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# Chemical compositions, antioxidant and antibacterial activities of *kepel* (*Stelechocarpus burahol*) fruit flesh and peel extracts

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**Abstract.** *Sundari D, Handayani DS, Suryanti V. 2023. Chemical compositions, antioxidant and antibacterial activities of* kepel *(*Stelechocarpus burahol*) fruit flesh and peel extracts. Biodiversitas 24: 4668-4675. Kepel (Stelechocarpus burahol* (Bl.) Hook f. & Th.) has been utilized by royalty in Java, Indonesia as perfume, deodorant and anti-aging agent. *S. burahol* fruit flesh has diuretic properties and can help avoid kidney irritation. *S. burahol* possesses anti-hyperuricemia and xanthine oxidase inhibitory properties, as well as antiseptic, anti-inflammatory, and antioxidant activity. Chemical contents and biological activities of *S. burahol* fruit and peel extracts were investigated in the present study. Based on GC-MS spectra, methanol fruits extract contains dodecanoic acid-1,2,3-propanetriyl ester. Methanol peel extract contains hexadecanoic acid methyl ester; 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene and dodecanoic acid-1,2,3-propanetriyl ester. Ethyl acetate peel extract contains dodecanoic acid-1,2,3-propanetriyl ester. LC-MS analysis revealed that ethyl acetate fruit flesh extract contains pelargonidin-malonylrhamnoside (anthocyanins), 8-epiiridodial glucoside tetraacetate (isoprenoid), epigallocatechin gallate (catechin) and 5-octadecenal (fatty aldehydes). Ethyl acetate fruit peel extract contains 1,5-dicaffeoylquinic acid (flavonoid), 1,6-di-o-galloylglucose (tannins), luteolin 7-*O*-glucoside (cynaroside), methyl hemiacetal in 3,4-DHPEA-EA (iridoids). Methanol extract of fruit flesh has strong antioxidant activity, whereas three other extracts have very strong antioxidant activities. *S. burahol* peel extract inhibited the *S. aureus* growth at a concentration extract of 15,000 to 25,000 ppm. These antioxidant and antibacterial activities are associated with the chemical composition of *S. burahol* fruit flesh and peel extracts. Further research into other biological functions and in vivo investigations are required for future studies.

**Keywords**: Antioxidant activity, antibacterial activity, *kepel*, *Stelepchocarpus burahol*

## INTRODUCTION

Natural product research continues to investigate a variety of lead structures that could be used as templates for the pharmaceutical industry's development of new drugs. Natural products are important sources of new pharmaceutical compounds (Najmi et al. 2022). A large number of active ingredients used in drug manufacturing are derived from plants (Osw and Hussain 2020; Khomsi et al. 2022). Medicinal plant extracts and essential oils derived from a wide range of plants have medicinal properties that can be used in phytotherapy, including antibacterial, antioxidant, antifungal, and pharmacological properties (Mssillou et al. 2020). Many drugs that have been synthesized from natural materials, including medicinal plants, are available as food supplements and alternative medicines (Sharma and Gupta 2015).

Natural polyphenolic compounds are of great interest because they have anticancer, anti-inflammatory, and antibacterial properties. Furthermore, phenolic compounds have potential applications in the industrial and pharmaceutical sectors. Plant-based antibiotics are being researched as potential replacements for traditional antibiotics. Significant research has been conducted to evaluate the antimicrobial impact of natural extracts, which have demonstrated the ability to inhibit the growth of a variety of harmful microorganisms (Abdel-Aty et al. 2019). Polyphenols are known as strong antioxidants that can protect cells from free radicals, which can lead to heart disease, cancer, and other diseases (Bensid et al. 2022). Antioxidants also have been used as food additives to prevent food degradation and reduce harmful effects on the human body (Bensid et al. 2022).

Indonesia is a tropical country known for its plant diversity. There are over 200,000 species of higher plants and more than 25% of the total number are flowering plants (Atun 2014). Natural products chemistry, which includes the isolation, characterization, and biological testing of organic compounds, is an interesting research field (Suryanti et al. 2020; Sianturi et al. 2023). *Stelechocarpus burahol* Hook F. & Th, known as Burahol or *kepel*, is an Annonaceous plant originally grown in Java, Indonesia. *S. burahol* can grow to be up to 25 meters tall and has a trunk diameter of 40 cm. The fruits of *S. burahol* grow on the larger branches in the lower part of the trunk. *S. burahol* has a round or even oval-shaped fruit with a diameter of 5-6 cm. The fruit stalk can grow to be up to 8 cm long. The seeds are pushing in shape, have 4-6 grains, and are 3 cm long (Shadrina et al. 2022).

*Stelechocarpus burahol* fruit is an identity flora of Daerah Istimewa Yogyakarta, Indonesia. Javanese princesses adored *S. burahol* fruit because it represented unity, mental and physical integrity. *S. burahol* fruit has long been used as an oral deodorant in Java. *S. burahol* fruit is also an antihyperuricemic and has been used traditionally to treat gout. This fruit was also believed to be used as contraception by Javanese princesses. The fruit is also considered to have the function of preventing kidney inflammation, while the leaves are also beneficial for lowering cholesterol levels. *S. burahol*'s fruit (pulp, seed and peel) had a potential pharmacological activity as an oral deodorant through its adsorbent function and probiotic activation (Darusman et al. 2012). Some biological activities of *S. burahol* leaves have been reported, namely anti-hyperuricemic, xanthine oxidase inhibitor and antioxidant activities (Sunarni 2007; Purwatiningsih et al. 2010; Suwandi et al. 2012). *S. burahol* fruit has been confirmed as anti-implantation, antiseptic and anti-inflammation (Sunardi et al. 2010; Pribadi et al. 2014; Suparmi et al. 2015).

Ethanol extract of *S. burahol* fruit contains flavonoids, alkaloids, saponins, polyphenols, terpenoids and quinones (Sunardi et al. 2010). The leaves (young and old) and seeds of *S. burahol* contain flavonoids. Ethanol extract from ripening *S. burahol* fruits reduces the number of implants and born fetuses of white rats. *S. burahol* fruit flesh serves two functions as an antifertility agent, such as anti-implantation and abortifacient. Fruit juice has wound healing activity because it contains saponins and flavonoids (Pribadi et al. 2014). Flavonoids of *S. burahol* leaves have antioxidant activity (Sunarni 2007). Although research on the bioactivities of leaves and fruit flesh of *S. burahol* has been evaluated, its diverse compound contents have not been studied yet. In this study, chemical components, antioxidant and antibacterial of *S. burahol* fruit flesh and peel are discussed.

