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Research Article

COMBINATION KARAS (*Aquilaria malaccensis* LAMK.) INFUSION AND ANTIBIOTICS AGAINST PATHOGENIC BACTERIA FROM DIABETIC FOOT ULCER (DFU)

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ABSTRACT

FICI used to describe the result of two compound antibacterial in resist bacterial growth so that FICI value characteristic can be known, there is synergistic, additive, indifferent, or antagonist. According to the background above this research purpose is to analyze FICI value from combination of antibiotic with karas infusion against bacteria isolate from DFU patient level III and IV Wagner. Subject taken from bacterial swab identification DFU level III and IV Wagner. This Research using Kirby-Bauer method with combination infusion with 25%;75%; 50%:50%; 75%:25% with Gentamicin, Tetracycline, and Ciprofloxacin. Based on this research combination from Karas: Gentamicin with 50%:50% showed inhibition zone (6.23+0.09 mm); Karas : Ciprofloxacin 50%:50% (6.37+0.21 mm), also 75%:25% for tetracycline (6.20+0.16) against *Bacillus cereus*. Combination and karas leaf infusion with the diameter zone is 50%:50% for Gentamicin (6,40+0,08 mm) and Ciprofloxacin (6.38+0.08 mm), also 75%:25% for Tetracycline (6.27+0.09 mm) against *Microbacterium hydrocarbonoxydans*. Combination Karas with the smallest diameter zone is 50%:50% with Gentamicin (6.42+0.08 mm), Tetracycline (6.17+0.05 mm), and Ciprofloxacin (6.33+0.12 mm) against *Clostridium sphenoides*. The last for *Streptococcus pyogenes* antibiotic combination Karas with the smallest diameter zone is 50%:50% for Gentamicin (9.50+0.16 mm) and Tetracycline (9.20+0.14 mm), also 75%:25% for Ciprofloxacin (10.45+0.27 mm). All the FICI value combination were 2 means indifferent characteristic against pathogenic bacteria. We conclude that *Aquilaria malaccensis* leaves infusion combination with antibiotics can be an alternative against infection bacteria resistant from DFU, however are necessary larger studies to prove their combination action.

Keywords: Karas Infusion, DFU patient swab, FICI, Indifferent

INTRODUCTION

DFU is a condition where the bacterial infection aggravate the damage in ulcer cell. The high blood sugar level in diabetic patient cause white blood cell decreasing significantly and decreasing interleukin inside the wound cell that grow the infection.[1] The prevalence about ulcer diabetic on foot globally reach 6.3%, and the prevalence in America, Europe, and Asian are 13%, 7.2% and 5.1%.[2] According the data that has been gather from *United Kingdom Prospective Diabetes Study* (UKPDS) shows that one of many chronic complications that has been experienced by Diabetes mellitus patient in Indonesia is ulcer diabetes on foot area with 15% percentage.[3] The survey result that been gathered on the field show that from 800 patients that get medical attention on a Special Clinic Injury Cure Kitamura in Pontianak City at least 470 patients were found with ulcer diabetes on foot area.[4] In handling ulcer diabetes infection by using antibiotic as the main therapy.[6] The main problem is antibiotic use make the healing process longer causing the antibiotic resistance. The antibiotic resistance cause by the wrong application antibiotic use or long term antibiotic medical treatment. So as the possibility organism resistance to one of antibiotic tend to build resistance to another antibiotic.[7] Other than that antibiotic resistance can be caused by wrong application of antibiotic or long term medical treatment by using single antibiotic.[8,9]

Karas is one of non-wood resources that have high economic value so that it has been developed in Indonesia by many individual. One of this kind of plants is *Aquilaria malaccensis.* This plants existence protected by Indonesian government. All this time karas only it's stem that have been harvested, however the leaves is not usable and only just wasted.[10]

