BIOTROPIA ISSN: 0215-6334 | e-ISSN: 1907-770X
The Southeast Asian Journal of Tropical Biology
Vol. 31 No. 2, 2024: 253 - 265
DOI: 10.11598/btb.2024.31.2.2165

Research Article

# THE POTENCY OF CINNAMON (*Cinnamomum burmanni* Blume) LEAF EXTRACT AS A BIOPRESERVATIVE AGENT FOR FOOD SAFETY OF *SATE LILIT*

Ida Bagus Gede Darmayasa[1]*, Yan Ramona[1,2], Anak Agung Ketut Darmadi[1], I Wayan Suanda[3] Ni Luh Hani[1], and Kalidas Shetty[4]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Udayana University, Bukit Jimbaran Campus, Badung 80361, Indonesia. [2]Integrated Laboratory for Biosciences and Biotechnology, Udayana University, Bukit Jimbaran Campus, Badung 80361, Indonesia. [3]Department of Biology Education, Faculty of Teacher Training and Education, University of PGRI Mahadewa, Denpasar 80235, Indonesia. [4]Global Institute of Food Security & International Agriculture, North Dakota State University, North Dakota, USA

## ARTICLE HIGLIGHTS

- Cinnamon leaf extract combats *E. coli* in traditional Balinese food.
- Natural preservative reduces harmful pathogens in sate lilit.
- Cinnamon extract offers a safer alternative to synthetic preservatives.
- Active compounds in cinnamon leaves inhibit bacterial growth.
- Effective biopreservative for enhancing food safety and quality.

**Article Information**

Received 16 January 2024
Revised 28 April 2024
Accepted 6 May 2024

***Corresponding author, e-mail:**
darmayasa@unud.ac.id

## ABSTRACT

*Escherichia coli* O157:H7 contamination of the meat used in preparing *sate lilit*, a favorite traditional food in Bali, Indonesia, has been a great concern for both local people and foreigners. Although *C. burmanni* has been included in its spice ingredients, active compounds that play a significant role in this pathogen have limitedly been elucidated. The main objectives of this research were to investigate the potency of this plant to control contaminants and elucidate possible compounds that prevent such contaminants by applying the disk diffusion method and LCMS analysis, respectively. The results showed that the leaf extract of this plant inhibited the *in vitro* growth of *E. coli* O157:7, with minimal inhibitory concentration (MIC) and $LC_{50}$ values of 4% and 2.59%, respectively. The LCMS analysis chromatogram showed that the plant extract's most active fraction produced nine peaks, representing nine possible active compounds. Among those, three compounds (*Azoxystrobin, Stigmatellin Y,* and *2-arachidonoyl glycerol*) were suspected of contributing to control contamination, especially by *E. coli* O157:H7.

**Keywords:** *2-arachidonoyl glycerol, azoxystrobin, biopreservative, cinnamon, e. coli O157:H7, phenolic compounds, sate lilit, stigmatellin*

## INTRODUCTION

Being one of the tourist islands in Indonesia, Bali is rich in various types of ethnic foods. Such kinds of foods (traditionally served along the streets in small stalls or modern restaurants) can easily be found in areas of tourist destinations in Bali. A specific characteristic of Balinese ethnic foods is the spicy ingredients used in the preparation. *Sate lilit* is a traditional Balinese Kebab and a favorite food in Bali, enjoyed by both locals and foreigners. *Sate lilit* has also been part of offerings in the Balinese religious rituals, such as temple festivals, Balinese wedding ceremonies, etc. The meat (beef, pork, chicken, or fish as the primary raw material) of the *sate lilit* is frequently contaminated by pathogenic microbes (Palupi et al, 2019) that can damage the quality of the *sate lilit*. These contaminated meats often become a medium for the pathogen to spread and cause food poisoning (foodborne disease outbreak).

Some researchers found that beef (a primary raw material of *sate lilit*) traded in traditional markets in Bali is frequently contaminated by pathogenic bacteria. For example, Purni et al. (2020) reported that 39 beef samples collected from some traditional markets located in Denpasar City, Badung, and Klungkung regencies were contaminated by *E. coli*. Among those samples, 25.6% were infected by *E. coli* O157:H7, the causative agent of hemorrhagic diarrhea, due to its ability to produce Shiga-like toxins (Patil et al., 2022). Similar results were also reported by Pinatih et al., (2021), who stated that this serotype of *E. coli* O157:H7 has been well-known to produce potent toxins identical to those

made by *Shigella dysentery* type 1, and therefore it is often referred to as *Shiga like a toxin*. As *sate lilit* is not fully cooked, it has the potential to spread pathogenic microbes that lead to an outbreak.

The application of synthetic chemical-based preservatives has not been encouraged to cope with such pathogenic microbial contamination due to their many harmful side effects on human health. According to the Indonesian Food and Drug Administration (BPOM), food poisoning was considered to be the highest case of poisoning in Indonesia in 2012. Approximately 66.7% of such poisoning cases were due to the consumption of foods containing poisonous preservatives, such as formalin, boric acid, or textile dyes (Utomo dan Kholifah, 2018). Based on those food poisoning cases due to the application of inappropriate preservative agents, the possible use of natural preservative agents extracted from plants has been intensively researched so that some alternative and safe food preservations can be invented.

The bark and leaf extracts of *C. burmanni* Blume have been applied as flavor enhancers in various foods in Indonesia. *C. burmanni* Blume is a wooden plant. Its height may reach 50 meters (Sujarwo and Keim, 2020). According to Djarot et al. (2023), this plant's leaves and bark contain high levels of flavonoids, saponin, tannin, and alkaloids. Mohamed et al. (2020) reported that the bark extract of this plant has the potential as an antibacterial agent. This is due to the antimicrobial effect of its essential oil, containing several active compounds, such as cinnamaldehyde, eugenol, cinnamic acid, and cinnamate (Plumeriastuti et al., 2019). The potential of *C. burmanni* essential oil to inhibit *Staphylococcus aureus, Enterobacter spp., Pseudomonas aeruginosa, and Candida albicans* was also reported by Novita and Sutandhio (2021). This background has opened the door for its use as an alternative agent in developing food preservatives.

Based on the above rationale, it is urgently needed to elucidate active compounds in the *C. burmanni* leaf extract that may play an essential role in controlling *E. coli* O157:H7 contamination before its use as an organic food preservative. This study focused on the leaf extract of the plant, because this part of the plant has been widely used as an important ingredient of *sate lilit* preparation in Bali. The results of our current research expectedly can be used to develop alternative food preservation methods to control pathogenic microbes (*E. coli* O157:H7 in particular) in ethnic Balinese food products.