## MATERIALS AND METHODS

### Materials

*Stelechocarpus burahol* fruit was obtained from Blitar, East Java, Indonesia. Chemicals were obtained from E-merck and used without further purification. *Staphylococcus aureus* (ATCC 31488), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATTC 15442) were collected from Medical Faculty, Universitas Sebelas Maret, Surakarta, Indonesia.

### Sample preparation

*Stelechocarpus burahol* fruit flesh (1 kg) was macerated with hexane for 24 h at room temperature, filtered and the filtrate was evaporated to obtain hexane extract at 45°C. Sample residue was then further macerated with ethyl acetate for 24 h at room temperature. The sample was then filtered and the filtrate was evaporated at 50°C to obtain ethyl acetate extract. Sample residue was further macerated with methanol for 24 h at room temperature. The sample was then filtered and the filtrate was evaporated at 55°C to obtain methanol extract. The same procedure was carried out for *S. burahol* peels. The resulting extracts were then subjected to TLC analysis using mixed solvents of hexane:ethyl acetate with a ratio of 7:3 and 5:5 v/v.

### Gas Chromatography-Mass Spectroscopy (GC-MS) of extracts

The extracts of *S. burahol* fruit flesh and peel were analyzed using GCMS-QP2010S Shimadzu. The GCMS was operated with column type Rtx 5 MS which had a column length of 30 cm and a diameter of 0.25 mm, ionizer type EI 70 EV, injector temperature of 300°C, column temperature of 70°C, split less injection method and helium carrier gas of 13.7 kPa.

### Liquid Chromatography-Mass Spectroscopy (LC-MS) of extracts

Ethyl acetate extract of *S. burahol* peel was analyzed by LC-MS with UPLC BEH C18 column, 1.7um, 2.1x50mm, polarity mode positive, source temp 500 C, capillary voltage 3.0 kV, cone voltage 30 V, mass range 50-1200, ethanol sample solvent with mobile phase A (water formic acid 0.1%) and B ($CH_3CN$ formic acid 0.1%).

### Determination of antioxidant activity

The antioxidant activity of fruit flesh and peel extracts was determined by the DPPH assay (Benchikh et al. 2022). Vitamin E was used as a positive control. Sample extracts were diluted with methanol to obtain a concentration of 100 ppm. Sample extracts were then made with a concentration of 5, 10, 15, 20, and 25 ppm in methanol. DPPH solution (1 mL, 0.2 mM) was added and the solutions were left for 30 mins. The samples were then measured for their absorbance at 517 nm. The antioxidant activity was calculated by Eq. 1, where $A_o$ is the absorbance of DPPH, $A_s$ is the absorbance of the mixture of DPPH and sample, and I is % inhibition. The $IC_{50}$ value (50% inhibition) is obtained from the percentage plot of the graph obtained from Eq. 1 (Feldberg et al. 2020).

$$\%I = \frac{(A_0 - A_s)}{A_0} \times 100 \quad (1)$$

### Antibacterial activity

Antibacterial activity was studied against gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*E. coli* and *P. aeruginosa*). The antibacterial activity test was performed using the disc diffusion method (Jusidin et al. 2022). The investigation was conducted in five different concentrations of 5, 10, 15, 20, 25 µg/mL. Sterile discs were filled with 20 µl of extract samples and placed on nutrient agar plates that had been cultured with test bacteria. The plate was incubated for 24 h at 35℃. The zone of inhibition was measured using a vernier capillary. Ethanol and chloramphenicol (30µg) were used as negative and positive control, respectively.

## RESULTS AND DISCUSSION

### Chemical compositions of extracts from fruit flesh and peel of *Stelechocarpus burahol*

The chemical contents of *S. burahol* fruit flesh and peel extracts were analyzed by GC-MS are presented (Table 1). No compound with a similarity index (SI) higher than 90 was detected for ethyl acetate fruit flesh extract. The analysis of the ethyl acetate extract of *S. burahol* peels and flash was also carried out by LC-MS analysis. The LC-MS analysis of fruit flesh and fruit peel *S. burahol* ethyl acetate extracts showed the presence of 20 and 14 peaks, respectively (Tables 2 and 3).

### Antioxidant activity of extracts

Antioxidant activities of fruit flesh and peel *S. burahol* extracts are shown in Table 4. The antioxidant activity of extracts was performed by the DPPH (1,1diphenyl-2-picrylhydrazyl) radical scavenging method. Vitamin E was used as a positive control.

### Antibacterial activity of extracts

Sample extracts were tested for activity against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and Gram-positive bacteria (*S. aureus*). Chloromphenicol was used as a positive control. Antibacterial activities of fruit flesh and peel *S. burahol* extracts are shown in Table 5.

### Discussion

GC-MS spectra analyzed showed that methanol extract of fruits includes dodecanoic acid-1,2,3-propanetriyl ester. Methanol peel extract includes hexadecanoic acid methyl ester; 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene; and dodecanoic acid-1,2,3-propanetriyl ester. Ethyl acetate peel extract contains dodecanoic acid-1,2,3-propanetriyl ester (Figure 1). Dodecanoic acid, 1,2,3-propanetriyl ester **(1)** is known as lauric acid ester (Ajibade et al. 2022). It is found in the leaves, fruit peel and bark. This compound is used as a conditioning agent in skin cosmetics, hepatoprotective, 5-α reductase inhibitor, flavor, hypocholesterolemic, lubricant, fragrance antioxidant and antibacterial (Achi and Ohaeri 2015; Sujatha et al. 2020; Kalasariya et al. 2022).

Hexadecanoic acid, methyl ester (methyl palmitate) **(2)** belongs to the fatty acid group. This compound has antibacterial activity (Astiti and Ramona 2021), anti-inflammatory (Vats and Gupta 2017), antifungal (Ogunlesi et al. 2010) and antioxidant (Kavitha and Uduman 2017). Compound 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22,-tetracosahexaene (squalene) **(3)** belongs to the class of triterpenes (Cappiello et al. 2020). Squalene can be found in shark liver oil, vegetable sources, microorganism sources, and squalene localization (Rosales-Garcia et al. 2017). Terpenes have anti-bacterial (Quaglio et al. 2020) and antidiabetic activities (Alqahtani et al. 2013).

Five compounds are identified from ethyl acetate extract of *S. burahol* fruit flesh which are eluted at 5.21; 5.47; 8.02; 11.50 and 16.24 minutes (Figure 2). The compounds that are eluted at 5.21 and 5.47 minutes have the same m/z of 503.6932 called Pelargonidin-malonylrhamnoside or Pelargonidin-succinyl-arabinoside (López-Fernández et al. 2020). *Pelargonidin*, peonidin, petunidin, delphinidin, cyanidin and malvidin are the most common *anthocyanidins* found in plants. Anthocyanins are pigments found in plants that are blue, crimson, or purple. They are most commonly found in flowers, fruits, tubers, and fruit peels. Anthocyanin appears as a red pigment in acidic conditions and as a blue pigment in alkaline conditions. These pigments are persistent in acid, at low temperatures, in the absence of light, oxygen and polyphenol oxidase enzyme. Because anthocyanins' structures are highly reactive, they serve as antioxidants (Delgado-Vargas et al. 2000). Anthocyanins' antioxidant action can help prevent cardiovascular disease, atherosclerosis, cholesterol, and colon cancer by preventing lipid oxidation and DNA mutation (Khoo et al. 2017).

