STUDY OF SECONDARY METABOLITES OF *Jatropha gossypifolia* LEAF EXTRACT AND ITS ACTIVITY AGAINST *Propionibacterium acne*

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Abstrak: Jarak merah merupakaan salah satu tanaman endemik Indonesia. Kandungan metabolit sekunder yang ada pada tanaman ini berpotensi untuk dijadikan sebagai antibakteri. Penelitian ini bertujuan untuk mengetahui potensi metabolit sekunder ekstrak jarak merah dalam menghambat pertumbuhan bakteri *Propinium acne*. Metode pengujian meliputi skrining fitokimia dan pengujian antibakteri dilakukan dengan metode sumuran. Hasil penelitian menunjukkan bahwa ekstrak daun jarak merah memiliki metabolit sekunder yaitu flavonoid, alkaloid dan steroid. Ekstrak etil asetat dan etanol daun jarak merah mampu menghambat pertumbuhan bakteri *Propionibacterium acne* dengan zona hambat berturut-turut sebesar 14,43 mm dan 9,37 mm.
Kata Kunci: antibakteri; *Jatropha gossypifolia*; *Propionibacterium acne*

Abstract: *Jatropha gossypifolia* is one of the endemic plants of Indonesia. The content of secondary metabolites in this plant has the potential to be used as an antibacterial. This study aims to determine the potential of secondary metabolites of *Jatropha gossypifolia* extract in inhibiting the growth of *Propinium acne* bacteria. Test methods include phytochemical screening and antibacterial testing carried out by the well method. The results showed that *Jatropha gossypifolia* leaf extract had secondary metabolites, namely flavonoids, alkaloids and steroids. Ethyl acetate and ethanol extract of *Jatropha gossypifolia* leaves were able to inhibit the growth of *Propionibacterium acne* with inhibition zones of 14.43 mm and 9.37 mm, respectively.

Keywords: antibacteri; *Jatropha gossypifolia*; *Propionibacterium acne*

INTRODUCTION

Propionibacterium acne is one of the bacteria that play a role in the pathogenesis of acne. These bacteria can break down lipids (sebum) on the skin into free fatty acids and glycerol. The free fatty acids produced will irritate and increase tissue inflammation accompanied by cell remodelling (Wijaya et al., 2018). In addition, the process of breaking down triglycerides will also produce hydrolytic enzymes. This enzyme can damage the sebaceous glands, causing inflammation. Activities in the sebaceous glands will affect the number of bacteria in the skin glands (Hafsari et al., 2015).

Jatropha gossypifolia is an endemic plant found in Indonesia. *J. gossypifolia* has been widely cultivated by the community both as ornamental and herbal plants. The stem of the *J. gossypifolia* plant is used as a laxative, while the sap contained in the stem has properties as a fever reducer and appetite enhancer. In addition, the leaves are efficacious as a pain reliever due to inflammation and fever. They also treat various skin disorders such as rashes, chickenpox, ringworm and itching. The community believes that the *J. gossypifolia* plant contains efficacious ingredients as herbal medicines (Pangestu, 2017).

Several researchers have researched the content contained in the *J. gossypifolia*. Some of them are research conducted by Torokano (2018) which states that secondary metabolites found in red distance after being identified using TLC are in the form of terpenoids, saponins, flavonoids, and alkaloids. Furthermore, according to Pangestu et al. (2017), *J. gossypifolia* leaf extract has antibacterial activity and is rich in antioxidants. Meanwhile, Dhale & Birari (2010) research stated that *J. gossypifolia* contains phenolic compounds, starch, protein, cellulose, saponins, tannins, and minerals such as calcium, potassium, and phosphorus. Chemical compounds that have been isolated from *J. gossypifolia* include *gossypiline, gossypifan, cyclogossine A, cyclogossine B isogadain, gossypidien, jatrophone, jatropholone, piperidine, citlatrione, arylnapthalene* dan *propacin* (Fatokun, 2016). Based on the above background, this study was conducted to determine the antibacterial activity of *J. gossypifolia* Linn against *Propionibacterium acne*. The antibacterial potential of *J. gossypifolia* against *Propionibacterium acne* so that it can be used as a natural acne medicine.

