Vol.7, No.1, 2022
e-ISSN 2502-4787

TESTING THE ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS OF *Tithonia diversifolia* LEAVES, FLOWERS AND ROOTS IN VARIOUS SOLVENT FRACTION

Indah Tri Susilowati[1*], Hartati Soetjipto[2], Susanti Pudji Hastuti[2]

[1]*Medical Laboratory Technology Diploma III Study Program, STIKES Nasional/Nasional Institute of Health Sciences, Jl. Solo-Baki Kwarasan Grogol, Sukoharjo 57552, Indonesia*
[2]*Faculty of Science and Mathematics, Satya Wacana Christian University, Jl. Diponegoro 52-60, Salatiga 50711, Indonesia*

E-mail: *indahtrisusilowati@gmail.com

Received: 15 September 2021. Accepted: 19 November 2022. Published: 31 Desember 2022

DOI: 10.30870/educhemia.v7i1.12403

Abstract: Medicinal plants are natural sources of effective antimicrobials from natural products. *Tithonia diversifolia* (*Asteraceae*) is a conventional herb that can be used for the treatment of malaria, wounds, and other diseases with biological properties such as antioxidant, antimalarial, antidiabetic, antibacterial, and anti-hyperglycemic. Research on *Tithonia diversifolia* leaf extract as an antibacterial has been widely carried out. However, its use as a natural antibacterial is rarely studied, especially on flowers and roots. This study aimed to test the antibacterial activity of leaf, flower, and root extracts of the *Tithonia diversifolia* plant in various solvent fractions. The methods used in this research were Antibacterial Testing with Agar Diffusion and Direct Bioautography Methods, while the solvent fractions used were petroleum ether, chloroform, and ethyl acetate. The results showed that the crude extract of *Tithonia diversifolia* leaves, flowers, and roots for petroleum ether, chloroform, and ethyl acetate fractions contained antibacterial compounds. They could inhibit the growth of *E. coli, P. aeruginosa, S. aureus*, and *B. subtilis* bacteria, with inhibitory activity in chloroform fraction, which was better than in petroleum ether and ethyl acetate fractions. The results of the bioautography test showed that the inhibition was good on gram-positive bacteria with the highest number of spots in the chloroform fraction for the crude leaf and flower extract samples.

Keywords: *Tithonia diversifolia*, Antibacterial, Agar Diffusion Method, Direct Bioautography Method

Abstrak: Tanaman obat merupakan sumber alami antimikroba yang efektif dari produk alami. *Tithonia diversifolia* (*Asteraceae*) adalah herbal konvensional yang dapat digunakan untuk pengobatan malaria, luka, dan penyakit lainnya dengan sifat biologis seperti antioksidan, antimalaria, antidiabetic, antibakteri, dan anti-hipergliseria Penelitian ekstrak daun Tithonia diversifolia sebagai antibakteri sudah banyak dilakukan tetapi pemanfaatannya sebagai antibakteri alami masih jarang diteliti terutama pada bagian bunga dan akar. Tujuan penelitian ini adalah menguji aktivitas antibakteri dari ekstrak daun, bunga dan akar tanaman Tithonia diversifolia dalam berbagai fraksi pelarut. Metode yang digunakan pada penelitian

ini adalah Pengujian Antibakteri dengan Metode Difusi Agar dan *Direct Bioautografi*, sedangkan fraksi pelarut yang dipakai adalah petrolium eter, kloroform dan etil asetat. Hasil penelitian menunjukkan bahwa ekstrak kasar daun, bunga dan akar Tithonia diversifolia untuk fraksi petroleum eter, kloroform dan etil asetat mengandung senyawa antibakteri yang bisa menghambat pertumbuhan bakteri *E. coli*, *P. aeruginosa*, *S. aureus*, dan *B. subtilis*, denan aktifitas penghambatan untuk fraksi kloroform lebih bagus dibanding dengan fraksi petroleum eter dan etil asetat. Hasi uji bioautografi menunjukkan bahwa penghambatan baik pada bakteri gram positif dengan jumlah spot paling banyak pada fraksi kloroform untuk sampel ekstrak kasar daun dan bunga.

