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**Original Research Article**

# Cytotoxic and growth inhibitory effects of the volatile oils of *Eugenia caryophyllus* (Myrtaceae), *Callistemon citrinus* (Myrtaceae) and *Zingiber officinale* (Zingiberaceae)

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## Abstract

**Purpose:** Search for medicinal plants with anti-tumor activity has become imperative due to the incidence and burden of various types of cancer. Some volatile oil-containing plants like *Eugenia caryophyllus* buds, *Callistemon citrinus* leaves and *Zingiber officinale* rhizomes are indicated in ethnomedicine for the treatment of tumor-related ailments. These necessitated the evaluation of the volatile oils for cytotoxic and anti-proliferative activities on tadpoles of *Ranniceps ranninus* and guinea corn (*Sorghum bicolor*) seeds respectively.
**Methods:** The volatile oils of the plants were obtained separately using Clavenger-type apparatus for at least 3 h. Using bench top assays, each volatile oil was examined for cytotoxicity test against tadpoles of *R. ranninus* at concentrations of 10, 20 and 40 µg/mL. Their antiproliferative potentials were assayed by their ability to inhibit the radicle growth of guinea corn (S. bicolor) seeds at concentrations ranging from 0.1 – 30 mg/mL.
**Results:** The volatile oil yields of the plants were 5.3, 0.6, and 0.16% v/w for *E. caryophyllus, C. citrinus* and *Zingiber officinale* respectively. At 20 µg/mL, the volatile oils of *E. caryophyllus* and *Z. officinale* exhibited 100% mortality on the tadpoles while the volatile oil of *C. citrinus* produced similar results at a concentration of 40 µg/mL. The LC50 were 12.8, 15.8 and 25.2 µg/mL respectively. On the growth inhibitory test, 100% reduction was observed with *E. caryophyllus* at 5 mg/ml while 68.59% and 44.08% reductions were produced by *Z. officinale* and *C. citrinus* oils respectively.
**Conclusion:** The results demonstrated that *E. caryophyllus*, *C. citrinus* and Z. *officinale* volatile oils may have cytotoxic and antiproliferative potentials as claimed in ethnomedicinal applications and may have therapeutic value in the management of tumor-related ailments.

**Keywords:** Cytotoxicity, growth inhibitory, *Eugenia caryophyllus*, *Callistemon citrinus*, *Zingiber officinale*

**Indexing:** Index Copernicus, African Index Medicus

## Introduction

Cancer is a major public health problem in the world with its global burden continuing to increase [1]. Current therapeutic approaches have had little impact on the overall survival in patients with advanced cases of this disease, and drug resistance as well as severe side effects and cost implications remain a significant obstacle to successful treatment. Natural products have played an important role as effective sources of antitumor agents. It is estimated that up to 30-40% of anticancer drugs used globally are derived from plant sources [2].

Essential oils are natural, volatile, and odorous molecules synthesized by the secretory cells of aromatic plants. They are complex mixtures of several components endowed with a wide range of biological activities including antiseptic, anti-inflammatory, spasmolytic, sedative, analgesic, and local anesthetic properties [3]. They are found in families such as Lauraceae, Lamiaceae, Asteraceae, Myrtaceae, Rutaceae as well as Conifers [4]. In ethnomedicine, essential oil-containing plants like *Eugenia caryophyllus* (Myrtaceae), *Callistemon citrinus* (Myrtaceae) and *Zingiber officinale* (Zingiberaceae) have been reportedly included in recipes used in the management of tumour-related ailments.

*E. caryophyllus* commonly called clove, is one of the most important herbs in traditional medicine [5]. It is cultivated in India, Madagascar, Sir Lanka, Indonesia and Southern China [6]. Clove essential oil is used in aromatherapy and to treat toothache in dental emergencies [7]. It is composed of different classes and chemical groups such as terpenes, sesquiterpenes and phenolic hydrocarbon compounds and possesses antibacterial, antifungal, insecticidal and antioxidant activities [8].

*C. citrinus* commonly called Bottle brush is a woody aromatic ornamental plant that grows in different tropical and subtropical regions of the world [9,10]. The leaf oils are known to have antimicrobial, fungitoxic, antinociceptive and anti-inflammatory activities [11,12]. Chemical investigations revealed that the flowers and leaves of the plant are rich in essential oils comprising of 1,8-cineole, α- and β-pinenes, α-terpineol, α-phellandrene, limonene, α-terpinene, linalool, trans-pinocarveol, terpinen-4-ol and geraniol [13-15].

