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Synergistic Antibacterial Effects of Combined Phytochemicals of Endophytic Fungi Extracts and Its Pure Compounds Isolated from Sungkai (*Peronema canescens*) Leaves

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ABSTRACT

In previous research, we reported the isolation of endophytic fungi from sungkai leaves (*Peronema canescens*) and the isolation of pure compounds accompanied by antioxidant and antibacterial activities. The development of drugs from natural ingredients is often effective at high concentrations. This can lead to adverse side effects caused by higher concentrations of a single ingredient. This limitation can be overcome by using a combination of pure extracts/compounds which allows for synergistic interactions with strong bioactivity at fairly low concentrations. It was discovered that mixtures of bioactive substances were more likely to prevent illness than single active molecules. The purpose of this study is to examine the interaction between endophytic fungus isolated from leaves (*P. canescens*) and purified chemicals that have significant antibacterial activity. Providing endophytic fungal extracts was carried out by re-isolating eight endophytic fungi from sungkai leaves, morphological characterization, cultivation and extraction. Isolation of endophytic fungi was carried out using the surface sterilization method, characterization using microscopic and macroscopic techniques, and extraction using the maceration method. Isolation of compounds using the column chromatography method and analysis using NMR spectroscopy. However, this study used five pure compounds from endophytic fungal extracts taken from stock compounds. Each endophytic fungi extract (PD1-PD8) and pure compound (C1-C5) were tested for antibacterial activity with the disc diffusion method using four test bacteria, namely, *E. coli*, *S. typhi*, *B. subtilis*, and *S. aureus*. Next, a combination of two and three component blends was carried out in a 1:1 ratio and the antibacterial activity was determined. The best combination of extracts is PD4+PD5+PD8 which comes from an additive effect and the best combination of pure compounds is C1+C2 which comes from an additive and synergistic effect. The additive and synergistic effects that occur in the combination of PD1+PD2 extract and the pure compound C3+C4 combination can apparently be used as an alternative formula compared to the single extract or the single pure compound. Thus, this combination formula is expected to help avoid undesirable side effects due to higher doses of single ingredients which need to be proven by in vivo tests.

1. INTRODUCTION

The herbal plant most frequently utilized to control the body's immune system in combating the COVID-19 virus is sungkai leaves (*Peronema canescens*). Many nations have made progress in carrying out preventative and therapeutic measures throughout time. Various studies related to sungkai leaves have been carried out. Research results prove that sungkai leaves are antibacterial against *Streptococcus mutants*, *Salmonella thyposa*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Shigella dysentriae* [1-4]. Sungkai leaves also have antioxidant and antithyrokinase activity because of their content, namely phenolics, tannins, alkaloids, steroids, saponins and flavonoids [5]. Apart from those bioactivities, it has also been reported that sungkai leaves can be used as anti-diabetic, anti-inflammatory and anti-cholesterol [6-8]. There are so many studies on the sungkai leaves which indicate that this plant has been proven to have medicinal properties due to its pharmacological effects.

Several research reported that sungkai leaves have great potential as an immunostimulant because in vitro it has been proven that this plant has very good and varied bioactivity. A typical compound from the sungkai, namely peronemin, has shown pharmacological effects as antiplasmodium, anticancer,

cytotoxic, antidiabetic, antioxidant, antibacterial, antityrosinase, antiparasitic, antihyperuricemic and anti-inflammatory. Based on these pharmacological effects, the sungkai leaves has great potential as an immunostimulant because several of these pharmacological effects are related to improving the immune system both directly and indirectly [9-11].

New medications are frequently discovered using natural compounds produced from endophytic fungus. There is now a lot of interest in studying endophytic fungal medicinal plants due to the tremendous potential for growth in the pharmaceutical, agricultural, and industrial domains [12]. Scientists have been interested in the characteristics of medicinal plants and their relationship to related endophytic fungus because these plants have the ability to synthesize natural bioactive chemicals. Fungi that grow within plants and are symbiotic with them benefit both parties. Endophytic fungi are a significant source of biologically active substances that can biosynthesize crucial "phytochemicals" for medicine and can do it more quickly than plants [13]. Antibacterial activity is present in the majority of the active substances extracted from endophytic fungus. Numerous endophytic fungus secondary metabolites have been researched for the creation of novel medicines because they have potent antibacterial properties. Most plants, especially those used as medicines, have endophytic fungus that have significant antioxidant activity [14, 15]. A number of bioactive secondary metabolites can be produced by endophytic fungi that have been identified from medicinal plants, including lignin, tannins, anthraquinones, xanthones, phenylpropanoids, steroids, isocoumarin derivatives, lactones, quinones, flavonoids, phenols, indole derivatives, and peptides. Additionally, its active element performs a variety of biological processes, including anti-inflammatory, anti-viral, anti-bacterial, and antioxidant effects [16-18].

The use of medications made from complex mixes of bioactive chemicals obtained from plants is more successful than the use of pure bioactive compounds, according to research in the literature. This occurs because of beneficial combination interactions between components in complex mixtures. According to data, adopting a mixture of bioactive substances rather than a single active molecule can help prevent disease resistance more effectively. The variety of structures and functional groups possessed by the combination of active compounds allows the combination effect to inhibit or kill target microbes more strongly. The target microbial species may have an impact on the combined effects in different ways [19].

The use of bioactive compounds in plant extracts is more widely used in medicine than single bioactive compounds. Crude extracts are a source of components that might change resistance, according to studies on the antibacterial properties of herbal remedies utilizing clinical isolates [20]. Since medicinal plant extracts may include hundreds or even thousands of distinct bioactive compounds in varying abundances, identifying the bioactive molecules responsible for a given biological action is extremely challenging. To overcome bacterial resistance, a mixture of crude extracts can be used. When a combination is made, there are four possible outcomes: indifference (when the combined product has no effect or has a weaker effect than the most potent individual component); additive effect (when the combined product is equal to the sum of the effects of each individual component); synergism (when the combined product outperforms the sum of the effects of each individual component); and antagonism (when the combined product has a strong negative effect) [19]. Something important and needs to be facilitated is finding the most effective combination among extract combinations to get the best activity.

The same applications as the host extract may be used to create endophytic fungal extracts that have been isolated from medicinal plants. New combination antimicrobial medicines will be created by examining the interactions between bioactive substances produced from endophytic fungal extracts of medicinal plants [19]. To develop this sungkai leaf endophytic fungus as a medicinal ingredient such as an immunostimulant, it is necessary to carry out in vivo tests using pure extracts/compounds with high activity. The objective of this research is to determine the effect of a combination extracts and pure compounds from endophytic fungi isolated from sungkai (*P. canescens*) leaves which provide high antibacterial activity. The findings of this study shed light on the interaction between sungkai leaf endophytic fungus extract and its pure compounds on antibacterial activity, including indifference, synergy, additive, or antagonism. It is also a consideration whether in further in vivo tests towards drug preparations a single extract, a combination of extracts, a single pure compound, or a combination of pure compounds will be used.