The compound eluted at 8.02 minutes has m/z 499.6910 which is called 8-Epiiridodial glucoside tetraacetate (**4**). This compound belongs to the isoprenoid (Noumi et al. 2020). This molecule was discovered as a secondary metabolite in the leaves of *Sphagneticola trilobata* J. F Pruski (Labhade et al. 2023). Methanolic extract of *S. trilobata* leaves has shown promise as a phytomedicine for the treatment of breast cancer. It also possesses antioxidant and antibacterial activity against gram-negative *E. coli* and *S. typh*i with comparable efficacy to commercial medications such as tetracycline, chloramphenicol, clindamycin, ciprofloxacin, ofloxacin, and ampicillin (Mardina et al. 2021).

**Table 1.** Chemicals content of fruit flesh and fruit peel *Stelechocarpus burahol* extracts analyzed GC-MS

| Sample | Rf (mins) | Peak number | % area | Molecule formula | Compound |
|---|---|---|---|---|---|
| Methanol fruit flesh extract | 21.94 | 6 | 20.36 | $C_{39}H_{74}O_6$ | Dodecanoic acid, 1,2,3-propanetriyl ester |
| | 14.42 | 1 | 0.70 | $C_{17}H_{34}O_2$ | Hexadecanoic acid, methyl ester |
| Methanol peel extract | 22.26 | 5 | 1.12 | $C_{30}H_{50}$ | 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22,-tetracosahexaene |
| | 22.79 | 8 | 32.64 | $C_{39}H_{74}O_6$ | Dodecanoic acid, 1,2,3-propanetriyl ester |
| Ethyl acetate fruit flesh extract | No compounds with similarity index (SI) higher than 90 | | | | |
| Ethyl acetate peel extract | 22.47 | 9 | 11.54 | $C_{39}H_{74}O_6$ | Dodecanoic acid, 1,2,3-propanetriyl ester |

**Table 2.** Chemical contents of fruit flesh of *Stelechocarpus burahol* ethyl acetate extracts by LC-MS

| Retardation time (mins) | m/z | Compound |
|---|---|---|
| 0.38 | 377.4181 | - |
| 3.97 | 701.8691 | - |
| 4.20 | 814.8267 | - |
| 5.21 | 503.6932 | Pelargonidin-malonylrhamnoside |
| 5.47 | 503.6932 | Pelargonidin-succinyl-arabinoside |
| 6.71 | 487.6845 | - |
| 7.44 | 682.05 | - |
| 8.02 | 499.6910 | 8-Epiiridodial glucoside tetraacetate |
| 8.56 | 655.1543 | - |
| 8.82 | 485.6835 | - |
| 9.81 | 467.6734 | - |
| 10.73 | 522.7379 | - |
| 11.50 | 457.6683 | Epigallocatechin gallate |
| 12.14 | 645.6318 | - |
| 12.89 | 671.8529 | - |
| 13.71 | 540.8167 | - |
| 14.19 | 871.8918 | - |
| 15.30 | 893.9038 | - |
| 15.97 | 967.9437 | - |
| 16.24 | 266.5307 | 5-Octadecenal |

**Table 3.** Chemical contents of the fruit peel of *Stelechocarpus burahol* ethyl acetate extracts by LC-MS

| Retardation time (mins) | m/z | Compound |
|---|---|---|
| 0.53 | 328.5641 | - |
| 4.25 | 702.8351 | - |
| 6.48 | 370.5178 | - |
| 7.41 | 516.5985 | 1 ,5-Dicaffeoylquinic acid (1,5 DCQA) |
| 8.55 | 483.6133 | Digalloyl glucose |
| 8.79 | 501.6231 | - |
| 9.78 | 566.8307 | - |
| 10.73 | 449.6640 | Luteolin 7-O-glucoside |
| 11.68 | 597.5370 | - |
| 11.86 | 411.6435 | Methyl hemiacetal in 3,4-DHPEA-EA |
| 13.80 | 782.8783 | - |
| 14.25 | 782.9474 | - |
| 15.80 | 820.0018 | - |
| 16.16 | 818.0697 | - |

**Table 4.** $IC_{50}$ values of fruit flesh and peel *Stelechocarpus burahol* extracts

| Sample | IC50 (ppm) | Antioxidant activity |
|---|---|---|
| Ethyl acetate fruit flesh extract | 19.33 | Very strong |
| Methanol fruit flesh extract | 11.99 | Very strong |
| Ethyl acetate peel extract | 19.19 | Very strong |
| Methanol peel extract | 71.16 | Strong |
| Vitamin E | 28.06 | Very strong |

**Table 5.** The inhibition evaluation of fruit flesh and peel *Stelechocarpus burahol* extracts

| Sample | Conc. (µg/mL) | Inhibition zone (mm) | | |
|---|---|---|---|---|
| | | ***E. coli*** | ***S. aureus*** | ***P. aeruginosa*** |
| Ethyl acetate fruit flesh extract | 5 | - | - | - |
| | 10 | - | - | - |
| | 15 | - | - | - |
| | 20 | - | - | - |
| | 25 | - | - | - |
| Methanol fruit flesh extract | 5 | - | - | - |
| | 10 | - | - | - |
| | 15 | - | - | - |
| | 20 | - | - | - |
| | 25 | - | - | - |
| Ethyl acetate peel extract | 5 | - | - | - |
| | 10 | - | - | - |
| | 15 | - | 6.35 | - |
| | 20 | - | 7.43 | - |
| | 25 | - | 7.80 | - |
| Methanol peel extract | 5 | - | - | - |
| | 10 | - | - | - |
| | 15 | - | - | - |
| | 20 | - | - | - |
| | 25 | - | - | - |
| Chloramphenicol | 30 | 32.65 | 27.10 | 30.25 |

The compound eluted at 16.24 minutes has m/z 457.6683 which is named Epigallocatechin gallate **(5)** (Abdallah et al. 2021). Epigallocatechin gallate (EGCG), also known as Epigallocatechin 3-gallate, is a gallic acid an epigallocatechin ester. EGCC is a kind of catechin found in tea leaves, banana leaves, and a variety of other type of plants. It is a powerful antioxidant with anti-cancer properties (Pyrko et al. 2007) and HIV inhibitory properties. EGCG has been linked to some disease prevention. It also has the potential to prevent chronic diseases like heart disease, diabetes and osteoporosis. It promotes weight loss, which increases fat burning.