Aquilaria spp have alkaloid, flavonoid, saponin, tannin, terpenoid, and phenol. *Aquilaria spp* has analgesic, antioxidant, anti-pyretic, anti-inflammatory, antimicrobial, and anti-hyperglycemic.[11,12] There are some *Aquilaria spp* species that have been reported has antibacterial activity is *Aquilaria crassna,*[12] *Aquilaria agallocha,*[13,14] and *Aquilaria subintegra.*[15]

Based on a previous research the plants that used to make an infusion been tested has a potential antibacterial. FICI value (Fractional Inhibitory Concentration Index) used to describe the result of two compound antibacterial in resist bacterial growth so that FICI value characteristic can be known, there is synergistic, additive, indifferent, or antagonist.[18,19] According to the background above this research purpose is to analyze FICI value from combination of antibiotic with karas infusion against resistance bacterial that found from like *Streptococcus pyogenes Sp , Microbacterium hydrocarbonoxydans Sp, Bacillus cereus Sp,* and *Clostridium sphenoides.*

MATERIALS AND METHODS

The material used were *Blood Agar Plate* (Himedia, India), *Mac Conkey Agar* (Oxoid, UK), *Salmonella Shigella Agar* (Oxoid, UK), *Triple Sugar Iron Agar* (Himedia, India), *Simon Sitrate agar* (Oxoid, UK), *Sulfur Indole Motility* (Himedia, India), Gentamicin 10 µg/disc (Pronadisa, Spain), Tetracycline 30

µg/disc (Pronadisa, Spain), Ciprofloxacin 5 µg/disc (Oxoid, UK), Clindamicin 10 µg/disc (Pronadisa, Spain), Cefotaxime 50 µg/disc (Pronadisa, Spain), and Imipenem 10 µg/disc (Oxoid, UK).

Karas Leaves Extraction

Add 50 gram of *Aquilaria malaccensis* Lamk. to the infusion pan, then add 100 ml distilled water, let it boil for about 15 minutes until it reach 90ºC. After that use funnel as the filter, add some hot water if the volume is insufficient through sample debris about 100 ml. close the tube with cotton and aluminum foil that has been sterilized in this research.[20]

Bacteria Media and Identification of Bacteria

Specimens of diabetic foot ulcers in sterile cotton sticks were planted in the media of Blood Agar Plate (BAP), Mac Conkey Agar (MCA). Bacterial planting was carried out directly on the media to be solid by the scratch method and incubated for 24 hours in an incubator at a temperature of 32-40ºC. Bacteria in sterilized swab were grown in blood agar, MC, and SSA with streak method. Incubation occurred in 24 hours with the temperature of 37ºC. The bacterial colony that has grown was separated and grown in NA.

Identification was done by Gram staining and biochemical test. The biochemical test included sugar fermentation test, carbohydrate fermentation test, motility test, indole test, H_2S producing test, urea test, oxidase and catalase test, and fermentative-obligative test.

Bacterial Tests

The bacteria were assessed against bacteria that isolated from DFU swab patients, maintained in BHI at -20°C; 300 mL of each stock-culture was added to 3 mL of BHI broth. Overnight cultures were kept for 24 h at 36°C ± 1°C and the purity of culture was checked after 8 hours of incubation. After 24 h of incubation, bacterial suspension (inoculum) was diluted with the sterile physiological solution, for the diffusion and indirect bioautographic test, to 10^8 CFU/ml (turbidity = McFarland barium sulfate standard 0,5).

Antibiotic Sensitivity Assay

This test was evaluated by Antibiotic diffusion disc test (Gentamicin, Tetracycline, Ciprofloxacin, Clindamicin, Cefotaxime, and Imipenem). The Antibiotics Disc were placed on the surface of the media. After 24 h of incubation, Inhibition zone were determined with CLSI standard.