*Z. officinale*, Ginger, is a rhizome used to treat vomiting, motion sickness, arthritis, cough and cold. It is also an antioxidant, antimicrobial, and antifungal agent [18]. Ginger is rich in both hydrophilic and hydrophobic constituents. The hydrophilic portion of ginger extract consists mostly of different types of poly-phenolic compounds [16]. The hydrophobic portion contains mainly different kinds of monoterpenes, oxygenated monoterpenes, sesquiterpenes, zingerone, paradols, gingerols and shogaols other than essential oils [17].

The inclusion of these plants in recipes for treating tumor related ailments prompted this work to examine the probable cytotoxic and growth inhibitory effects of the respective essential oils on tadpoles of *Raniceps ranninus* and *Sorghum bicolor*, guinea-corn radicles.

## Methods

### Collection and identification of plants

The leaves of *C citrinus* were collected from the premises of University of Benin, while *E. caryophyllus* buds and *Z. officinale* rhizomes were purchased from a local market in Benin City. Their identities were confirmed by Dr A. Bamidele of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. Voucher numbersUBHC384, UBHC383 and UBHZ384 were assigned for *E. caryophyllus*, *C. citrinus* and Z*. officinale* respectively.

### Extraction of the plant material

About 1.5 kg of each of the plant materials were extracted by hydro-distillation method with the aid of a Clavenger-type apparatus to obtain their essential oils. The yields of the oils were noted while the equivalent weights were also determined. The volatile oils obtained were collected into glass sample bottles and kept in the refrigerator maintained at 4 °C.

### Sources and identification of the tadpoles of *Raniceps ranninus*

Tadpoles (5-6 days old) were harvested from toad colonies in small water settlements around the Faculty of Pharmacy, University of Benin. They were identified as the tadpoles of *Raniceps ranninus* by Professor M. Aisien, Animal Parasitologist, Department of Animal and Environmental Biology, Faculty of Science, University of Benin, Benin City, Nigeria.

### Source and preparation of the guinea corn (*Sorghum bicolor*)

Guinea corn obtained from a local market in Benin was cleansed with absolute alcohol to remove the preservative. 100 mL of water was added and immediately decanted along with the seeds floating. The submerged seeds were adjudged viable. These were dried on filter papers before use.

### Determination of the cytotoxic effects of the essential oils on tadpoles (*Raniceps ranninus*)

Following the method described in the literature [19], ten (10) tadpoles were selected in 50 mL capacity beakers containing 15 mL of the water from the source of the tadpoles, which was made up to 49 ml with distilled water. The volume was made up to 50 ml with 0.5, 1, and 2 mg/mL of each of the oils previously prepared by mixing them with 0.1 mL of tween 80 and water, thereby making concentrations of 10, 20, and 40 μg/mL respectively. The experiment was carried out in triplicates for each concentration. The controls for each of the experiments were not treated. The mortality rates of the tadpoles were observed for 24 h.

**Determination of the growth inhibitory effects of the essential oils on guinea corn (*Sorghum bicolor*)**

About 10 ml of 1, 2, 5, 10, 20 and 30 mg/mL of *E. caryophyllus*, *C. citrinus*, and *Z. officinale* oils prepared by dissolution in 0.1 mL of tween 80 and water, were poured into 9-cm-wide glass Petri dishes under laid with cotton wool and filter paper (Whatman No 1). Lower concentrations of 0.1, 0.25 and 0.5 mg/ml were also included for *E. caryophyllus* oil. Twenty (20) viable seeds were spread on each plate and incubated in a dark environment. The lengths (mm) of the radicles emerging from the seeds were taken at 24, 48, 72 and 96 h. The control seeds were treated with 10 mL of 2% tween 80 in distilled water. The experiments were carried out in triplicates.

**Statistical analysis**

All data obtained were expressed as mean ± SEM and analyzed with one way Analysis of Variance (ANOVA) using SPSS 21. $P < 0.05$ was regarded as significant

## Results

The volatile oil yields of the plants were 5.3, 0.6, and 0.16%v/w with equivalent weights of 1.07, 0.89, and 0.87g per mL for *E. caryophyllus*, *C. citrinus* and *Zingiber officinale* respectively.