RESEARCH METHODS

This research was conducted in August 2021. This research consisted of two stages, first is the phytochemical test of *J. gossypifolia* leaf extract (Shaikh & Patil, 2020). Furthermore, the antibacterial activity was tested using the diffusion method using a paper disk. In this method, bacteria are inoculated into the agar medium and then put a paper disk soaked with the test sample into the media according to a predetermined area. The advantages of the disc paper diffusion method are that it is fast, easy and inexpensive (Sariadji & Sembiring, 2019).

RESULT AND DISCUSSION

Based on the research on *J. gossyfolia* extract obtained from three types of solvents, namely ethanol, ethyl acetate and n-hexane. With weight yield, 49.6891 grams, 33.9001 grams, and 15.1947 grams, respectively. The yield results obtained indicate that the higher the percentage of the extract, the more active compounds will diffuse into the solvent (Putri et al., 2013; Herdiana & Aji 2020). The yields was then tested for phytochemicals. The results showed a positive outcome from the flavonoid test, namely a colour change from brownish yellow to red (Lisi et al., 2017). The colour change is caused because the reagent can hydrolyze O-glycosyl in the structure of flavonoid compounds until the glycosyl is replaced by H+ ions (Sukarno, 2017).

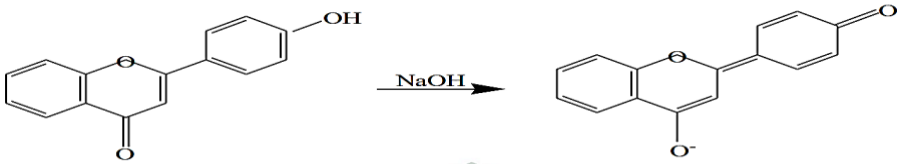

Figure 1. The reaction of flavonoids with NaOH

Tannin group was identified using 5% $FeCl_3$ and 1% $FeCl_3$ reagents. The formation of a dark blue or green-black colour indicates the presence of tannin compounds in the red distance sample (Setiawan et al., 2017). The reaction between tannins and FeCl3 can be seen in the Figure 2.

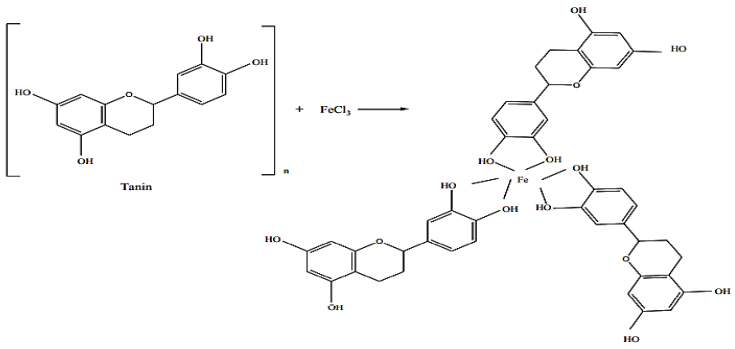

Figure 2. Reaction of tannin with $FeCl_3$

Identify terpenoid and steroid compounds in *J. gossypifolia* leaf extract by reacting the sample with Liebermann-Burchard reagent, 1% $FeCl_3$ and concentrated H_2SO_4. Suppose the sample changes colour to blackish-blue or blackish green after reacting with 1% $FeCl_3$ and Liebermann-Burchard (Setiawan et al., 2017) and is brownish yellow after being reacted with concentrated H_2SO_4. In that case, the sample is positive for terpenoid compounds. Meanwhile, if the colour obtained is light green or a green ring is formed, the positive sample contains active steroid compounds (Lisi et al., 2017). Description of the chemical reactions that occur in terpenoids and steroids shown in figure 3.