## MATERIALS AND METHOD

### Extraction of *C. burmanni* Blume leaves

Mature leaves (located on rows of 4-9 from tips of branches) of *C. burmanni* Blume were collected from Bedugul village, Tabanan-Bali. These leaf samples were chopped, air dried at ambient temperature (25 – 33°C), powdered with a warring blender, macerated in 96% ethanol (Sigma Aldrich, Germany) in a ratio of 1:10 w/v, and filtered with 4 layers of Whatman no. 2 filter papers (pore size 8 µm). The filtrate was then evaporated in a vacuum rotary evaporator at 40°C to obtain crude extract/ pellet of the leaf extract and stored at 4°C before being used in the subsequent experiments. The crude extract obtained from this evaporation was considered to be 100% concentration.

### Preparation of *C. burmanni* Blume crude extract

The crude extract of the plant leaves was diluted with 96% ethanol (Sigma-Aldrich, Germany) to obtain final concentrations of 20% (w/v), 15% (w/v), 10%, and 5% (w/v) by applying the following dilution formula:

$$V_1 . M_1 = V_2 . M_2$$

Where:

$V_1$ = Volume of the most concentrated extract (mL)

$V_2$ = Volume of the adjusted extract concentration (mL)

$M_1$ = Concentration of the most concentrated extract (% w/v)

$M_2$ = The adjusted extract concentration (% w/v).

### Preparation of *E. coli* O157:H7 cell suspension

The strain of *Escherichia coli* O157:H7 was obtained from the stock culture collection of the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Udayana University. Pure culture of *E. coli* O157:H7 (1 loopful) was inoculated into 100 mL of Nutrient Broth medium (Oxoid) and incubated for 24 hours at 37°C. The density of this bacterial suspension was then adjusted to 0.5 McFarland scale or equal to $10^8$ cells/mL.

## Bioassy (*in vitro* inhibition tests) of the *C. burmanni* Blume leaf extract on *E. coli* O157:H7

*In vitro* inhibition tests of the *C. burmanni* Blume leaf extract on *E. coli* O157:H7 were conducted by applying the method of Kirby Bauer (1966) with minor modifications. Suspension of *E. coli* O157:H7 amounted at 200 µL was homogenized in 15 mL nutrient agar medium (Oxoid) and poured into a sterile petri dish to produce bacterial lawn. Once the mix was settled, a preliminary bioassay was conducted on the crude extract of this plant at high concentrations (25%, 50%, and 75% w/v) to determine anti-bacterial activity of the crude extract. The lowest concentration of this crude extract that inhibited the bacterial lawn was then used as a reference to determine the crude extract concentrations applied in the main experiments. As the inhibition zone already happened at 25% (w/v), the *C. burmanni* Blume leaf extract concentrations used in the experiments were 5, 10, 15, and 20% w/v. In these bioassays, paper disks previously deposited with 20 µL *C. burmanni* Blume leaf extract at those various concentrations (5, 10, 15, and 20% w/v) were placed at equidistance on the surface of this bacterial lawn to allow active compounds of the plant extract to diffuse. The same volume of 96% ethanol (Sigma-Aldrich, Germany) and 1% w/v of chloramphenicol (a product of Kalbe Farma, Indonesia) in paper disks served as negative and positive controls, respectively. Four replicates were prepared in this bioassay to obtain representative data. All plates were incubated for 24 hours at 37°C and measured for formation of inhibition zones around the paper disks, previously deposited with the plant extract. Measurements of inhibition zones around each paper disk were conducted from 4 different angles and the results were averaged.

## Determination of minimal inhibitory concentration (MIC) value of the *C. burmanni* Blume leaf extract on *E. coli* O157:H7

MIC value of the leaf plant extract was determined by applying the same method (Kirby and Bauer, 1966) mentioned above. The concentrations of the plant extract were adjusted from 0% to 5% w/v with interval increment of 1% w/v. Four replicates were prepared in this bioassay to obtain representative data. All plates were incubated for 24 hours at 37°C and measured for formation of inhibition zones around the paper disks (CT0998B) deposited with the plant extract. Measurements of inhibition zones around each paper disk were conducted from 4 different angles and the results were averaged.

## Determination of *Lethal Concentration* 50% ($LC_{50}$) of the *C. burmanni* Blume leaf extract on *E. coli* O157:H7

Bacterial suspension prepared above was diluted in serial dilution method so that cell with density of $10^3$ cells/mL was obtained. A volume of 100 µL of this diluted cell suspension (100 cells) was exposed in Eppendorf tubes for 15 minutes to the *C. burmanni* Blume leaf extract of the same concentrations with those applied in the MIC test. These exposed cell suspensions were then homogenized in nutrient agar medium (Oxoid), poured into petri dishes, incubated at 37°C for 24 hours, and counted for colonies appeared on the surface of the medium. The results were then plotted in a graph that shows relationship between total colony counted and concentration of *C. burmanni* Blume leaf extract (% b/v). The value of the $LC_{50}$ was determined from the curve or calculated using the equation of the regression.

## Phytochemical analysis of the *C. burmanni* Blume leaf extract

### Alkaloid test

Leaf extract of the *C. burmanni* Blume was dissolved in 10 mL of chloroform-ammonia solution (Sigma-Aldrich, Germany), added with 0.5 mL 1N $H_2SO_4$ (1 N), homogenized, and let it to form 2 layers. The upper layer was collected and added with 1 drop of Meyer reagent (Sigma-Aldrich, Germany). The formation of precipitation indicates positive result (Dey et al., 2020).

### Flavonoid and phenolic tests

These tests followed the method specified in Aryal et al. (2019). The *C. burmanni* Blume leaf extract was dissolved in 70% ethanol, heated, and filtered. The filtrate was then placed on a plate with Mg and 1N HCL (Merck) for the flavonoid test and $FeCL_3$ (Merck) for the phenolic test. The formation of red color and green to purple ring indicated positive results for flavonoid and phenolic tests, respectively.

### Steroid and terpenoid tests

The *C. burmanni* Blume leaf extract was added with chloroform, heated for 10 minutes, placed on a plate, and added with Liebermann-Burchard reagent. A positive result for the terpenoid test was indicated by formation of red, pink, or violet color, while the steroid test was indicated by the formation of green or pink color (Malik, 2023).

### Saponin test

A weight of 0,1g leaf extract of the *C. burmanni* Blume was dissolved in 5 mL hot distilled water and shaken for 10 seconds. A positive test for saponin test was indicated by the formation of stable foam for 10 seconds (Depkes RI, 2009).

### Tannin test

Leaf extract of the *C. burmanni* Blume amounted to 0,1 g and was added with $FeCl_{3,}$ (Merck) and then it was left to react for 5 minutes. The positive test was indicated by the formation of a blue color (Sri Kosnayani et al., 2019).