2. METHODOLOGY

2.1 Materials

Sungkai leaves were collected in May 2023 in the Palembang, Indonesia. The plant was then assigned the number 302/UN9.1.7/4/EP/2021 in the Laboratory of Biosystematic, University of Sriwijaya. Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), MHA (Muller Hinton Agar) from Oxoid, alcohol 70%, NaOCl solution from Onemed, ethyl acetate of technical grade and distilled before use, aquabidest, methanol, DMSO, paper dish, tetrasiklin, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus*, pure compounds C1-C5 from previous research.

2.2 Methods

2.2.1 Isolation of endophytic fungi

P. canescens fresh leaves were rinsed in distilled water for a minute before being air dried. Leaf surfaces were disinfected by soaking them in 70% ethanol ± 1 minute, 1% sodium hypochlorite ± 1 minute, and then sterile distilled water before washing. The cleaned leaves are divided into pieces of 0.5 cm^2 in size. In petri plates using sterile PDA medium containing chloramphenicol, layers of leaves were produced. The plates were incubated for three days at room temperature (26°C-28°C). Endophytic hyphae that develop from plant tissue have their tips clipped off and put to a petri plate with fresh PDA medium. The plates were kept for seven days at room temperature (26°C-28°C). All isolated endophytic fungus were described, including colony shape, asexual/sexual spores, and mycelium under a microscope [21].

2.2.2 Identification of endophytic fungi morphologically

Endophytic fungi are identified using phenotypic traits. With 1000X magnification, microscopic characteristics were

observed using the slide culture method. The phenotypic characteristics that emerge are then identified by comparing them with a number of sources (books and journals) [22-24].

2.2.3 Cultivation and extraction

Endophytic fungal cultures grown on PDA media were divided into tiny (6 mm in diameter) pieces and then put into three PDB medium-filled Erlenmeyer flasks of 300 mL each. The incubation process was place under static circumstances for 30 days at room temperature. After the biomass has been filtered, the liquid broth containing secondary metabolites is partitioned in ethylacetate (ratio 1:1) and evaporated to provide a concentrated extract of ethylacetate used rotary evaporator (Buchi R300+V300 with interface I300 Pro-F305) [25]. Ethyl acetate is a semi-polar solvent. Several studies have revealed that semi-polar solvents are able to bind polar and non-polar compounds so that more complex compounds can be obtained.

2.2.4 Antibacterial activity

The petri dish containing agar media is inoculated with 0.1 mL of test bacteria and spread evenly to cover the surface of the agar. Petri plates with agar material were filled with regular paper discs (6 mm in diameter), then the test sample was injected at a concentration of 400 µg/disc and tetracycline antibiotics at a concentration of 30 µg/disk. After 24 - 48 hours, a clear area was observed around the disc paper. If the diameter of the clear area formed is greater than the negative control (solvent), then the extract has antibacterial activity. The criteria for the diameter of the inhibition zone for each sample tested against standard antibiotics are grouped as weak, medium and strong using the following equation [26]:

$$\text{Weak: } \frac{A}{B} \times 100\% < 50\%;\ \text{Moderate: } 50\% < \frac{A}{B} \times 100\% < 70\%$$
$$\text{Strong: } \frac{A}{B} \times 100\% > 70\%$$

where, A: Inhibition zone (mm) of sample; B: Inhibition zone (mm) of antibiotic (Control +).

By dilution in EtOH to a concentration series, the pure compound's minimum inhibitory concentration (MIC) value was calculated: 200; 100; 50; 25; 12.5; 6.25; and 3.125 µg/mL. The MIC value was determined from the smallest concentration that still provides an inhibition zone of > 9 mm [26]. Dilution in EtOH to a concentration series was used to determine the pure compound's minimum inhibitory concentration (MIC) value: 200; 100; 50; 25; 12.5; 6.25; and 3.125 µg/mL. The MIC value was determined from the smallest concentration that still provides an inhibition zone of > 9 mm [16].

2.2.5 Combination of endophytic fungi extracts and its pure compounds

Each endophytic fungal extract (PD1-PD8) and pure compound (C1-C5), made to a concentration of 4% (400 µg/dish) in 2 mL. The combination effect test was carried out by combining two or three extract components in a ratio of 1: 1. Five combinations of extracts were prepared through tests 1-5. Antibacterial activity was measured for each test using the Kirby Bauer method (2x repetitions). Each inhibitory zone diameter was measured and the % antibacterial activity was calculated. The combination effects were grouped into four according to Simoes et al. [21], namely indifference, additive, synergism, and antagonism.

Indifference: activity of CP = activity of the most active IC
Additive: activity of CP = sum of activities of each IC
Synergism: activity of CP > activity of the most active IC
Antagonism: activity of CP < activity of least active IC
CP = combination products
IC = individual components

3. RESULTS AND DISCUSSION

Our previous research has found 16 endophytic fungi from sungkai leaves. We have selected eight endophytic fungi to proceed to the combination effect testing stage. In our previous article we used stocks of endophytic fungal cultures stored in the laboratory to test the combined effect of antioxidant activity. In this study, we prepared eight endophytic fungi from direct isolation from fresh sungkai leaves. We did this to find out whether these endophytic fungi could be found again from sungkai leaves at different times and seasons. Morphological characterization and identification of endophytic fungi are shown in Figures 1-2 and Tables 1-2.

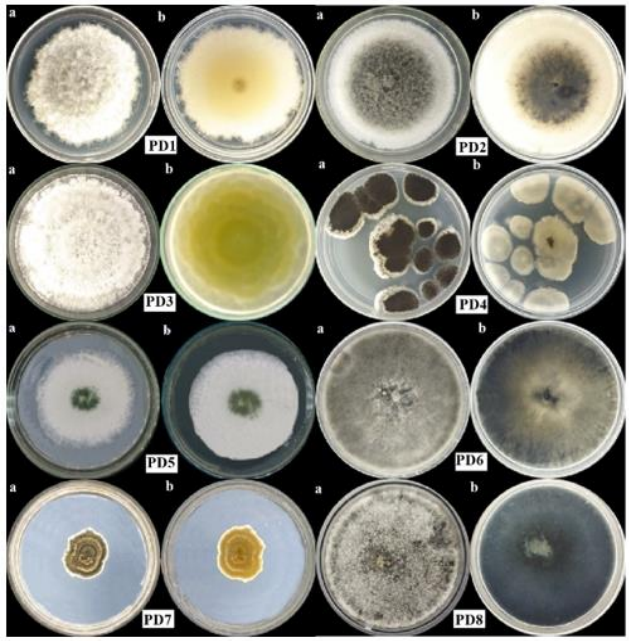


Figure 1. Endophytic fungus colonies' morphological characteristics in *Peronema canescens* leaves: Macroscopic (a: front view; b: reverse view)

