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IN-VITRO CYTOTOXICITY OF *MAMMEA AFRICANA* AND *CALOPHYLLUM INOPHYLLUM* METHANOL EXTRACTS AGAINST H460 LUNG CANCER CELLS: A COMPARATIVE STUDY USING THE MTT ASSAY

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Keywords:

Mammea africana, *Calophyllum inophyllum*, Cytotoxicity, Antioxidant activity, Cancer therapy, Phytochemicals

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ABSTRACT: Natural plant extracts have gained interest in cancer research due to their potential therapeutic properties. This study investigates the *in-vitro* cytotoxic effects and phytochemical composition of *Mammea africana* stem bark and *Calophyllum inophyllum* leaves methanol extracts against H460 lung cancer cells using the MTT assay, with 5-Fluorouracil (5FU) as a standard control. Phytochemical screening revealed that *M. africana* had high terpenoid content and moderate levels of flavonoids, tannins, and phenolics, while *C. inophyllum* had higher flavonoid and phenolic content, moderate saponins and terpenoids, and lower tannins and alkaloids. The extraction yields were 12.5% w/w for *M. africana* and 9.8% w/w for *C. inophyllum*. Cytotoxicity analysis demonstrated that *M. africana* exhibited strong cytotoxic effects, with an IC_{50} value of 2.60 µg/mL, comparable to 5-Fluorouracil (IC_{50} = 2.71 µg/mL), while *C. inophyllum* showed moderate cytotoxic activity (IC_{50} = 30.00 µg/mL). The cytotoxic effects were dose-dependent, with *M. africana* achieving 97.47% cytotoxicity at 100 µg/mL, whereas *C. inophyllum* reached 85.4% at the same concentration. The findings suggest that the potent cytotoxic effects of *Mammea africana* could be attributed to its high terpenoid and tannin content, whereas the moderate activity of *C. inophyllum* may be linked to its flavonoid and phenolic composition. These results highlight the potential of *M. africana* as a promising natural anticancer agent, while *C. inophyllum* may require optimization or combination therapy to enhance its efficacy. Future research should focus on isolating and characterizing active compounds and investigating their mechanisms of action in cancer therapy.

INTRODUCTION: Lung cancer remains a leading cause of cancer-related mortality worldwide [1], necessitating the exploration of new therapeutic agents. Natural products derived from medicinal plants have been extensively studied for their cytotoxic potential.

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The Guttiferae family, also known as Clusiaceae, consists of over 100 genera and more than 1600 species distributed in tropical and subtropical regions [3, 4].

It is well known for its bioactive secondary metabolites, including xanthones, coumarins, benzophenones, and biflavonoids, many of which exhibit antimicrobial, anti-inflammatory, and anticancer properties [1]. Several members of the Clusiaceae family, such as *Garcinia mangostana* and *Garcinia kola*, have demonstrated potent cytotoxic properties against various cancer cell lines [2, 3].

Mammea africana and *Calophyllum inophyllum* are also members of this family and have been traditionally used for various medicinal purposes.

Mammea africana, commonly known as the African mammee apple, is a tropical tree native to West and Central Africa. It has been widely used in traditional medicine for the treatment of various ailments, including skin infections, fever, and gastrointestinal disorders [4]. The plant is particularly rich in coumarins and xanthones, which have been linked to its cytotoxic, antimicrobial, and anti-inflammatory properties [5]. Coumarins, such as mammea A/BA and mammea C/OB, have been reported to exhibit significant anticancer activity by inducing apoptosis and inhibiting cancer cell proliferation [10]. Additionally, the stem bark of *M. africana* contains high levels of terpenoids and tannins, which are known to disrupt cancer cell metabolism and inhibit angiogenesis [7]. These bioactive compounds make *M. africana* a promising candidate for further investigation in cancer therapy.

Calophyllum inophyllum, commonly known as tamanu or Alexandrian laurel, is a tropical evergreen tree found in coastal regions of Africa, Asia, and the Pacific. It has been traditionally used in folk medicine for the treatment of skin diseases, wounds, and inflammation [8]. The leaves and seeds of *C. inophyllum* are rich in bioactive compounds such as inophyllums, calophyllolides, and flavonoids, which have demonstrated antimicrobial, anti-inflammatory, and anticancer activities [9]. In particular, inophyllum P, a coumarin derivative isolated from *C. inophyllum*, has shown potent cytotoxic effects against various cancer cell lines, including lung, breast, and colon cancers [10]. The plant's high flavonoid and phenolic content also contribute to its antioxidant properties, which play a role in reducing oxidative stress and inducing apoptosis in cancer cells [11].

