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Original Research Article

Antioxidant, Anti-inflammatory, Antiviral and Anticancer Potentials of Zingiberaceae Species Used as Herbal Medicine in Indonesia

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ABSTRACT

The Zingiberaceae family comprises 50 genera and 1300 species in South and Southeast Asia. Indonesia is one of the countries that have the greatest diversity of plant species from the Zingiberaceae family. This research aims to do phytochemical screening and bioassay of extracts of nine ginger species (*Z. officinale var. Rubrum, Z. officinale Roscoe., Z. purpureum, C. longa, C. heyneana, C. purpurascens, C. aeruginosa, C. xanthorrhiza*, and *B. rotunda*) from Indonesia. The dried powder rhizome of each species was extracted by maceration using methanol. The dry methanol extract obtained was tested for phytochemical screening and bioactivities. Phytochemical screening and activity assay were done using established methods. All extracts contained phenolics and flavonoids, with TPC values range 10–11 mg GAE/g. *C. longa* extract has the highest total flavonoid content (TFC = 1.720 QE/g). *Z. officinale* Roscoe exhibited the highest antioxidant activity against DPPH (IC_{50}= 233.0 ± 7.9 µg/mL) and ABTS (IC_{50}= 320.2 ± 27.4 µg/mL) radicals. *C. xanthorrhiza* extract showed the best anti-inflammatory activity with an IC_{50} value of 1.7 ± 0.0 µg/mL. Meanwhile, *Z. officinale var. Rubrum, Z. officinale* Roscoe, and *B. rotunda* exhibited the best cytotoxic activity against HeLa cells with respective IC_{50} values: 7.3 ± 0.3; 11.9 ± 1.3, 11.1 ± 1.4, and 13.7 ± 0.8 µg/mL. *C. longa* extract, also exhibited the most potent anti-dengue viral activity (IC_{50} value of 13.1 ± 0.2 µg/mL). This study's findings unravel the potential of the *Zingiberaceae* family as a source of antioxidant, anti-inflammatory, anticancer, and anti-dengue agents.

***Keywords*:** Bioactivity screening, Phytochemical screening, Total phenolic contents, Total Flavonoid contents, *Zingiberaceae.*

Introduction

Indonesia has the world's largest diversity of medicinal plants, with as many as 6000 species.[1] Local people have used medicinal plants as an alternative to herbal medicine for generations. The advantages of herbal medicines (natural products) are that they are considered less harmful and have abundant natural availability.[2] It is inseparable from several secondary metabolites isolated from medicinal plants.[3-5] Medicinal plant species generally contain secondary metabolites, such as steroids, phenolics, tannins, alkaloids, anthocyanins, flavonoids, and saponins. Previous studies have shown promising bioactivity of this group of compounds as antioxidants,[6] anti-inflammatory,[7] anticancer,[8] and anti-dengue agents.[9]

Lifestyle patterns with increased disease prevalence have a strong correlation. For example, consuming foods with preservatives, carbonated drinks, cigarette smoke, and pollution are implicated in many diseases. These factors cause increased oxidative stress and damage to cell metabolism.

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Some reactive molecules such as hydroxyl ($^{*}OH$), peroxide (ROO^{*}), and superoxide (O_2^{*-}) radicals can cause severe damage to cells and body tissues.[2] In addition, this condition causes increased levels of ROS (reactive oxygen species), which can cause inflammation and cell damage leading to cancers, as reported in some studies.[10]

Therefore, oxidative stress has become a major concern for researchers to find sources of antioxidants from natural products or synthetic materials in overcoming various diseases related to oxidative stress (inflammation and cancer).

