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Antioxidant And Antibacterial Activities Of Methanol Extract Of Fig Fruit And Leaves (*Ficus Carica* L.)

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Antioxidant, Antibacterial , Fig Plants

A b s t r a c t

Background: *Fig plants (Ficus carica L.) have phytochemical contents such as polyphenols, flavonoids, and anthocyanins which are relatively high. The content of this active compound can be used as an antioxidant and antibacterial.* ***Objectif****: The study aims to determine the antioxidant and antibacterial activity of methanol extracts fruit and leaves of fig (Ficus carica* L.*).* ***Methods :*** *DPPH method was carried out to determine the antioxidant fruit and leaves of fig (Ficus carica L.) by observing the change in colour of the test sample after incubation with DPPH solution using a UV-Vis spectrophotometer at a wavelength of 517 nm. The antibacterial test uses disc paper diffusion test to see the inhibition zone formed and continued with the MIC and MKC tests.* ***Results :*** *The antioxidant test results showed the IC50 values in methanol extracts of fig fruits were 13.402 ppm and 7.9875 ppm for fig leaves. From the IC50 value, both the fruit and fig leaves are classified into a solid antioxidant compound. The antibacterial test results showed that the highest inhibitory diameter was found at a 100% concentration of 28.5 mm. The quantitative antibacterial activity test showed that the methanol extract of fig fruit had a MIC value at a concentration of 80% and a MKC value at a concentration of 100%. The optimal strength as an antibacterial from fruit extracts and leaves is 100% concentration.* ***Conclusion :*** *The results showed the methanol extract of fig (Ficus carica L.) has potential as an antioxidant and antibacterial compound.*

INTRODUCTION

Antioxidants are compounds that can slow down, delay or inhibit an oxidation reaction. Antioxidant compounds themselves can fight free radicals or Reactive Oxygen Species (ROS), usually formed from the body's metabolic processes. Free radicals themselves are known to cause arteriosclerosis, ageing and cancer caused by tissue damage due to oxidation.[1]

Antibacterial is a compound that can kill non-pathogenic bacteria and pathogenic bacteria. Antibacterial compounds usually have toxic but selective properties which can kill parasites but do not occur in the host.[2] One of the natural ingredients that can be used as a source of antioxidants and antibacterial compounds is figs (*Ficus carica L.*).

One of the common pathogenic bacteria found in human respiratory tract infections is *Streptococcus pneumonia*e, a gram-positive bacterium that inhabits normal flora in the upper respiratory tract.[3] Pathogenic bacteria are bacteria that can infect and cause disease for living things that are their hosts.[4] *Streptococcus pneumonia*e which is not common in humans, causes respiratory infections such

as pneumonia, otitis, sinusitis, bronchitis, meningitis. Pneumonia is included in the Acute Lower Airway Infection located in the lung parenchyma cause high mortality.[5] One of the common pathogenic bacteria found in human respiratory tract infections is *Streptococcus pneumonia*e, a gram-positive bacterium that inhabits normal flora in the upper respiratory tract.[3] Pathogenic bacteria are bacteria that can infect and cause disease for living things that are their hosts.[4] *Streptococcus pneumonia*e which is not common in humans, causes respiratory infections such as pneumonia, otitis, sinusitis, bronchitis, meningitis. Pneumonia is included in the Acute Lower Airway Infection located in the lung parenchyma cause high mortality.[5] The second highest cause of under-five mortality in 2007 reached 30,470 under-five (15.5% x 196.579) or if an average of 83 under-five died every day due to pneumonia.[6]

The use of antibiotics as a countermeasure can lead to bacterial immunity against the antibiotics themselves if their use is not as recommended, this results in an increase in the death rate due to infection. Currently, treatment and control of infectious diseases by bacteria are difficult to overcome because combined antibiotic drugs also causes antibiotic resistance.[7]

Figs (*Ficus carica* L.) is a plant that has been used by the community as food and medicine for centuries.[8,9.] The benefits of the fruit, roots and leaves of *Ficus carica L.* are efficacious as a source of medicine in various diseases such as digestive disorders, diarrhoea, sore throat, cough, bronchitis, inflammation, cardiovascular system disorders and cancer.[8-12.]