The compound eluted at 11.50 minutes has m/z 266.5307 which is called 5-Octadecenal **(6).** This molecule is a member of the organic compound class known as fatty aldehydes (Akar 2021). Octadecenal possesses insecticidal effects and is employed as a pheromone by various insect species. It has antimicrobial activity against *P. aeruginosa*. The compound inhibits bacterial growth by binding to the pyruvate form of the enzyme pyruvate: ferredoxin oxidoreductase. This binding inhibits pyrite oxidation in bacterial cells, which is required for energy production, and prevents the formation of an enzyme-substrate complex (Tang et al. 2010).

Four compounds are identified from ethyl acetate *S. burahol* fruit peel extract which are eluted at 7.41; 8.55; 10.73 and 11.86 minutes (Figure 3). Compound eluted at 8.55 minutes has m/z 516.5985 namely 1,5-Dicaffeoylquinic acid (1,5 DCQA) **(7)** (Costea et al. 2022). Dicaffeoylquinic acid is a flavonoid, polyphenolic molecule, found in plants such as fennel and coffee. 1,5 DCQA, also known as cynarin, belongs to the class of organic compounds known as quinic acids and derivatives. 1,5 DCQA are considered to be good for human health because of their anti-inflammatory and antioxidant capabilities. 1,5 DCQA is a powerful and harmless HIV-1 replication inhibitor. 1,5 DCQA is an antioxidant component of *Cynara cardunculus* leaves. 1,5 DCQA is an α-glucosidase inhibitor from the root of *Dorema ammoniacum* D. Don which has potential as an antidiabetic agent (Etemadi-Tajbakhsh et al. 2020).

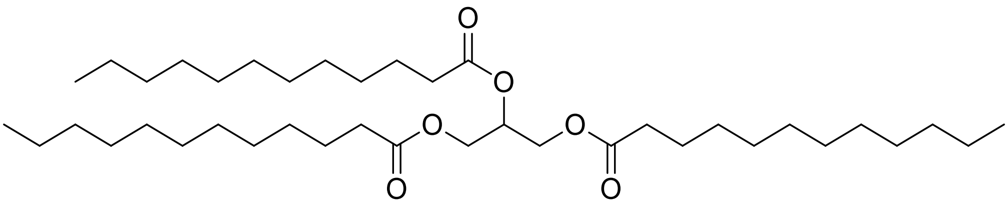
The compound eluted at 8.79 minutes has m/z 483.6133 and was identified as Digalloyl glucose **(8)** (Bao et al. 2018). 1,6-di-o-galloylglucose is a very weakly acidic compound that is slightly soluble (in water). This compound is a member of tannins. Tannins are naturally occurring polyphenols that can be categorized into four main classes: hydrolysable tannin, condensed tannins, complex tannins and phlorotannins. Tannic acid has anticarcinogenic and antimutagenic activity, particularly in the presence of other procarcinogenic insults (Gray et al. 2005). At low levels in the diet, tannic acid has been proposed as a chemopreventative agent for colon cancer. Digalloyl glucose isomers in *Acacia nilotica* pods have antioxidant and anti-inflammatory activity (Salem et al. 2011).

The compound eluted at 10.73 has m/z 449.6640 and is identified as Luteolin 7-O-glucoside known as Cynaroside **(9)** (Pedan et al. 2019). Luteolin 7-O-beta-D-glucoside is a glycosyloxyflavone that is luteolin substituted by a beta-D-glucopyranosyl moiety at position 7 via a glycosidic linkage. It is a flavonoid oxoanion obtained by deprotonation of the 5-hydroxy group of luteolin 7-O-beta-D-glucoside. Luteolin is found in high concentration in anises (*Pimpinella anisum*), Mexican oreganos (*Lippia graveolens*), and wild celeries (*Apium graveolens*) and in a lower concentration in olives (*Olea europaea*), celery leaves (*Apium graveolens* var. secalinum), and lentils (*Lens culinaris*). It has also been detected, but not quantified in, several different foods, such as hard wheat (*Triticum durum*), prairie turnips (*Pediomelum esculentum*), rice (*Oryza sativa*), common sages (*Salvia officinalis*), and green bell peppers (*Capsicum annuum*). Luteolin is an anti-inflammatory and anti-oxidative agent (De Stefano et al. 2021). The hydroxyl moieties and double bonds are important structural features in this compound and are associated with their biochemical and biological activities, like antioxidant and anti-inflammatory activity.

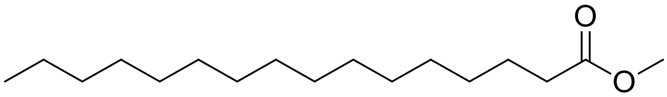
The compound eluted at 11.86 minutes has m/z 411.6435 and is known as methyl hemiacetal in 3,4-DHPEA-EA **(10)** (Celano et al. 2018). 3,4-DHPEA-EA is the major form of the oleuropein-aglycone and is found in olives. 3,4-DHPEA-EA is effective against *S. aureus* and *S. epidermidis* (Bisignano et al. 2014). 3,4-DHPEA-EA belongs to the class of organic compounds known as iridoids. These are monoterpenes containing a skeleton structurally characterized by the presence of a cyclopentane fused to a pyran (forming a 4,7-dimethylcyclopenta[c]pyran), or a derivative where the pentane moiety is open. Iridoids from various plant families, including *Rubiaceae*, *Plantaginaceae*, *Scrophulariaceae*, offer a wide range of biological effects, namely antileishmanial, anticancer, antiplasmodial, antimalarial and anti-inflammatory potency (Hussain et al. 2019).

