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# Indonesian Wild Ginger (Zingiber sp) Extract: Antibacterial Activity against Mycoplasma gallisepticum

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***Abstract:*** *Lempuyang gajah (Zingiber zerumbet (L.) Smith), lempuyang pahit (Zingiber amaricans BL.), and lempuyang wangi (Zingiber aromaticum Vahl.) are used as traditional medicine (jamu) in Indonesia. It is also used for treatment of microbial infections, helps to increase appetite and stimulate digestion in chickens. Information on their uses are available, but only limited in the scientific data on their bioactivity. The study was conducted on the antibacterial effect of organic extracts of these plants with Mycoplasma gallisepticum as the agent of chronic respiratory disease in chickens. Juice and extracts of fresh and dried rhizome are evaluated through the disc diffusion assay and minimum inhibitory concentration. Oxytetracyclin (30 μg) are used as standards. All extracts are individually exhibited as antibacterial activity against Mycoplasma gallisepticum (7 ± 0.11 mm to 21 ± 0.86 mm). The minimum inhibitory concentration (MIC) determination of plants extracts are ranged from 7.8 mg/ml to 31.2 mg/ml. The preliminary results suggested promising antibacterial properties of wild ginger from Indonesia, and probably could be used in management of chronic respiratory disease in chickens.*

***Keywords –*** *Wild ginger, chronic respiratory disease, mycoplasma gallisepticum*

## I. INTRODUCTION

Indonesia has three species of wild gingers (*Zingiber zerumbet (L.) Smith, Zingiber amaricans BL.*, and *Zingiber aromaticum Vahl.*), with respective local names such as lempuyang gajah, lempuyang pahit, and lempuyang wangi. The difference between them are the sizes of each part of the plant, where *Zingiber amaricans* is slightly smaller than *Zingiber zerumbet* and *Zingiber aromaticum*. Rhizome of *Zingiber zerumbet* L. Sm. is bigger in size, yellow fleshed, and efficacious as an appetite stimulant. Rhizome of *Zingiber amaricans* BL. is smaler in size, yellow with a bitter taste and is used to increase appetite. Rhizome of *Zingiber aromaticum* has fraganted flesh, and efficacious as slimming agent [21]. The three wild ginger have been used traditionally as one of the components in the prescription of jamu (cabepuyang) and it can substitute each other [14]. There are limited publications on *Zingiber amaricans*, and *Zingiber aromaticum*. *Zingiber zerumbet* has been used continuously as a subject for further investigations. Phytochemical investigations on this plant have revealed the isolation of several sesquiterpenes [6,23], flavonoids [9], tannins [11] and aromatic compounds. The volatile oil of rhizome contains zerumbone, humulene, camprene, α-caryophyllene and champhene [2].

*Mycoplasma gallisepticum* (MG) is a simple prokaryote that functions as the major pathogen of chronic respiratory disease (CRD) in fowls. The disease is prevalent in commercial poultry farms with weak health control measures, CRD retard growth, decreased laying rate, and lower feed conversion ratio [22]. This is due to CRD that is estimated up to billions of rupiahs in Indonesia. Respiratory disturbances, excretion of nasal exudates, coughing, sneezing and hyperaemic of the conjunctiva are very often seen as the clinical signs of MG infection [10,17]. Chronic respiratory disease not only affects respiratory system but also reproductive system in the layers. Mostly Broilers and layer chicks in the age group of 4-8 weeks are affected [15]. In general, the objective of this study is to determine the rhizomes of *Zingiber zerumbet (L.) Smith, Zingiber amaricans BL.,* and *Zingiber aromaticum Val.,* as antimicrobial agent against *Mycoplasma gallisepticum*.

## II. MATERIAL AND METHODS

### 2.1 Plant materials and preparation

The rhizomes of *Zingiber zerumbet (L.) Smith, Zingiber amaricans BL.*, and *Zingiber aromaticum Vahl.* that were used in this study were obtained from the Research Institute for Medicinal and Aromatic Plants Bogor, Indonesia. Healthy and matured rhizomes of *Zingiber amaricans* Vahl., *Zingiber zerumbet* L., and *Zingiber aromaticum* L were collected, washed, then sliced into small pieces, dried under a shade to protect the thermo labile components if present from being chemically transformed. Dried rhizomes were grounded into powder using a grinder. After the rhizomes have been dried, they were kept in a proper container until the time of extraction.