### Elucidation of active compounds contained in the Leaf extract of the *C. burmanni* Blume

Fractionation and purification processes of active compounds contained in the crude extract of *C. burmanni* Blume were conducted by applying column chromatography and thin layer chromatography (TLC), respectively. A 1g crude extract previously obtained was fractionated in column chromatography, where 300 g silica gel 60 (Merck, CT1.07733) was used as the stationary phase. The silica gel was first suspended in an eluent of n-hexane (Merck) as the mobile phase of the system. This silica gel suspension was poured into a column and acclimatized for 24 hours. The crude extract (1g) was dissolved in 1 mL, added with 1g silica gel, poured into the previously prepared column (length and diameter of 300 mm and 20 mm, respectively), and then eluted with a gradual change in the polarity of solvents. A volume of 25 mL *eluent* (with fractions of active compounds) flowing along the column was collected, evaporated, and analysed with a thin layer chromatography (TLC). Fractions with the same Rf values were composited and tested for their inhibitory activity against *E. coli* O157:H7 by disc diffusion (Kirby-Bauer, 1966). Fractions with the ability to produce inhibition zones were subsequently analysed using the LCMS (*Liquid Chromatography Mass Spectroscopy*, Shimadzu VP series SPD-10A VP Uv Vis Detector HPLC LC MS Chromatography) method, and the peaks that appeared were aligned with those produced by known compounds following the application of the MassLynx V4.1 software linked with the ChemSpider program.

### Data analysis

Quantitative data was analyzed with *analysis of variance* (ANOVA) at $p<0.05$ using *SPSS software for Windows version* 17.0. When significant difference was indicated at $p<0.05$, the statistical test was further conducted by applying the *Duncans Multiple Range Test* (DMRT) at $p<0.05$.

## RESULTS AND DISCUSSIONS

### *In vitro* inhibitory activity of *C. burmanni* Blume on *E. coli* O157:H7

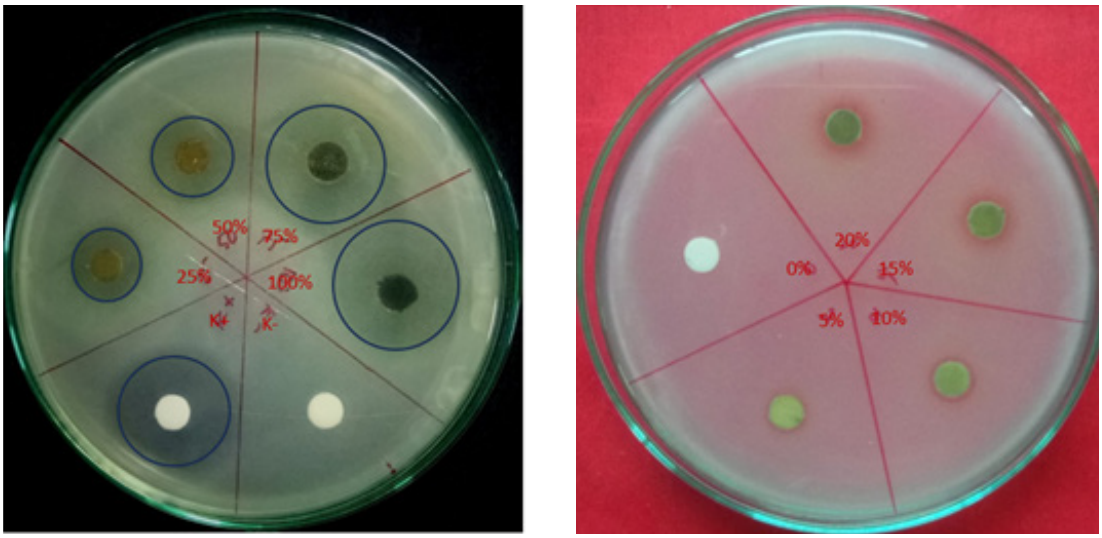
All concentrations applied in the preliminary bioassay experiments were found to inhibit the growth of *E. coli* O157:H7 with various inhibitions. The diameter of inhibition zones was proportional to crude extract concentrations (Table 1 and Figure 1). These results provided us with information on the toxicity level of this plant crude extract on the *E. coli* O157:H7. As the negative control did not inhibit the growth of the *E. coli* O157:H7, the clear zones formed around the disks deposited with the plant crude extract were confirmed to be due to active compounds contained in the plant crude extract. As shown in Table 1, the diameter of inhibitions of the plant crude extract applied at concentrations of between 25% w/v – 100% w/v was slightly different from that produced by positive control, although they are statistically different at $p<0.05$. This indicated that the toxicity level of the crude extract was comparable with that of the 1% w/v of chloramphenicol.

The *C. burmanni* Blume leaf crude extract also inhibited the growth of *E. coli* O157:H7 when applied at concentrations lower than 25% w/v. The results are shown in Table 2 and Figure 1B. The inhibitory effect of the plant extract was still observed when the extract was applied at 5%w/v.

Table 1 Average diameter of inhibition of the *C. burmanni* Blume leaf extract in the preliminary determination of appropriate concentrations to control *E. coli* O157:H7

| Extract concentrations (%) | Diameter of inhibition zones* |
|---|---|
| 100 | 21.08±0.04$^{e}$ |
| 75 | 19.53±0.05$^{d}$ |
| 50 | 18.02±0.05$^{c}$ |
| 25 | 17.21±0.06$^{b}$ |
| 0 | 0.00 ±0.00$^{a}$ |
| Positive control | 20.66±0.05$^{f}$ |

*Values in Table 1±standard deviations are averages of 4 replicates. Values followed by different letters are statistically different at p<0.05 based on DMRT analysis following Anova. Positive control is 1% chloramphenicol.




A B

Figure 1 Diameters of inhibition zones of the *C. burmanni* Blume leaf extract on *E. coli* O157:H7 obtained from preliminary bioassay (high concentrations of the plant extract) on nutrient agar medium (A); Diameters of inhibition zones of the *C. burmanni* Blume leaf extract on *E. coli* O157:H7 obtained from the main experiment of bioassay (concentrations of 5, 10, 15, and 20% w/v) on nutrient agar medium (B).

The result shown in Table 2 was consistent with those found in the preliminary study of the extract bioassay (Table 1). This led us to investigate the minimum inhibitory concentration (MIC) of the *C. burmanni* Blume leaf extract on *E. coli* O157:H7 by applying the same method with adjusted concentrations of between 0% and 5% w/v with an increment concentration of 1%, and the results are shown in Table 3. Based on this bioassay, the MIC value of the extract could be claimed at 4%, although it might fall between 3 and 4% w/v if the bioassay was continued by applying the plant extract concentrations between this range.

As the scientific evidence of this plant's antimicrobial activity is still limited, we focused our study on elucidating active compounds (with the potential to inhibit the growth of *E. coli* O157:H7) contained in this plant extract. *In vitro* bioassays of this plant extract in our study showed that the crude extract of this plant inhibited the growth of this pathogen when applied at a low concentration of 5% or more (Tables 1- 3 and Figure 1). Scientists such as Parisa et al. (2019) also reported a similar phenomenon, who found *in vitro* microbial activity of this plant extract inhibitory against *E. coli* and *Staphylococcus aureus*. Didehdar et al. (2022) have also recently reviewed extensively the potential of this plant as a new therapeutic agent to inhibit biofilm formation by pathogenic fungi or bacteria in the early stages of their infection. This indicates that active compounds inhibitory to pathogenic bacteria or fungi exist in this plant extract.