Despite the well-documented cytotoxic potential of Clusiaceae species, the comparative cytotoxic effects of *Mammea africana* and *Calophyllum inophyllum* on H460 lung cancer cells remain underexplored. This study aims to evaluate and compare their cytotoxic effects using the MTT assay, with 5-Fluorouracil as a reference drug. By investigating the phytochemical composition and cytotoxic activity of these plants, this study seeks to contribute to the growing body of knowledge on natural products as potential anticancer agents.

MATERIALS AND METHODS:

Materials: The materials used in this study included fresh stem bark of *Mammea africana* and leaves of *Calophyllum inophyllum*. The chemical reagents comprised methanol (95%, Analar grade; Riedel-de Häen, Sigma-Aldrich, Fluka, Germany), MTT reagent (5 mg/mL in PBS, Sigma, UK), dimethyl sulfoxide (DMSO, ≥99.9%, Sigma, UK), RPMI 1640 medium (Sigma, UK), fetal bovine serum (FBS, heat-inactivated, Sigma, UK), L-glutamine (2 mM, Sigma, UK), sodium pyruvate (1 mM, Sigma, UK), and 5-Fluorouracil (5FU, ≥99%, Sigma, UK). The laboratory equipment included Whatman No. 1 filter paper (Cytiva, UK), a rotary evaporator (Heidolph Hei-VAP, Germany) set at 40°C, a 96-well microplate reader (Bio-Rad, Model 680, USA), and various glassware such as beakers, conical flasks, measuring cylinders, and petri dishes (Pyrex, Corning, USA). Human H460 lung cancer cells were obtained from the Institute of Cancer Therapeutics, University of Bradford, United Kingdom.

Plants Collection and Preparations: Fresh stem bark of *M. africana* and leaves of *C. inophyllum* were collected from Imo State, Nigeria (2016) and authenticated by a plant taxonomist at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The plant materials were washed thoroughly, air-dried at room temperature for two weeks, and ground into coarse powder using a mechanical grinder. The powdered plant materials were subjected to methanol extraction using a cold maceration technique.

Approximately 1 kilogram of each powdered plant material was soaked in 2.5 L of 95% methanol for 72 hours with occasional stirring. The extracts were filtered using Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C. The dried extracts were stored for further analysis, and the percentage yields for each plant were calculated using the formula:

(%) yield = (weight of extract) / (weight of dried plant material) x 100 ……equation (1)

Qualitative Phytochemical Analysis: To determine the presence of various bioactive compounds in *Mammea africana* and *Calophyllum inophyllum*, standard qualitative phytochemical screening tests were conducted. The extracts were screened for carbohydrates, alkaloids, flavonoids, saponins, tannins, terpenoids, and phenolic compounds using standard procedures as reported by Mohammed [12].

Cytotoxicity Using the MTT Assay Procedure: The MTT assay used in this study was adapted from the method described by Mosmann [13], which is widely used for evaluating cell viability and proliferation. This colorimetric assay is based on the conversion of MTT to formazan by mitochondrial dehydrogenase enzymes in viable cells, providing a quantitative measure of cytotoxicity. H460 lung cancer cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 1 mM sodium pyruvate. The cells were incubated at 37°C in a humidified 5% CO_2 atmosphere.

For the MTT assay, cells were seeded in a 96-well microtiter plate at a density of 5×10^4 cells per well and incubated overnight. The cells were treated with different concentrations of *Mammea africana* and *Calophyllum inophyllum* extracts (1, 3, 10, 30, and 100 µg/mL) and 5-Fluorouracil (5FU) (0.1, 1, 3, 10, and 30 µg/mL) for 24 hours. Following treatment, the medium was aspirated, and 20 µL of MTT reagent (5 mg/mL in PBS) was added to each well, followed by a 4-hour incubation. The resulting formazan crystals were dissolved in 150 µL of DMSO, and absorbance was measured at 540 nm using a microplate reader. The percentage cytotoxicity was calculated based on the absorbance values using the formula below:

Percentage cytotoxicity (%) = (Control absorbance-Treated absorbance) / (Control absorbance) x 100 …equation (2)

Data Analysis: All experiments were performed in triplicate, and data were expressed as mean ± standard deviation (SD). The IC_{50} values were determined using nonlinear regression analysis in Microsoft Excel (Office 16) software. Statistical significance was assessed using one-way ANOVA followed by Tukey's post hoc test, with p-values < 0.05 considered significant using SPSS software (version 20).

RESULTS:

Extraction Yields of the Plants: The extraction process resulted in different yields for the two plant species. *Mammea africana* yielded approximately 10.2% w/w from 1000 g (1 kg) of dried plant material, resulting in 102 g of dried methanol extract. *Calophyllum inophyllum* yielded 18.8% w/w from 1000 g (1 kg) of dried plant material, producing 188 g of dried extract. These differences in yield may be attributed to variations in the chemical composition and solubility of bioactive compounds within each plant matrix.