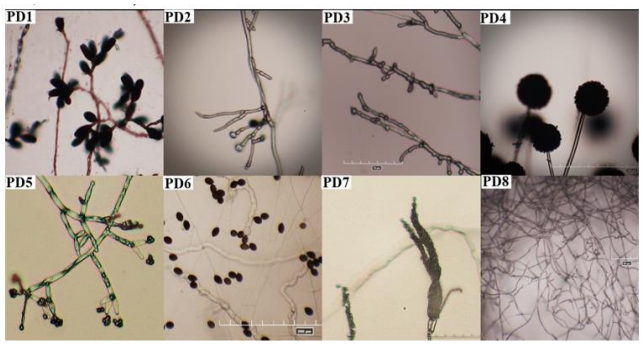


Figure 2. Characteristics of endophytic fungus isolated from *Peronema canescens* leaves at the microscopic level (1000X magnification)

Table 1. Endophytic fungus colonies isolated from *Peronema canescens* leaves (seven days old in PDA medium)

Code	Surface Colony	Reverse Colony	Structure	Elevation	Pattern	Exudate Drops	Radial Line	Concentric Circle
PD1	White	Beige	Cottony	Umbonate	Radiate	-	-	√
PD2	White to grey	White to grey	Cottony	Umbonate	Zonate	-	-	√
PD3	White	White	Cottony	Verrucose	Flowery	-	√	√
PD4	Black	White	Powder	Umbonate	Spread	-	-	-
PD5	White greentint	White greentint	Cottony	umbonate	Radiate	-	√	-
PD6	Grey	Grey	Cottony	Umbonate	Radiate	-	√	-
PD7	Green	Green	Velvety	Umbonate	Zonate	-	-	-
PD8	White to grey	Black	Cottony	Umbonate	Radiate	-	-	-

Table 2. Microscopic characteristics and Identification of endophytic fungi isolated from *Peronema canescens* leaves

Isolate	Spore	Shape	Hyphae	Characteristic	Identification
PD1	Conidia	Subglobose	Septate	Conidiophores hyaline, branched	*Neopestalotiopsis* sp.
PD2	Conidia	subglobose	Septate	Conidiophores hyaline, branched, phialides short	*Colletotrichum sp*
PD3	Spore	Subglobose	Septate	Sporangial type and the number of antheridia per oogonium and oospore type	*Pythium longifolium*
PD4	Conidia	Subglubose	Septate	Phialides radiate around antire vesicle and are biseriate, with the metulae twice as long as the phalides	*Aspergilus Niger*
PD5	Spore	Globose	Septate	Conidiophores hyaline, phialides short and thick, globose	*Trichoderma asperellum*
PD6	Conidia	Globose	Septate	Conidiophores hyaline, simple, inflated globosely	*Lasiodiplodia theobromae*
PD7	Spore	Globose	Septate	Conidia pale green, dark brown in mass, subglobose, minutely echinulate on the surface	*Penicillium oxalium*
PD8	Spore	Subglobose	Septate	The hyphae of fluffy and white in the early stage of growth	*Trichoderma* sp.

3.1 Antibacterial activity test of extract and combination effect

Eight endophytic fungi (PD1-PD8) have been isolated and characterized from sungkai leaves. The MIC value was used to examine the antibacterial efficacy of each endophytic fungus ethyl acetate extract (Table 3). The MIC value was also determined for a pure compound that had been previously isolated, namely 4-hydroxybenzoic acid (C1) isolated from *Trichoderma asperellum* [27]; 3-methyl-3,4-dihydro-1H-isochromene-1,8(7H)-dione (C2) and 3-hydroxy-4(hydroxy(4-hydroxyphenyl)methyl)-ɣ-butyrolactone (C3) isolated from *Pythium periplocum* [25]; 3-(2,6-dihydroxyphenyl)-2-hydroxyacrylic acid (C4) isolated from *Penicillium oxalicum* [28]; and 3-benzyl-2,6-dihydroxy-1,4,11,13-tetramethyl-5-methylene-12,15-dioxo-14-oxabicycloheptadeca-8,16-diene-7-carbo-xylic acid (C5) isolated from *Aspergillus niger* [29] listed in Table 3.

Endophytic fungi extract were obtained through a maceration process and evaporated using a rotary evaorator to obtain concentrated extracts. The combination of extracts is carried out by mixing two or three extracts with a 1:1 composition and the antibacterial activity of the combination product is determined. The antibacterial activity of each individual extract and the effect of the combination of extracts at a concentration of 4% were determined by measuring the diameter of the inhibition zone and calculating the % antibacterial activity. The results of the antibacterial activity test for individual extracts and combinations of extracts are shown in Figure 3.

The MIC values in Table 3 show that compounds C2 and C3 are produced by the same endophytic fungus, namely PD6. Compound C3 had the strongest antibacterial activity on all tested bacteria, followed by compound C2. Compound C4 has moderate antibacterial activity isolated from the endophytic fungus PD7 with weak antibacterial activity. Compounds C1 and C5 have weak antibacterial activity, but compound C5 is produced by the endophytic fungus PD4 which has strong antibacterial activity. This needs to be studied in the future to prove whether there is a synergistic effect in individual PD4 extracts on the chemical compounds they contain.

Table 3. MIC values of the ethyl acetate extract of the endophytic fungus *P. canescens* and its pure compounds against four test bacteria with the tetracycline as positive control

Sample	MIC Value (µg/mL)			
	E. coli	***S. aureus***	***S. thypi***	***B. subtilis***
PD 1	50	50	50	50
PD 2	50	50	50	50
PD 3	> 100	> 100	> 100	> 100
PD 4	25	25	25	25
Compund C5	> 100	> 100	> 100	> 100
PD 5	> 100	> 100	> 100	> 100
Compund C1	> 100	> 100	> 100	> 100
PD 6	50	100	50	50
Compund C2	50	50	100	50
Compund C3	25	50	50	50
PD 7	> 100	> 100	100	> 100
Compund C4	100	100	100	100
PD 8	50	100	100	50
Control (+)	3.125	3.125	3.125	3.125

Research reveals that purified phenolic compounds have high MIC values against *S. aureus* and *E. coli* bacteria, but the combination of these compounds in grape extract greatly increases the antibacterial activity with reduced MIC values [30]. This synergistic effect might result from the combined action of the compounds in the extract, rather than from the action of a single compound, in which case each of the identified compounds contributes to this effect and the multi-objective effect is produced rather than just one compound being in charge of the observed synergistic effect. A similar thing might also happen to the C5 compound which is purified

with a high MIC value, but if the C5 compound is in the PD4 extract, a synergistic effect occurs due to the combined action of the compounds contained in the PD4 extract, thereby reducing the MIC value of the PD4 extract.