Previous studies have examined the phytochemical content and various bioactive compounds in the plant *Ficus carica L.* There are polyphenol, flavonoid, and anthocyanin compounds that have the ability as antioxidants and antibacterial agents.[8,10,11,13-16.] These phenolic compounds have an important role as antioxidants and antibacterial agents.[17] Figs (*Ficus carica L.*) itself has a phenolic content equal to 1,090-1,110 mg / 100g of fresh fruit.[18] The greater the phenolic compounds' range, the greater the antioxidant and antibacterial activity.[17] Based on this background, a study was carried out to determine the action of the methanol extract of fig (*Ficus carica* L.) as an antioxidant and antibacterial compound.

RESEARCH METHOD

The materials used in this study were Figs (*Ficus carica L.*), methanol, sterile aquades, 5% DMSO, FeCl3 1%, paper disks, Agar Nutrient Media (NA), Nutrient Borth (NB) media, ampicillin, pure culture of *Streptococcus pneumonia*, DPPH, Methanol PA. While the tools used in this study are beaker glass, plastic wrap, funnel, filter paper, stirring glass, stirrer, oven, analytical balance, rotary evaporator, measuring cup, Erlenmeyer, knife, blender, sieve, dropper, aluminium foil, autoclave plastic, autoclave, ose needle, test tube rack, vortex, test tube, bunsen, laminar airflow (LAF), incubator, fat cotton, petri dish, callipers, micropipette, label paper, UV-Vis spectrophotometer, Glass Cuvette, 50 ml Pumpkin, Vial Bottle, Watch Glass.

Plant identification at the Kebun Raya Purwodadi Pusat Penelitian Botani, Lembaga Ilmu Pengetahuan Indonesia (LIPI), Purwodadi showed that the type of plant used as material for this study was *Ficus carica L*. **Extraction**

Fruit and leaves powder was extracted by maceration method using methanol as a solvent with a ratio between solvent and powder 4: 1. 250 grams of fig fruit and leaves powder were soaked with 1000 mL methanol for 2x24 hours by stirring 1x24 hours for 10 minutes. The results of the marinade are then filtered to separate the residue from the filtrate. The extract of fig fruit and leaves extract was then concentrated with a rotary evaporator for 2 hours. The extraction results that have been carried out on the extract of fig extracts obtained thick and dark brown concentrated extract.

Antioxidant Test

Prepare a sample of 200 ppm master extract test figs with methanol PA and then do dilution with concentrations of 3, 6,12.5, 25, 50, 100, 200, 400, 800, 1000, 2000 ppm each of 5 ml each in methanol PA. Prepare a solution of 100 ppm DPPH stock. The solution of DPPH stock is prepared by dissolving 5 mg of DPPH solid into 50 ml of methanol PA. A comparison solution was then prepared, a control solution containing 3 ml of methanol PA and 1 ml of a 100 ppm DPPH solution. For the test sample, each 1 ml sample solution + 1 ml DPPH solution + 2 ml PA methanol is prepared. Then, it was incubated for 30 minutes at 27 ℃ until the discolouration of DPPH activity occurred. All samples were extracted set samples tested for their absorbance values using a UV-Vis spectrophotometer at a wavelength of 517 nm.

Determination of the IC50 value

Antioxidant testing of figs Fruit and leaves (*Ficus carica L.*) by DPPH method was conducted by observing the change in colour of the test sample after incubation with DPPH solution. If all DPPH electrons' reaction is paired with electrons in the extracted sample, there will be a change in the colour of the piece from dark purple to bright yellow. Then the absorbance value was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm.

Antibacterial Test Rejuvenation of *Streptococcus pneumonia*e bacteria

Nutrient Agar (NA) Nutrient Media is heated, then poured into a Petri dish until solid. Pure cultures of *Streptococcus pneumonia*e bacteria were inoculated onto NA media. Media that have been inoculated and incubated for 24 hours until bacteria grow.

Manufacture of Antibacterial Test Extract Concentrations

The methanolic extract of figs was weighed on an analytical balance according to the concentration used, and then the extract was dissolved with 10 ml of 5% DMSO solution.