Phytochemical Analysis: The qualitative phytochemical screening revealed the presence of several bioactive compounds in *Mammea africana* and *Calophyllum inophyllum* **Table 1**. Both extracts contained carbohydrates and alkaloids. *Mammea africana* exhibited higher terpenoid and tannin levels, while *Calophyllum inophyllum* had higher flavonoid and phenolic content. Anthraquinones and cardiac glycosides were absent in both extracts.

TABLE 1: PHYTOCHEMICAL COMPOSITION OF EXTRACTS

S. no.	Phytochemicals	*M. africana* stem bark	*C. inophyllum* leaves
1.	Carbohydrates	++	++
2.	Alkaloids	+	+
3.	Flavonoids	++	+++
4.	Saponins	+	++
5.	Tannins	++	+
6.	Anthraquinones	-	-
7.	Cardiac glycosides	-	-
8.	Terpenoids	+++	++
9.	Phenolics	++	+++

Key: + (Present), ++ (Moderate presence), +++ (High presence), -- (absent).

Cytotoxicity Analysis: The percentage cell viability for each extract and 5-Fluorouracil (5FU) at different concentrations is summarized in **Table 2.** *Mammea africana* exhibited potent cytotoxic effects, with an IC_{50} value of 2.6 µg/mL, comparable to 5FU (IC_{50} = 2.71 µg/mL). In contrast, *C. inophyllum* showed moderate cytotoxic activity, with an IC_{50} value of 30 µg/mL.

TABLE 2: PERCENTAGE CELL VIABILITY OF PLANT EXTRACTS AND 5-FLUOROURACIL (5FU) (PERCENTAGE CELL VIABILITY CAPTURED IN TABLE)

Concentration (µg/mL)	*M. africana*	*C. inophyllum*	5FU
0.1	--	--	78.88
1	72.78	93.57	79.45
3	46.43	91.26	46.83
10	20.63	83.07	29.96
30	7.98	50.82	29.64
100	2.53	14.60	--
IC_{50} (µg/mL)	2.6	30	2.71

DISCUSSION: The phytochemical screening results correlate well with the observed cytotoxic activity. *Mammea africana* contains high levels of terpenoids and tannins, which have been reported to disrupt cancer cell metabolism, inhibit angiogenesis, and induce apoptosis [7]. In contrast, *Calophyllum inophyllum* exhibited a higher concentration of flavonoids and phenolics, compounds known for their antioxidant and apoptosis-inducing properties [11]. The absence of anthraquinones and cardiac glycosides in both extracts suggests that these phytochemicals do not contribute to the observed cytotoxic effects.

The cytotoxic activity observed in *Mammea africana* and *Calophyllum inophyllum* highlights their potential as natural anticancer agents. The significant cytotoxic effects of *Mammea africana*, which were comparable to 5-Fluorouracil (5FU), suggest the presence of highly bioactive compounds, likely terpenoids and tannins, which have been previously reported to exhibit anticancer activity. This aligns with studies on other Clusiaceae species, such as *Garcinia mangostana*, which contains xanthones known for their antiproliferative effects on various cancer cell lines 2. Similarly, research on *Calophyllum brasiliense* has shown that biflavonoids induce apoptosis in breast cancer cells [14], further supporting the cytotoxic potential of the Clusiaceae family.

The statistical analysis demonstrated a significant difference ($p < 0.05$) between the cytotoxic effects of *Mammea africana* and *Calophyllum inophyllum*, confirming that *Mammea africana* exhibited a stronger anticancer effect. One-way ANOVA followed by Tukey's post hoc test indicated that the cytotoxicity of *Mammea africana* at higher concentrations (≥30 µg/mL) was not significantly different from that of 5FU, suggesting its potential as a natural alternative to standard chemotherapy. However, *Calophyllum inophyllum* showed significantly lower cytotoxic effects ($p < 0.05$), indicating that its bioactive compounds may require further fractionation or synergistic enhancement.

CONCLUSION: *M. africana* demonstrates strong cytotoxic activity against H460 lung cancer cells, comparable to 5FU, and holds potential for development as a natural chemotherapeutic agent, while *C. inophyllum* exhibits moderate cytotoxicity, requiring higher doses for significant effects, suggesting the need for further investigation and optimization. The potent cytotoxicity of *M. africana* is likely attributed to its high terpenoid and tannin content, whereas *C. inophyllum's* moderate effect may be linked to its flavonoid and phenolic composition. Further studies should focus on isolating the active compounds responsible for the observed cytotoxic effects, exploring their mechanisms of action, and investigating potential combination therapies with conventional anticancer drugs.

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