### 2.2 Extraction of plant materials

**Ethanol extracts of fresh and dried rhizome**

The fresh and powdered dried rhizomes (5 g) were extracted with 50 ml ethanol from solid to solvent with a ratio of 1:10 (w/v). The extracts were concentrated under vacuum at 40-50ºC using a rotary evaporator. Extracts were stored at 20 ºC and were freshly dissolved in suitable solvents prior to screening for antimicrobial activity.

**Fresh rhizome juice**

100 g of healthy and matured rhizomes were washed with water and cut into small pieces and liquidized using electrical blender without adding any solvent. Rhizome juice were collected in a clean airtight bottle, and stored for antibacterial activity test (500 ml/0.5 kg of rhizome; 100% fresh rhizome juice).

### 2.3 Phytochemical screening

The ethanol extracts and juice were used for the screening. The dried extracts were first reconstituted in the respective solvents used for their extraction and then tested by standard phytochemical method for the presence of alkaloid, flavonoid, tannin, saponin , andterpenoids [13,5].

**Alkaloids test**: 0.5 g of the extract was added to 5 ml of 1% aqueous HCl on a steam bath and filtered. 1 ml of the filtrate was treated with a few drops of Draggendorf's reagent and another 1 ml filtrate was treated with Wagner's reagent. The formation of precipitates was an indication of the presence of alkaloids.

**Flavonoids test**: 0.5g of the extract was dissolved in 2 ml of dilute NaOH solution. A few drops of concentrated $H_2SO_4$ were then added. Formation of yellow solution confirmed the presence of flavonoids.

**Tannin test**: 0.5 g of the extract was stirred with 10 ml of distilled water. The mixture was filtered and a few milliliters of 5% ferric chloride were added to the filtrate. A green coloration showed the presence of tannin.

**Terpenoids test**: 5 ml of rhizome extract was mixed in 2 ml of chloroform and concentrated 3 ml of $H_2SO_4$. A reddish brown coloration at the interface showed the presence of terpenoids.

**Saponins test**: 15 ml of distilled water was added to the extract and shaken vigorously until formation of a stable persistent froth which indicated the presence of saponins.

### 2.4 Microorganisms

Bacteria strains were obtained from standard laboratories of the Indonesian Research Center for Veterinary Science. Antibacterial activity of the plant extracts were investigated using *Mycoplasma gallisepticum* R-strain. The culture of bacteria was sub-cultured on PPLO nutrient agar (difco).

### 2.5 Screening of extracts for antibacterial activity

The disc diffusion method (Kirby Bauer) was used to study the antimicrobial activity. The agar plate was inoculated with freshly grown bacterial culture of approximately $10^8$ CFU/ml for *Mycoplasma gallisepticum*. About 50 µl of each plant extract was loaded in a sterile filter paper disc (6 mm) and placed on plate. The pure solvents in equal volume were served as negative control and oxytetracyclin (30 µg) antibiotic disc was used as a positive control. The plate was incubated at 37$^o$C for 24–48 hours. Evaluation of antibacterial activity was measured showing the diameter of the zones of inhibition against the tested bacteria. Each method in this experiment was repeated 3 times.

**2.6 Determination of minimum inhibitory concentration (MIC)**

The minimum inhibitory concentration of the ethanol extracts that showed inhibition in the antimicrobial screening was used. The MIC was carried out by preparing the dried plant extracts in different concentrations, 125 mg/ml, 62.5 mg/ml, 31.2 mg/ml, 15.6 mg/ml, 7.8 mg/ml, 3.9 mg/ml, and 1.9 mg/ml. Extracts mixed with PPLO agar were allowed to solidify and then inoculated with bacteria culture. The bacteria plates with plant extracts were incubated for 24–48 hours at 37$^{o}$C. Minimum inhibitory concentration is defined as the lowest concentration of the extract that inhibits visible growth of the microorganism in plate.