Table 2 Inhibitory effect of the *C. burmanni* Blume leaf extract on *E. coli* O157:H7 when applied at low concentration (between 5% - 20% w/v) on nutrient agar medium

| Extract concentrations (%) | Diameters of inhibition zones* |
|---|---|
| 20 | 11.07± 0.05$^{e}$ |
| 15 | 9.05± 0.05$^{d}$ |
| 10 | 8.24± 0.05$^{c}$ |
| 5 | 7.56± 0.06$^{b}$ |
| 0 | 0.00 ± 0.00$^{a}$ |
| Positive control | 20.66± 0.06$^{f}$ |

*Values in Table 1±standard deviations are averages of 4 replicates. Values followed by different letter(s) are statistically different at p<0.05 based on DMRT analysis following Analysis of variance (ANOVA). The positive control is 0.15 mg chloramphenicol.

Table 3 Averages of inhibition zone diameters of *C. burmanni* Blume leaf extract on *E. coli* O157:H7 when applied at a concentration of between 0% - 5% w/v on nutrient agar medium to determine MIC value.

| Extract concentrations (%) | Diameters of inhibition zones* |
|---|---|
| 5 | 7.56± 0.06$^{b}$ |
| 4 | 6.11± 0.02 $^{c}$ |
| 3 | 0.00±0.00 $^{a}$ |
| 2 | 0.00± 0.00 $^{a}$ |
| 1 | 0.00± 0.00 $^{a}$ |
| 0 | 0.00 ± 0.00$^{a}$ |

*Values in Table 1±standard deviations are averages of 4 replications. Values followed by different letter(s) are statistically different at p<0.05 based on DMRT analysis following Analysis of variance (ANOVA).

This plant extract appeared to be highly potent as its MIC value was very low (at 4% w/v concentration, which was deposited on filter paper at a volume of 20 µL). When applied at 100% concentration, the *C. burmanni* Blume leaf extract produced a diameter of inhibition higher than 20 mm, as shown by our study's positive control (1% chloramphenicol). Based on the diameter of the inhibition zone, the effectiveness of this plant crude extract in controlling *E. coli* O157:H7 can be categorized as very high, according to the criteria made by Rastina et al. (2018). In all cases (Table 1 – 3), the negative control did not show an inhibition zone, indicating that the inhibition phenomenon shown by the crude extract of this plant (applied at various concentrations) must be due to the presence of compounds inhibitory against *E. coli* O157:H7.

*Cinnamomum burmanni* Blume (in Bali, traditionally named *kayu manis*) can abundantly be found in Indonesia as it is widely used as a component in many traditional cuisines in this country, including in the ethnic foods of Bali. In Bahasa, this plant is known as *cinnamon* or *Padang cinnamon*. This plant has been included in many popular Indonesian foods or beverages, such as rendang (the most popular slow-cooked meat from Padang, west Sumatra), Bali coffee, satay (skewered and grilled meat), Balinese spice paste, which is used as a base for a wide range of dishes, and sambal (spicy chili pastes or sauce which is a staple in Indonesian and Balinese cuisines). Recently, *C. burmanni* Blume has also been included in the production of herbal tonics. The main role of this plant part in all those traditional foods is to enhance their flavor and balance the aroma of the foods. Many people also believe that this plant has antimicrobial activity, so it is widely used as an alternative organic preservative agent to lengthen food shelf life.

## Determination of $LC_{50}$ value of the *C. burmanni* Blume extract on *E. coli* O157:H7

A lethal concentration of 50% ($LC_{50}$) is the concentration of the plant extract that kills 50% of bacterial population following exposure to such plant extract. The curve estimated this value (total viable cells vs extract concentration). The results are shown in Figure 2. The number of cell death was proportional to the increment of the plant extract concentration. At 5% w/v, this plant extract totally eliminated/killed the *E. coli* O157:H7 (Figure 2). The linear regression of the curve produces a

formula of Y = -18.886X + 95.937, with $r^2$ value of 0.9908. From Figure 2 or the formula generated from the curve, the $LC_{50}$ value of the extract fell between 2 and 3% w/v (approximately 2.59% w/v, based on calculation using the displayed formula).

The values of the $LC_{50}$ have been commonly used to assess the potential toxicity of substances exposed to living organisms (Gupta, 2020). According to this scientist, the values of $LC_{50}$ could help scientists determine safe exposure levels of toxic substances for types of organisms, specifically humans. These values are often used to establish permissible exposure limits for chemicals and pollutants (Wolf & Segner, 2023). Therefore, $LC_{50}$ values for toxic substances, including those produced by plants, are important to be determined prior to their application to control undesired microbes. This is aimed at reducing negative impacts following the application of those substances in any aspect of our lives.

## Phytochemical analysis of the *C. burmanni* Blume leaf extract

From this analysis, the leaf extract of *C. burmanni* Blume contains several groups of compounds, such as alkaloid, steroid, phenolic, saponin, and flavonoid. In our study, terpenoids and tannin were not detected, and the results are shown in Table 4. These groups of compounds are very common in plant extract and may play an important role in inhibiting the growth of microbes. Inhibition zones formed on the lawn of *E. coli* O157:H7 might have been due to the presence of these groups of compounds.