Dengue Hemorrhagic Fever (DHF) is an endemic disease that has caused many deaths in various countries in Africa and Southeast Asia.[11-13] DHF is caused by the DENV virus that spreads through the bite of the *Aedes aegypti* and *Aedes albopictus* mosquitoes. The total number of cumulative DHF cases in 2022 was 21,689, an increase of 68.7% from 2021.[14] Based on this trend, it is expected that DHF cases may increase every year. Therefore, finding alternative treatments that can minimize cases of death by DHF is necessary. Several reports showed that secondary metabolites (flavonoids and alkaloids) could inhibit the replication of the DENV virus.[15-16]

This study focused on medicinal plant species from the *Zingiberaceae* family, which have been widely applied as herbal medicine by the Indonesian people since ancient times. The *Zingiberaceae* family comprises 50 genera and 1300 species in South and Southeast Asia.[17] Plants from the *Zingiberaceae* family grow in the tropics and subtropics. Indonesia is one of the countries that have the greatest diversity of plant species from the *Zingiberaceae* family. Other countries with the greatest species diversity from this family are Malaysia, Brunei Darussalam, Singapore, and Papua New Guinea.[19]

This family consists of a wide variety of very useful species. Several species are used as spices and are also used for medicine.[18] Species from the *Zingiberaceae* family commonly found in Indonesia are *Zingiber officinale var. Rubrum, Zingiber officinale* Roscoe, *Zingiber purpureum, Curcuma longa, Curcuma heyneana, Curcuma purpurascens, Curcuma aeruginosa, Curcuma xanthorrhiza,* and *Boesenbergia rotunda*. In addition, it has been known that several of these species have good bioactivity as an antioxidant,[19] anti-inflammatory,[20] anticancer,[21] and anti-dengue.[22]
In this work, the bioactivity screening of nine extracts from *Z. officinale var. Rubrum, Z. officinale* Roscoe, *Z. purpureum, C. longa, C. heyneana, C. purpurascens, C. aeruginosa, C. xanthorrhiza*, and *B. rotunda* of the *Zingiberaceae* family from Indonesia was carried out. The extracts' phytochemical screening, Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC) were evaluated, as well as their antioxidant, anti-inflammatory, anticancer against HeLa and PC3 cells, and anti-dengue potentials.

Materials and Methods

Sample Preparation of Zingiberaceae Family
The *Zingiberaceae* family plants used in this study include *Zingiber officinale* var. Rubrum, *Zingiber officinale* Roscoe, *Zingiber purpureum, Curcuma longa, Curcuma heyneana, Curcuma purpurascens, Curcuma aeruginosa, Curcuma xanthorrhiza,* and *Boesbergia rotunda*, obtained from Mount Lawu, Jogorogo, Ngawi, East Java, Indonesia, in June 2021. The plant specimens were determined as members of the Zingiberaceae family by YayasanGenerasiBiologi Indonesia with respective voucher numbers as follows:

326/02.Genbinesia/2022 : *Z. officinale* Roxb. var. Rubrum Roscoe
327/02.Genbinesia/2022 : *Z. officinale* Roscoe.
328/02.Genbinesia/2022 : *Z. purpureum* Roscoe
329/02.Genbinesia/2022 : *C. longa* L.
330/02.Genbinesia/2022 : *C. heyneana*Valeton&Zijp.
331/02.Genbinesia/2022 : *C. purpurascens* Blume
332/02.Genbinesia/2022 : *C. aeruginosa* Roxb.
333/02.Genbinesia/2022 : *C. xanthorrhiza*Roxb
334/02.Genbinesia/2022 : *Boesenbergia rotunda* (L.) Mansf.

The rhizomes of the plants were washed and dried, respectively. A total of 1 Kg of dry powder rhizome of each species was extracted by maceration using 3 L of methanol for 24 hours and repeated three times. The methanol extract of each sample was concentrated using a rotary vacuum evaporator at 50ºC to obtain concentrated crude extract.

Phytochemical Screening
Qualitative phytochemistry screening was conducted based on the reaction to the reagents.[23] Phytochemical screenings were carried out, such as steroid, phenolic, tannin, alkaloid, flavonoid, and saponin compounds.

