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COMPARATIVE *IN-VITRO* ANTICANCER EFFICACY EVALUATION: *CASSIA ABBREVIATE* MEDIATED COPPER-DOPED MAGNESIUM OXIDE NANOPARTICLES VS CRUDE AQUEOUS BARK EXTRACT.

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ABSTRACT: Background: In Zimbabwe, green synthesis of nanoparticles has recently drawn much attention due to its eco-friendliness, biocompatibility, and capability to augment the healing properties of medicinal plants. *Cassia abbreviate* (Muremberembe) is a cardinal Zimbabwean traditional medicinal plant used to manage various conditions. However, there is limited scientific evidence on the antiproliferative activity of both *C. abbreviate* extracts and *C. abbreviate*-mediated nanoparticles against cancer cell lines. Therefore, this study compared the *in-vitro* antiproliferative efficacy of an aqueous bark extract of *C. abbreviate* and biosynthesized copper-doped magnesium oxide nanoparticles mediated by *C. abbreviate* against the human breast cancer cell line HCC1937. **Methods:** Cytotoxicity assays were performed using the well-known and standardized Sulforhodamine B (SRB) colorimetric assay method over a concentration range (1). IC₅₀ values were calculated using dose-response curve trend-line equations. **Results:** Both treatments significantly inhibited proliferation of HCC1937 breast cancer cells in a concentration-dependent manner ($P < 0.0001$). Cu₂O–MgO NPs showed higher antiproliferative activity than the aqueous bark extract with a lower IC₅₀ value (35.08 µg/mL vs. 61.43 µg/mL). **Conclusion:** *Cassia abbreviate*-mediated Cu₂O–MgO nanoparticles demonstrated significantly greater antiproliferative activity than the crude aqueous bark extract against HCC1937 breast cancer cells, highlighting their potential as promising candidates for further anticancer development.

INTRODUCTION: *Cassia abbreviate* Oliv. (Muremberembe) is one of the medicinal plants widely used in Zimbabwean traditional medicine.

It contains various bioactive phytochemicals, namely flavonoids, tannins, phenolics, alkaloids, and anthraquinones, all of which have been reported to possess anticancer activities².

On the other hand, the application of plant-synthesized metal oxide nanoparticles has recently gained significant attention as an eco-friendly method to enhance the therapeutic potential of medicinal plants. The Copper-doped Magnesium oxide nanoparticles (Cu₂O–MgO NPs) exhibit

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specific physicochemical properties, including a larger surface area³, improved uptake by cells, as well as the ability to generate reactive oxygen species, which may be beneficial for their anticancer effects.

Cassia abbreviata: *Cassia abbreviata* Oliv. is a member of family Caesalpiniaceae, and one of the 600 species in the genus *Cassia*². This genus has been of scientific interest because of its wide range of biological, pharmacological and medicinal properties reported from various parts of the world. *C. abbreviata* is known in Zimbabwe as Muremberembe and has been used for the treatment of many ailments for many years⁴. Ethnobotanical information suggests that the roots are usually crushed and boiled in water to make medicines to treat diarrhoea, constipation, sexually transmitted diseases and as an aphrodisiac⁵. The bark is also extensively used, especially in the treatment of abdominal pain by soaking it in water and giving it orally for a number of days. However, the use of leaf infusions for fever and other symptoms in malaria control has been reported in several communities in Africa, and the species continued to play an important role in traditional healthcare systems. *Cassia abbreviata* is a shrub or medium sized tree, up to 10 metres in height, botanically. Features yellowish green compound leaves with 5-12 pairs of leaflets, and a light brown bark with a rounded crown. It has large, sweetly scented yellow flowers which turn brown with age. The fruit is in the form of a dark-brown, cylindrical and hanging pod, when mature⁶.



FIG. 1: FLOWERING CASSIA ABBREVIATA PLANT

Green Synthesis: Among green synthesis strategies, the most extensively studied and versatile is plant-mediated synthesis. Secondary

metabolites in plants include a wide range of compounds such as alkaloids, flavonoids, terpenoids, phenols, tannins, and saponins⁷. These compounds can reduce metal ions to nanoparticles and also provide stability to prevent aggregation of the particles formed. The process is usually as simple as adding a plant extract to an aqueous metal salt solution, at room temperature, and observing nanoparticle formation minutes to hours later, usually signaled by a color change⁸. Subsequent studies have proved that almost all parts of the plant can be used as a bio-reducing agent leaves, roots, stems, fruits, seeds, flowers, or bark.