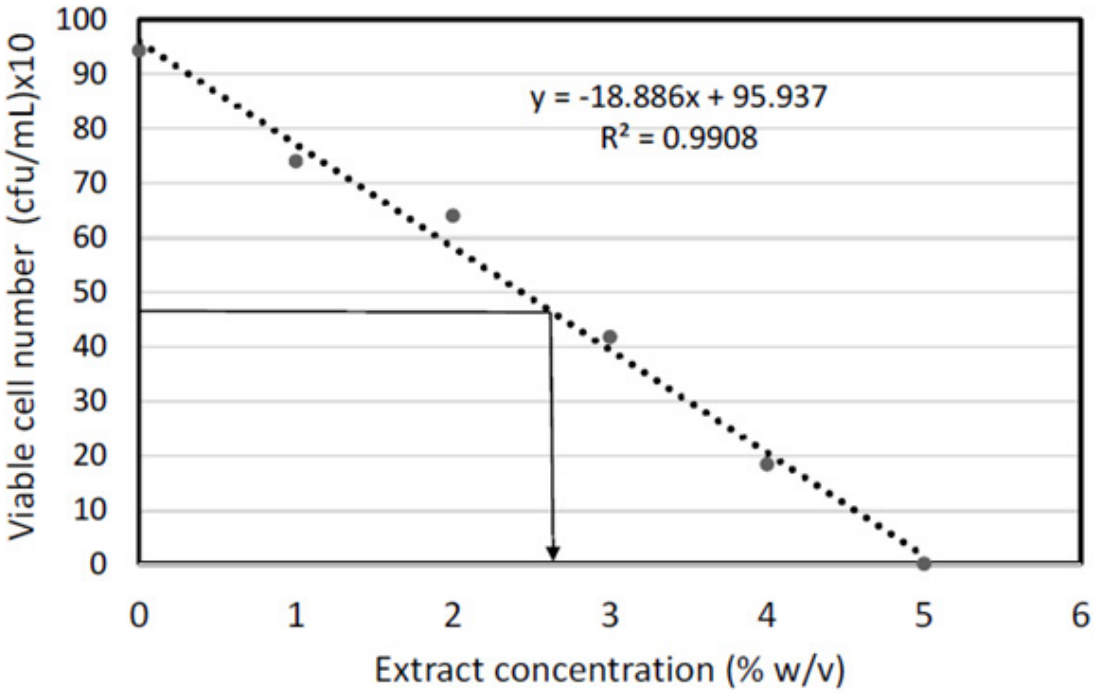



Figure 2 Estimation of $LC_{50}$ value of the *C. burmanni* Blume leaf extract on *E. coli* O157:H7

Table 4 Groups of compounds detected in the leaf extract of *C. burmanni* Blume

| Types of compounds tested | Results* |
|---|---|
| Alkaloids | + |
| Steroid | + |
| Terpenoid | - |
| Phenolic | + |
| Saponin | + |
| Flavonoid | + |
| Tannin | - |

* + = detected/present; - = not detected/absent

The phytochemical tests analyzed the active compounds that might have played important roles in inhibiting *E. coli* O157:H7 *in vitro* growth. These included alkaloids, steroids, phenolics, saponins, and flavonoids (Table 4). Our results are in line with those reported by Raji et al. (2019), Ullah et al. (2020), and Huang et al. (2022). The mechanisms by which these compounds control microbial growth have also been extensively reviewed, such as by Yuan et al. (2021), Santiago et al. (2021), Tungmunnithum et al. (2018), and Kavya et al. (2021). The most common mechanism reviewed and reported is interference with the function of the cell membrane that leads to the release of important components (including proteins) from the cytoplasm of microbial cells. Other mechanisms have also been reported, such as enzyme inhibition, DNA binding, oxidative stress, iron chelation, or antioxidant activity of those compounds to interfere with microbial growth. According to Raji et al. (2019), such compounds can also affect the swarming motility of bacterial cells.

The level of impact of alkaloid, steroid, phenolic, saponin, and flavonoid compounds to interfere with microbial growth is determined by many factors. These include types and concentrations of those compounds or microbial resistance developed over time (Palupi et al., 2019; Biharee et al., 2020). Alkaloids, for example, may act in three different modes of action (disrupting cell membranes, interfering with enzyme activity, or binding on the DNA of the targeted cells (Jubair et al., 2021). Conversely, phenolic compounds may generate reactive oxygen species (ROS) that lead to oxidative damage to cellular components, such as proteins, lipids, and DNA. Oxidative damages caused by these ROS certainly result in cell death (Hajam et al., 2023).

## Fractionation and elucidation of active compounds contained in the leaf extract of *C. burmanni* Blume)

On the completion of fractionation, 12 fractions were obtained, but only 4 of those fractions (fractions 3, 4, 5, and 6) showed inhibitory activity on *E. coli* O157:H7 (Table 5). As shown in Table 5, fraction 4 produced the biggest potential to inhibit the growth of this bacterial strain with a diameter inhibition of 25.23 mm (Table 5 and Figure 3).

It is clearly demonstrated in figure 3 that some fractions inhibited the growth of *E. coli* O157:H7, which is indicated by clear zones around the paper disks deposited with each fraction. These results indicate that some active plant extract compounds exist in the fractions showing positive results in this *in vitro* bioassay (Table 5). In the subsequent stage of our research, only fraction 4 (F4) was elucidated in the *Liquid Chromatography-Mass Spectroscopy* (LCMS) analysis as it produced the highest inhibition zone in the *in vitro* bioassay, with a diameter inhibition zone of 25.23 mm (Table 5 and Figure 3).

In the LCMS analysis, fraction 4 of the plant extract was the main focus of the analysis as it gave the highest diameter of inhibition on this bacterium (Table 5). The chromatogram of this analysis (Figure 4) shows that nine peaks appeared in the chromatogram to represent compounds of *N-(4-Methoxyphenyl)-4-methyl-1-piperazinecarbothioamide; azoxystrobin; 2-(2-Cyano-benzylsulfanyl)-4,6-diphenyl-nicotinonitrile; difenoconazole; Octoxinol-2; N-(4-Butylphenyl)-11-[4-(dimethylamino)phenyl]-10-methyl-8-phenyl-8,11 dihydropyrazolo [3',4':4,5] pyrimido [1,2-a] quinoxalin-6-amine; Stigmatellin Y; 2-arachidonoyl glycerol;* and *1-(Butylamino)-3-methyl-2-octylpyrido[1,2-a]benzimidazole-4-carbonitrile.* The chemical structures of these compounds are shown in Table 6.

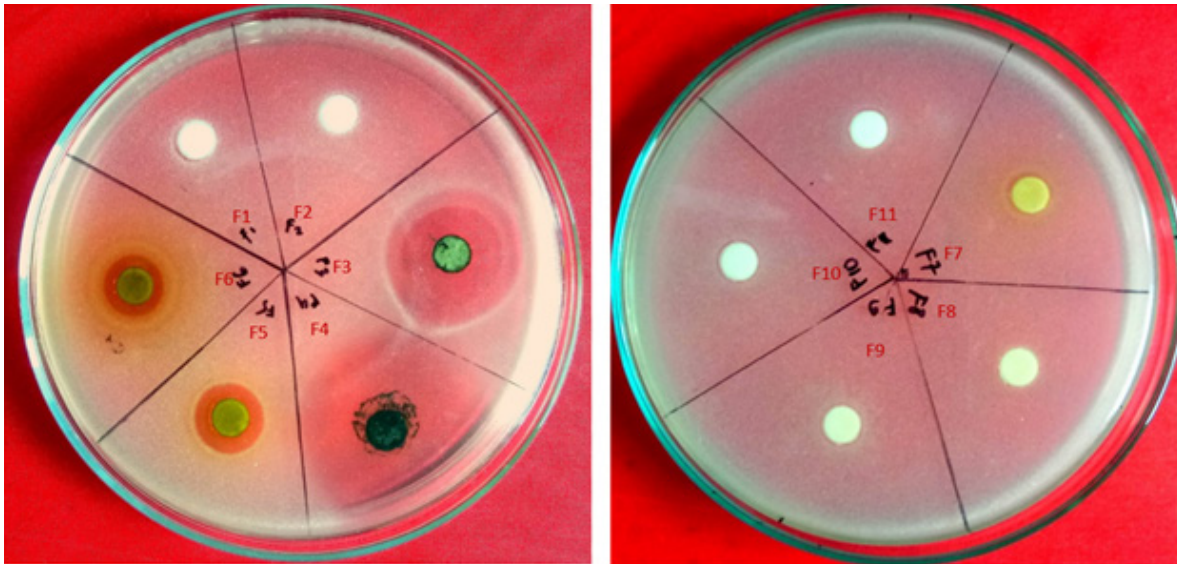
Some compounds detected in our current study have been reported to have antimicrobial activity. *Azoxystrobin* was reported by Sun et al. (2023) to inhibit the growth of *Rhizoctonia solani*, a fungal pathogen attacking tobacco plants. These authors also found this compound to affect the composition and diversity of microbes in tobacco plants. *Stigmatellin Y*, as detected in our study, has also been reported to have antimicrobial activity. Boopathi et al. (2022) reported that this compound plays an important role in interfering with communication (*quorum sensing*) among cells of *Pseudomonas aeruginosa cells*, resulting in its use as an anti-virulence Pseudomonas. The compound of *2-arachidonoyl glycerol* is another compound with the ability to inhibit the growth of microbes. According to Chouenard et al. (2013), such compounds can stimulate neutrophils (a type of white blood cell) to release an antimicrobial effector that leads to growth inhibition on several bacterial species, such as *Escherichia coli* and