Methanol extract of fruit flesh has strong antioxidant activity, whereas three other extracts have very strong antioxidant activities. A very strong antioxidant has an $IC_{50}$ of less than 50 ppm, a strong antioxidant has an $IC_{50}$ of 50-100 ppm, a moderate antioxidant has an $IC_{50}$ of 100-150 ppm, and a weak antioxidant has an $IC_{50}$ of 150-200 ppm (Bahriul et al. 2014). These antioxidant activities are related to the secondary metabolite contents of extracts. As confirmed by LC-MS, the ethyl acetate fruit flesh extract contains pelargonidin-malonylrhamnoside (anthocyanins), 8-epiiridodial glucoside tetraacetate (isoprenoid); epigallocatechin gallate (catechin) and 5-octadecenal (fatty aldehydes). The ethyl acetate fruit peel extract contains 1,5-dicaffeoylquinic acid (flavonoid), 1,6-di-o-galloylglucose (tannins), luteolin 7-*O*-glucoside (cynaroside), methyl hemiacetal in 3,4-DHPEA-EA (iridoids). The ethyl acetate extract of fruit flesh and peel has active compounds as antioxidants. The compounds have functional groups that are responsible for antioxidant activity, such as free hydroxyl group on the aromatic ring and conjugated double-bond systems (Sofyan et al. 2017). The aromatic nature of phenol allows for any radical or ion formed on the hydroxyl group to be resonance stabilized. The antioxidant activity is affected by the number and the arrangement of the hydroxyls on the aromatic ring. Naturally occurring antioxidant compounds are β-carotene, 1ascorbic acid, alkaloids, saponins, and tannins, as well as phenolics such as flavonoids, cinnamic acid derivates, coumarin, and tocopherols (Suryanti et al. 2016; Suryanti et al. 2021; Suryanti et al. 2022). Secondary metabolite structure modification is frequently used to improve antioxidant activity (Suryanti et al. 2018).

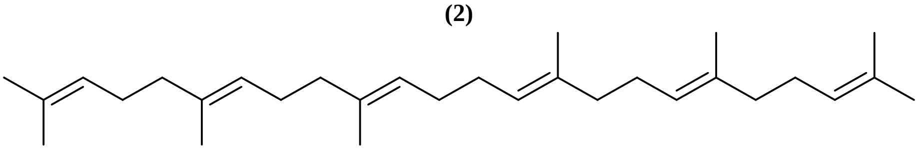
*Stelechocarpus burahol* fruit peel extract inhibited the *S. aureus* growth at a concentration extract of 15 to 25 µL. However, ethyl acetate extract of *S. burahol* fruit peel, methanol fruit flesh extract and methanol fruit peel extract did not show growth inhibition of *E. coli*, *S. aureus* and *P. aeruginosa*. These results indicated that the ability of antibacterial activity against bacteria is related to the differences in cell wall structure, cell physiology and metabolism between Gram-negative and Gram-positive bacteria (Zhu et al. 2018). The presence of phenols, flavonoids, alkaloids, saponins, and tannins is associated to impart bioactivate properties of plants**.** Tannins, which have phenolic hydroxyls in their structures, inhibit the growth of microorganisms including bacteria, fungi and yeast (Farha et al. 2020). Phenolic compounds in ethyl acetate extract of *S. burahol* fruit peel are responsible for inhibiting *S. aureus*. These results are consistent with the previous findings that phenolic compounds have stronger antibacterial activity in gram-positive bacteria (Aldulaimi 2017).



**(1)**

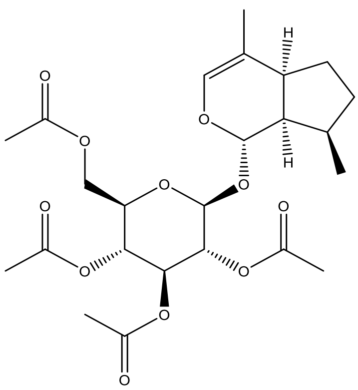


**(2)**

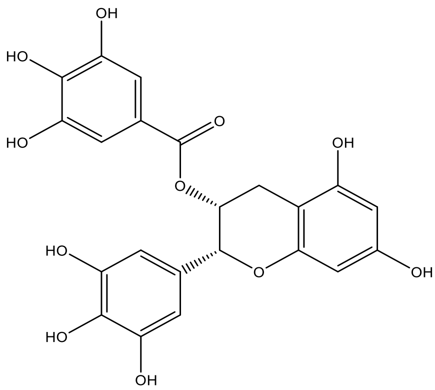


**(3)**

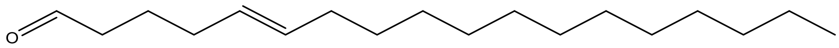
**Figure 1.** Compounds in fruit flesh and fruit peel *Stelechocarpus burahol* extracts analyzed by GC-MS



**(4)**

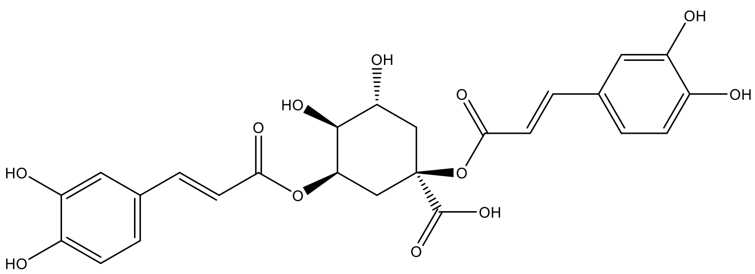


**(5)**



**(6)**

**Figure 2.** Compounds in ethyl acetate extract of *Stelechocarpus burahol* fruit flesh analyzed by LC-MS



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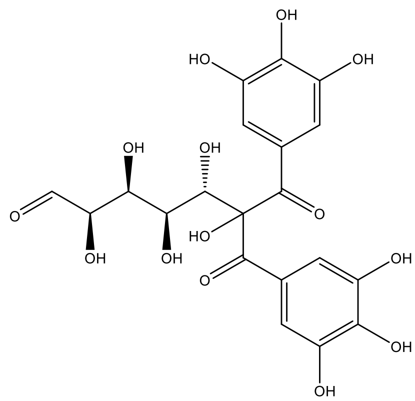

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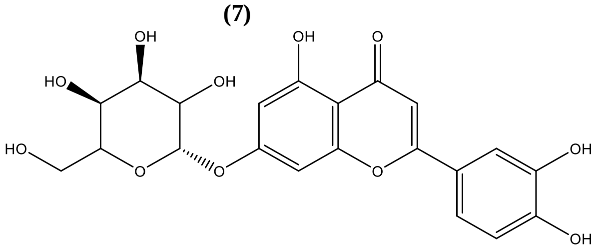

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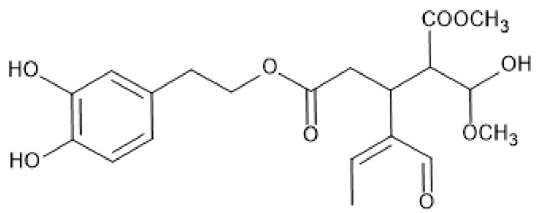

**(10)**

**Figure 3.** Compounds in ethyl acetate extract of *Stelechocarpus burahol* fruit peel analyzed by LC-MS

GC-MS spectra revealed that methanol fruit extract includes dodecanoic acid-1,2,3-propanetriyl ester. Methanol fruit peel extract includes hexadecanoic acid methyl ester; 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22 tetracosahexaene and dodecanoic acid-1,2,3-propanetriyl ester. Ethyl acetate fruit peel extract contains dodecanoic acid-1,2,3-propanetriyl ester. LC-MS confirmed that ethyl acetate fruit flesh extract contains Pelargonidin-malonylrhamnoside (anthocyanins), 8-epiiridodial glucoside tetraacetate (isoprenoid), epigallocatechin gallate (catechin), and 5-octadecenal (fatty aldehydes). Ethyl acetate fruit peel extract includes 1,5-dicaffeoylquinic acid (flavonoid), 1,6-di-o-galloylglucose (tannins), luteolin 7-O-glucoside (cynaroside), and methyl hemiacetal in 3,4-DHPEA-EA (iridoids). Methanol extract of fruit flesh shows high antioxidant activity, while three other extracts have extremely high antioxidant activity. At concentrations ranging from 15,000-25,000 ppm, *S. burahol* fruit peel extract inhibited the *S. aureus* growth.