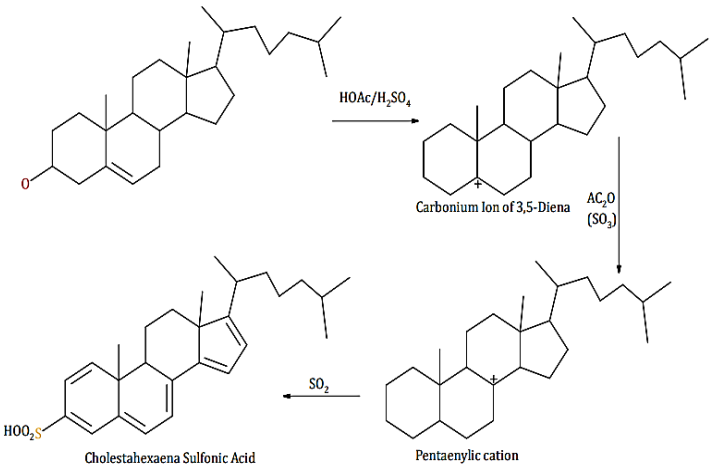

Figure 3. Reaction of terpenoid dan steroid with *Liebermann-burchard* (Habibi et al., 2018)

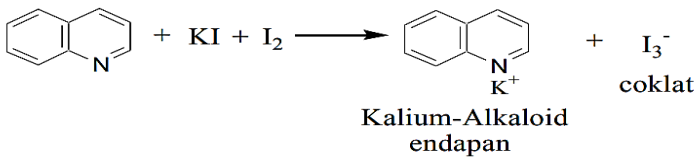

Figure 4. Reaction of alkaloid with Wagner (Marliana et al., 2005)

Group of alkaloid compounds was identified by adding Dragendorf and Wagner reagents. The results obtained were positive if there was a brownish-yellow precipitate

(Wagner) (Marliana et al., 2005). The reaction between alkaloids and Wagner's reagents can be seen in the Figure 4. While the reaction between the group of alkaloid compounds and Dragendorff's reagent will produce a colour change to reddish-brown (Lisi et al., 2017). Reaction between alkaloid compounds and Dragendorff's reagent can be seen in Figure 5.

$$Bi(NO_3)_3 + 3KI \rightarrow BiI_3 + 3KNO_3$$
$$BiI_3 + KI \rightarrow K[BiI_4]$$

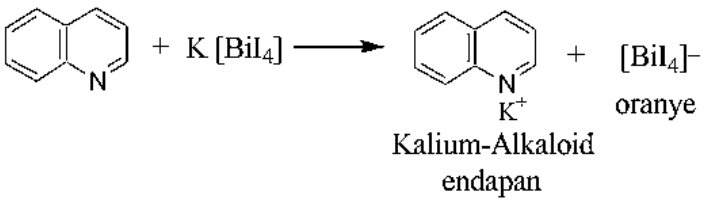

Figure 5. Reaction alkaloid with Dragendorff (Masrliana et al., 2005)

Alkaloid test also uses Mayer's reagent, which is characterized by a change in colour to light yellow or the presence of a white precipitate (Ikalinus et al., 2015). Reaction between alkaloid compounds and Mayer's reagent can be seen in Figure 6.

$$HgCl_2 + 2KI \rightarrow HgI_2 + 2KCl$$
$$HgI_2 + 2KI \rightarrow K_2[HgI_4]$$

Kalium tetraiodomerkurat(II)

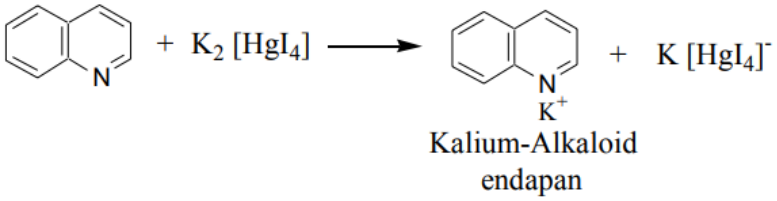

Figure 6. Reaction of alkaloid with Mayer (Marliana et al., 2005

Based on the results obtained, it is known that *J. gossypifolia* leaves contain active compounds that are polar, semi-polar and non-polar. The results of this study are by the theory of Torokano (2018) that *J. gossypifolia* leaves contain several groups of compounds, namely terpenoids, saponins, phenolics, steroidal alkaloids and flavonoids. The active compounds in the ethanol extract of *J. gossypifolia* leaves are flavonoids, terpenoids, steroids, tannins, and alkaloids. This compound is soluble in ethanol because it contains a polar hydroxyl group attached to an aromatic ring. Generally, there are more than one –OH group in flavonoids. The more -OH groups bonded to the aromatic groups, the higher their solubility in polar solvents (Nuraeni & Darwiati, 2021). Tannins belong to a class of polyphenolic compounds with the –OH functional group, so these compounds can be extracted using polar solvents (Nuraeni & Darwiati, 2021). This theory is by the research results obtained, namely discovering tannin compounds in ethanol extracts.