Test for Steroids: To 1 mL of the extract was added acetic anhydride followed by concentrated H_2SO_4. The formation of blue and green colours indicates a positive test for steroids.

Test for Phenolics: One mL extract plus 10 drops of 1% $FeCl_3$. The presence of green, red, purple, blue, or dark black indicates the presence of phenolic compounds.

Test for Tannins: One mL of extract plus five drops of 10% NaCl, then filtered and added 1% gelatin and 10% NaCl. The formation of a white precipitate indicates the presence of tannins.

Test for Alkaloids: About 1 mL of extract was mixed with 2 mL of 2N HCl and then shaken and filtered. The filtrate was used to test for alkaloids using different alkaloidal reagents (Meyer's, Dragendorff's and Wagner's reagents). Yellow, red and brown or reddish precipitates indicate the presence of alkaloids to Meyer's, Dragendorff's and Wagner's reagents, respectively.

Test for Flavonoids: About 1 mL of extract was mixed with 3 mL of 70% ethanol, then shaken, heated, and then shaken again, and then filtered. To the filtrate obtained was then added 0.1 grams of Mg and two drops of concentrated HCl. The formation of red colour indicates the presence of flavonoids.

Test for Saponins: To 1 mL of extract was added to 2 mL of distilled water while shaking for 1 minute, and then two drops of 1N HCl were added. The formation of foams that persist for about 7 min indicates the presence of saponins.

Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC)
The determination of the TPC and TFC in nine plant extracts from the *Zingiberaceae* family was based on a previous method with a few modifications.[24] For the TPC determination, 25uL extract of each sample was transferred to a 96-well microplate, followed by 25 µL of Folin-Ciocalteau reagent and 75 µL of distilled water. The mixture was incubated in the dark for 5 minutes. Then, 100 µL of 7% Na_2CO_3 solution was added and incubated for 90 min. The absorbance was measured using a microplate reader at a λ_{max} of 753 nm. The TPC value was expressed in mg GAE/g extract.
The determination of TFC in the nine samples followed the procedure described for TPC.[24]. In this determination, 10 µL ($AlCl_3$ 10%) was transferred into the 96-well microplate containing 50 µL extract of each sample, followed by 150 µL (ethanol 96%) and 10 µL ($NaCH_3COO$ 1 M). The mixture was incubated for 40 minutes in a dark room. The absorbance measurements were carried out using a microplate reader at a λ_{max} of 430 nm. The TFC value was expressed in mg QE/g extract.

Antioxidant Assay
The antioxidant activity screening of the nine samples was done using the DPPH radical method previously described.[25] The methanol solution of 125 µM DPPH (250 µL) was added to 40 µL of each extract in a 96-well microplate. The mixture was incubated for 30 minutes in a dark room. The absorbance of the solution was measured with a microplate reader at a λ_{max} of 515 nm.
The antioxidant test using the ABTS radical method was carried out based on a previous method.[26] The ABTS solution (200 µL) was added to a 96-well microplate, followed by 10 µL of each extract. The mixture was incubated for 7 min and kept away from light. The absorbance of the mixture was measured at a λ_{max} of 730 nm.

ROS Anti-inflammatory Assay
The anti-inflammatory potential of the samples was carried out using the Luminol-enhanced chemiluminescence ROS anti-inflammatory method previously described.[27] A total of 25 µL of blood diluted with HBSS++ (Hanks Balanced Salt Solution, containing calcium chloride and magnesium chloride) was placed in a 96-well plate, and 25 µL of sample extract with various concentrations (1.0; 10.0, and 100.0 µg/mL) was added. The mixture was incubated at 37ºC for 15 minutes in the luminometer thermostat chamber. Afterwards, 25 µL of serum opsonized zymosan (SOZ) and 25 µL of intracellular reactive oxygen species detecting probe were added to each well. The blank used was the HBSS++ solution. ROS levels were recorded in luminometers in relative light units (RLU).