**Cytotoxic effects of the essential oils**

The three volatile oils were observed to exhibit varying concentration-dependent cytotoxic effects on the tadpoles. At the end of the 24-h period, clove oil was observed to exhibit the highest cytotoxicity on the tadpoles producing a mortality of 23.33 ± 13.33% at a concentration of 10 μg/ml, which increased to 100% with 20 μg/ml. Ginger oil also produced high cytotoxic effects as it recorded 100% mortality at a concentration of 20 μg/ml.

No mortality was observed with *C. citrinus* oil at lower concentrations of 10 and 20 μg/ml. However, 100% mortality was obtained at 40 μg/ml (Figure 1). No death was observed with the controls ($P < 0.001$). An $LC_{50}$ of 12.8 and 15.8 and 25.2μg/ml were obtained for *E. caryophyllus* buds, *Z. officinale* rhizome and *C. citrinus* leaves essential oils respectively.

**Antiproliferative effects of the essential oils on guinea corn radicle length**

There was a general increase in the lengths of the seed radicles within the incubation period of 24-96 h which was more obvious in the control. The oils were observed to elicit concentration-dependent reductions in the length of the radicles that emerged from the guinea corn seeds. Here again, the essential oil of clove showed the highest growth inhibitory effects.

The average radicle length of the control seeds at 24 h was 3.57 ± 0.3 mm, while the average length in the seeds treated with 1.0 mg/ml of clove oil was 0.18 ± 0.06 mm, indicating 94.96% reduction in length. After 96 h, the control seeds produced an average length of 36.3 ± 3.24 mm, whereas the seeds treated with 0.5 and 1.0 mg/ml of the oil showed average lengths of 9.17 ± 2.24 and 0.62 ± 0.09 mm, also indicating 74.74 and 98.29% reductions, respectively.

Concentration of 5 mg/mL completely inhibited the growth of the radicle (Figure 2). The different concentrations of *Callistemon citrinus* and *Zingiber officinale* essential oils were equally observed to inhibit the growth of the seed radicles. Ginger oil was observed to be more effective. An average length of 14.90 ± 0.57 mm in the controls at 96 h was reduced to 4.68 ± 0.54 and 0.50 ± 0.23 mm in seeds treated with 5 and 10 mg/ml concentrations, showing 68.59 and 96.64%, reductions respectively (Figure 3).

With similar concentrations, *Callistemon citrinus* oil reduced the control lengths of 13.68 ± 0.20 at 96 h to 7.65 ± 1.05 and 4.87 ± 0.40 mm, indicating 44.08 and 64.40% reductions, respectively (Figure 4).The variations in length were found to be significantly different from the controls at $P<0.05$.

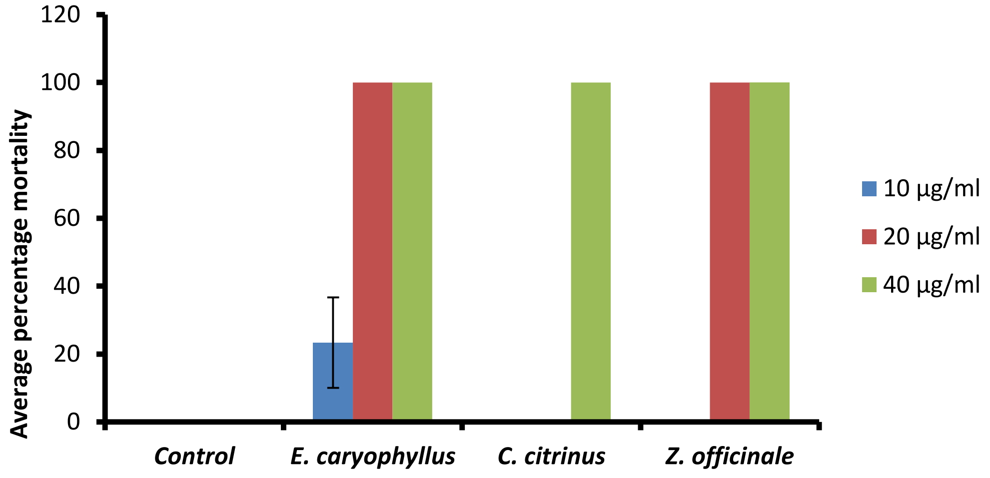


**Figure 1:** Effects of the essential oils of *Eugenia caryophyllus*, *Callistemon citrinus*, and *Zingiber officinale* on tadpole's mortality. n= 3