The mechanisms of plant-mediated synthesis are rather intricate and species-dependent. Generally, in most cases, more than one phytochemical takes part in the process of reduction and capping; different compounds are responsible for size, shape, and surface properties. The flavonoids and terpenoids are mainly responsible for the primary reduction; proteins and polysaccharides play the role of capping agents in most cases, which does not allow nanoparticle aggregation and gives stability for a long time⁹. The plant-mediated synthesis can be said to be a highly flexible process because nanoparticle properties can be adjusted with ease by simply changing the plant species.

In-vitro Cytotoxicity Bioassays using Human Cell Lines: Researchers frequently use in vitro bioassays with human cell lines in pharmaceuticals, biomedicine, toxicology, and nanotechnology to determine the safety and biological activity of drugs, natural products, and nanomaterials and study their possible effectiveness in controlled experimental conditions. Human cell lines refer to cell aggregate systems developed from human tissue that are capable of being cultured in the laboratory¹⁰. Cancer-derived human cell lines are used for the determination of cytotoxicity, proliferation or apoptosis of cells, uptake of compounds by cells, oxidative stress, and other important biological responses since they provide a reliable and cost-effective alternative to such studies on normal cells and tissues as well as animals¹¹. This testing can replace animal experiments. The most common human cell lines are cancer cell lines, such as MCF-7, HCC1937 HeLa, HepG2, A549, and Caco-2, and normal lines

like HEK293 and HDF¹². *In-vitro* bioassays involve treating a cultured monolayer of cells with various concentrations of the test substance and subsequently measuring the response of the cells¹³. Parameters that can be determined, depending on the objective of the study, are as follows: cell survival, growth, membrane integrity, metabolic activity, oxidative stress, gene expression, and modes of cell death¹⁴.

Most popular tests for cell toxicity include Sulforhodamine B (SRB), MTT, XTT, Alamar Blue, and Neutral Red Uptake. Most popular tests for cell toxicity include Sulforhodamine B (SRB), MTT, XTT, Alamar Blue, and Neutral Red Uptake¹⁵. These are commonly used in cancer research to determine the IC₅₀, the concentration required to inhibit cell growth by 50%. Other assays include measurement of lactate dehydrogenase (LDH) release for cell membrane damage, Annexin V/Propidium Iodide staining for cell death, assays for reactive oxygen species (ROS) for oxidative stress, and flow cytometry for the cell cycle¹⁶.

Suphordamine Assay: The Suphorhodamine B (SRB) assay is a quantitative colorimetric assay used to assess the *in-vitro* anticancer activity of the target analyte by quantifying cytotoxicity in cultured cancer cell lines¹⁷. The Suphorhodamine B (SRB) assay relies on the capability of the SRB dye to attach to electrostatically and in a pH-dependent manner to positively charged amino

acids in protein chains. Under mildly acidic conditions, SRB binds to positively charged amino acids in protein chains in cells fixed with trichloroacetic acid (TCA)¹⁸. The dye can then be quantitatively extracted from the cells and solubilized for optical density (OD) measurement with weak bases such as Tris. As the binding of SRB occurs in stoichiometric ratios, the quantity of dye extracted from stained cells is directly proportional to the cell mass¹⁹.

METHODS AND MATERIALS:

Plant Collection: *Cassia abbreviata* commonly known as sjambok pod were collected from Murehwa Mashonaland East, Zimbabwe (Coordinates: 17.464°S, 31.802°E) during the month of October-November 2025 after thorough examination of the surroundings. Samples of the plants were authenticated and identified at the National Herbarium and Botanical Gardens, by the Research Officer and was identified as *Cassia abbreviata* which belongs to the Fabaceae-Caesalpinioideae family.

Plant Preparation and Extraction: The tree bark of *Cassia abbreviata* was carefully washed to remove soil and dirt, and then dried in the shade at room temperature to a constant weight. No direct sunlight was exposed to avoid degradation of heat and light-sensitive phytochemicals. The dried bark was then ground to fine powder by a laboratory Termarmill.



FIG. 2: FINE CASSIA ABBREVIATA TREE BARK POWDER FROM THE TERMARMILL

200 g of the powdered material was placed in 1 L of purified reverse osmosis (RO) water and heated in a water bath for 8 hours. The mixture was cooled and equilibrated to the room temperature after extraction. Particulate matter was removed from the extract by filtering through sterile 0.45 µm

membrane filters into a 500 mL volumetric flask. The filtrate was centrifuged for 5 minutes to remove any remaining debris. The supernatant was then freeze-dried to yield a dry extract powder. The lyophilized extract was kept in airtight amber glass containers at -15°C for future studies⁵.