Anticancer Assay
The anticancer activity of the plant extracts from the *Zingiberaceae* family was carried out using the MTT method. The cancer cell cultures (HeLa and PC3) were placed in 96-well plates, and 100 µL of sample extract was added and incubated for 24 h. Then, 100 µL of MTT reagent (5 mg/mL PBS) was added to each well and incubated for 2-3 hours in a 5% CO2 incubator to form formazan crystals. After incubation, 100 µL SDS (10% in 0.1 N HCl) was added and incubated at room temperature without light for 24 hours. The absorbance was measured using an ELISA reader at a λ_{max} of 550 nm.[28-30]

Anti-DENV Assay
Anti-DENV test of the plant extracts was carried out using the Viral ToxGlo assay method. Vero cells (ATCC® CCL-81TM) were placed in a 96-flat bottom luminescence microplate. Each sample extract (25

µL) was added to the microplate at various concentrations. Then, 25 µL of DENV-2 stock (KT012509) Surabaya isolates with a 2 x 10^3 FFU/mL concentration were added. The incubation was conducted for 48 hours at 37ºC, with 5% CO_2. After incubation, 100 µL of Viral ToxGlo assay reagent was added and incubated for 10 min at 37ºC, 5% CO_2. The absorbance measurement was done using a GloMax reader on the luminescence menu.

Statistical Analysis

The data was repeated thrice (n = 3), and Origin®2018 software was used to perform calculations and analysis.

Results and Discussion

Phytochemical screening for secondary metabolites

In this work, nine plant extracts from the *Zingiberaceae* family were obtained from Mount Lawu, Jogorogo, Ngawi, East Java, Indonesia, at an altitude of 611 masl (Figure 1). The study aimed to identify the content of secondary metabolites in the rhizome extracts and their bioactivity. The local communities use these plant extracts for wound infections, fever, and malaria. Several applications of plant rhizome are closely related to bioactivity, such as antioxidant, anti-inflammatory, anticancer, and anti-dengue. Therefore, the extraction process was focused on that part of each plant of the *Zingiberaceae* family.

The phytochemical screening of the nine plant extracts revealed the presence of different secondary plant metabolites: steroid, phenolic, tannin, alkaloid, flavonoid, and saponin (Table 1). Tannins and alkaloids were present in all the samples investigated; however, only *C. longa* extract showed positive results for the array of secondary metabolites investigated.

Table 1: Phytochemical constituents of the *Zingiberaceae* family from Indonesia

***Zingiberaceae*species**	**Steroid**	**Phenolic**	**Tannin**	**Alkaloid**	**Flavanoid**	**Saponin**
Z. officinale var. Rubrum	-	+	+	+	+	-
Z. officinale Roscoe	-	-	+	+	+	+
Z. purpureum	+	+	+	+	+	-
C. longa	+	+	+	+	+	+
C. heyneana	-	-	+	+	+	+
C. purpurascens	-	-	+	+	+	+
C. aeruginosa	+	-	+	+	+	+
C. xanthorrhiza	+	-	+	+	-	+
B. rotunda	+	+	+	+	-	-

(+) positive samples contain secondary metabolites, (-) negative samples contain secondary metabolites

