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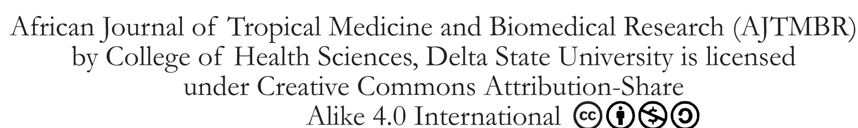

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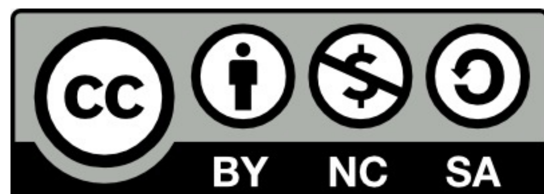

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Assessment of Cytotoxicity and Growth Inhibitory Effects of Methanol Extract of *Ageratum conyzoides* Linn.

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ABSTRACT

Introduction: Evaluating cellular cytotoxicity is a crucial step in the development of specific anticancer therapies. As a medicinal plant, *Ageratum conyzoides* is well-known for its abundance of bioactive constituents, anti-inflammatory and anti-bacterial properties. In this study, its effect was evaluated to confirm if its methanol extract exhibits cytotoxic and growth-inhibitory properties, with potential relevance to oncology.

Materials and Methods: Extraction of 1000 g of dried leaf material with methanol yielded 100 g of crude extract (10 %), indicating a high content of methanol-soluble bioactive compounds. The cytotoxic potential of the extract was assessed using *Ranicep ranninus* tadpoles as a preliminary model.

Results: The study's findings demonstrated a clear concentration-dependent increase in mortality, with significant effects observed at 160 and 320 µg/mL and a moderate LC_{50} value of 100.76 µg/mL, confirming the extract's capacity to impair cell viability. Additionally, the extract exhibited significant inhibitory effects on the radicle growth of *Sorghum bicolor*, with both dose- and time-dependent responses. Higher concentrations (8 -16 µg/mL) and prolonged exposure periods resulted in marked suppression of radicle elongation, as reflected by percentage inhibition and IC_{50} values.

Conclusion: These findings suggest that the extract can interfere with cell division and proliferation, processes analogous to those driving uncontrolled growth in cancer cells. Collectively, this is an indication that *A. conyzoides* methanol extract possesses compounds with both cytotoxic and antiproliferative activity. These effects in non-cancerous models suggest preliminary potential as natural anticancer sources agents.

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INTRODUCTION

In biological sciences, cytotoxicity refers to the capacity of a substance to damage cells or induce cell death and is a fundamental concept in toxicology, pharmacology, and biomedical research.[1] Cytotoxicity assessment is routinely performed to evaluate the effects of chemical substances, pharmaceutical agents, or environmental pollutants on cell survival and viability.[2] Such agents may induce cell death through mechanisms such as necrosis or apoptosis, underscoring the need to ensure that potential therapeutic compounds are effective while remaining safe for patients. Because substances vary greatly in their cytotoxic potential, systematic evaluation is essential before clinical or environmental application.[3] Indeed, many chemotherapeutic drugs currently used in

cancer treatment exert their effects through well-established cytotoxic mechanisms.[4-6] Growth inhibition, by contrast, describes the suppression or reduction of cell, tissue, or organismal growth following exposure to a given substance.[7] Growth inhibition assays are widely applied in biological and pharmacological research to assess the activity of chemical compounds, plant extracts, or drugs against targets such as cancer cells, microorganisms, or developing plant tissues.[8,9]

Medicinal plants remain a major source of novel therapeutic agents,[10-11] with ongoing research focused on identifying bioactive lead compounds and evaluating the efficacy of whole-plant preparations.[12] Numerous studies demonstrate that medicinal plants contain compounds with antibacterial, anti-inflammatory, antioxidant, and anticancer activities,[13-15] and several naturally derived molecules have played key roles in modern anticancer drug development.[16] Consequently, scientific evaluation of plants used in traditional medicine is critical to establish safety standards and minimise risks associated with toxic herbal products.[17] Historically, plants have served as reservoirs of unique secondary metabolites capable of treating a wide range of diseases,[18,19] and many contemporary pharmaceuticals are plant-derived.[20] Rigorous assessment of traditionally used plants is therefore necessary to identify compounds that are both safe and effective, while reducing potential toxicity.[13]

Ageratum conyzoides is a medicinal plant widely used in traditional healthcare systems worldwide, with applications varying by region. In Nigeria, it is known as *Imi esu* among the Yoruba people.[21-23] In India, it is used as a bactericidal, anti-dysenteric, and anti-lithic agent, while in parts of Asia, South America, and Africa, aqueous extracts are commonly employed for antibacterial purposes.[24] In Central Africa, the plant is used to treat pneumonia and to promote wound and burn healing,[25] as well as for managing fever, rheumatism, headaches, and colic. Phytochemical studies reveal that *A. conyzoides* contains diverse secondary metabolites, including terpenoids, flavonoids, chromenes, alkaloids, coumarins, and lignans.[26] Its essential oil is rich in biologically active compounds such as quercetin derivatives, chromenes, phytosterols, and saponins,[27] highlighting its pharmacological potential. This study therefore evaluated the cytotoxic and growth-inhibitory effects of the methanol extract of *Ageratum conyzoides*.