Overall, the chemical contents of methanol and ethyl acetate extract differ due to the differing polarity of the solvents, which affects the antioxidant and antibacterial activities. These findings encourage us to pursue further biological activities and perform in vivo research in future investigations.

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## REFERENCES

Abdallah MS, Mustafa M, Nallappan MA, Choi S, Paik JH, Rusea G. 2021. Determination of phenolics and flavonoids of some useful medicinal plants and bioassay-guided fractionation substances of *Sclerocarya birrea* (A. Rich) Hochst stem (bark) extract and their efficacy against *Salmonella typhi*. Front Chem 9: 670530. DOI: 10.3389/fchem.2021.670530.

Abdel-Aty AM, Bassuiny RI, Barakat AZ, Mohamed SA. 2019. Upgrading the phenolic content, antioxidant and antimicrobial activities of garden cress seeds using solid-state fermentation by *Trichoderma reesei*. J Appl Microbiol 127 (5): 1454-1467. DOI: 10.1111/jam.14394.

Achi N, Ohaeri O. 2015. GC-MS Determination of bioactive constituents of the methanolic fractions of *Cnidoscolus aconitifolius*. British J Pharm Res 5 (3): 163-172. DOI: 10.9734/bjpr/2015/13893.

Ajibade MA, Akhigbemen AM, Okolie NP, Ozolua RI. 2022. Methanol leaf extract of *Paullinia pinnata* exerts sleep-enhancing and anticonvulsant effects via a mechanism involving the GABAergic pathway. Epilepsy Res 183: 106943. DOI: 10.1016/j.eplepsyres.2022.106943.

Akar Z. 2021. Chemical compositions by using LC–MS/MS and GC–MS and antioxidant activities of methanolic extracts from leaf and flower parts of *Scabiosa columbaria* subsp. *columbaria* var. *columbaria* L. Saudi J Biol Sci 28: 6639-6644. DOI: 10.1016/j.sjbs.2021.07.039.

Aldulaimi OA. 2017. General overview of phenolics from plant to laboratory, good antibacterials or not. Pharmacogn Rev 11 (22): 123-127. DOI: 10.4103/phrev.phrev_43_16.

Alqahtani A, Hamid K, Kam A, Wong KH, Abdelhak Z, Razmovski-Naumovski V, Chan K, Li KM, Groundwater PW, Li GQ. 2013. The pentacyclic triterpenoids in herbal medicines and their pharmacological activities in diabetes and diabetic complications. Curr Med Chem 20: 908-931. DOI: 10.2174/0929867311320070007.

Astiti NPA, Ramona Y. 2021. GC-MS analysis of active and applicable compounds in methanol extract of sweet star fruit (*Averrhoa carambola* L.) leaves. Hayati J Biosci 28 (1): 12-22. DOI: 10.4308/hjb.28.1.12.

Atun S. 2014. Metode isolasi dan identifikasi struktural senyawa organik bahan alam. Jurnal Konservasi Cagar Budaya 8 (2): 53-61. DOI: 10.33374/jurnalkonservasicagarbudaya.v8i2.132. [Indonesian]

Bahriul P, Rahman N, Diah AWM. 2014. Uji aktivitas antioksidan ekstrak daun salam (*Syzygium polyanthum*) dengan menggunakan 1,1-difenil-2- pikrilhidrazil. Jurnal Akademika Kimia 3: 143-149. [Indonesian]

Bao Y, Qu Y, Li J, Li Y, Ren X, Maffucci KG, Li R, Wang Z, Zeng R. 2018. In vitro and in vivo antioxidant activities of the flowers and leaves from *Paeonia rockii* and identification of their antioxidant constituents by UHPLC-ESI-HRMSn via pre-column DPPH reaction. Molecules 23 (2): 392. DOI: 10.3390/molecules23020392.

Benchikh F, Benabdallah H, Amira H, Amira I, Mamache W, Amira S. 2022. Free radical scavenging, metal chelating and antiperoxidative activities of *M. communis* berries methanol extract and its fractions. Turk J Agric Food Sci Technol 10 (6): 1089-1094. DOI: 10.24925/turjaf.v10i6.1089-1094.5062.

Bensid A, El Abed N, Houicher A, Regenstein JM, Özogul F. 2022. Antioxidant and antimicrobial preservatives: Properties, mechanism of action and applications in food–a review. Crit Rev Food Sci Nutr 62 (11): 2985-3001. DOI: 10.1080/10408398.2020.1862046.

Bisignano C, Filocamo A, Ginestra G, Giofre, SV, Navarra M, Romeo R, Mandalari G. 2014. 3,4-DHPEA-EA from *Olea europaea* L. is effective against standard and clinical isolates of *Staphylococcus* sp. Ann Clin Microbiol Antimicrob 13: 24. DOI: 10.1186/1476-0711-13-24.

Cappiello F, Loffredo MR, Plato CD, Cammarone S, Casciaro B, Quaglio D, Mangoni ML, Botta B, Ghirga F. 2020. The revaluation of plant-derived terpenes to fight antibiotic-resistant infections. Antibiotics 9 (6): 325. DOI: 10.3390/antibiotics9060325.

Celano R, Piccinelli AL, Pugliese A, Carabetta S, Di Sanzo R, Rastrelli L, Russo M. 2018. Insights into the analysis of phenolic secoiridoids in extra virgin olive oil. J Agric Food Chem 66 (24): 6053-6063. DOI: 10.1021/acs.jafc.8b01751.

Costea L, Chițescu CL, Boscencu R, Ghica M, Lupuliasa D, Mihai DP, Deculescu-ioniță T, Duțu LE, Popescu ML, Luță EA, Nițulescu GM, Olaru OT, Gîrd CE. 2022. The polyphenolic profile and antioxidant activity of five vegetal extracts with hepatoprotective potential. Plants 11 (13): 1680. DOI: 10.3390/plants11131680.