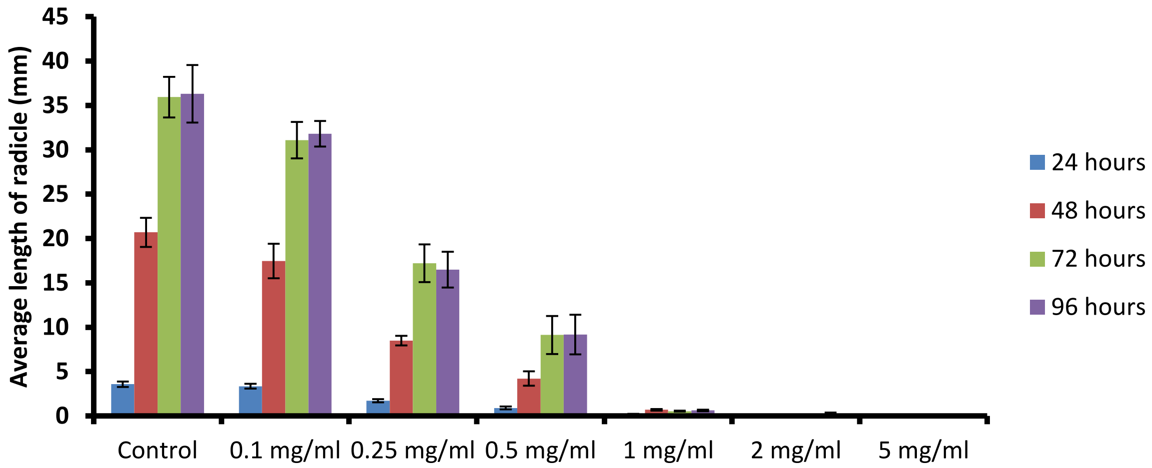


**Figure 2:** Inhibitory effects of the essential oil of *Eugenia caryophyllus* buds on length of *Sorghum bicolor* radicle. n=3

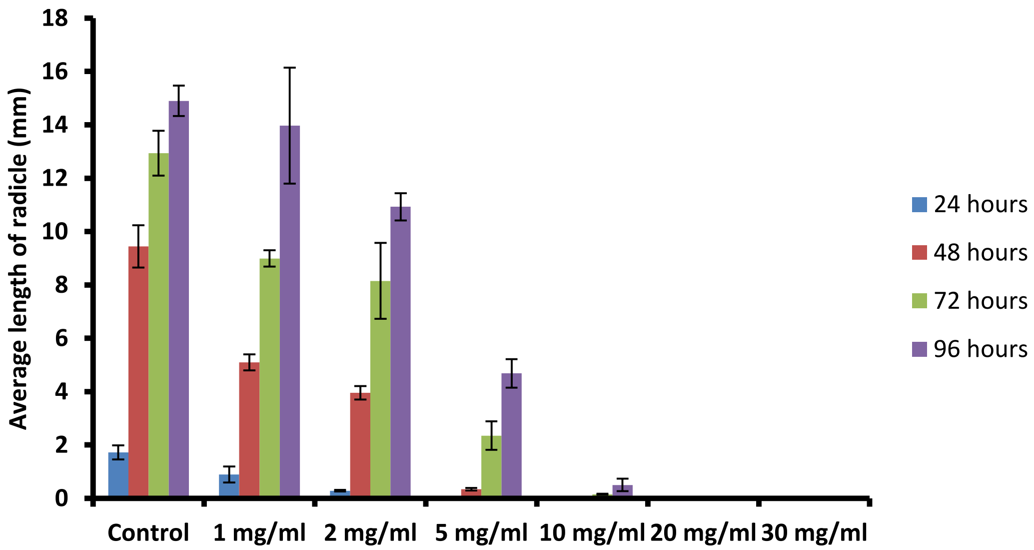


**Figure 3:** Inhibitory effects of the essential oil of *Zingiber officinale* rhizome on length of *Sorghum bicolor* radicle. n=3