Antibacterial Activity Test

Nutrient Agar (NA) media were poured into 20 ml Petri dishes mixed with 200 µl bacterial suspensions that had been measured with a light wave spectrophotometer of 600 nm with absorbance results of 0.1, and then the media was shaken slowly until the homogeneous media was allowed to solidify. Disk paper is soaked in fig fruit and leaves extract which has been diluted according to concentration. Disc paper is placed on the surface and incubated for 24 hours in an incubator of 37 ° C until an inhibition zone is formed. Antibacterial test carried out by repetition three times. To determine the antibacterial activity of the inhibition zone formed is measured by callipers, the area of the inhibitory zone formed is measured by the formula:

ZH = ZK - DC

Information :

ZH = Inhibition Zone

ZK = Overall Zone

DC = Disc Diameter

Minimum Inhibitory Concentration Test (MIC)

The Minimum Inhibitory Concentration Test (MIC) is carried out by a dilution method in which bacteria are planted on liquid media. Nutrient Borth (NB) is placed on a test tube as a normal test medium. The positive control was filled with 1 ml of NB, and 1 ml of *Streptococcus pneumoniae* suspension and the negative control was filled with 1 gram of extract each fig and 1 ml of NB. In another test tube filled with 1 ml of sterile NB and added 0.5 ml of *Streptococcus pneumoniae* bacterial suspension and each repeated three times. Before incubating the test solution, the absorbance was measured with a spectrophotometer at a wavelength of 600 nm. Furthermore, it was incubated at 37^{o}C for 24 hours, and the absorbance value was tested again. The formula measures the

MIC value:
MIC = OD after - OD before
Konsentrasi yang digunakan yaitu 1, 10, 20, 30, 40, 50, 50, 70, 80, 90 dan 100 dengan perbandingan control positif antibiotic ampicillin, suspense bakteri dan control negative dengan ekstrak buah dan daun tin

Minimum Kill Concentration Test (MKC)

Determination of the MKC test value with the whole solution stage from the MIC test was retested by taking one dose of each test solution then planted on the media on NA (Nutrient Agar) agar. All
cultures were incubated for 24 hours at 37^{o}C. After the incubation period can be read the results see the growth of bacteria on the media. The media concentration that did not show bacterial growth was MKC, which is the smallest concentration that could kill the test bacteria.[19]

The concentrations used were 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% with a positive control ratio of ampicillin antibiotics, bacterial suspense and negative control with fig fruit and leaves extracts.

RESULT AND DISCUSSION

Antioxidant

DPPH absorption method is one of the methods used in testing antioxidant activity. This method is relatively simple, it does not cost a lot, and the processing time is quite short. DPPH acts as a free radical where this test is based on the sample's ability as an antioxidant that can counteract free radicals.[20] The study results showed that the samples of both the fruit methanol extract and the fig leaves that had reacted with DPPH experienced colour changes from purple to light purple to yellow. The colour change effected by DPPH has captured hydrogen atoms from antioxidant compounds in the sample.[21]

Based on the data that has been obtained at each concentration that has been tested using a UV-vis spectrophotometer with a wavelength of 517 nm and analyzed using linear regression where the figs fruit and leaves methanol extract produces the equation y and IC50 values as follows:

Table 1. IC50 Values (*Ficus carica* L.)

Ekstract	Linear Regression	IC50 Value
Fruit	y = 3.6868x + 0.5895	13.402
Leaves	y = 7.2556x - 7.1555	7.9875

The value of IC50 in figs fruit methanol extract was 13,402 ppm while in fig leaves methanol extract was 7,9875 ppm. The IC50 value is included in the category of solid antioxidant activity because of the IC50 value <50 ppm.[22] Based on IC50 values produced, methanolic extracts of fig leaves showed better antioxidant activity in figs. This can occur because the quercetin content of fig leaves which is thought to act as an antioxidant is higher than the quercetin content of figs.Quercetin is a large group of antioxidants that are included in the flavonol derivative, in which flavonols themselves are part of flavonoids.[23] Quercetin acts as an antioxidant by cutting the chain oxidation reaction in free radicals or capturing electrons in free radicals.[24]

Free radicals are atoms which are reactive and less stable. In achieving stability, free radicals have electrons which will interact by getting a pair. If free radicals do not get a partner and continuously take place, it can lead to a disease such as cancer. Therefore, the body needs an antidote to free radicals in the form of antioxidants, with IC50 values that have been obtained so that it can be said that the methanol extract of fruit and foliage in this study has the potential as an antioxidant.