Initial Cell Recovery and Expansion: Initial Cell Recovery and Expansion was executed as recommended by ²⁰. Take the cryovial containing frozen cells out of the liquid nitrogen or the -80°C freezer while making sure to wear appropriate PPE and using tweezers. Immediately thaw the vials in a preheated water bath at 37°C for around 2 minutes or until there is a small ice crystal remaining. Place the contents of the vial inside a sterile 20 mL conical tube with 11 mL of pre-warmed complete media. Centrifuge for 3 minutes at room temperature at a speed of 1500 rpm. Gently aspirate the supernatant to remove the DMSO. Redisperse the pellet in 10 mL of appropriate complete media and transfer to a tissue culture-treated 10 cm dish. Incubate in a 37°C , 7% CO_2 incubator. Monitor cell attachment and morphology every day. When the culture reaches 95% confluence, proceed to the assay protocol.

Procedure for Sulforhodamine B (SRB)

Cytotoxicity Assay: The human breast cancer cell line HCC1937 (Catalogue No. 305064 from Cytion) was used to determine the cytotoxic activity of the extracts of the bark of *Cassia abbreviata* and the synthesized Cu_2O – MgO nanoparticles using the Sulforhodamine B (SRB) assay ²¹. The HCC1937 cells were grown in triplicate in sterile 96-well microplates at a seeding density of 2×10^3 cells/mL in RPMI-1640 culture medium (supplemented with 1 mM L-glutamine and sodium bicarbonate) and 10% heat-inactivated fetal bovine serum (FBS). Cell attachment was allowed by incubating the cells overnight at 37°C in a 7% CO_2 humidified atmosphere.

After the incubation, the cells were treated with different concentrations (0.1–100 $\mu\text{g/mL}$) of *Cassia abbreviata* extracts and Cu_2O – MgO nanoparticles in 1% (v/v) dimethyl sulfoxide (DMSO). Wells containing cells treated with 1% DMSO only served as the negative control. The treated cells were then cultured for 96 hours in the same conditions. Cells were fixed at the end of the treatment with 50% (w/v) trichloroacetic acid (TCA) and incubated at 4°C for 1 hour. The plates were then gently dipped in purified RO water and washed four times to remove excess TCA and medium residues and then dried on sterile filter paper at room temperature. After that, 100 μL of 0.4% (w/v) of Sulforhodamine B (SRB) solution

was added to each well and the plates were stained with the solution for 1 hour at room temperature. A 1% (v/v) acetic acid washing of the plates four times was followed by air drying to remove excess dye. For solubilisation of protein-bound dye, 100 μL of 10 mM Tris base solution (pH 10.7) was added to each well and the plates shaken on an orbital shaker for 15 minutes at room temperature. The absorbance measurements were performed at 510 nm with Agilent Synergy HTX Microplate Reader. Cell viability was obtained as a percentage of the untreated control cells and half-maximal inhibitory concentration (IC_{50}) values were calculated by non-linear regression analysis using scatter regression analysis with exponential trendline.

The % inhibition of cell growth was calculated using the formula:

$$\% \text{ Cell Growth} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Negative Control}} \times 100\%$$

$$\% \text{ Inhibition} = 100 - \% \text{ Cell Growth}$$

Objectives of the Study: To assess the comparative *in-vitro* antiproliferative efficacy evaluation of aqueous *Cassia abbreviata* pulp bark extract versus biosynthesized Copper-doped Magnesium Oxide nanoparticles (Cu_2O – MgO NPs) in human cancer cell lines, in terms of cell viability inhibition (IC_{50}).

Statistical Analysis: Data are presented as mean, standard deviation from 3 independent experiments. Variance analysis was determined by one-way ANOVA at a P value of <0.05 ; analysis of the dose-dependent activity of the drug extracts was performed by regression scatter with an exponential trend line at 95% confidence interval.

RESULTS AND DISCUSSION: Table 1 and 2 show the mean absorbance values for HCC1937 breast cancer cells treated with *Cassia abbreviata* bark extracts and Cu_2O – MgO nanoparticles, respectively. The average absorbance values for untreated control, extract from *Cassia abbreviata* bark and Cu_2O – MgO nanoparticles are presented in Table 3. Absorbance (measured by Sulforhodamine B (SRB) assay) is directly proportional to the number of viable cells, therefore lower values of absorbance are indicative of increased cytotoxic activity.