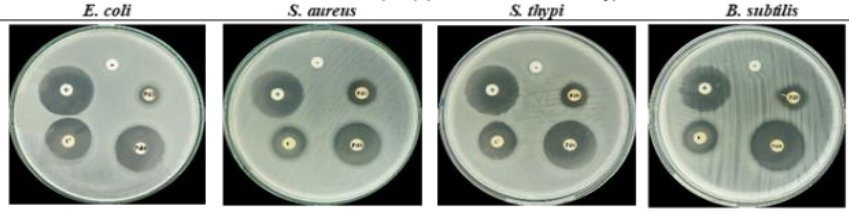
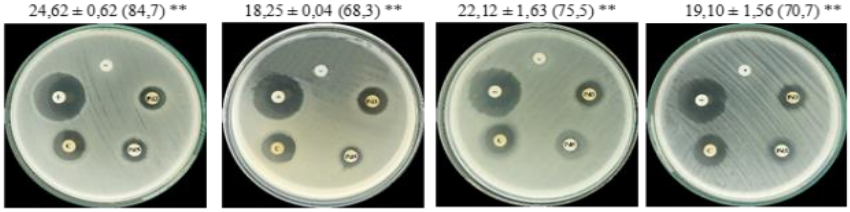
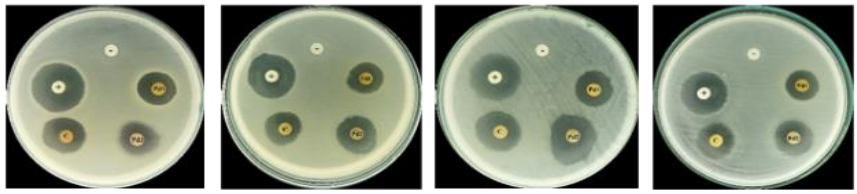
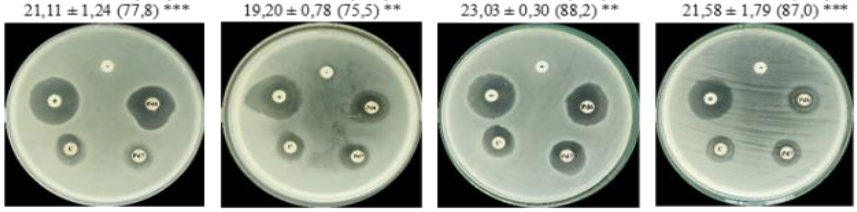
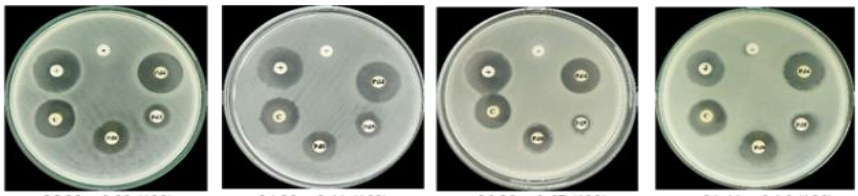
Sample	Inhibition zone (mm) (% Antibacterial Activity)			
	E. coli	*S. aureus*	*S. thypi*	*B. subtilis*
Test 1				
Control (+)	29,08 ±0,62 (100)	26,71 ± 0,30 (100)	29,28 ± 0,18 (100)	27,01 ± 0,02 (100)
Control (-)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
PD3	10,53 ± 0,32 (36,2)	15,06 ± 0,28 (56,4)	14,85 ± 1,45 (50,7)	15,52 ± 1,20 (57,5)
PD4	26,64 ± 0,64 (91,6)	24,58 ± 0,09 (92,0)	28,80 ± 0,07 (98,4)	29,15 ± 1,70 (107,9)
Combination (C)	24,62 ± 0,62 (84,7) **	18,25 ± 0,04 (68,3) **	22,12 ± 1,63 (75,5) **	19,10 ± 1,56 (70,7) **
Test 2				
Control (+)	28,13 ± 0,05 (100)	26,20 ± 0,44 (100)	27,65 ± 0,76 (100)	26,98 ± 0,05 (100)
Control (-)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
PD3	14,13 ± 0,87 (50,2)	16,11 ± 0,87 (61,5)	14,56 ± 0,02 (52,7)	14,58 ± 0,95 (54,0)
PD5	14,08 ± 0,83 (50,1)	12,20 ± 0,78 (46,6)	15,41 ± 1,96 (55,7)	16,01 ± 1,43 (59,3)
Combination (C)	17,38 ± 0,27 (61,8) ***	17,77 ± 0,23 (67,8) ***	16,13 ± 0,92 (58,3) ***	18,32 ± 0,34 (67,9) ***
Test 3				
Control (+)	27,13 ± 0,04 (100)	25,44 ± 0,14 (100)	26,11 ± 0,18 (100)	24,81 ± 0,67 (100)
Control (-)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
PD1	19,35 ± 0,72 (71,3)	18,82 ± 0,12 (74,0)	19,76 ± 1,36 (75,7)	18,07± 0,65 (72,8)
PD2	20,83 ± 1,43 (76,8)	22,24 ± 0,99 (87,4)	24,99 ± 0,07 (95,7)	20,07± 0,72 (80,9)
Combination (C)	21,11 ± 1,24 (77,8) ***	19,20 ± 0,78 (75,5) **	23,03 ± 0,30 (88,2) **	21,58 ± 1,79 (87,0) ***
Test 4				
Control (+)	26,15 ± 0,09 (100)	28,86 ± 0,05 (100)	25,24 ± 0,02 (100)	23,66 ± 0,09 (100)
Control (-)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
PD6	25,10 ± 0,53 (96,0)	20,42± 1,33 (70,8)	23,45 ± 0,55 (92,9)	16,10 ± 0,55 (68,0)
PD7	14,69 ± 1,71 (56,2)	15,49 ± 0,21 (53,7)	20,09 ± 0,57 (79,6)	15,97 ± 0,12 (67,5)
Combination (C)	17,07 ± 1,27 (65,3) **	15,77 ± 0,21 (54,6) **	17,74 ± 1,34 (70,3) ****	15,12 ± 0,19 (63,9) ****
Test 5				
Control (+)	25,23 ± 0,09 (100)	24,39 ± 0,11 (100)	26,20 ± 0,67 (100)	21,11 ± 0,16 (100)
Control (-)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
PD4	23,38 ± 1,27 (92,7)	22,82 ± 0,19 (93,6)	20,80 ± 0,99 (79,4)	21,95 ± 1,24 (104,0)
PD5	14,16 ± 0,05 (56,1)	14,10 ± 0,02 (57,8)	10,77 ± 0,27 (41,1)	14,57 ± 1,04 (69,0)
PD8	19,28 ±1,38 (76,4)	16,63 ± 0,21 (68,2)	16,94 ± 0,97 (64,7)	20,48 ± 0,92 (97,0)
Combination (C)	21,65 ± 0,09 (85,8) **	21,22 ± 0,90 (87,0) **	20,10 ± 0,14 (76,7) **	21,07 ± 1,10 (99,8) **

Figure 3. Effect of a combination of *P. canescens* endophytic fungus extracts on antibacterial activity
Note: *indifference; **additive; ***synergism; ****antagonism

The simplest form of determining the effect of a combination is through a diffusion test. Either in a well or on a disc, each independent test sample (A or B) is inserted. The inhibition zone of the combination (A + B) is compared with an independent test sample after being put on a separate disc. A synergistic interaction will take place if the zone of inhibition at A + B is bigger than it is at A or B. An antagonistic interaction will take place if the zone of inhibition on A plus B is smaller than on A or B alone. Although straightforward, this test is only used as a qualitative reference and needs further investigation because it depends on several factors that may affect the outcomes. It should only be used to identify the samples that need additional investigation [31, 32]. Some studies contend that "synergistic" interactions between the molecules produce more potent products, making combinations of natural goods more effective than pure substances. The phenomenon of antagonism also occurs in mixtures of natural products, where the effect of the active

element is masked by other compounds in the complex mixture [33].