Terpenoids from the monoterpene group are polar compounds. This compound can be dissolved in ethanol solvent because it has glycoside bonds which tend to be polar. In contrast, steroids are soluble in ethanol. However, these secondary metabolites are generally semi-polar and non-polar (Khair et al., 2017) because ethanol is a universal solvent. It can attract polar and non-polar compounds (Purgiyanti et al., 2018). Alkaloids

are secondary metabolites that are semi-polar. This compound was found in the ethanol extract because ethanol is a universal solvent.

Secondary metabolites contained in the ethyl acetate extract of *J. gossypifolia* leaves are flavonoids, tannins, terpenoids, alkaloids and steroids. As with ethanol solvent, ethyl acetate also attracts both polar and non-polar active compounds. This is because ethyl acetate has a broad polarity range (Putri et al., 2013). The active compounds produced from n-hexane extract are flavonoids, alkaloids and steroids. At the same time, steroids and flavonoids are soluble in n-hexane solvent because flavonoids and steroids are classified as secondary metabolites, semi-polar and non-polar (Khair et al., 2017). Based on the results of the phytochemical test obtained, it can be concluded that the secondary metabolite compounds contained in the *J. gossypifolia* leaf extract are more polar and semi-polar than non-polar secondary metabolites (Kasminah, 2016).

The mechanism of antibacterial action can be in several ways: destroying cell structure, changing cell permeability, inhibiting enzyme work, changing protein molecules, and inhibiting nucleic acid synthesis (Rollando, 2019). Antibacterial testing in this study was carried out by the disc diffusion method. The working principle of this method is the absorption of the extract by disc paper (Novita, 2016). *Propionibacterium acnes* is a gram-positive bacteria that grows optimally at 37^{o}C. The characteristics of Propionibacterium acne are rod-shaped with white colonies (Hafsari et al., 2015). These bacteria are motile so that in the rejuvenation process, bacteria that grow do not follow the shape of the scratch but grow evenly over the entire surface of the agar medium.

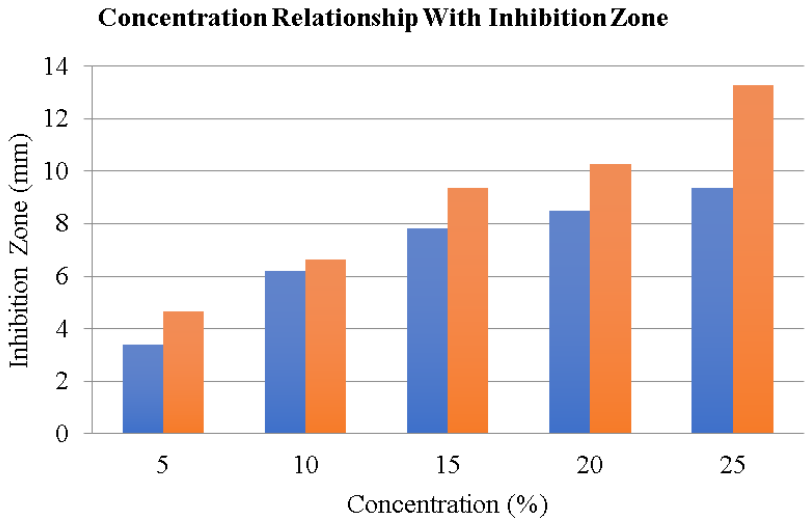

Figure 7. Antibacterial activity *of Jatropha gossyfipolia* ethanol extract (Orange bar is inhibition zone of 24 hour incubation and blue bar is inhibition zone of 48 hour incubation)

In this study, tetracycline HCl was used as a positive control because it is one of the antibiotics used to treat acne and has high antibacterial activity. The structure of the tetracycline consists of four rings joined by two conjugated bonds. The substitution on the ring improves the pharmacokinetic properties and the different spectrum of activity against bacteria. Tetracyclines were initially used to treat infections with gram-positive and gram-negative bacteria and some protozoa (Wasitaningrum, 2009).