Karas Infusion and Antibiotic Assay with FICI (Fractional Inhibitory Concentration Index)

Sensitivity test of antibiotic and antibacterial combination with karas leaf infusion by using Kirby-Bauer method. Subject that have been incubated then inoculating on media like Mueller Hinton Agar (MHA). The previous bacterial subject in suspension form with the same turbidity standard content Mc Farland. Concentration equalization of microorganism by using $BaCl_2$ 1% and H_2SO_4 1% solvent. The standard that used was Mc Farland with 0.5 standard point.[21]

Bacterial plantation on MHA media by using gores method with ose needle. Then put the antibiotic disk and incubate it with 37ºC temperature for 24-48 hours. Sensitivity stipulation antibiotics observed by zone resistor. The resistor be marked on the clean area around antibiotic disk. The zone resistor diameter then compared with the data from *Clinical Laboratory Standard Institute* (CLSI) to decide whether the antibiotic has sensitivity characteristic, intermediate, or resistance.[21]

Preliminary test antibacterial by using karas leaves infusion (*Aquilaria malaccensis* Lamk.) to identify karas leaves infusion have antibacterial activity, and to analyze minimum concentration that could inhibit bacterial growth for variation mold karas leaf infusion, then by combining antibiotic and karas leaf infusion with differences of 50%:50%, 25%:75%, and 75%:25%.

FICI value (Fractional Concentration Inhibitory Index) from karas infusion and antibiotics were calculated with this formula[22]:

$$FICI = FIC_A + FIC_B$$

$$= \frac{\text{MIC infusion combination}}{\text{MIC infusion}} + \frac{\text{MIC antibiotic combination}}{\text{MIC antibiotic}}$$

FICI value is ≤ 0,5, show that the combination between extract and antibiotic has synergy effect, additive if more than 0,5 and less than 1, no differences if more than 1 and less than 4, and contrast if more than 4.[18]

RESULTS AND DISCUSSION

Karas Leaves Extraction

Karas leaves extraction method (*Aquilaria malaccensis* Lamk.) by using infusion method. The advantages of infusion method is cost effective, minimum tools. The disadvantages from method is the bacterial can easily contaminated with other bacterial and sometime because of the solvent, and then essences production was not stable. Infusion method choice is to pull the active substance in karas leaves. The active substance suspected to be inside karas leaves have potential as antibacterial is flavonoid, alkaloid, saponin.[19] Thus useful substance is a natural antibacterial that can be easily vapored in water, other than that this method choice has been adjusted with people habit on produce leaves or medical plants by boiling it.

Infusion production is put 50 gram leaves in infusion pan, and then add a solvent there is aqua pro injection about 100 ml. The purpose of this powder damping is to make the solvent enter the pores of *Aquilaria malaccensis* Lamk. powder that have been dry and change it with the solvent. After that, heated it for 15 minutes. The time calculation after the temperature reach 90ºC. Next phase is to distil it with funnel, volume deficiency can be added with hot water through subject grout infusion about 100 ml. And then close the tube with cotton and aluminum foil that have been sterilize previously in this research.

Infusion making can be done quickly before the test start. Do this to prevent fungus growth, bacterial or mold than can be intervene the test result. Aqua pro injection selection as the solvent agent or essence solvent because of the *Aquilaria malaccensis* Lamk. powder that been used has substance compound with polar characteristic. Another advantage is its low price, nontoxic, not easily vaporize, not inflammable, and can be access anywhere. Meanwhile the disadvantage as the solvent is that it can easily grow mold and fungus which can affect outcome of result.