## III. RESULTS

The dried and fresh powder samples of wild ginger were extracted with ethanol solvent using the maceration method. After the complete extraction, the ethanol solvent was evaporated using rotary evaporator producing semisolid mass crude extracts, followed by dissolving in dimethyl sulfoxide (DMSO) for antibacterial activity test.

**3.1 Phytochemical screening**

The phytochemical analysis of ethanol extract, juice from the fresh rhizome of *Zingiber zerumbet (L.) Smith, Zingiber amaricans BL.,* and *Zingiber aromaticum Vahl.* showed the presence of alkaloids, flavonoids, tannins, and terpenoids (Table 1). The secondary metabolite of *Zingiber zerumbet (L.) Smith, Zingiber amaricans BL.,* and *Zingiber aromaticum Vahl.* were observed to be higher in ethanol extract compared to juice.

**Table 1** Phytochemical analysis of ethanol extract, and juice from the fresh and dried rhizome of *Zingiber zerumbet (L.) Smith, Zingiber amaricans BL.,* and *Zingiber aromaticum Vahl.*

| **Plant** | **Ethanol extracts** | **Biochemical** | | | | |
|---|---|---|---|---|---|---|
| | | Alkaloids | Flavonoids | Saponins | Tannins | Terpenoids |
| ***Zingiber zerumbet (L.) Smith*** | Dried rhizome | + | + | - | + | ++ |
| | Fresh rhizome | + | + | - | + | ++ |
| | Juice | + | + | - | + | + |
| ***Zingiber amaricans BL.*** | Dried rhizome | + | + | - | + | ++ |
| | Fresh rhizome | + | + | - | + | ++ |
| | Juice | + | + | - | + | + |
| ***Zingiber aromaticum Vahl.*** | Dried rhizome | + | + | - | + | ++ |
| | Fresh rhizome | + | + | - | + | ++ |
| | Juice | + | + | - | + | + |

(++) = strongly present, (+) = poorly present, (-) = absent

**3.2 In vitro antibacterial activity**

The in vitro antibacterial activity of ethanol extracts and juice from fresh and dried rhizome against *Mycoplasma gallisepticum* were quantitatively assessed as present or absent of inhibition zones. The different crude extracts from dried and fresh rhizomes of wild gingers exhibited antibacterial potential against *Mycoplasma gallisepticum* at four concentrations of 500 mg/ml, 250 mg/ml, 125 mg/ml and 62.5 mg/ml with dimethyl sulphoxide (DMSO). Table 2 shows the diameter of zones of inhibition of bacterial growth at various concentrations of the fresh rhizome juice, and also the fresh and dried rhizome ethanol extracts. From the table below, it is shown that the *Zingiber amaricans BL*. has the widest diameter of zone of inhibition of 21± 0.86 mm on the dried rhizome of ethanol extract with a concentration of 500 mg/ml, but based on the average ethanol extracts of fresh rhizome has the widest diameter of zone inhibition.

**Table 2** Antimicrobial activity of different crude extracts of *Zingiber zerumbet (L.) Smith, Zingiber amaricans BL.,* and *Zingiber aromaticum Vahl.* against *Mycoplasma gallisepticum*

| Plant | Concentration (mg/ml) | Zone of inhibition (mm) | | |
|---|---|---|---|---|
| | | Fresh rhizome | Dried rhizome | Juice |
| *Zingiber zerumbet (L.) Smith* | 500 | 19 ± 0.65 | 17 ± 0.37 | 8 ± 0.59 |
| | 250 | 16 ± 1.56 | 16 ± 1.18 | 7.9 ± 0.64 |
| | 125 | 9 ± 0.73 | 15 ± 0.5 | 7.8 ± 0.83 |
| | 62.5 | 8 ± 0.47 | 10 ± 0.10 | 7.7 ± 0.29 |
| *Zingiber amaricans BL.* | 500 | 19 ± 0.89 | 21 ± 0.86 | 9 ± 0.44 |
| | 250 | 16 ± 1.41 | 13 ± 0.51 | 8.5 ± 1.08 |
| | 125 | 11 ± 0.29 | 11 ± 0.39 | 8 ± 1.34 |
| | 62.5 | 9 ± 0.71 | 10 ± 2.19 | nd |
| *Zingiber aromaticum Vahl.* | 500 | 20 ± 0.43 | 13 ± 0.04 | 9 ± 0.38 |
| | 250 | 19 ± 0.87 | 11 ± 1.61 | 8 ± 0.41 |
| | 125 | 18 ± 1.66 | 8 ± 0.65 | 8 ± 0.43 |
| | 62.5 | 15 ± 0.27 | nd | 7 ± 0.11 |
| Standard (oxytetracyclin) | 30 µg | 28 ± 2.93 | | |