*Staphylococcus aureus.* This result partially explains or elucidates the mechanism by which both leaf *C. burmanni* Blume crude extract and its fractions inhibited the *in vitro* growth of *E. coli* O157:H7 in our study. In other words, these three compounds (*Azoxystrobin, Stigmatellin Y,* and *2-arachidonoyl glycerol*) contributed in the growth inhibition of the targeted bacterium (*E. coli* O157:H7)

The roles of the other six compounds to inhibit microbial growth have not been reported. They probably contribute to inhibiting the growth of microbes, although their contribution needs to be further elucidated.

Table 5 Inhibitory activity of each fraction in the fractionation of the crude leaf extract of *C. burmanni* Blume on *E. coli* O157:H7

| Fractions | Diameters of inhibition zones on *E. coli* O157:H7 (mm) |
|---|---|
| F1 | 0.00 |
| F2 | 0.00 |
| F3 | 15.23 |
| F4 | 25.23 |
| F5 | 18.05 |
| F6 | 9.40 |
| F7 | 0.00 |
| F8 | 0.00 |
| F9 | 0.00 |
| F10 | 0.00 |
| F11 | 0.00 |
| F12 | 0.00 |




A B

Figure 3 Inhibition zones of each fraction of *C. burmanni* Blume on *E. coli* O157:H7 on nutrient agar medium

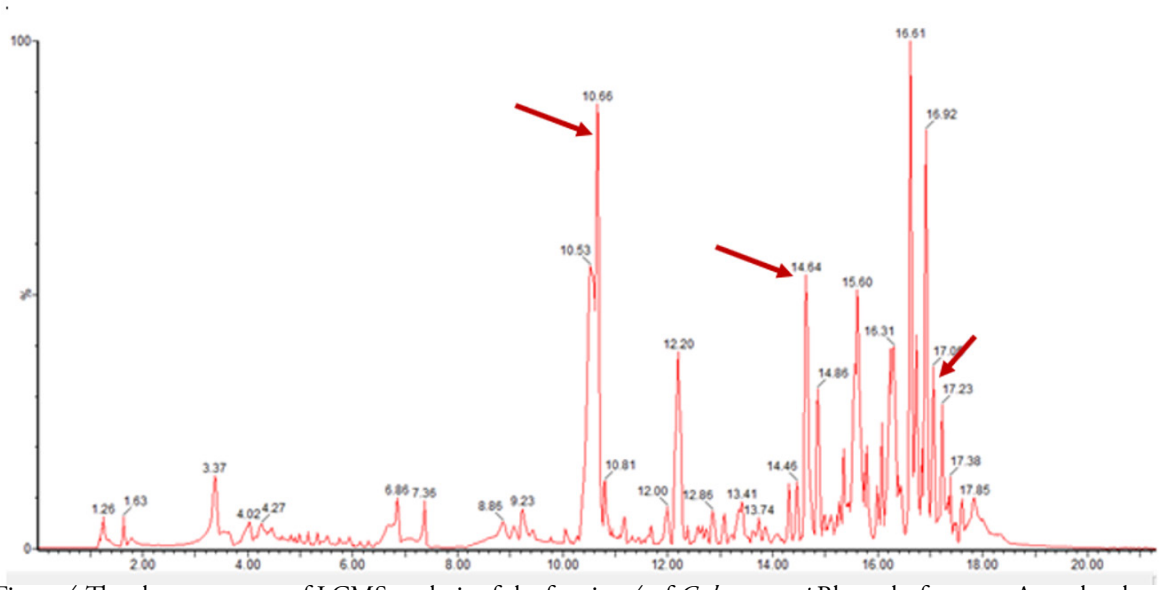
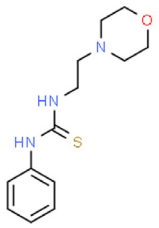
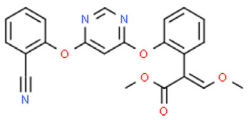
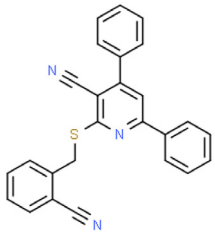
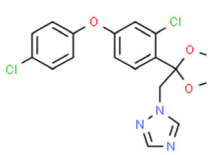
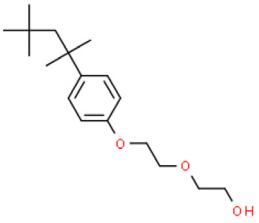
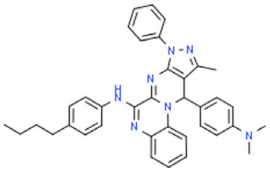
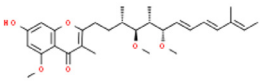
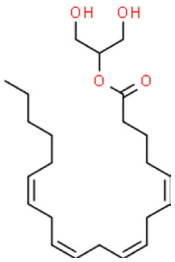
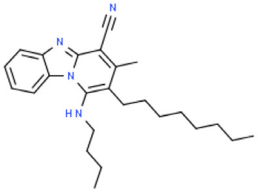



Figure 4 The chromatogram of LCMS analysis of the fraction 4 of *C. burmanni* Blume leaf extract. Arrowheads point out the suspected active compounds (*Azoxystrobin, Stigmatellin Y,* and *2-arachidonoyl glycerol*) appeared at retention times of 10.06, 14.64, and 17.05, respectively, that might have contribution to inhibiting the *in vitro* growth of *E. coli* 0157:H7.