Table 1: Inhibition Zone Diameter Combination of Karas Leaf Infusion and Antibiotics

Microbacterium hydrocarbonoxydans

Antibiotics	Inhibition Zone Diameter (n=3, x±SD)		
	50%:50%	25%:75%	75%:25%
Gentamicin 0.25 mg/ml	6.40±0.08	7.22±0.82	6.82±0.49
Tetracycline 0.5 mg/ml	6.67±0.42	7.02±0.55	6.27±0.09
Ciprofloxacin 0.25 mg/ml	6.38±0.08	6.77±0.17	6.47±0.12

Bacillus cereus

Antibiotics	Inhibition Zone Diameter (n=3, x±SD)		
	50%:50%	25%:75%	75%:25%
Gentamicin 0.5 mg/ml	6.23±0.09	7.00±0.08	6.60±0.29
Tetracycline 0.5 mg/ml	6.55±0.39	7.13±0.99	6.20±0.16
Ciprofloxacin 0.25 mg/ml	6.37±0.21	7.87±0.39	8.17±0.09

Clostridium sphenoides

Antibiotics	Inhibition Zone Diameter (n=3, x±SD)		
	50%:50%	25%:75%	75%:25%
Gentamicin 0.5 mg/ml	6.42±0.08	6.42±0.06	6.72±0.49
Tetracycline 0.5 mg/ml	6.17±0.05	6.53±0.29	6.48±0.10
Ciprofloxacin 0.25 mg/ml	6.33±0.12	7.07±0.74	7.17±0.17

Streptococcus pyogenes

Antibiotics	Inhibition Zone Diameter (n=3, x±SD)		
	50%:50%	25%:75%	75%:25%
Gentamicin 0.5 mg/ml	9.50±0.16	10.82±0.20	11.88±0.32
Tetracycline 0.25 mg/ml	9.20±0.14	10.80±0.30	11.68±0.85
Ciprofloxacin 0.25 mg/ml	10.47±1.24	11.50±0.33	10.45±0.27

Table 2: Results of Determination of FICI Value in Combination of Karas Leaf Infusion and Antibiotics against *Streptococcus pyogenes, Microbacterium hydrocarbonoxydans, Bacillus cereus,* and *Clostridium sphenoides*

Combination		FIC_A	FIC_B	FICI	Interpretation
Karas leaf infusion (102.4 mg/mL)	Gentamicin	1	1	2	indifferent
	Tetracycline	1	1	2	indifferent
	Ciprofloxacin	1	1	2	indifferent

Figure: 1 Diameter of the *Streptococcus pyogenes* bacterial inhibition zone

Figure: 2 Diameter of the *Microbacterium hydrocarbonoxydans* bacterial inhibition zone

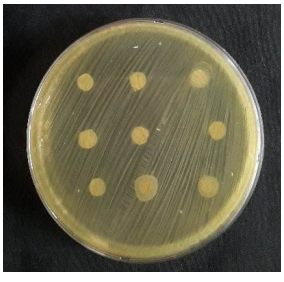

Figure: 3 Diameter of the *Bacillus cereus* bacterial inhibition zone

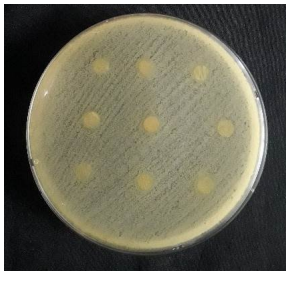

Figure: 4 Diameter of the *Clostridium sphenoides* bacterial inhibition zone

The Combination Test of Karas Leaf Antibacterial Infusion and Antibiotic

The sensitivity test was done by suing Mueller-Hinton Agar (MHA) method and antibiotic disk. The antibiotic that tested on bacterial is gentamicin, ciprofloxacin, and tetracycline. MHA use because this media has been recommended by FDA and WHO to test aerob antibacterial and anaerob antibacterial facultative to food and clinic material. Apart from this agar media is proven to give good result. This antibiotic disk was chosen because it's more practical and it isn't necessary to make antibiotic suspension.

According to the previous test on karas leaf infusion, there's some minimum infusion concentration that could have potential as antibacterial is 102.4 mg/ml, and the bacterial combination can be tested with antibiotics like gentamicin, tetracycline, and ciprofloxacin. According to the isolation bacterial test on ulcer diabetes leg there's many types of pathogen bacterial in it, but this research only use 4 pathogenic bacteria from the isolation result like *Streptococcus pyogenes, Microbacterium hydrocarbonoxydans, Bacillus cereus,* and *Clostridium sphenoides*. The result graph can be seen on picture 1,2,3, and 4 according to the bacterial position.