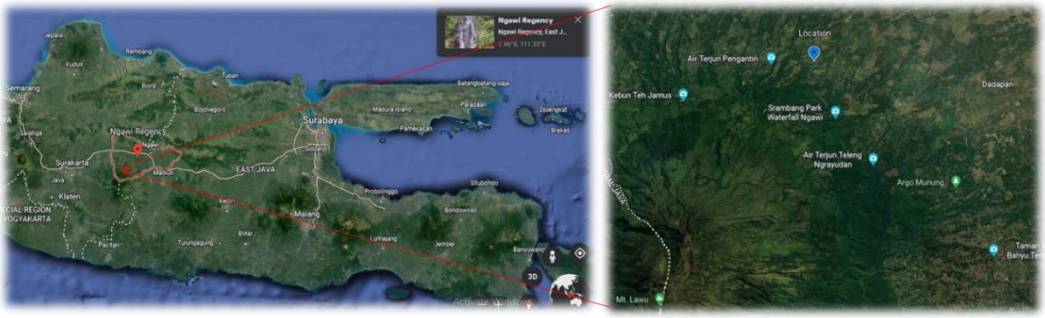

Figure 1: Sampling locations of nine *Zingiberaceae* plant species

Total phenolic contents (TPC) and total flavonoid contents (TFC)

TPC and TFC are initial indicators in identifying levels of bioactive compounds that are beneficial to humans. The bioactive compounds in high amounts in plants informed their use in developing herbal medicines. Several reports show that the correlation between TPC and TFC is closely related to antioxidant, anti-inflammatory, and anticancer activities.[31-32]

TPC was used to determine the content of phenolic compounds in plant extracts expressed in gallic acid equivalents per gram of extract (GAE/g extract).[31] Conversely, TFC was used to determine the total flavonoid content in selected plant extracts. The TFC value was expressed in quercetin equivalents per gram of extract (QE/g extract).[31] TPC and TFC were measured in the nine selected extracts from the *Zingiberaceae* family (Figures 2 and 3).

The nine extracts have the same TPC values ranging from 10-11 mg GAE/g extract. However, of the nine extracts, *C. longa* extract had the highest TFC value (1.72 ± 0.09 mg QE/g extract) compared to others. The other extracts have TFC values ranging from 0.45 – 0.44 mg QE/g extract. These results suggest that the content of flavonoids is not dominant in each extract from the *Zingiberaceae* family. It is similar to reports from previous research on these plants, which showed higher TPC than TFC.[33] It is hoped that the analysis of these variables will provide an initial indicator to see the potential of *Zingiberaceae*family plants as a source of herbal medicine.

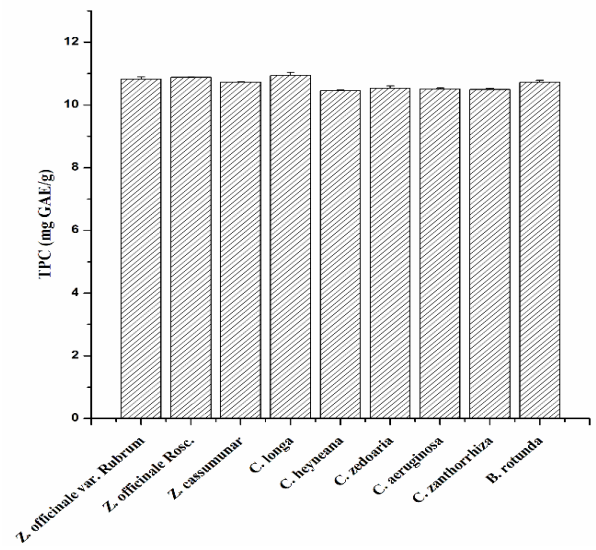

Figure 2: Comparison of total phenolic contents (TPC) in nine plant extracts from the *Zingiberaceae* family

Antioxidant activity

The antioxidant activity of nine extracts from the *Zingiberaceae* family was investigated. The antioxidant activity was tested by two methods, namely DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2-casino-bis (3-ethylbenzothiazoline-6-sulfonic acid). The DPPH assay method determines antioxidant activity based on the DPPH radical reduction reaction that leads to the formation of diphenylhydrazine.[34] Meanwhile, the ABTS assay method determines antioxidant activity based on the oxidation reaction of potassium persulfate with ABTS diammonium salt.[33] The comparison of the IC_{50} values of the extracts against DPPH and ABTS radicals is presented in Table 2. The best potential antioxidant activity against DPPH radicals with the smallest IC_{50} value is the *Z. officinale*Rosc extract with an IC_{50} value of 233.0 ± 7.9 µg/mL. Similar to the DPPH test, *Z. officinale* Roscoe shows the smallest IC_{50} values (320.2 ± 27.4 µg/mL) against ABTS radicals.