Based on the data shown in Figure 7, it can be seen that the inhibitory activity of this antibiotic is included in the category of powerful inhibition, which is 20.44 mm at 24 hours of incubation and 24.51 mm at 48 hours of incubation. The mechanism of action of

tetracycline is by inhibiting the protein synthesis of the 70s ribosomal subunit and 80s ribosomal subunit. The effect of tetracycline on tRNA-ribosomes can be seen by inhibiting the binding of aminosial-tRNA to the recipient receptor on the ribosome (Wasitaningrum, 2009).

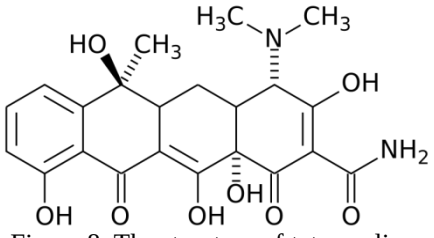

Figure 8. The structure of tetracyclines

Tetracyclines do not directly inhibit the peptide assembly or translocation step but inhibit the termination of the peptide chain at the termination codon. The mechanism for penetrating tetracycline to enter bacterial cells is the same as inhibiting protein synthesis plus structural modifications to inhibit protein synthesis (Wasitaningrum, 2009). The negative control used was dimethyl sulfoxide (DMSO). DMSO is a solvent that can dissolve polar and non-polar compounds. In addition, DMSO does not inhibit bacterial growth, so it does not interfere with the results of antibacterial activity testing (Utami, 2011).

In ethanol extract, a minor inhibition zone diameter was obtained at a 5% concentration of 3.79 mm with an incubation time of 24 hours. The highest inhibition concentration against *P. acne* was obtained at a 25 % concentration of 9.37 mm. The inhibition zone formed at a concentration of 5% was included in the category of weak inhibition. In comparison, the inhibition zones obtained at concentrations of 10%, 15%, 20% and 25% were included in moderate growth inhibition. The result follows the theory put forward by Pangestu (2017), that the inhibition zone with a diameter of <5 mm is included in the weak category, while the inhibition zone with a diameter of 5 to 10 mm is categorized as a moderate zone of inhibition.

Based on the graph in Figure 7, it can be seen that the antibacterial activity of the ethanol extract increased at 48 hours of incubation. The lowest inhibition zone diameter was obtained at a concentration of 5%, which was 4.66 mm. Meanwhile, the largest inhibition zone was obtained at a concentration of 25% and was included in the strong category. The increase in the inhibition area indicated that the antibacterial activity of the ethanol extract of red castor leaf was bactericidal. Bactericide is a type of antibacterial capable of killing bacteria (Rollando, 2019).

Furthermore, the inhibition zone with an incubation time of 24 hours obtained from the ethyl acetate extract (Figure 9) is shows the smallest inhibition zone diameter was obtained at a concentration of 5% at 5.47 mm and the largest inhibition zone at a concentration of 25%, which was 14.43 mm. These results show that the ethyl acetate extract had a higher inhibition zone than ethanol extract and n-hexane extract. The inhibition zones formed at concentrations of 5%, 10%, and 15% ethyl acetate extract were included in the moderate inhibition category, while concentrations of 20% and 25% were categorized in the strong category (Pangestu, 2017).

In Figure 9, it is known that during the 48-hour incubation time, the inhibition zone of the ethyl acetate extracts decreased by 3.74 mm, 5.81 mm, 7.15 mm, 8.47 mm, and 12.17 mm, respectively. The decrease that occurred during the 48-hour incubation

time indicated that the antibacterial activity of the ethyl acetate extract of *J. gossypifolia* leaf was bacteriostatic. Bacteriostatic is a type of antibacterial that can only inhibit the growth of bacteria (Rollando, 2019).