Table 6 Chemical structures of compounds detected in fraction 4 of the *C. burmanni* Blume leaf extract following application of *LCMS MassLynx V4.1* software, which is linked to *ChemSpider* reference.

| No | Retention time (Rt) | Molecular structure | Identified compound | Molecular formula | Molecular weight |
|---|---|---|---|---|---|
| 1 | 10,53 |  | N-(4-Methoxyphenyl)-4-methyl-1-piperazinecarbothioamide | C13H19N3OS | 266,1372 |
| 2 | 10,66 |  | azoxystrobin | C22H17N3O5 | 404,1246 |
| 3 | 10,81 |  | 2-(2-Cyano-benzylsulfanyl)-4,6-diphenyl-nicotinonitrile | C26H17N3S | 404,1237 |
| 4 | 12.20 |  | difenoconazole | C19H17N3O3Cl2 | 406,0725 |

| No | Retention time (Rt) | Molecular structure | Identified compound | Molecular formula | Molecular weight |
|---|---|---|---|---|---|
| 5 | 13.41 |  | OCTOXYNOL-2 | C18H30O3 | 295,2273 |
| 6 | 14.46 |  | N-(4-Butylphenyl)-11-[4-(dimethylamino)phenyl]-10-methyl-8-phenyl-8,11-dihydropyrazolo[3',4':4,5] pyrimido[1,2-a] quinoxalin-6-amine | C37H37N7 | 508,3189 |
| 7 | 14.64 |  | Stigmatellin Y | C29H40O6 | 485,2903 |
| 8 | 17.05 |  | 2-arachidonoylglycerol | C23H38O4 | 391,2848 |
| 9 | 17.05 |  | 1-(Butylamino)-3-methyl-2-octylpyrido[1,2-a] benzimidazole-4-carbonitrile | $C_{25}H_{34}N_4$ | 391,2862 |

## CONCLUSION

Leaf extract of *C. burmanni* Blume has the potential to control contamination of *E. coli* O157:H7 as it shows inhibitory activity in the serial *in vitro* bioassays, with minimal inhibitory concentration (MIC) and $LC_{50}$ values of 4% w/v and 2.59% w/v, respectively. The leaf crude extract of this plant contains groups of chemical compounds belong to alkaloids, steroids, phenolics, saponins, and flavonoid. Fraction of the extract with the highest inhibition zone (fraction 4) contains 9 compounds, namely *N-(4-Methoxyphenyl)-4-methyl-1-piperazinecarbothioamide; azoxystrobin; 2-(2-Cyano-benzylsulfanyl)-4,6-diphenyl-nicotinonitrile; difenoconazole; Octoxinol-2; N-(4-Butylphenyl)-11-[4-(dimethylamino)phenyl]-10-methyl-8-phenyl-8,11 dihydropyrazolo [3',4':4,5] pyrimido [1,2-a] quinoxalin-6-amine; Stigmatellin Y; 2-arachidonoyl glycerol;* and *1-(Butylamino)-3-methyl-2-octylpyrido[1,2-a]benzimidazole-4-carbonitrile.* Among these compounds, three (*Azoxystrobin, Stigmatellin Y,* and *2-arachidonoyl glycerol*) probably contributed to inhibiting the *in vitro* growth of *E. coli* O157:H7.

## REFERENCES

Aryal S, Baniya M K, Danekhu K, Kunwar P, Gurung R, Koirala N. 2019. Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. *Plants*, *8*(4): 96. DOI: https://doi.org/10.3390/plants8040096

Biharee A, Sharma A, Kumar A, Jaitak V. 2020. Antimicrobial flavonoids as a potential substitute for overcoming antimicrobial resistance. *Fitoterapia*, *14:* 104720. DOI: https://doi.org/10.1016/j.fitote.2020.104720

Boopathi S, Vashisth R, Manoharan P, Mohanti, Jia AQA, Sivakumar R, Arockiaraj R. 2022. *Bacillus subtilis* BR4 derived stigmatellin Y interferes Pqs-PqsR mediated quorum sensing system of *Pseudomonas aeruginosa*. *J. of Basic Microbiology*:1-14. DOI: http://dx.doi.org/10.1002/jobm.202200017

Chouinard F, Turcotte C, Guan X, Larose MC, Pirier S, Bouchard L, Provost V, Flamand L, Grandvaux N, Flamad N. 2013. 2-Arachidonoyl-glycerol- and arachidonic acid-stimulated neutrophils release antimicrobial effectors against *E. coli*, *S. aureus*, HSV-1, and RSV *Journal of Leukocyte Biology* 93: 267-276. DOI: https://doi.org/10.1189%2Fjlb.0412200

Dey P, Kundu A, Kumar A, Gupta M, Lee BM, Bhakta T, Dash S, Kim HS. 2020. Analysis of alkaloids (indole alkaloids, isoquinoline alkaloids, tropane alkaloids). In *Recent Advances in Natural Products Analysis*: 505–567, Elsevier. DOI: https://doi.org/10.1016/B978-0-12-816455-6.00015-9

Didehdar M, Chegini Z, Tabaeian S P, Razavi S, Shariati A. 2022. Cinnamomum: The New Therapeutic Agents for Inhibition of Bacterial and Fungal Biofilm-Associated Infection. *Frontiers in Cellular and Infection Microbiology*, *12*. DOI: https://doi.org/10.3389/fcimb.2022.930624

Djarot P, Yulianita Y, Utami N F, Putra AM, Putri YIM, Muhardianty SM, Suciyani TA, Syaepulrohman A. 2023. Bioactivities and Chemical Compositions of Cinnamomum burmannii Bark Extracts (Lauraceae). *Sustainability*, *15*(2), 1696. DOI: https://doi.org/10.3390/su15021696

Gupta PK. 2020. Principles of Toxicology. In *Problem Solving Questions in Toxicology:* (pp. 27–45). Springer International Publishing. DOI: https://doi.org/10.1007/978-3-030-50409-0_3

Hajam YA, Lone R, Kumar R. 2023. Role of Plant Phenolics Against Reactive Oxygen Species (ROS) Induced Oxidative Stress and Biochemical Alterations. In *Plant Phenolics in Abiotic Stress Management*: 125–147). Springer Nature Singapore. DOI: https://doi.org/10.1007/978-981-19-6426-8_7

Huang W, Wang Y, Tian W, Cui X, Tu P, Li J, Shi S, Liu X. 2022. Biosynthesis Investigations of Terpenoid, Alkaloid, and Flavonoid Antimicrobial Agents Derived from Medicinal Plants. *Antibiotics*, *11*(10), 1380. DOI: https://doi.org/10.3390/antibiotics11101380

Jubair N, Rajagopal M, Chinnappan S, Abdullah NB, Fatima A. 2021. Review on the Antibacterial Mechanism of Plant-Derived Compounds against Multidrug-Resistant Bacteria (MDR). *Evidence-Based Complementary and Alternative Medicine*: 1–30. DOI: https://doi.org/10.1155/2021/3663315