Absorbances of *Cassia abbreviata* Treated wells:

TABLE 1: ABSORBANCES AT 510NM OF CASIA ABBREVIATA EXTRACT-TREATED WELLS CONTAINING HCC1937 CELLS

Concentrations (µg/ml)	0.1	10	20	30	40	60	80
	0.2905	0.2456	0.2403	0.2199	0.2149	0.1789	0.0701
	0.2911	0.25	0.2315	0.2208	0.2167	0.1792	0.0698
	0.2954	0.2483	0.2363	0.2216	0.218	0.1758	0.0697
Average Absorbance	0.2923	0.2480	0.2360	0.2208	0.2165	0.1780	0.0699

p-Value < 0.0001

Absorbances of Cu₂O-MgO NPs Treated wells:

TABLE 2: ABSORBANCES AT 510NM OF CU₂O-MGO NPS EXTRACT-TREATED WELLS CONTAINING HCC1937 CELLS

Concentrations (µg/ml)	0.1	10	20	30	40	60	80
	0.2102	0.1998	0.1907	0.1573	0.1319	0.1201	0.078
	0.2194	0.2004	0.19	0.1499	0.1322	0.1194	0.0773
	0.2156	0.1985	0.1905	0.1502	0.133	0.1198	0.0772
Average Absorbance	0.2151	0.1996	0.1904	0.1525	0.1324	0.1198	0.0775

p-Values < 0.0001

Average Absorbances for the Control, Cu₂O-MgO NPs and *Cassia abbreviata* Extracts:

TABLE 3: AVERAGE ABSORBANCES AT 510NM OF CU₂O-MGO NPS, CASSIA ABBREVIATA AND CONTROL EXTRACT-TREATED WELLS CONTAINING HCC1937 CELLS

Concentration(ug/ml)	0.1	10	20	30	40	60	80
Cu ₂ O-MgO NPs	0.2151	0.1996	0.1904	0.1525	0.1324	0.1198	0.0775
<i>Cassia abbreviata</i>	0.2923	0.2480	0.2360	0.2208	0.2165	0.1780	0.0699
Control	0.3137	0.3137	0.3137	0.3137	0.3137	0.3137	0.3137

% Inhibition Results:

TABLE 4:% INHIBITION RESULTS

Concentration(ug/ml)	0.1	10	20	30	40	60	80
Cu ₂ O-MgO NPs	68.56	63.62	60.69	48.60	42.20	38.18	24.71
<i>Cassia abbreviata</i>	87.00	79.05	75.24	70.38	69.03	56.73	22.27

% Cell Growth:

TABLE 5: %CELL GROWTH RESULTS

Concentration(ug/ml)	0.1	10	20	30	40	60	80
Cu ₂ O-MgO NPs	31.44	36.38	39.31	51.40	57.80	61.82	80.74
<i>Cassia Abbreviata</i>	13.00	20.95	24.76	29.62	30.97	43.27	77.73

Cytotoxicity Results for *Cassia abbreviata* Bark Extracts:

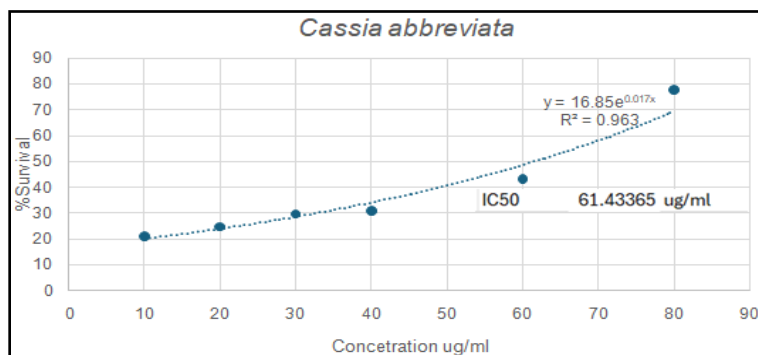


FIG. 3: CYTOTOXICITY OF CASSIA ABBREVIATA BARK EXTRACTS IN HCC1937 TRIPLE-NEGATIVE BREAST CANCER CELL LINE

Cytotoxicity Results for Copper-doped Magnesium Oxide Nanoparticles:

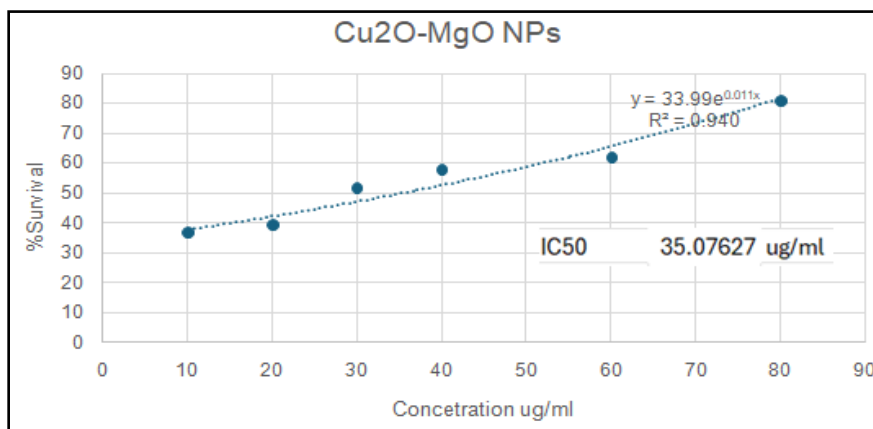


FIG. 4: CYTOTOXICITY OF CASSIA ABBREVIATA BARK EXTRACTS IN HCC1937 TRIPLE NEGATIVE BREAST CANCER CELL LINE

The cells treated with Cu₂O-MgO nanoparticles had significantly lower absorbance values at all the concentrations tested when compared to the cells treated with *Cassia abbreviata* bark extract, showing that cell viability was greatly inhibited by the nanoparticles. The differences among the treatment groups were statistically significant ($P < 0.0001$) using one-way analysis of variance (ANOVA) to determine whether any effects were due to random variation. The absorbance values were used to determine the percentage inhibition of cell growth. The bark extract of *Cassia abbreviata* and Cu₂O-MgO nanoparticles showed concentration-dependent cytotoxic activity against the breast cancer cell line (HCC1937). As the concentration of the cells increased, the cell proliferation was increasingly inhibited; and as the concentration of the cells decreased, the inhibitory effect also decreased. This dose-dependent response is shared by many anti-cancer drugs and implies that treatments have a cytotoxic effect that is dose-dependent.

Nevertheless, Cu₂O-MgO nanoparticles always generated more growth inhibition than the bark extract of *Cassia abbreviata* at all the concentrations evaluated. The increased cytotoxic effect of these nanoparticles could be explained by their nanoscale size and large surface area, their ability to penetrate cells, and their capacity to generate reactive oxygen species (ROS) that induce oxidative stress, DNA damage, mitochondrial dysfunction, and apoptosis in cancer cells. Half-maximal inhibitory concentration (IC₅₀) values were estimated from computer-generated dose-

response curves using the equations of the fitted trend lines in Microsoft Excel. The aqueous bark extract of *Cassia abbreviata* had an IC₅₀ value of 61.43 µg/mL, while the Cu₂O-MgO nanoparticles had an IC₅₀ value of 35.08 µg/mL against the breast cancer cells HCC1937. The cytotoxicity of the nanoparticles was found to be much higher than that of the crude extract, as lower IC₅₀ values indicate greater potency in promoting cytotoxicity. The enhanced biological activity of the active ingredients in the nanoparticles is attributed to the synthesis method, which is more effective for the Cu₂O-MgO nanoparticles. In addition, the observed cytotoxicity of the *Cassia abbreviata* extract may be attributable to bioactive phytochemicals, including flavonoids, tannins, phenolic compounds, alkaloids, and anthraquinones, which have been reported to exert antiproliferative and pro-apoptotic effects against cancer cell lines²². All in all, the results suggest that both treatments exhibit anticancer activity in the human breast cancer cell line HCC1937, with Cu₂O-MgO nanoparticles being highly effective compared to the crude aqueous bark extract.

CONCLUSION: The biosynthesized Cu₂O-MgO nanoparticles showed much higher antiproliferative activity against the HCC1937 human breast cancer cell line compared to the aqueous bark extract of *Cassia abbreviata* as indicated by their lower IC₅₀ value (35.08 µg/mL vs. 61.43 µg/mL). This proves that green-synthesized nanoparticles are better in inhibiting the growth of breast cancer cells than the crude plant extract. Most probably, the enhanced anticancer activity is due to a synergistic effect

between copper, magnesium oxide, and the bioactive phytochemicals from *C. abbreviata* that participated in the synthesis. This study, therefore, brings to light the potential of green nanotechnology to improve bioactivity and pharmacological efficacy of traditional medicinal plants, such as *Cassia abbreviata*, and make them better sources of effective low-cost remedies in rural and resource-limited settings in Zimbabwe.

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CONFLICTS OF INTEREST: None

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