The plant extracts studied showed potential antioxidant properties due to the phenolic content contained therein. Specifically, the extract of *Z. officinale* Roscoehas better antioxidant activity in counteracting DPPH and ABTS radicals than the other extracts. It is possible because other antioxidant components in plant tissues are responsible for unusual redox processes.[31] Some components reported are tannin, anthocyanins, alkaloids, and pro-anthocyanins.[36-37]

Table 2: Comparison of antioxidant activity of plant extracts using DPPH and ABTS methods

***Zingiberaceae* species**	**IC_{50} ± SE (µg/mL)**	
	DPPH	**ABTS**
*Z.officinale*var. Rubrum	250.2 ± 5.1	380.8 ± 17.2
*Z.officinale*Roscoe	233.0 ± 7.9	320.2 ± 27.4
Z. purpureum	622.8 ± 27.7	461.6 ± 2.6
C. longa	366.0 ± 0.6	419.9 ± 17.7
C. heyneana	NA	NA
C. purpurascens	383.1 ± 8.6	359.6 ± 0.9
C. aeruginosa	NA	NA
C. xanthorrhiza	NA	476.1 ± 11. 2
B. rotunda	745.5 ± 33.5	838.4 ± 13.9
Vitamin C	20.3 ± 3.70	29.5 ± 4.21

NA, not applicable

Anti-Inflammatory Activity

The anti-inflammatory process of measuring plant extracts was seen from the level of ROS (Reactive Oxygen Species) recorded in the luminometer. The results showed that five of the nine plant extracts tested for anti-inflammatory activity have promising activity. *C. xanthorrhiza*extract showed the best activity with an IC_{50} value of 1.7 ± 0.0 µg/mL, followed by *C. longa* extract (2.4 ± 0.0 µg/mL), *Z. purpureum* (6.0 ± 0.5 µg/mL), and *Z. officinale* var. Rubrum (6.1 ± 1.3 µg/mL). Meanwhile, *Z. officinale* var. Rubrum showed moderate antioxidant activity (19.8 ± 2.4 µg/mL). The remaining extracts, *C. heyneana, C. purpurascens, C. aeruginosa,* and *B. rotunda,* did not show anti-inflammatory activity. The IC_{50} values for each extract are presented in Table 3.

Table 3: Comparison of anti-inflammatory activity of plant extracts

***Zingiberaceae* species**	**IC_{50} ± SD (µg/mL)**
Z. officinale var. Rubrum	19.8 ± 2.4
Z. officinale Roscoe	6.1 ± 1.3
Z. purpureum	6.0 ± 0.5
C. longa	2.4 ± 0.0
C. heyneana	NA
C. purpurascens	NA
C. aeruginosa	NA
C. xanthorrhiza	1.7 ± 0.0
B. rotunda	NA
Ibuprofen	11.2 ± 1.7

NA, not applicable

This study has shown that the intake of antioxidants can reduce the natural risk of several diseases, such as inflammation and cancer. Traditionally, local Indonesians use *C. xanthorrhiza* and *C. longa*powder as medicine to enhance the healing of wounds. These benefits cannot be separated from the activity of *C. xanthorrhiza* and *C. longa* as potential anti-inflammatory agents (Table 3). Anti-inflammatory testing aims to test the potential of plant extracts on the physiological response to tissue injury triggered by physical contact, chemical toxicity, and microbial agents.[38] Determination of ROS levels helps determine the effectiveness of plant extracts from the *Zingiberaceae* family on inflammatory cell activity. It should be noted that ROS (reactive oxygen species) radicals result from cell metabolism

that causes inflammation. The phenolic compounds in each extract can reduce ROS levels so that cells are protected from excessive inflammatory processes.