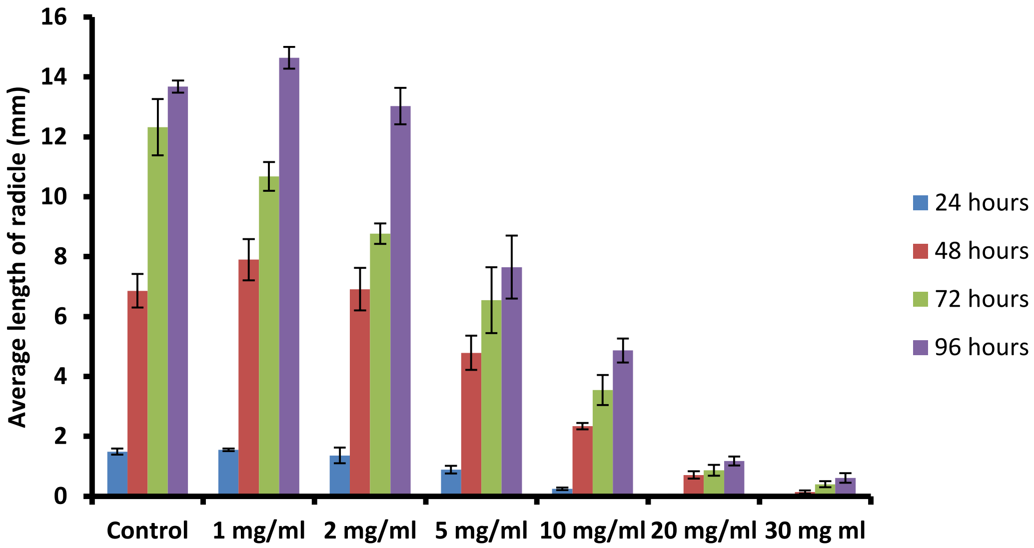


**Figure 4:** Inhibitory effects of the essential oil of *Callistemon citrinus* leaves on length of *Sorghum bicolor* radicle. n=3

## Discussion

Radicles of germinating seeds, mortality of tadpoles, and lethality of extracts on Brine shrimp (*Artemisia salina*) are examples of bench-top assays used as indicators of potentially promising antitumor agents by researchers [20,21]. These methods are highly valued because they are simple, rapid, reproducible, and material and time saving. They can also be carried out in labs where appropriate human cell lines are not readily available.

Cancer cells are known to have highly proliferative capacities which explain the use of *S. bicolor* seeds whose meristematic cells are prone to proliferation under favourable conditions [21]. The concentration dependent reduction in lengths of the treated radicles could have been due to an interference of some biochemical processes which could have affected the mitotic process in the developing radicle. A growth inhibition of 100% was observed at 5 mg/ml concentration for clove oil showing a very high level of activity compared to 68.59 and 44.08% reduction for ginger and *C. citrinus* oil respectively.

The activities of plant extracts in effecting any therapeutic or biological changes are a direct function of the chemical constituents inherently present in them [19]. *E. caryophyllus* oil is a mixture of different constituents, with three main active ingredients being eugenol, caryophyllene, and aceteugenol [5,6]. These could have been responsible for the observed cytotoxic and growth inhibitory activities. Zingiberene, β-sesquiphellandrene, and β-pinene are the major constituents found in ginger oil and have been linked to various medicinal uses of ginger [22].

Cancer is the second largest single cause of death claiming over six million lives every year worldwide [23]. It is a global health concern that causes mortality in both children and adults. Though studies have been done using these oils against some human cancer cell lines, a lot still needs to be done considering the fact that there are more than 100 distinct types and subtypes of cancer which can be found within specific organs [24]. These oils may be potential sources of natural anti-cancer compounds and therefore play important roles in human health. More studies are currently in progress using cancer cell lines to further validate the findings of this work.

## Conclusion

This study demonstrates that *E. caryophyllus, Z. officinale* and *C. citrinus* oil possess cytotoxic and growth inhibitory properties and are ideal candidates for further antitumor studies using human cancer cell lines.

## Conflict of Interest

No conflict of interest is associated with this work.

## Contribution of Authors

We declare that this study was done by the authors stated in this article and all liabilities pertaining to claims relating to the content of this work will be borne by us. BAA conceived, designed, supervised the study and contributed in the manuscript write-up, ROI collected the plants, carried out the laboratory work and also contributed in the manuscript write-up.

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