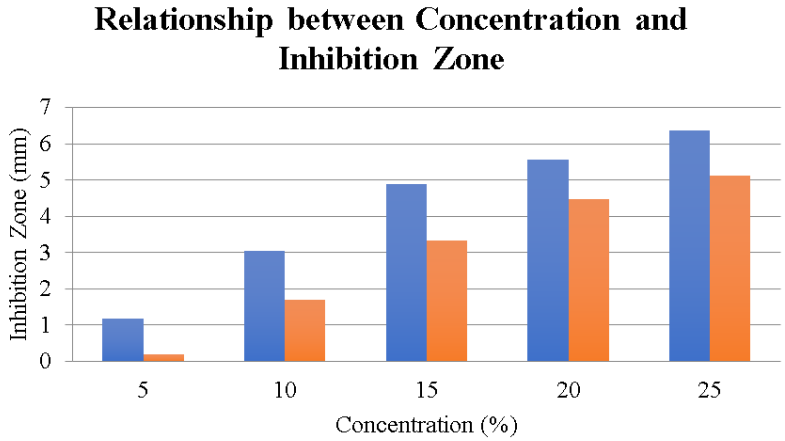

Figure 9. Antibacterial activity of *Jatropha gossyfipolia* ethyl acetatel extract (Orange bar is inhibition zone of 24 hour incubation and blue bar is inhibition zone of 48 hour incubation)

Ethanol and ethyl acetate extract had a higher inhibition zone diameter than the n-hexane extract. The result is due to the ability of the two solvents to attract polar compounds. *Propionibacterium acnes* is a gram-positive bacteria with a cell wall composed of single-layered peptidoglycan and low lipid content of about 1 to 4% (Pelczar & Chan, 1988; Pratiwi, 2014). Generally, gram-positive bacteria have an opposite cell wall. So it tends to be easily penetrated by polar compounds and will be difficult to penetrate by non-polar compounds (Hidayatullah, 2001; Pratiwi, 2014). The differences in the compounds that make up gram-positive and negative bacteria can be seen in the Figure 10.

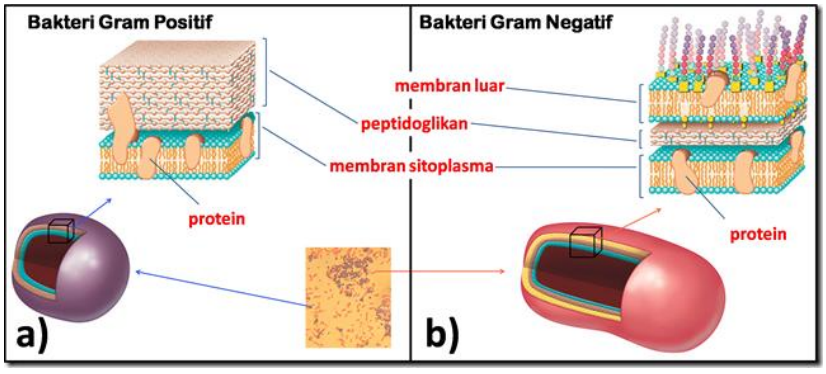

Figure 10. Gram positive and negative bacteria

The inhibition zones obtained from the n-hexane extract of *J. gossypifolia* leaves with an incubation period of 24 hours with 5%, 10%, 15%, 20%, and 25% are 1.17 mm, 3.04, 4.88 mm, 5.56 mm, and 6.37 mm. The most significant inhibition zone was obtained at a concentration of 25% and was included in the medium category (Pangestu, 2017). Figure 11 illustrates that the concentration of the test extract is directly proportional to the diameter of the inhibition zone formed. The results obtained following the theory put forward by Warokka (2016) that the greater concentration of the test extract, the greater the antibacterial activity. Antibacterial activity of n-hexane extract at 48 hours of

incubation decreased by 0.18 mm, 1.68 mm, 3.34 mm, 4.47 mm, and 5.12 mm, respectively. The decrease that occurred during the 48-hour incubation time indicated that antibacterial activity of the n-hexane extract of *J. gossypifolia* leaves was bacteriostatic. Bacteriostatic is an antibacterial whose growth inhibition is only temporary (Septiani et al., 2017).