The effects of the blend of endophytic fungal extracts as shown in Figure 3 can be classified as indifference (0%), additive (60%), synergism (30%), and antagonism (10%). The data shows that the combined effect of sungkai leaf endophytic fungus extract is dominated by an additive effect, namely 60%. The additive effect provides strong category antibacterial activity (% Antibacterial Activity: 70 – 100%) reaching 45%. In fact, the best extract combination in this study for all tested bacteria was PD4+PD5+PD8 (1:1) which was an additive effect. The synergism effect is 30% which is generally expected from the combination effect, but in this study only 10% gave a strong category. This best combination of extracts can be used as a formula for further research into in vivo immunostimulant tests.

According to research, the type, genus, species, and strain of the target microorganism, the botanical source of the plant, the bioactive compounds it contains, as well as the harvest time, stage of development, or extraction method, and its chemical properties (concentration, pH value, and lipophilicity), all affect how effective plant compounds are at combating bacteria. Three probable mechanisms of action of plant extracts and their main ingredients include inhibition of bacterial growth or survival, concentrating on bacterial virulence factors, or increasing the efficiency of antibiotics as resistance-modifying agents. Inhibiting DNA/RNA synthesis and function, interfering with intermediate metabolism, disrupting membrane structure and function (including efflux systems), and causing cytoplasmic components to congeal are some of the methods used to stop bacterial growth [32]. After NMR spectroscopy analysis, the endophytic fungus extract of sungkai leaves showed that it contained phenolic compounds. Phenolic compounds reveal a variety of structures, such as the existence, number, and location of substitute the groups of hydroxyls, and the length of the saturated side chain that gives this compound its antibacterial properties. This is based on the finding that phenolic-acid inhibits the action of ribonucleic-acid-reductase, an enzyme-required for DNA synthesis, thus causing the failure of bacterial DNA synthesis. The antibacterial agent of citric acid is caused by the physical and chemical properties, which reduce the extracellular aggregates production and the surface bacterial cell walls hydrophobicity. Antibacterial compounds can kill bacteria by working on cell walls of bacterial, plasma membranes, protein synthesis, or the metabolism of nucleic acid [34, 35].

3.2 Pure compounds and their combination as antibacterial

The theory underlying the effectiveness of these plant compounds against bacteria can be applied to the effectiveness of endophytic fungi against bacteria. This hypothesis is based on the idea that when two actors work together, their combined actions are more successful than the sum of their separate actions. The same thing can be found in the combined effect of pure compounds from endophytic fungi as shown in Figure 4. The pure compound was obtained from the column chromatography process of endophytic fungal extract. After that, identify the pure compound using NMR spectroscopy analysis. However, this research used existing stocks of five compounds. The five test combinations of pure compounds from endophytic fungi against the four test bacteria in Figure 4 can be classified as indifference (0%), additive (60%), synergism (40%), and antagonism (0%). Additive effects also dominate the combined effects of these pure compounds and 40% of them are in the strong category. Furthermore, the synergism effect takes up 40%, all the combinations provide strong category antibacterial activity. The best combination of pure compounds in this study for all test bacteria was C1+C2 (1:1) which is a combination of additive and synergistic effects.

In the research [36] revealed that the phenolic compounds obtained from Olive Mill Wastewater (OMW) showed that Instead of employing only one component as an antibacterial agent, the natural mixture of several compounds proved preferable. The bioactivity of the individual phenolic components used in his study was found to be extremely low and required high concentrations exceeding 1000 µg mL^{-1} per component to inhibit the growth of the four test bacteria (*S. pyogenes*, *S. aureus*, *E. coli*, and *K. pneumoniae*). But all four bacterial strains were totally inhibited by OMW that had been enhanced with particular combinations of phenolic compounds at low combined concentrations of 50/50-200/400 g mL^{-1}. Due to the synergistic impact produced by utilizing several components, including OMW/hydroxytyrosol, OMW/gallic, and gallic/hydroxytyrosol, the combination of various phenolic compounds was successful against four different bacterial strains.

Because plants naturally possess antibacterial qualities, the possibility of employing antibacterial medications derived from them is becoming increasingly important as the problem of bacterial resistance worsens. Direct impact on bacteria, synergistic activity with other plants, or synergistic activity with antibiotics are all potential causes of this activity. Numerous scientific investigations have amply established the in vitro synergistic efficacy of active plant components against multidrug-resistant microorganisms [37].

Numerous phytochemical components found in medicinal plants have the potential to be investigated as treatments for various ailments. The current hunt for novel bioactive metabolites from endophytic fungus of medicinal plants is driven by the potential of bioactive chemicals and the rising demand for therapeutic medicines [38, 39]. One of the most important sources of discovery and molecular diversity for novel medications is thought to be natural compounds generated from endophytic fungus. Therefore, categorizing the pharmacological effects of endophytic fungal extracts and their metabolites can help to ensure their continued use as active medicinal ingredients. It can also provide information about the bioprospects of endophytic fungi as biological entities [40, 41].

Advances in research on the synergistic effects of medicinal plants can be applied to synergistic effects on endophytic fungi. The potential for developing novel antibacterial medicines generated from endophytic fungi for different therapies, including the treatment of infections, may expand with continued study into the synergistic effects of endophytic fungi. However, little is known about the processes behind these synergistic effects. It will only be feasible to create new generations of standardized and efficient preparations with appropriate comprehension of these systems. Testing various activities, toxicity, and in vivo bioavailability will also help decide whether or not they are actually relevant for the provision of drugs in the future.