Anticancer activity

Anticancer activity was tested against HeLa and PC3 cell lines using the MTT method. The MTT method works based on the conversion of the tetrazolium salt (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide or commonly called the MTT salt) to formazan by the succinate dehydrogenase enzyme in the mitochondria.[39] A plant extract is active against cancer cells if it has IC_{50} < 20 µg/mL, moderately active if 20 µg/mL < IC_{50} > 100 g/mL, and inactive if IC_{50} >100 µg/mL.[40]

Z. officinale var. Rubrum extract shows the highest anticancer activity against the HeLa cell line with an IC_{50} value of 10.4 ± 1.6 µg/mL (Table 4). This was followed by *Z. officinale* Roscoe and *B. rotunda* active against HeLa cells with IC_{50} values of 14.9 ± 0.8 and 18 ± 1.4 µg/mL, respectively. Besides, *C. longa* and *C. xanthorrhiza* showed moderate activity against HeLa cells (IC_{50} = 20.4 ± 1.6 and 21.1 ± 0.7 µg/mL, respectively). Meanwhile, *Z. purpureum*, *C. heyneana*, *C. purpurascens*, and *C. aeruginosa* were inactive against HeLa cells. *C. longa* exhibited the highest anticancer activity against the PC3 cell line with an IC_{50} value of 7.3 ± 0.3 µg/mL. The other plant extracts with strong cytotoxicity against the PC3 cell line are *Z. officinale* var. Rubrum, *Z. officinale* Roscoe, and *C. xanthorrhiza* with IC_{50} values of 11.9 ± 1.3, 11.1 ± 1.4, and 13.7 ± 0.8 µg/mL, respectively (Table 4). Extracts of *B. rotunda* showed moderate activity. The *Z. purpureum*, *C. heyneana*, *C. purpurascens*, and *C. aeruginosa* extracts were inactive against PC3 cells.

The significant anticancer activity of *C. longa* extracts against the PC3 cancer cells with IC_{50} < 10.0 µg/mL may be due to the higher TFC content than other extracts (Figure 3). Previous reports indicate that flavonoids play an active role as an anticancer.[41-43] However, several other compounds in the extract, such as alkaloid, phenolic, and curcumin, may have anticancer activity.

Table 4: The anticancer activity of plant extracts against HeLa and PC3 cell lines

***Zingiberaceae* species**	**IC_{50} ± SD (µg/mL)**	
	HeLa cell line	**PC3 cell line**
Z. officinale var. Rubrum	10.4 ± 1.6	11.9 ± 1.3
Z. officinale Roscoe	14.9 ± 0.8	11.1 ± 1.4
Z. purpureum	Inactive	Inactive
C. longa	20.4 ± 1.6	7.3 ± 0.3
C. heyneana	Inactive	Inactive
C. purpurascens	Inactive	Inactive
C. aeruginosa	Inactive	Inactive
C. xanthorrhiza	21.1 ± 0.7	13.7 ± 0.8
B. rotunda	18 ± 1.4	21.3 ± 0.3
Doxorubicine	2,68 ± 0,61	1.9 ± 0.08

Anti-Dengue

The analysis process was carried out by looking at the values of several variables, such as cytotoxic concentration (CC_{50}), minimum inhibition concentration (IC_{50}), and selectivity index (SI). The evaluation criteria for extracts with good anti-dengue activity are indicated by SI values > 10.[22.] A higher SI value shows the extract's ability to kill the virus before the host cell. Thus, this selectivity value becomes an important variable to be measured. In detail, the results of the analysis are provided in Table 5. It can be seen that the inhibitory value of *C. longa* (IC_{50}: 13.1 ± 0.2 µg/mL) is the most effective among the nine extracts tested. However, all extracts showed a low selectivity value of < 10.