Darusman HS, Rahminiwat M, Sadiah S, Batubara I, Darusman LK, Mitsunaga T. 2012. Indonesian *Kepel* fruit (*Stelecchocarpus burahol*) as oral deodorant. Res J Med Plants 6 (2): 180-188. DOI: 10.3923/rjmp.2012.180.188.

De Stefano A, Caporali S, Di Daniele N, Rovella V, Cardillo C, Schinzari F, Minieri M, Pieri M, Candi E, Bernardini S, Tesauro M, Terrinoni A. 2021. Anti-inflammatory and proliferative properties of luteolin-7-O-glucoside. Intl J Mol Sci 22: 1321. DOI: 10.3390/ijms22031321.

Delgado-Vargas F, Jiménez AR, Paredes-López O, Francis FJ. 2000. Natural pigments: Carotenoids, anthocyanins, and betalains - characteristics, biosynthesis, processing, and stability. Crit Rev Food Sci Nutr 40 (3): 173-289. DOI: 10.1080/10408690091189257.

Etemadi-Tajbakhsh N, Faramarzi MA, Delnavazi MR 2020. 1,5-dicaffeoylquinic acid, an α-glucosidase inhibitor from the root of *Dorema ammoniacum* D. Don. Res Pharm Sci 15 (5): 429-436. DOI: 10.4103/1735-5362.297845.

Farha AK, Yang QQ, Kim G, Li HB, Zhu F, Liu HY, Gan RY, Corke H. 2020. Tannins as an alternative to antibiotics. Food Biosci 38: 100751. DOI: 10.1016/j.fbio.2020.100751.

Feldberg L, Schuster O. Elhanany E. Laskar O, Yitzhaki S, Gura S. 2020. Rapid and sensitive identification of ricin in environmental samples based on lactamyl agarose beads using LC-MS/MS (MRM). J Mass Spectrom 55 (1): e4482. DOI: 10.1002/jms.4482.

Gray JP, Mishin V, Heck DE, Laskin JD. 2005. Dietary tannic acid stimulates production of reactive oxygen intermediates and growth factor and inflammatory gene expression in human colon tumor cells. Am Assoc Cancer Res 46: 5181.

Hussain H, Nazir M, Green IR, Saleem M, Raza ML. 2019. Therapeutic potential of iridoid derivatives: Patent review. Inventions 4 (2): 29. DOI: 10.3390/inventions4020029.

Jusidin MR, Othman R, Shaleh SRM, Ching FF, Senoo S, Oslan SNH. 2022. In vitro antibacterial activity of marine microalgae extract against *Vibrio harveyi*. Appl Sci 12 (3): 1148. DOI: 10.3390/app12031148.

Kalasariya SH, Pereira L, Patel BN. 2022. Biologically active components for cosmeceutical use extracted from *Chaetomorpha aerea*. Tradit Med Res 7 (4): 35. DOI: 10.53388/tmr20220326002.

Kavitha R, Uduman MAM. 2017. Identification of bioactive components and its biological activities of *Abelmoschas moschatus* flower extrtact-a GC-MS study. IOSR J Appl Chem 10 (11): 19-22.

Khomsi ME, Imtara H, Kara M, Hmamou A, Assouguem A, Bourkhiss B, Tarayrah M, AlZain MN, Alzamel NM, Noman O, Hmouni D. 2022. Antimicrobial and antioxidant properties of total polyphenols of *Anchusa italica* Retz. Molecules 27 (2): 416. DOI: 10.3390/molecules27020416.

Khoo HE, Azlan A, Tang ST, Lim SM. 2017. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res 61 (1): 1361779. DOI: 10.1080/16546628.2017.1361779.

Labhade S, Jain S, Chitlange S, Sharma S. 2023. LC/MS characterization of secondary metabolites of *Sphagneticola trilobata* J. F Pruski leaves 14 (3): 1278-1283. DOI: 10.47750/pnr.2023.14.03.171.

López-Fernández O, Domínguez R, Pateiro M, Munekata PES, Rocchetti G, Lorenzo JM. 2020. Determination of polyphenols using Liquid Chromatography-tandem Mass Spectrometry technique (LC-MS/MS): A review. Antioxidants 9 (6): 479. DOI: 10.3390/antiox9060479.

Mardina V, Ilyas S, Halimatussakdiah H, Harmawan T, Tanjung M, Yusof F. 2021. Anticancer, antioxidant, and antibacterial activities of the methanolic extract from *Sphagneticola trilobata* (L.) J. F pruski leaves. J Adv Pharm Technol Res 12 (3): 222-226. DOI: 10.4103/japtr.JAPTR_131_21.

Mssillou I, Agour A, El Ghouizi A, Hamamouch N, Lyoussi B, Derwich E. 2020. Chemical composition, antioxidant activity, and antifungal effects of essential oil from *Laurus nobilis* L. flowers growing in Morocco. J Food Qual 2020: 8819311. DOI: 10.1155/2020/8819311.

Najmi A, Javed SA, Al Bratty M, Alhazmi HA. 2022. Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents. Molecules 27 (2): 349. DOI: 10.3390/molecules27020349.

Noumi E, Snoussi M, Anouar EH, Alreshidi M, Veettil VN, Elkahoui S, Adnan M, Patel M, Kadri A, Aouadi K, De Feo V, Badraoui R. 2020. HR-LC-MS-based metabolite profiling, antioxidant, and anticancer properties of *Teucrium polium* l. methanolic extract: Computational and in vitro study. Antioxidants 9: 1089. DOI: 10.3390/antiox9111089.

Ogunlesi M, Okiei W, Osibote EA. 2010. Analysis of the essential oil from the leaves of *Sesamum radiatum*, a potential medication for male infertility factor, by Gas Chromatography-Mass Spectrometry. Afr J Biotechnol 9 (7): 1060-1067. DOI: 10.5897/ajb09.941.

Osw PS, Hussain FHS. 2020. Isolation of kaempferol 3-O-rutinoside from Kurdish plant *Anchusa italica* Retz. and bioactivity of some extracts. Eurasian J Sci Eng 6 (2): 141-156. DOI: 10.23918/eajse.v6i2p141.

Pedan V, Popp M, Rohn S, Nyfeler M, Bongartz A. 2019. Characterization of phenolic compounds and their contribution to sensory properties of olive oil. Molecules 24 (11): 2041. DOI: 10.3390/molecules24112041.