According to the result of karas leaf infusion and gentamicin, tetracycline, and ciprofloxacin are shown in table 1. The determination test of minimum resistor concentration antibacterial to the each 4 bacterial shown different data analysis. On *Bacillus cereus* bacterial antibiotic combination and karas leaf infusion with the smallest resistor zone diameter was 50%:50% for gentamicin and ciprofloxacin and 75%:25% for tetracycline. On *Microbacterium hydrocarbonoxydans* bacterial antibiotic combination and karas leaf infusion with the smallest resistor diameter zone was 50%:50% for gentamicin and ciprofloxacin, and 75%:25% for tetracycline. On *Clostridium sphenoides* antibiotic combination and karas leaf infusion with the smallest resistor diameter zone was 50%:50% for gentamicin, tetracycline and ciprofloxacin, and lastly on *Streptococcus pyogenes* antibiotic combination and karas leaf infusion with the smallest resistor diameter zone was 50%:50% for gentamicin and tetracycline, and 75%:25% for ciprofloxacin.

FICI Value Measurement Combination of Karas leaf Infusion and Antibiotic

FICI value is helpful to interpretation the combination of two combination antibacterial in resistant bacterial growth. In this research FICI value determination was used karas leaf infusion minimum resistor concentration and minimum antibiotic resistor concentration.

Determination test for FICI value of karas leaf infusion with antibiotic to *Streptoccus* pyogenes, *Microbacterium hydrocarbonoxydans, Bacillus cereus,* and *Clostridium sphenoides* are shown in table 2, which shows that the combination of karas leaf infusion with antibiotic resulted 2 FICI value therefore not showing differences (*indifferent)*. This show that antibacterial combination strength doesn't have any significant value if compare with karas leaf infusion with antibiotic.

Based on the research of Rakholiya and Chanda study conclude that ketapang leaves combination extract (*Terminalia catappa)* and gentamicin resulting characteristic (antibiotic strength) no difference to bacterial *Staphyococcus aureus*. But, combination of papaya leaves extract (*Carica papaya*) and gentamicin resulting characteristic (antibacterial strength) synergy along with bacterial *Stayphylococcus aureus*.[23] Rosato *et al* conclude that some combination of essential oil and gentamicin to bacterial *Escherichia coli* have a different effect. Combination of *Origanum vulgare* and gentamicin to bacterial *Escherichia coli* have a different effect with FICI value of 0.65. The combination of *Aniba rosaeodora* and gentamicin to bacterial *Escherichia oil* have a synergy effect along with FICI value of 0.35. The combination of *Melaleuca alternifolia* and gentamicin to *Escherichia coli* have a synergy effect along with FICI value of 0.49. The combination of *Pelargonium graveolens* and gentamicin to bacterial *Escherichia coli* have a synergy effect along with FICI value of 0.30.[24] This show that the combination between karas leaf infusion combination (*Aquilaria malaccensis* Lamk.) and antibiotic can resulting characteristic (antibacterial strength) with many differences. The secondary metabolism compound with different kind in plant extract can be affecting antibacterial activity too after the combine it with antibiotic.

CONCLUSION

From this study it can be concluded that minimum resistor concentration from karas leaf infusion combination (*Aquillaria malaccensis* Lamk.) and antibiotic can slow the bacterial growth *Streptococcus pyogenes, Microbacterium hydrocarbonoxydans, Bacillus cereus,* and *Clostridium sphenoides* with zone resistor of 7.63 + 0.35 mm. Fractional Inhibitory Concentration Index (FICI) from the combination of karas leaf infusion (*Aquilaria malaccensis* Lamk) and antibiotic was found to be 2 combination that show characteristic (antibacterial strength) no differences (indifferent).

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