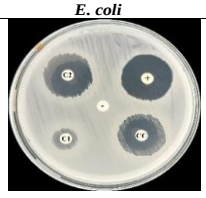
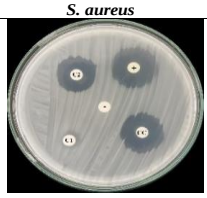
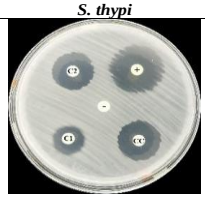
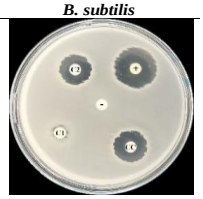
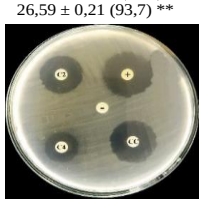
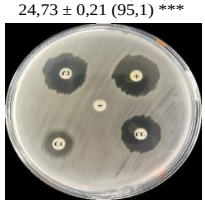
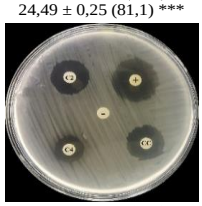
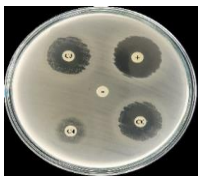
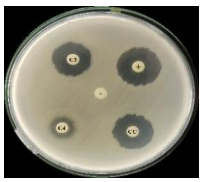
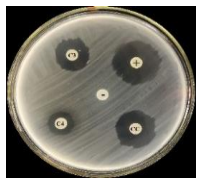
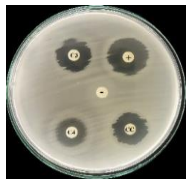
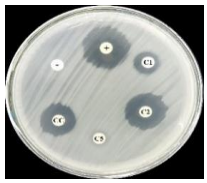
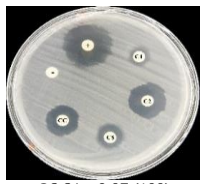
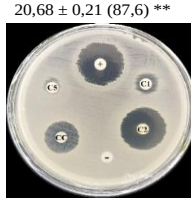
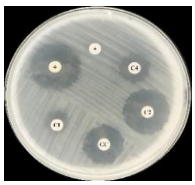
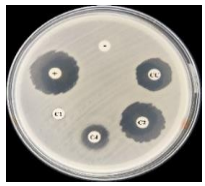
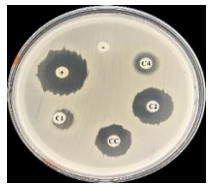
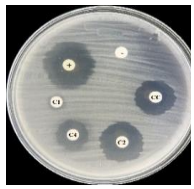
Sample	Inhibition zone (mm) (% Antibacterial Activity)			
	E. coli	*S. aureus*	*S. thypi*	*B. subtilis*
Test 1				
Control (+)	28,38 ± 0,04 (100)	26,00 ± 0,05 (100)	30,18 ± 0,04 (100)	24,87 ± 0,04 (100)
Control (-)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
C1	13,92± 0,07 (49,0)	10,20 ± 0,07 (39,2)	17,41 ± 0,07 (57,7)	8,73 ± 0,11 (35,1)
C2	27,33 ± 0,07 (96,3)	21,28 ± 0,04 (81,8)	23,28± 0,14 (77,1)	18,56 ± 0,39 (74,6)
Comb. (CC)	26,59 ± 0,21 (93,7) **	24,73 ± 0,21 (95,1) ***	24,49 ± 0,25 (81,1) ***	19,39 ± 0,32 (78,0) ***
Test 2				
Control (+)	27,66 ± 0,18 (100)	25,34 ± 0,04 (100)	25,42 ± 0,05 (100)	24,66 ± 0,04 (100)
Control (-)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
C2	22,98 ± 0,14 (83,1)	23,50 ± 0,07 (92,72	20,93 ± 0,04 (82,3)	20,93 ± 0,07 (84,9)
C4	18,59 ± 0,07 (67,2)	15,53 ± 0,07 (61,3)	16,17 ± 0,07 (63,6)	17,11 ± 0,21 (69.4)
Comb. (CC)	25,21 ± 0,18 (91,1) ***	21,77 ± 0,18 (85,9) **	20,32 ± 0,04 (79,9) **	23,39 ± 0,35 (94,8) ***
Test 3				
Control (+)	27,04 ± 0,10 (100)	24,15 ± 0,05 (100)	27,90 ± 0,05 (100)	23,62 ± 0,05 (100)
Control (-)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
C3	24,36 ± 0,07 (90,1)	21,26 ± 0,07 (88,0)	19,71 ± 0,07 (70,6)	20,86 ± 0,14 (88,3)
C4	16,93 ± 0,04 (62,6)	14,08 ± 0,07 (58,3)	15,28 ± 0,35 (54,8)	18,36 ± 0,49 (77,7)
Comb. (CC)	25,21 ± 0,04 (93,2) ***	21,85 ± 0,11 (90,5) ***	23,77 ± 0,07 (85,2) ***	20,68 ± 0,21 (87,6) **
Test 4				
Control (+)	24,73 ± 0,04 (100)	26,58 ± 0,05 (100)	26,64 ± 0,07 (100)	26,41 ± 0,05 (100)
Control (-)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
C1	10,64 ± 0,04 (43,0)	14,41 ± 0,04 (54,2)	12,16 ± 0,04 (45,6)	12,86 ± 0,07 (48,7)
C2	22,62 ± 0,04 (91,5)	22,54 ± 0,28 (84,8)	21,59 ± 0,04 (81,0)	25,65 ± 0,14 (97,1)
C5	11,23 ± 0,07 (45,4)	8,14 ± 0,04 (30,6)	16,09 ± 0,07 (60,4)	10,12 ± 0,11 (38,3)
Comb. (CC)	17,10 ± 0,04 (69,1) **	19,44 ± 0,04 (73,1) **	18,53 ± 0,11 (69,6) **	19,33 ± 0,07 (73,2) **
Test 5				
Control (+)	28,44 ± 0,09 (100)	27,17 ± 0,09 (100)	27,71 ± 0,05 (100)	28,08 ± 0,13 (100)
Control (-)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
C1	13,69 ± 0,07 (48,1)	7,72 ± 0,04 (28,4)	11,79 ± 0,49 (42,5)	9,33 ± 0,14 (33,2)
C2	25,97 ± 0,07 (91,3)	24,86 ± 0,18 (91,5)	21,98 ± 0,42 (79,3)	23,33 ± 0,14 (83,1)
C4	17,19 ± 0,11 (60,4)	20,16 ± 0,14 (74,2)	14,68 ± 0,18 (53,0)	21,44 ± 0,39 (76,4)
Comb. (CC)	22,86 ± 0,11 (80,4) **	15,73 ± 0,07 (57,9) **	19,39 ± 0,04 (70,0) **	17,86 ± 0,21 (63,6) **

Figure 4. Effect of a combination of pure compounds from the endophytic fungus *P. canescens* on antibacterial activity
Note: * indifference; ** additive; *** synergism; **** antagonism

4. CONCLUSIONS

The results obtained in this study support the fact that the antibacterial activity of the pure extract/compound combination is difficult to predict from the activity of the single pure extract/compound. This uncertainty is caused by the diverse structures of compounds and secondary metabolites so that there are many possible reactions that can occur. The combination of sungkai leaf endophytic fungal extracts provides additive, synergistic and antagonistic effects. Meanwhile, the combination of pure compounds provides additive and synergistic effects. The best combination in this study was chosen from the highest % antibacterial activity of the combination effect, which can be used as a formula for further research into in vivo tests. The best combination of extracts is PD4+PD5+PD8 which comes from an additive effect and the best combination of pure compounds is C1+C2 which comes from an additive and synergistic effect. Thus, this combination formula is expected to help avoid unwanted side effects due to higher doses of single ingredients. The additive and synergistic effects that occur in the combination of PD1+PD2 extract and the pure compound C3+C4 combination can apparently be used as an alternative formula compared to the single extract or the single pure compound.