Pribadi P, Latifah E, Rohmayanti R. 2014. Pemanfaatan perasan buah *Kepel* (*Stelechocarpus burahol* (Blume) Hook & Thomson) sebagai antiseptik luka. Pharmaciana 4 (2): 177-183. [Indonesian]

Purwatiningsih, Hakim AR, Purwantini, I. 2010. I. Antihyperuricemic activity of the *kepel* [*Stelechocarpus buraho*l (BI.) Hook. F. &Th.] leaves extract and xanthine oxidase inhibitory study. Intl J Pharm Pharm Sci 2 (2): 123-127.

Pyrko P, Schöntha AH, Hofman FM, Chen TC, Lee AS. 2007. The unfolded protein response regulator GRP78/BiP as a novel target for increasing chemosensitivity in malignant gliomas. Cancer Res 67 (20): 9809-9816. DOI: 10.1158/0008-5472.CAN-07-0625.

Quaglio D, Corradi S, Erazo S, Vergine V, Berardozzi S, Sciubba F, Cappiello F, Crestoni ME, Ascenzioni F, Imperi F, Delle Monache F, Mori M, Loffredo MR, Ghirga F, Casciaro B, Botta B, Mangoni ML. 2020. Structural elucidation and antimicrobial characterization of novel diterpenoids from *Fabiana densa* var. ramulosa. *A*CS Med Chem Lett 11 (5): 760-765. DOI: 10.1021/acsmedchemlett.9b00605.

Rosales-Garcia T, Jimenez-Martinez C, Davila-Ortiz G. 2017. Squalene extraction: Biological sources and extraction methods. Intl J Environ Agric Biotechnol 2 (4): 1662-1670. DOI: 10.22161/ijeab/2.4.26.

Salem MM, Davidorf FH, Abdel-Rahman MH. 2011. In vitro anti-uveal melanoma activity of phenolic compounds from the Egyptian medicinal plant *Acacia nilotica*. Fitoterapia 82 (8): 1279-1284. DOI: 10.1016/j.fitote.2011.08.020.

Shadrina AN, Widyanengsih E, Eiko NB, Putri NA, Sulastri N, Dzulfiana N, Rajebi O, Sulvita W. 2022. Analisis fitokimia dan aktivitas farmakologi tanaman *kepel* (*Stelechocarpus burahol*) terhadap beberapa penyakit : Review. Jurnal Buana Farma 2 (3): 14-21. DOI: 10.36805/jbf.v2i3.546. [Indonesian]

Sharma SB, Gupta R. 2015. Drug development from natural resource: A systematic approach. Mini-Rev Med Chem 15 (1): 52-57. DOI: 10.2174/138955751501150224160518.

Sianturi GLR, Trisnawati EW, Koketsu M, Suryanti, V. 2023. Chemical constituents and antioxidant activity of Britton's wild petunia (*Ruellia brittoniana*) flower. Biodiversitas 24 (7): 3665-3672. DOI: 10.13057/biodiv/d240703.

Sujatha S, Sara SC, Gayathiri M, Roselin IR, Ruby RGD. 2020. Analysis of bioactive compounds present in methanolic extract of *Phymatosorus scolopendria* (Burm. F.) Pic. Serm. through gas chromatography and mass spectroscopy. Intl J Pharm Sci Res 11 (7): 3294-3299. DOI: 10.13040/IJPSR.0975-8232.11(7).3294-99.

Suwandi AO, Pramono S, Mufrod. 2012. Pengaruh konsentrasi ekstrak daun *Kepel* (*Stelechocarpus burahol* (BL) Hook f. & Th.) terhadap aktivitas antioksidan dan sifat fisik krim. Majalah Obat Tradisional 17 (2): 27-33. [Indonesian]

Sofyan A, Widodo E, Natsir H. 2017. Bioactive component, antioxidant activity, and fatty acid profile of red Beewort (*Acorus* sp.) and white Beewort (*Acorus calamus*). Jurnal Teknologi Pertanian 18 (3): 173-180. [Indonesian]

Sunardi C, Sumiwi SA, Hertati A. 2010. Penelitian antiimplantasi ekstrak etanol daging buah burahol (*Stelechocarpus burahol* Hook F. & Thomson) pada tikus putih. Majalah Ilmu Kefarmasian 7 (1): 1-8. [Indonesian]

Sunarni T. 2007. Antioksidan-free radical scavenging of flavanoid from the leaves of *Stelechocarpus burahol* (Bl.) Hook f. & Th.). Indonesian J Pharm 18 (3): 111-116.

Suparmi S, Isradji I, Yusuf I, Fatmawati D, Ratnaningrum I H, Fuadiyah S, Wahyuni II, Rahmah DA. 2015. Anti-implantation activity of *kepel* (*Stelechocarpus burahol*) pulp ethanol extract in female mice. J Pure Appl Chem Res 4: 94-99. DOI: 10.21776/ub.jpacr.2015.004.03.220.

Suryanti V, Marliyana SD, Putri H.E. 2016. Effect of germination on antioxidant activity, total phenolics, β-carotene, ascorbic acid and α-tocopherol contents of lead tree sprouts (*Leucaena leucocephala* (lmk.) de Wit). Intl Food Res J 23 (1): 167-172.

Suryanti V, Wibowo FR., Khotijah S, Andalucki N. 2018. Antioxidant activities of cinnamaldehyde derivatives. IOP Conf Ser Mater Sci Eng 333 (1): 012077. DOI: 10.1088/1757-899X/333/1/012077.

Suryanti V, Kusumaningsih T, Marliyana SD, Setyono HA, Trisnawati EW. 2020. Identification of active compounds and antioxidant activity of teak (*Tectona grandis*) leaves. Biodiversitas 21 (3): 946952. DOI: 10.13057/biodiv/d210313.

Suryanti V, Marliyana SD, Rohana GL, Trisnawati EW, Widiyanti. 2021. Bioactive compound contents and antioxidant activity of fermented lead tree (*Leucaena leucocephala* (lmk.) de wit) seeds. Molekul 16 (3): 192-199. DOI: 10.20884/1.jm.2021.16.3.756.

Suryanti V, Sariwati A, Sari F, Handayani DS, Risqi HD. 2022. Metabolite bioactive contents of *Parkia timoriana* (DC) Merr seed extracts in different solvent polarities. HAYATI J Biosci 29 (5): 681-694. DOI: 10.4308/hjb.29.5.681-694.

Tang M, Bie Z, Wu M, Yi H, Feng J. 2010. Aroma characterization of "Flavor No. 3" melon using headspace-solid phase microextraction combined with gas chromatography-mass spectrometry. Acta Hortic 871: 513-518. DOI: 10.17660/ActaHortic.2010.871.71.

Vats S, Gupta T. 2017. Evaluation of bioactive compounds and antioxidant potential of hydroethanolic extract of *Moringa oleifera* Lam. from Rajasthan, India. Physiol Mol Biol Plants 23 (1): 239-248. DOI: 10.1007/s12298-016-0407-6.