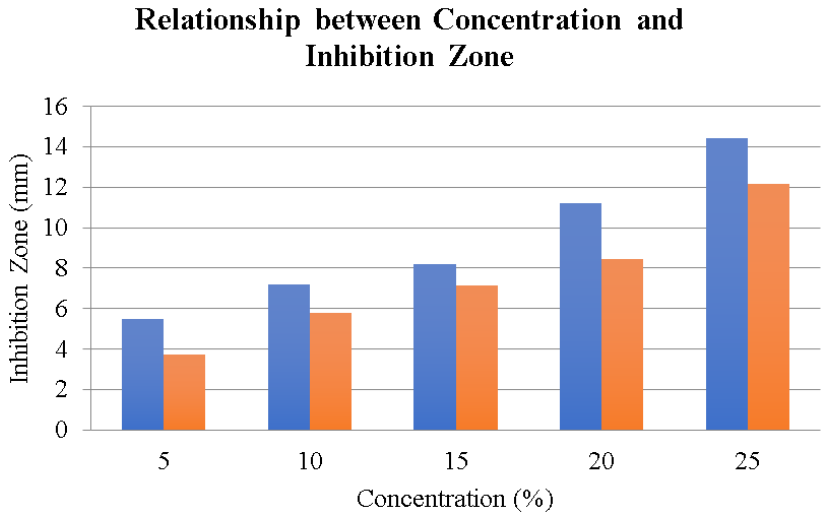

Figure 11. Antibacterial activity of *Jatropha gossypifolia* n- hexane extract (Orange bar is inhibition zone of 24 hour incubation and blue bar is inhibition zone of 48 hour incubation)

Based on the results obtained, it can be concluded that among the three types of extracts used, the one with the highest inhibitory power was ethyl acetate extract with a concentration of 25%, namely 14.43 mm at 24 hours incubation and 12.17 at 48 hours incubation. However, the antibacterial activity of the ethyl acetate extract was only temporary or bacteriostatic. In contrast, the antibacterial activity of the ethanol extract within 24 hours of incubation was highest at a concentration of 25% with an area of inhibition of 9.37 mm and increased during incubation for 48 hours. The highest antibacterial activity of the ethanol extract of *J. gossypifolia* leaves was at incubation for 48 hours of 13.29 mm. Antibacterial activity of n-hexane extract with an incubation time of 24 hours was highest at a concentration of 25% with a diameter of 6.37 mm and 5.12 at an incubation time of 48 hours. The formation of the inhibition zone was due to the antibacterial activity of secondary metabolite compounds contained in red castor leaf extract (*J. gossypifolia* Linn.). These secondary metabolites include tannins, flavonoids, terpenoids, alkaloids and steroids.

The mechanism of inhibition of tannin compounds as an antibacterial is by forming complexes with other compounds or elements. Tannins are polyphenolic compounds that bind to proteins by forming hydrogen bonds between the phenol groups of tannins and the carboxyl groups (aromatic and aliphatic) of proteins. Strong bonds between tannins and proteins will affect protein metabolism (Fahey & Berger, 1988; Hidayah, 2016). Structure of the tannin compound shown in Figure 12.

Tannins can precipitate proteins with several functional groups to form powerful complex bonds with protein molecules. This bond causes the protein cannot be degraded by microbes (Fahey & Berger, 1988 in Hidayah 2016). Tannins are highly reactive with bacterial cell walls and extracellular enzymes produced by bacteria. This interaction will inhibit nutrient transport into cells, thereby inhibiting the growth of organisms (McSweeny et al., 2001 in Hidayah, 2016).

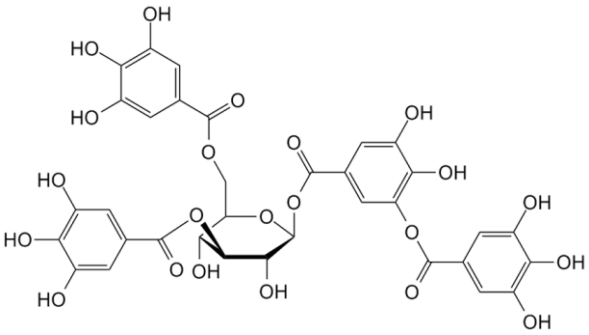

Figure 12. Structure of tannin

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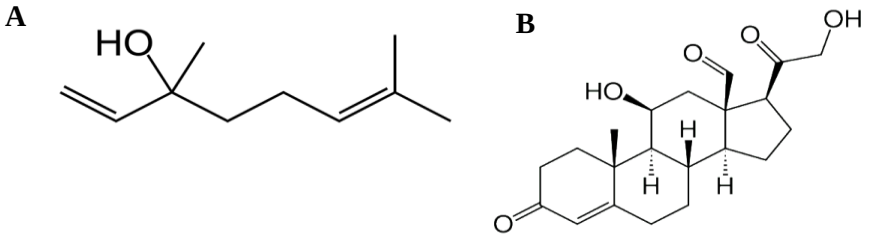