Antibacterial

In the methanol extract of the fruit and fig leaves, each concentration showed different results in the inhibition zone formed, the higher the concentration, the greater the inhibition zone formed. This happens because it is influenced by the content of secondary metabolites which have an antibacterial role.[25]

The ability as an antibacterial for pathogenic bacteria Streptococcus pneumonia has been shown in both methanol extracts of figs fruit and leaves. The difference in terms of the inhibitory zone's diameter is due to the content of different secondary metabolite compounds in figs fruit and leaves. Secondary metabolites in figs fruit such as phenols and flavonoids while in fig leaves such as phenols, flavonoids and tannins. Although secondary metabolite content in fig leaves more, it does not produce a higher inhibition zone than figs fruit. This is because the phenol content of fresh figs is 1,090-1,110 mg / 100g.[18] According to Pelczar and Chan (2008), one compound that can act as an antibacterial for pathogenic bacteria by damaging bacterial cell walls is phenol.[26]

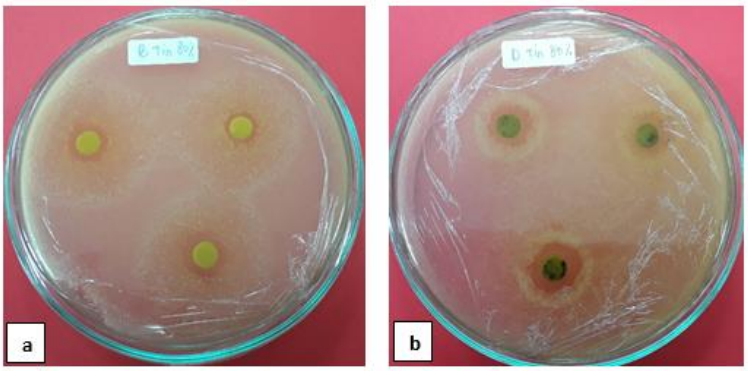

Figure 2. Comparison of inhibition zone diameters in methanol extracts of figs fruit (a) and leaves (b)

The comparison of inhibition zones that were formed on the methanol extract of fruit and leaves at each concentration can be seen in Figure 1. As follows:

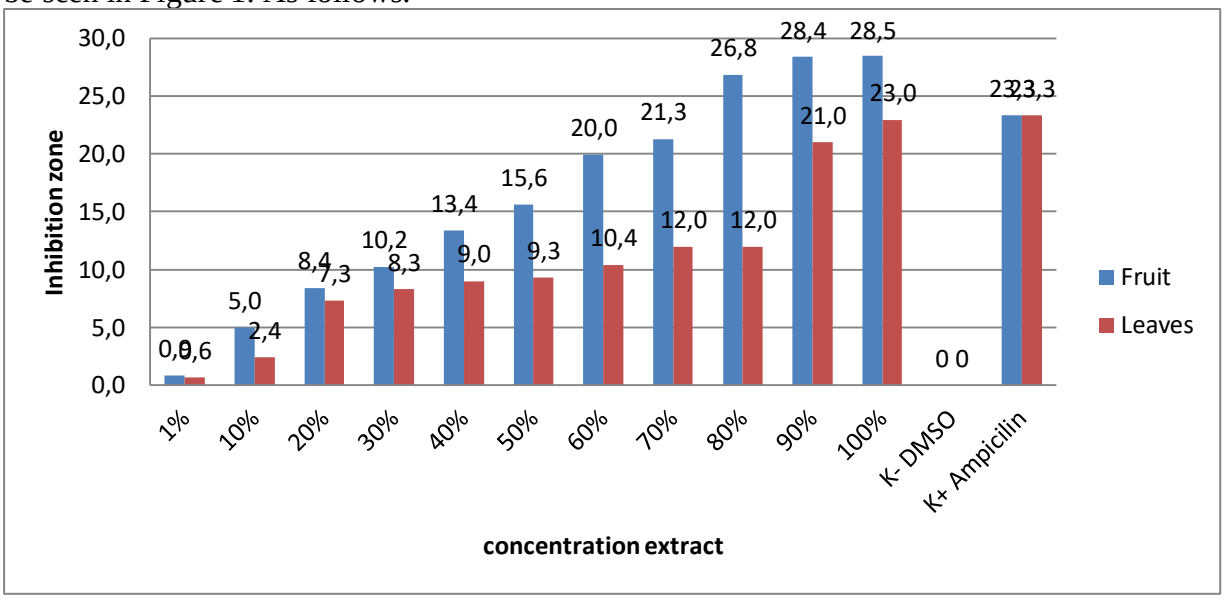

Figure 1. Comparison of inhibition zones in methanol extracts of fruit and tin leaves