DENV virus can thrive in various cells, such as mammalian and insect cells. In this work, we investigated the inhibitory activity of the DENV virus on the Vero cells of each extract. The *C. longa* extract showed significantly higher anti-dengue activity against the DENV virus than other extracts (Table 5). It may be due to the high TFC content of *C. longa* compared to other extracts (Figure 3). This statement is supported by a previous study which explains that flavonoid compounds have good activity in inhibiting the DENV virus.[15]*C. longa* is a curcumin plant group with a class of curcumin compounds with promising dengue virus inhibitory activity.[22] Unfortunately, the selectivity of *C. longa* shows a negative value. It has a CC_{50} value, which is similar to the IC_{50} value. The same results also apply to all plant extracts from the *Zingiberaceae* family, which show low selectivity values of 0.2-0.9 (SI < 10).

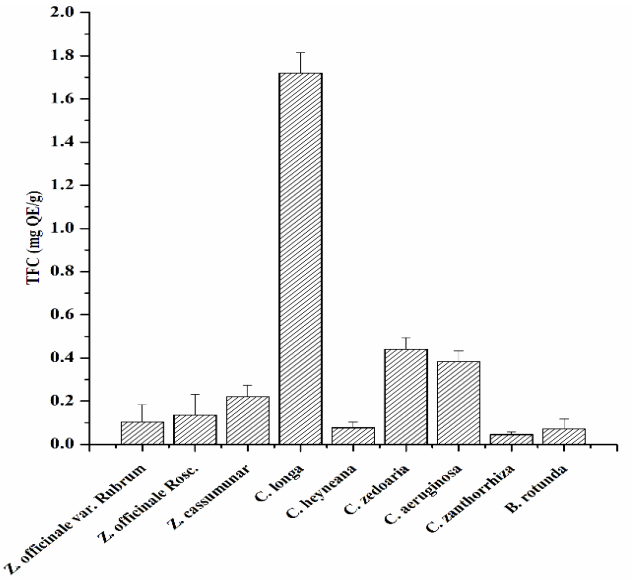

Figure 3: Comparison of total flavonoid contents (TFC) in nine plant extracts from the *Zingiberaceae* family.

Table 5: Comparison of the anti-dengue activity of plant extracts

***Zingiberaceae* species**	**CC_{50} ± SD (µg/mL)**	**IC_{50} ± SD (µg/mL)**	**SI ± SD**
Z. officinale var. Rubrum	14.2 ± 0.0	23.9 ± 3.0	0.6 ± 0.0
Z. officinale Roscoe	22.2 ± 0.1	24.4 ± 0.5	0.9 ± 0.0

Z. purpureum	17.4 ± 0.1	45.4 ± 5.8	0.3 ± 0.0
C.longa	12.5 ± 0.1	13.1 ± 0.2	0.9 ± 0.0
C. heyneana	21.2 ± 0.2	46.5 ± 4.7	0.4 ± 0.0
C. purpurascens	6.7 ± 0.0	25.4 ± 1.6	0.2 ± 0.0
C. aeruginosa	15.4 ± 0.0	23.8 ± 2.2	0.6 ± 0.0
C. xanthorrhiza	15.6 ± 0.1	22.1 ± 1.3	0.7 ± 0.0
B. rotunda	26.1 ± 0.0	27.3 ± 0.3	± 0.0

Conclusion

The bioactivity test revealed differences in the activity of each extract from nine plants of the Zingiberaceae family that play an active role as an antioxidant, anticancer, anti-inflammatory, and anti-dengue agents. The Zingiberaceae family is rich in phenolic compounds with various bioactivities that can be developed as raw materials for herbal medicines. This study provides useful information for the wider application of the Zingiberaceae family in managing various diseases, including cancers, inflammations, etc. Additionally, this study provides a crucial foundation for future studies on herbal medicine development.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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