MATERIALS AND METHODS

Collection and authentication of plant materials

Fresh leaves of *A. conyzoides* were obtained from the surroundings of campuses II (5.79093 ° N, 6.09847 ° E) and III (5.78072 ° N, 6.10169 ° E) of Delta State University, Abraka, Nigeria (Figure 1). Preliminary identification and authentication of the plant were carried out by Akinnibosun Henry Adewale at the Department of Plant Biology and Biotechnology, Herbarium Unit, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria. A voucher specimen was deposited in the herbarium with the reference number UBH-A344 (*Ageratum conyzoides* Linn). The freshly collected leaves were thoroughly rinsed under running tap water to eliminate dirt and debris, after which they were air-dried at ambient laboratory temperature (23–29 °C) in the Department of Medical Biochemistry laboratory, Delta State University, Abraka. Drying was continued for 21 days until a constant weight was achieved, following the methods described by Usin and Ugwu[28] and Odeghe *et al.*[29] The dried leaves were then ground into a fine powder and stored in airtight containers at 4 °C prior to extraction.

Preparation and extraction of plant materials

The harvested plant material was washed and allowed to air-dry at ambient temperature for 14 days. Thereafter, the dried sample was milled into a fine powder using an electric grinding blender. A measured quantity of the powdered leaves (1000 g) was subjected to methanol extraction using a Soxhlet apparatus, following the method previously described by Usin and Ugwu.[28] The resulting extract was concentrated on an electrothermal constant water bath to obtain a greenish semi-solid residue, which was subsequently preserved in a refrigerator at 4 °C for subsequent analyses.

Sources of tadpoles (*Raniceps ranninus*)

Tadpoles were collected from toad breeding sites in small water bodies within Campus II (5.79093 ° N, 6.09847 ° E) of Delta State University, Abraka, Nigeria. Species identification was carried out by the Department of Animal and Environmental Biology, Faculty of Sciences, Delta State University, Abraka. Following identification, tadpoles aged 5 - 6 days were excluded from the study.

Evaluation of cytotoxic activity of *A. conyzoides* fractions on tadpole (*Raniceps ranninus)*

The cytotoxic activity of *A. conyzoides* fractions was assessed using a procedure adapted from Ayinde *et al.*[30] with minor modifications. Ten tadpoles were introduced into 250 mL beakers containing 15 mL of water obtained from the original tadpole habitat, which was then diluted with distilled water to a volume of 49 mL. The final volume was adjusted to 50 mL by adding 0.5, 1, 2, 4, or 8 µL of the fractions prepared in 5 % dimethyl sulfoxide (DMSO) in water, corresponding to final concentrations of 20, 40, 80, 160, and 320 µg/mL, respectively. Control groups were treated accordingly, and tadpole mortality was monitored over a period of at least 24 hours.

Group 1: Tadpoles + 5 % DMSO in distilled water.

Group 2: Tadpoles + 20 µg/mL of methanol extract

Group 3: Tadpoles + 40 µg/mL of methanol extract

Group 4: Tadpoles + 80 µg/mL of methanol extract

Group 5: Tadpoles +160 µg/mL of methanol extract

Group 6: Tadpoles + 320 µg/mL of methanol extract

The experiment was carried out in triplicate.

Sources of guinea corn (*Sorghum bicolor*)

Guinea corn was obtained from the Abraka Main Market and was cleansed with absolute ethanol. The viability of the seeds was determined by their ability to remain submerged in water.[31] The seeds that remained submerged in water were selected and dried for use in this study.

Evaluation of growth inhibitory activity of *A. conyzoides* fractions on guinea corn radicle length

Precisely 10 mL of the fractions at concentrations of 1, 2, 4, 8, and 16 µg/mL, prepared in 5 % DMSO in water, were dispensed into 9 cm Petri dishes lined with cotton wool and Whatman No. 1 filter paper. Seed viability was assessed by pre-soaking twenty seeds in 50 mL of distilled water, after which they were evenly distributed on each Petri dish and incubated in a dark cabinet. The lengths (cm) of the emerging radicles were measured at 24, 48, 72, and 96 hours. Control seeds received 10 mL of 5 % dimethyl sulfoxide in distilled water without any extract, in accordance with the method described by Ayinde *et al.*[30]

Group 1: Guinea corn seeds + 5 % DMSO in distilled water.