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REFERENCES

[1] Ibrahim, A., Kuncoro, H. (2012). Identifikasi metabolit sekunder dan aktivitas antibakteri ekstrak daun sungkai (Peronema canescens Jack.) Terhadap Beberapa Bakteri Patogen. Journal of Tropical Pharmacy and Chemistry, 2(1): 8-18. https://doi.org/10.25026/jtpc.v2i1.43

[2] Faurizia, D., Fitriani, L., Harmoko, H., Nopiyanti, N. (2021). Pengaruh Antibakteri Sari Pati Daun Sungkai (Peronema canescens Jack) Terhadap Zona Hambat Shigella dysentriae. Nusantara Hasana Journal, 1(7): 33-37.

[3] Alkausart, A., Asra, R., Fauziah, F. (2022). Overview of phytochemicals and pharmacological activities of Sungkai (Peronema canescens Jack): Popular plants in Indonesia during the Covid-19 Pandemic. IOSR Journal of Pharmacy and Biological Sciences, 17(2): 17-29. https://doi.org/10.9790/3008-1702011729

[4] Fadriyanti, O., Afriza, D., Chaerunnisa, C. (2023). Biological effect of peronema canescens jack on the surface changes of alginate mold. Journal of Syiah Kuala Dentistry Society, 8(1): 1-8. https://doi.org/10.24815/jds.v8i1.33001

[5] Fadlilaturrahmah, F., Putra, A.M.P., Rizki, M.I., Nor, T. (2021). Uji aktivitas antioksidan dan antitirosinase fraksi n-butanol daun sungkai (peronema canescens jack.) secara kualitatif menggunakan kromatografi lapis tipis. Journal of Pharmascience 8(2): 90-98. https://doi.org/10.20527/jps.v8i2.11160

[6] Latief, M., Sari, P.M., Fatwa, L.T., Tarigan, I.L., Rupasinghe, H.P.V. (2021). Antidiabetic activity of Sungkai (Peronema canescens Jack) leaves ethanol extract on the male mice induced alloxan monohydrate. Pharmacology Clinical Pharmaeutical Research, 6(2): 64-73. https://doi.org/10.15416/pcpr.v6i2.31666

[7] Latief, M., Tarigan, I.L., Sari, P.M., Aurora, F.E. (2021). Aktivitas antihiperurisemia ekstrak etanol daun sungkai (Peronema canescens jack) pada mencit putih jantan. Pharmacon: Journal Farmasi Indonesia, 18(1): 23-37. https://doi.org/10.23917/pharmacon.v18i01.12880

[8] Muharni, M., Ferlinahayati, F., Yohandini, H., Riyanti, F., Pakpahan, N.A.P. (2021). The anticholesterol activity of betulinic acid and stigmasterol isolated from the leaves of Sungkai (Paronema Canescens Jack). International Journal of Applied Pharmaceutics, 13(2): 198-203. https://doi.org/10.22159/ijap.2021v13i2.40372

[9] Rahardhian, M.R.R., Susilawati, Y., Sumiwi, A., Muktiwardoyo, M., Muchtaridi. (2022). A review of Sungkai (Peronema Canescens): Traditional usage, phytoconstituent, and pharmacological activities. International Journal of Applied Pharmaceutics, 14 (Special issue no 5): 15-23. https://doi.org/10.22159/ijap.2022.v14s5.24

[10] Nurjannah, S., Arum, D., Tarigan, I.L., Latief, M. (2023). Anti-inflammatory prediction of peronemin compounds from Sungkai (Peronema Canescens Jack) and their derivatives. Al Ulum Journal of Sains Dan Teknologi, 9(2): 59-65. https://doi.org/10.31602/jst.v9i2.11338

[11] Dillasamola, D., Aldi, Y., Kurniawan, H., Jalius, I.M. (2021). Immunomodulator effect test of Sungkai leaves (Peronema canescsens Jack) ethanol extract using carbon clearance methode. International Conference on Contemporary Science and Clinical Pharmacy, 40(2): 1-6. https://doi.org/10.2991/ahsr.k.211105.001

[12] Wen, J., Okyere, S.K., Wang, J., Huang, R., Wang, Y., Liu, L., Nong, X., Hu, Y. (2023). Endophytic fungi isolated from Ageratina Adenophora exhibits potential antimicrobial activity against multidrug-resistant staphylococcus aureus. Plants, 12(3): 12-24. https://doi.org/10.3390/plants12030650

[13] Wen, J., Okyere, S.K., Wang, S., Wang, J., Xie, L., Ran, Y., Hu, Y. (2022). Endophytic fungi: An effective alternative source of plant-derived bioactive compounds for pharmacological studies. Journal of Fungi, 8(2): 23-35. https://doi.org/10.3390/jof8020205

[14] Elfita E., Muharni M., Munawar M., Aryani S. (2012). Secondary metabolite from endophytic fungi aspergillus niger of the stem bark of kandis gajah (Garcinia griffithii). Indonesian Journal of Chemistry, 12(2): 195–200. https://doi.org/10.22146/ijc.21363

[15] Shen, N., Chen, Z., Cheng, G., Lin, W., Qin, Y., Xiao, V., Chen, H., Tang, Z., li, Q., Yuan, M., Bu, T. (2023). Diversity, chemical constituents and biological activities of endophytic fungi from Alisma orientale (Sam.) Juzep. Frontier Microbiology, 14(1): 1-16. https://doi.org/10.3389/fmicb.2023.1190624

[16] Hemaiswarya, S., Poonkothai, M., Raja, R., Anbazhagan, C. (2009). Comparative study on the antimicrobial activities of three Indian medicinal plants. Egyptian Journal of Biology, 11(1): 52-57.