Kavya NM, Adil L, Senthilkumar P. 2021. A Review on Saponin Biosynthesis and its Transcriptomic Resources in Medicinal Plants. *Plant Molecular Biology Reporter 39*(4) 833–840. DOI: https://doi.org/10.1007/s11105-021-01293-8

Malik, S K. 2023. Qualitative and Quantitative Estimation of Terpenoid Contents in Some Important Plants of Punjab, Pakistan. *Pakistan Journal of Science 69*(2): 150 - 154. DOI: https://doi.org/10.57041/pjs.v69i2.364

Mohamed EA, Abdur R, Sadeek AMM. 2020. Cinnamon bark as antibacterial agent: A mini-review. *GSC Biological and Pharmaceutical Sciences 10*(1): 103–108. DOI: https://doi.org/10.30574/gscbps.2020.10.1.0012

Novita BD, Sutandhio S. 2021. The Effect of *Cinnamomum burmannii* Water Extraction Against *Staphylococcus aureus*, *Enterobacter* spp., *Pseudomonas aeruginosa*, and *Candida albicans*: *In Vitro* Study. *Folia Medica Indonesiana 55*(4), 285. DOI: https://doi.org/10.20473/fmi.v55i4.24449

Palupi MF, Wibawan IWT, Sudarnika E, Maheshwari H, and Darusman HS. 2019. Prevalence of mcr-1 Colistin Resistance Gene in *Escherichia coli* Along the Broiler Meat Supply Chain in Indonesia. *Biotropia 26*(2), 143–153. DOI: https://doi.org/10.11598/btb.2019.26.2.1054

Parisa N, Islami RN, Amalia E, Mariana M, Rasyid RSP. 2019. Antibacterial Activity of Cinnamon Extract (*Cinnamomum burmannii*) against *Staphylococcus aureus* and *Escherichia coli In Vitro*. *Bioscientia Medicina : Journal of Biomedicine and Translational Research 3*(2): 19–28. DOI: https://doi.org/10.32539/bsm.v3i2.85

Patil S, Lopes BS, Liu S, Wen F. 2022. Emergence of Shiga toxin-producing *Escherichia coli* O157:H7 in paediatric patients in Shenzhen, China. *The Lancet Microbe 3*(11): e809. DOI: https://doi.org/10.1016/S2666-5247(22)00223-3

Pinatih KJP, Suardana IW, Widiasih DA, Suharsono H. 2021. Shiga-Like Toxin Produced by Local Isolates of *Escherichia coli* O157:H7 Induces Apoptosis of the T47 Breast Cancer Cell Line. *Breast Cancer: Basic and Clinical Research 15*: 117822342110101. DOI: https://doi.org/10.1177/11782234211010120

Plumeriastuti H, Budiastuti B, Effendi M, Budiarto B. 2019. Identification of bioactive compound of the essential oils of *Cinnamomum burmannii* from several areas in Indonesia by gas chromatography-mass spectrometry method for antidiabetic potential. *National Journal of Physiology, Pharmacy and Pharmacology 0*,1. DOI: https://doi.org/10.5455/njppp.2019.9.1236702022019

Purni NW, Kawuri R, Darmayasa IBG. 2020. Elimination of *Escherichia coli* O157:H7 Isolated from Beef which was Collected from Slaughtering House and Traditional Markets. *Jurnal Metamorfosa* 7(2):199-204. DOI: 10.24843/metamorfosa.2020.v07.i02.p08

Raji P, Samrot AV, Keerthana D, Karishma S. 2019. Antibacterial Activity of Alkaloids, Flavonoids, Saponins and Tannins Mediated Green Synthesised Silver Nanoparticles Against *Pseudomonas aeruginosa* and *Bacillus subtilis*. *Journal of Cluster Science 30*(4): 881–895. DOI: https://doi.org/10.1007/s10876-019-01547-2.

Rastina, Mirnawati S, Ietje M. 2015. Antibacterial Activity of Ethanol Extract of *Murraya koenigii* Leaves on *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas* sp. *Jurnal Kedokteran Hewan* 9(2): 185-188. DOI: https://doi.org/10.21157/j.ked.hewan.v9i2.2842

Santiago LÂM, Neto RN M, Santos Ataíde AC, Fonseca DCSC, Soares EFA, de Sá Sousa JC, Mondego-Oliveira R, Ribeiro RM, de Sousa Cartágenes MS, Lima-Neto LG, Carvalho RC, de Sousa EM. 2021. Flavonoids, alkaloids and saponins: are these plant-derived compounds an alternative to the treatment of rheumatoid arthritis? A literature review. *Clinical Phytoscience 7*(1): 58. DOI: https://doi.org/10.1186/s40816-021-00291-3

Sri Kosnayani A, Badriah L, El Akbar RR, Kurnia Hidayat, A. 2019. A Qualitative Analysis of Tannin Type and Tannin Content in Meniran Tea (Phyllanthus Niruri Linn.) with Permanganometry Method. *Proceedings of the 5th International Conference on Health Sciences (ICHS 2018)*. DOI: https://doi.org/10.2991/ichs-18.2019.4

Sujarwo W, Keim AP. 2020. *Cinnamomum burmanni (Nees & T.Nees) Blume Lauraceae*: 1 – 7. DOI: https://doi.org/10.1007/978-3-030-14116-5_174-1

Sun M, Wang H, Shi C, Li J, Cai L, Xiang L, Liu T, Goodwin PH, Chen X, Wang L. 2023. Effect of azoxystrobin on tobacco leaf microbial composition and diversity. *Frontiers in Plant Science 13*. DOI: https://doi.org/10.3389/fpls.2022.1101039

Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. 2018. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines 5*(3): 93. DOI: https://doi.org/10.3390/medicines5030093

Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, Emwas AH, Jaremko M. 2020. Important Flavonoids and Their Role as a Therapeutic Agent. *Molecules 25*(22): 5243. DOI: https://doi.org/10.3390/molecules25225243

Utomo D, Kholifah S. 2018. Tests of Boracks dan Formalin on Snacks Collected from Food Stalls Around the University of Yudharta Pasuruan. *Teknologi Pangan: Media Informasi Dan Komunikasi Ilmiah Teknologi Pertanian 9*(1). DOI: https://doi.org/10.35891/tp.v9i1.933

Wolf JC, Segner HE. 2023. Hazards of current concentration-setting practices in environmental toxicology studies. *Critical Reviews in Toxicology 53*(5): 297–310. DOI: https://doi.org/10.1080/10408444.2023.2229372

Yuan G, Guan Y, Yi H, Lai S, Sun Y, Cao S. 2021. Antibacterial activity and mechanism of plant flavonoids to gram-positive bacteria predicted from their lipophilicities. *Scientific Reports 11*(1): 10471. DOI: https://doi.org/10.1038/s41598-021-90035-7