Group 2: Guinea corn seeds + 1 μg/mL of methanol extract
Group 3: Guinea corn seeds + 2 μg/mL of methanol extract
Group 4: Guinea corn seeds + 4 μg/mL of methanol extract
Group 5: Guinea corn seeds +8 μg/mL of methanol extract
Group 6: Guinea corn seeds +16 μg/mL of methanol extract

RESULTS

Percentage yield methanol extract of *A. conyzoides* leaf

The percentage yield of the methanol extract of *Ageratum conyzoides* leaf is presented in Table 1. It shows that the extraction of 1000 g of plant material with methanol produced 100 g of crude extract, giving a percentage yield of 10 %. This indicates that methanol was an effective solvent for extracting soluble bioactive constituents from the plant material, suggesting a relatively high abundance of methanol-soluble compounds in the sample.

Cytotoxic effect of the methanol extract of *A. conyzoides* on *Ranicep ranninus* (tadpole)

The results from Tables 2 and 3 show that the methanol extract exerted a clear, concentration-dependent cytotoxic effect on *Ranicep ranninus* tadpoles. Both cytotoxic response and percentage mortality increased steadily with rising extract concentration, with statistically significant effects observed at 160 and 320 μg/mL when compared with the control. The absence of mortality in the D.H_2O control confirms that the observed effects were due to the extract. The LC_{50} value of 100.76 μg/mL indicates moderate cytotoxic potency of the methanol extract.

Growth inhibitory effect of the methanol extract of *A. conyzoides* of *S. bicolor* (guinea corn) radicle.

The results in Tables 4 and 5 indicate that the methanol extract of *Ageratum conyzoides* affected the radicle growth of *Sorghum bicolor* in a concentration- and time-dependent manner. While the control showed normal progressive radicle elongation, exposure to the extract led to significant growth inhibition at several concentrations, particularly at higher doses (8 - 16 μg/mL) and longer exposure periods. The percentage inhibition data further confirm this trend, with positive inhibition values increasing over time and corresponding IC_{50} values demonstrating measurable phytotoxic activity.

DISCUSSION

Cancer is fundamentally characterised by uncontrolled cell proliferation, resistance to cell death, and sustained growth signalling.[32] Therefore, substances capable of inducing cytotoxicity and inhibiting growth in biological models are of considerable interest as potential anticancer agents.[33] The extraction yield of 10 % obtained using methanol suggests that the plant leaf is rich in methanol-soluble secondary metabolites. Methanol is known to extract compounds such as flavonoids, alkaloids, terpenoids, and phenolic compounds, many of which have been widely reported to possess anticancer properties.[34,35] The appreciable yield observed in this study indicates a high likelihood that the extract contains sufficient quantities of these bioactive compounds capable of exerting biological effects, including cytotoxicity against rapidly dividing cells. The cytotoxicity assay using *Ranicep ranninus* tadpoles revealed a clear concentration-dependent toxic effect of the methanol extract, with significant mortality at higher concentrations and an LC_{50} value of 100.76 μg/mL. Although tadpoles are not cancer cells, this model serves as a preliminary biological system to evaluate general cytotoxic potential.[36]

Furthermore, the growth inhibitory effect of the methanol extract on *Sorghum bicolor* radicle elongation reinforces its relevance to cancer growth suppression. Guinea corn radicle growth depends on active cell division, elongation, and differentiation, processes that are also exaggerated in cancer cells.[37,38] The significant reduction in radicle length at higher extract concentrations and longer exposure times indicates that the extract interferes with cellular proliferation.[39] In cancer treatment, inhibiting cell cycle progression and suppressing proliferative capacity are key therapeutic goals. The observed concentration- and time-dependent growth inhibition, therefore, suggests that the extract may contain anti-proliferative agents capable of limiting abnormal cell growth. The percentage inhibition and IC_{50} values further support this interpretation, as increasing inhibition over time reflects sustained suppression of growth-related processes. In cancer models, such sustained inhibitory effects are desirable, as they imply the ability of a compound to restrain tumour expansion rather than producing only transient effects continuously. The presence of both growth-stimulatory effects at very low concentrations and inhibitory effects at higher concentrations is also consistent with the behaviour of many anticancer agents, which may exhibit hormetic responses depending on dose.

CONCLUSION

The present study suggests that the methanol extract of *Ageratum conyzoides* leaf could exhibit cytotoxic and growth-inhibitory properties, which are relevant in the field of oncology. Even though these findings are based on non-cancer biological models, they provide preliminary evidence that the extract might contain bioactive compounds capable of inducing cell death and suppressing proliferation, which are the two critical targets in cancer treatment. However, further studies using established cancer cell lines, apoptosis assays, and molecular mechanism analyses are necessary to validate the anticancer potential of this extract and to identify the specific compounds responsible for these effects.

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