Figure 13. Structure of terpenoid (A) and steroid (B)

Terpenoids are compounds that contain only carbon, hydrogen and oxygen. Terpenoids and steroids are generally fat-soluble and are present in the cytoplasm of plant cells. The presence of phenol groups in terpenoids and steroids will also interfere with the peptidoglycan components of bacterial cells. The cell layer is not formed entirely and damages the bacterial wall by breaking the peptidoglycan bonds (Egra et al., 2019). Terpenoids and steroids can also bind to transmembrane proteins (porins) outside the cell wall. The reaction between terpenoids and porins will cause damage to porins and cell permeability. If cell permeability is damaged, bacterial growth will be inhibited and even cause the death of microbes (Cowan, 1999; Torokano, 2018).

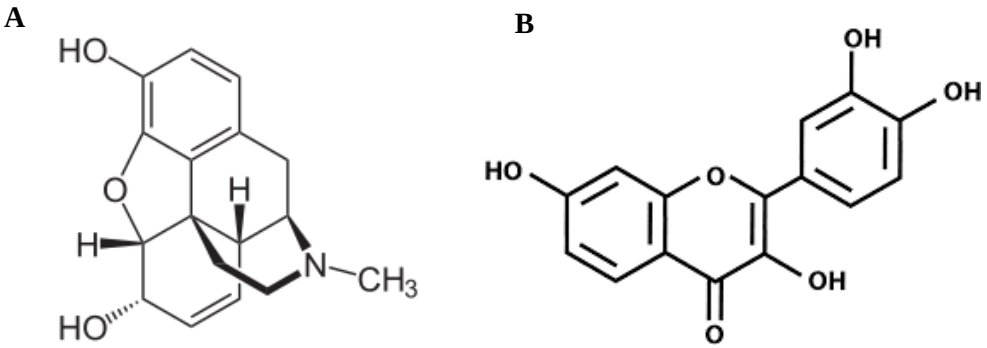

Figure 14. Structure of alkaloid (A) and flavonoid (B)

Mechanism of alkaloids in inhibiting bacterial growth is by damaging cells and disrupting primary and energy metabolism (ATP) in bacteria (Sari & Mursiti, 2016). The difference in polarity between the lipids that make up bacterial cells and the alcohol groups in the alkaloid compounds will cause damage to the bacterial cells. Therefore, inhibition of ATP synthesis affects the metabolic processes of microorganisms, which can cause biological death (Yan et al., 2021). Flavonoids inhibit the growth or kill microorganisms by damaging the permeability of the cell membrane and cytoplasm of a microbe (Hafsari et al., 2015). Flavonoid compounds can penetrate polar peptidoglycan because flavonoids are also polar. In addition, phenol compounds damage the bacterial wall by breaking the peptidoglycan bond (Pelczar & Chan, 1988; Ayen et al., 2017). Robinson (1995) also explained that the mechanism of inhibition of bacteria by phenolic compounds is thought to be interfering with the peptidoglycan constituent components of bacterial cells so that the cell layer is not entirely formed. Besides, alkaloid compounds work by inhibiting cell wall synthesis (Ayen et al., 2009; Lamothe et al., 2009).

Instability of the cell wall causes the function of selective permeability, active transport function, and control of the protein composition of bacterial cells to be disturbed, causing bacterial cells to lose shape and lysis (Pelczar and Chan, 1988 in Ayen et al., 2017). In addition, flavonoids can also inhibit bacterial motility. The -OH group in the structure of flavonoid compounds causes changes in organic components and nutrient transport, eventually leading to toxic effects on bacteria (Egra et al., 2019).

CONCLUSION

Secondary metabolites contained in the ethanol extract and ethyl acetate extract of red jatropha leaf (*Jatropha gossypifolia* L.) are flavonoids, tannins, terpenoids, alkaloids and steroids. The n-hexane extract is in the form of flavonoids, alkaloids and steroids. While the optimum concentration of red jatropha leaf extract which killed *Propionibacterium acne* for ethyl acetate extract, was 25% (14.43 mm at 24 hours and 12.17 at 48 hours) and optimum antibacterial activity of the ethanol extract was at concentration of 25% (9.37 mm at 24 hours and 13.29 mm at 48 hours).

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