[17] Oktiansyah, R., Juliandi, B., Widayati, K., Juniantito, V. (2018). Neuronal cell death and mouse behaviour induced by Bee Venom. Tropical Life Sciences Research, 29(2): 1-11. https://doi.org/10.21315/tlsr2018.29.2.1

[18] Fuego, B.N., Romano, K.G., Pinlac, C.D., Lirio, G.A.C. (2021). Evaluation of the antimicrobial activity of endophytic fungus isolated from *Cocos nucifera* (L.) cotyledon against medically-important pathogens. Journal Bioscience Medical, 9(01): 86-97. https://doi.org/10.4236/jbm.2021.91007

[19] Vaou, N., Stavropoulou, E., Voidarou, C.C., Tsakris, Z. (2022). Interactions between medical plant-derived bioactive compounds: Focus on antimicrobial combination effects. Multidisciplinary Digital Publishing Institute, 9(2): 1-23. https://doi.org/10.3390/antibiotics11081014

[20] Kapeua-Ndacnou, M., de Abreu, L.M., de Macedo, D.M., da Nóbrega, T.F., Pereira, C.M., Evans, H.C., Barreto, R.W. (2023). Assessing the biocontrol potential of clonostachys species isolated as endophytes from coffea species and as mycoparasites of hemileia rusts of coffee in Africa. Journal of Fungi, 9(2): 248. https://doi.org/10.3390/jof9020248

[21] Simoes, M., Bennett, R.N., Rosa, E.A. (2009). Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms. Natural Product Reports, 26(6): 746-757. https://doi.org/10.1039/b821648g

[22] Pitt, J.I., Hocking, A.D. (2009). Fungi and Food Spoilage.

New York: Springer.
[23] Walsh, T.J., Hayden, R.T., Larone, D.H. (2018). Larone's Medically Important Fungi. John Wiley & Sons.
[24] Watanabe, T. (2010). Pictorial Atlas of Soil and Seed Fungi. CRC Press, Boca Raton. https://doi.org/10.1201/EBK1439804193
[25] Oktiansyah, R., Widjajanti, H., Setiawan, A., Elfita, E. (2024). Antioxidant and antibacterial activity of endophytic fungi isolated from fruit of Sungkai (Peronema canescens). Science and Technology Indonesia, 9(1): 17-27. https://doi.org/10.26554/sti.2024.9.1.17-27
[26] Ding, Z., Tao, T., Wang, L., Zhao, Y., huang, H., Zhang, D., Liu, M., Wang, Z., Han, J. (2019). Bioprospecting of novel and bioactive metabolites from endophytic fungi isolated from rubber tree ficus elastica leaves. Journal Microbiol Biotechnology, 29(5): 731-738. https://doi.org/10.4014/jmb.1901.01015
[27] Oktiansyah, R., Widjajanti, H., Setiawan, A., Nasution, S.Sa.A., Mardiyanto, M., Elfita. (2023). Antibacterial and antioxidant activity of endophytic fungi extract isolated from leaves of Sungkai (Peronema canescens). Science Technology Indonesia, 8(2): 170-177. https://doi.org/10.26554/sti.2023.8.2.170-177 1
[28] Elfita, E., Oktiansyah, R., Mardiyanto, M., Widjajanti, H., Setiawan, A. (2022). Antibacterial and antioxidant activity of endophytic fungi isolated from Peronema canescens leaves. Biodiversitas, 23(9): 4783-4792. https://doi.org/10.13057/biodiv/d230946
[29] Oktiansyah, R., Elfita, E., Widjajanti, H., Setiawan, A., Mardiyanto, M., Nasution, S.S. (2023). Antioxidant and antibacterial activity of endophytic fungi isolated from the leaves of sungkai (Peronema canescens). Tropical Journal of Natural Product Research, 7(3): 2596-2604. https://doi.org/10.26538/tjnpr/v7i3.20
[30] El-Hawary, S.S., Moawad, A.S., Bahr, H.S., Abdelmohsen, U.R., Mohammed, R. (2020). Natural product diversity from the endophytic fungi of the genus Aspergillus. Royal Science of Chemistry Journal, 10(37): 22058-22079. https://doi.org/10.26554/sti.2023.8.2.170-177
[31] Pezzani, R., Salehi, B., Vitalini, S., Iriti, M., Zuniga, F.A., Sharifi-Rad, J., Martorell, M., Martins, N. (2019). Synergistic effects of plant derivatives and conventional chemotherapeutic agents: An update on the cancer perspective. Medical Journal, 55(4): 1-16. https://doi.org/10.3390/medicina55040110
[32] Ocana, A., Amir, E., Yeung, C., Seruga, B., Tannock, I.F. (2012). How valid are claims for synergy in published clinical studies. Ann Oncol Journal, 23(8): 2161-2166. https://doi.org/10.1093/annonc/mdr608
[33] Franco, J.L.M., Giulianotti, M.A., Welmaker, G.S., Houghten, R.A. (2013). Shifting from the single to the multitarget paradigm in drug discovery. Drug Discovery Today, 18: 495-501. https://doi.org/10.1016/j.drudis.2013.01.008
[34] Abdelhakim B., Chamkhi I., Balahbib A., Rebezov M., Shariati M.A., Wilairatana P., Mubarak M.S., Benali T., Omari N.E. (2022). Mechanisms of medicinal plant bioactive compounds against bacteria. Molecules 27(1484): 1-29. https://doi.org/10.3390/molecules27051484
[35] Burel, C., Kala, A., Purevdorj-Gage, L. (2021). Impact of pH on citric acid antimicrobial activity against Gram-negative bacteria. Letters in Applied Microbiology, 72(3): 332-340. https://doi.org/10.1111/lam.13420
[36] Stefanović, O.D. (2018). Synergistic activity of antibiotics and bioactive plant extracts: A study against gram-positive and gram-negative bacteria. Bacteria Pathogen. Intechopen, 4(8): 237-243. https://doi.org/10.5772/intechopen.72026
[37] Azaizeh, H., Tafesh, A., Najami, N., Jadoun, J., Halahlih, F., Riepl, H. (2011). Synergistic antibacterial effects of polyphenolic compounds from olive mill wastewater. Evidence-based Complement. National Library Medicine, 8(2): 24-32. https://doi.org/10.1155/2011/431021
[38] Lu, S., Qiu, Y., Ni, D., He, X., Pu, J., Zhan, J. (2020). Emergence of allosteric drug-resistance mutations: New challenges for allosteric drug discovery. Drug Discov Today, 25(1): 177-184. https://doi.org/10.1016/j.drudis.2019.10.006
[39] Adeleke, B.S., Babalola, O.O. (2021). Pharmacological potential of fungal endophytes associated with medicinal plants: A review. Journal Fungi, 7(2): 1-16. https://doi.org/10.3390/jof7020147
[40] Cruz, J.S., Da Silva, C.A., Hamerski, L. (2020). Natural products from endophytic fungi associated with rubiaceae species. Journal Fungi, 6(3): 1-26. https://doi.org/10.3390/jof6030128
[41] Sanhueza, L., Melo, R., Montero, R., Maisey, K., Mendoza, L., Wilkens, M. (2017). Synergistic interactions between phenolic compounds identified in grape pomace extract with antibiotics of different classes against Staphylococcus aureus and Escherichia coli. PloS One, 12(2): 1-15. https://doi.org/10.1371/journal.pone.0172273