Statistical testing is done in stages after the results obtained from the inhibition zone formed from the Shapiro-Wilk normality test to Mann-Whitney. The final results of statistical tests using Mann-Whitney obtained p value> 0.05. This value indicates that the inhibition zone's diameter between the two concentrations in the sample of both methanol extracts of fig fruit and leaves did not differ significantly. Mann-Whitney test results obtained showed that the higher concentration of extracts tested could affect the resulting inhibition zone. Extract concentration is related to metabolite compounds that act as an antibacterial, where the content of these compounds at high concentrations will be far more than low concentrations.[27]

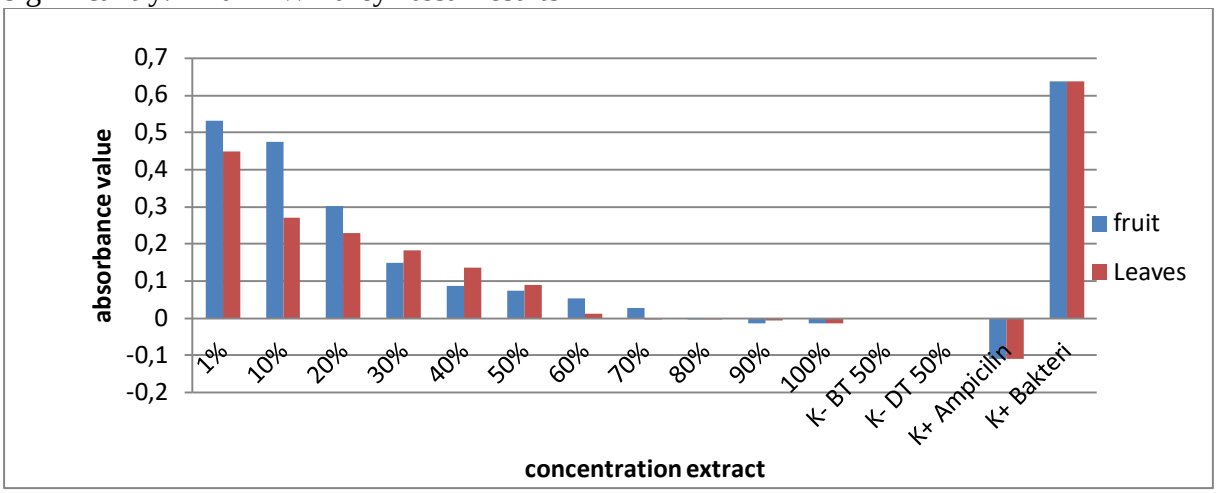

Figure 3. absorbance value of MIC test

The MIC test was statistically tested by using Paired Sample T-Test and obtained a p-value of 0.00 in both samples. This indicates a significant difference in absorbance values. The absorbance value in the MIC test has increased and decreased due to bacterial growth and death caused by the extracted test. The results obtained in the MIC test, the higher the concentration, the lower the absorbance value, and the lower the concentration, the higher the absorbance. In this case, the bacterial growth activity is still visible. The difference in the effect obtained at each concentration can occur because of the lower the concentration, the lower the secondary metabolite level as antibacterial, so that the ability to inhibit it is getting weaker.[27] The concentrations that were determined as the minimum inhibitory concentration (MIC) in this study were 80% concentration in the methanol extract of figs and 70% in the methanol extract of figs leaves.

The MKC test (The minimum concentration of kills) aims to determine the minimum concentration that can kill the pathogenic bacteria Streptococcus pneumoniae in the methanol extract of the fruit and leaves of fig. The test results show that both the methanol extract of figs fruit and leaves the concentration set as MKC is a concentration of 100%. This can be said as MKC because at this concentration there was no bacterial colony growing on the media, while concentrations below 100% found a white, cloudy bacterial colony (Figure 4).In the MKC test, the two extracts had the ability to kill pathogenic *Streptococcus pneumoniae* bacteria. This ability is none other than due to the presence of secondary metabolites in the form of quite high phenols. In figs plants the content of phenol derivatives that can act as antibacterials, namely flavonoids, tannins, polyphenols, and anthocyanins.[3,5,6,8-11.]

CONCLUSIONS

A methanol extract of fig fruit and leaves with IC50 values of 13,402 ppm and 7,9875 ppm, had very strong antioxidant activity. Figs fruit extracts and leaves also can be antibacterial as indicated by inhibition zone values, MIC and MKC. The highest inhibitory diameter was found at a concentration of 100% of 28.5 mm. The optimal level as an antibacterial from fruit extracts and leaves is 100% concentration.

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