nd = no detection.
*Values are represented as the mean ± S.D. of three experiments.

**3.3. Minimum Inhibitory Concentration**

The MIC was determined for the ethanol extracts of dried and fresh rhizome because the extracts showed higher zone inhibition compared to juice. As shown in Table 3, display of strong inhibition of ethanol extracts of dried rhizome against *Mycoplasma gallisepticum* and their respective MIC values were 7.8 mg/ml. The ethanol extracts of fresh rhizome had inhibitory activity against *Mycoplasma gallispeticum* and their MIC values were ranged from 15.6 to 31.2 mg/ml.

**Table 3** Minimum inhibitory concentration of different crude extracts of *Zingiber zerumbet (L.) Smith, Zingiber amaricans BL.,* and *Zingiber aromaticum Vahl.* against *Mycoplasma gallisepticum*

| Plant | Minimum inhibitory concentration (mg/ml) | |
|---|---|---|
| | Fresh rhizome | Dried rhizome |
| *Zingiber zerumbet (L.) Smith* | 15.6 | 7.8 |
| *Zingiber amaricans BL.* | 15.6 | 7.8 |
| *Zingiber aromaticum Vahl.* | 31.2 | 7.8 |

## IV. DISCUSSION

The phytochemical screening in the present study has revealed the presence of alkaloids, flavonoids, tannins, and terpenoids. Phytochemical constituents in the plant samples are known to be biologically active compound and they are responsible for different activities such as antimicrobial, antifungal, anti-inflammatory, antipyretic, and antioxidant [20,12,9,16,18]. All secondary metabolite components displayed antimicrobial properties through different biological mechanisms [2,4].

Generally, the antimicrobial activity of plant crude extracts depends on the type and the dose of extracts. It has been reported that different solvent extraction systems can contribute significantly to differences in the antibacterial activities of the extracts [7, 16, 8], which holds true in the present study also. The ethanol extracts of rhizome (dissolved in DMSO) has greater antibacterial activity than fresh rhizome juice and ethanol extract of fresh rhizome showed higher antibacterial potential than the corresponding ethanol extract of dried rhizome. It can be therefore inferred that the active principles of the plant may be more soluble in ethanol [3]. The zone of inhibitions that were created by the three types of extracts increased in size with the increase of the extract concentrations. A low level of activity at a low extract concentration may suggest that the concentrations of the active constituent in the extracts are too low for any appreciable antibacterial activity [3]. The antibacterial action could be related to their chemical components in the crude extracts. The most numbers of bioactive compounds that is present in the crude extracts is the zerumbone one of the compound under sesquiterpenoid group [2,6]. It has got strong antimicrobial activity against bacterial and fungal [19]. Further studies on zerumbone as an antimycobacterial agent are to be continued for a better understanding on the properties of zerumbone. Besides that, more in vivo studies should be conducted for a better understanding of their mechanism of action as an anti-chronic respiratory disease in chickens.

## V. CONCLUSION

The result of this study indicated that ethanol extract is a better choice than juice for the extraction of active ingredients of these plants. The observed inhibition of *Mycoplasma gallisepticum* by ethanol extracts of fresh and dried rhizome showed that the active ingredients for the antibacterial effect best extracted in fresh rhizome with ethanol. *Zingiber zerumbet (L.) Smith, Zingiber amaricans BL.,* and *Zingiber aromaticum Vahl.* showed that they have great potential as remedies for chronic respiratory disease.

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