-
Cardioglycoside	-	-	-

4.5. XRD Analysis

XRD is used to determine the crystalline nature of the material or sample. Figures 5, 6, and 7 above show the crystalline nature of the nanoparticles formed due to their sharpness, indicating the formation of nanoparticles.

4.6. S.E.M. (Scanning Electron Microscope)

S.E.M. is a test process that scans a sample with an electron beam to produce a magnified image for analysis. The method, also known as S.E.M. analysis, is used very effectively in the microanalysis in solid inorganic materials. For example, plate 1 and 2 above shows a magnified image of copper oxide and zinc oxide nanoparticles, indicating nanoparticle formation.

5. Conclusion

Green synthesis of nanoparticles (zinc oxide N.P.s and copper oxide N.P.s) used in this research work is eco-friendly and non-toxic. It involves less usage of chemical reagents compared to other methods of nanoparticle synthesis. Furthermore, phytochemicals in the bark extract help synthesized metal oxide nanoparticles by inducing oxidation and reduction reactions. Synthesis conditions were optimized, and resultant nanoparticles were characterized using U.V. - Visible and Fourier-transformed infrared (FT-IR) spectroscopy. XRD and S.E.M. Both nanoparticles synthesized were crystalline with zinc oxide nanoparticles showing better antihelminthic activity in Rat than the copper nanoparticle.

Corresponding Author:
Gongden J. J., PhD
Department of Chemistry, University of Jos, Plateau State, Nigeria
E-mail: gongdenjj@gmail.com

References:

1. Hernandezbattez, A; Gonzalez, R.; Viesca, J.; Fernandez, J.; Diazfernandez, J.; MacHado, A.; Chou, R.; Riba, J. (2008). "CuO, ZrO2 and ZnO nanoparticles as an antiwear additive in oil lubricants". Wear. 265 (3–4): 422–428.
2. Marcel, D. L. (2006). Zinc Oxide (Zinc White): Pigments, Inorganic, 1 ″in Ullmann's Encyclopedia of Industrial Chemistry.
3. Mohanpuria, P., Rana, N.K., Yadav, S.K., (2008). Biosynthesis of nanoparticles: technological concepts and future applications. J. Nanopart. Res. 10, 507–517.
4. Özgür, Ü. Alivov, Ya. I.; Liu, C.; Teke, A.; Reshchikov, M. A.; Doğan, S.; Avrutin, V.; Cho, S.-J.; Morkoç, H. (2005). "A comprehensive review of ZnO materials and devices". Journal of Applied Physics. 98 (4): 041301.
5. Vimala, K., Sundarraj, S., Paulpandi, M., Vengatesan, S., & Kannan, S. (2014). Green synthesized doxorubicin loaded zinc oxide nanoparticles regulates

the Bax and Bcl-2 expression in breast and colon carcinoma. Process Biochemistry, 49(1), 160-172.

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