Kata kunci: Tithonia diversifolia, Antibakteri, Metode Difusi Agar, metode Direct Bioautografi

INTRODUCTION

Scientists have carried out many studies on the determination of antimicrobial properties of many natural products: however, further research in this area is still promising due to phenomena such as drug resistance and the emergence of new diseases (Gutierrez et al., 2015). Medicinal plants are natural sources of effective antimicrobials from natural products. The use of medicinal plants worldwide predates the introduction of antibiotics and other modern drugs (Alaofin and Onifade, 2019). Research on the use of natural ingredients, namely the lipid extract of *Sargassum polycystic* methanol phase, can inhibit the growth of gram-positive bacteria, *Bacillus cereus* and *Staphylococcus aureus* (Panjaitan & Warganegara, 2018).

One plant that has been researched as a medicinal plant is *Tithonia diversifolia* (*Asteraceae*). *Tithonia diversifolia* (*Asteraceae*) is a conventional herb that can be used for the treatment of malaria, wounds and other diseases (Tagne et al., 2018). Phytochemical studies have shown that the *Tithonia diversifolia* plant contains alkaloids, tannins, flavonoids, saponins, terpenoids and phenols in the leaves, roots and stems (Olayinka et al., 2015), with biological properties such as antioxidant, antimalarial, antidiabetic, antibacterial and anti-hyperglycemic (Dlamini et al., 2020).

Methanol extract from *Tithonia diversifolia* leaf is active against *B. cereus* and *S. aureus* with MIC values of 500 and 1000 µg/mL, respectively (Maregesi et al., 2008). In addition, at a dose of 80 mg/mL, the methanolic extract of *Tithonia diversifolia* leaves is highly active against *E. coli* and *B. subtilis*, with average diameters of inhibition zones (MIC) of 20.33 mm and 23 mm, respectively (Gutierrez et al., 2013). Pathogen growth is inhibited by

dichloromethane extract of *T. diversifolia* leaves at concentrations higher than 25 mg/mL. E. coli is found to be the least sensitive to different plant extracts from *T. diversifolia* (Douglas, and Janet, 2016).

Research on *Tithonia diversifolia* leaf extract as an antibacterial has been widely carried out but its use as a natural antibacterial is still rarely studied, especially on the flowers and roots. Based on that background, the purpose of this study was to test the antibacterial activity of the leaf, flower and root extracts of *Tithonia diversifolia* in various solvent fractions.

METHOD

The plant material used was *Tithonia diversifolia* taken from Salatiga, and the parts used in this research were leaves, flowers and roots. As for the chemicals used included: Petroleum ether, chloroform, ethyl acetate and 80% methanol (v/v). The reagents used for TLC visualization were iodonitrotetrazolium chloride (5 mg/ml) bacterial staining reagent, the bacteria used were *Escherichia coli* (0091-IFO), *Pseudomonas aeruginosa* (FNCC 0063), *Staphylococcus aureus* (ID 784) and *Bacillus subtilis* (ATCC 6051), the media used to grow bacteria were Nutrient Agar (NA), Nutrient Broth (NB) and Mueller Hinton (MH) agar.

Extraction Stage

100 grams of dried samples of leaves, flowers and roots of *Tithonia diversifolia* with water content of 12.06% for leaves, 12.54% for flowers and 11.52% for roots were macerated with 80% methanol (v/v) for 24 hours and repeated for 3 times. After the maceration done, it was filtered then the resulting filtrate was concentrated using a rotary evaporator until the volume was approximately 100 ml. The filtrate was extracted using petroleum ether, the obtained petroleum ether fraction was then mixed and added with anhydrous sodium sulfate, filtered and then concentrated using a rotary evaporator. The concentrated fraction was subsequently dried using nitrogen gas. The rest of the extracted filtrate was then re-partitioned with chloroform and ethyl acetate in the same way, so that three fractions were obtained, namely petroleum ether, chloroform and ethyl acetate from leaf, flower and root extracts of *Tithonia diversifolia*, respectively. Each fraction was subjected to phytochemical screening to determine the content of active compounds including sterol and triterpene, saponin and tannin,

flavonoid and alkaloid tests (Ahmed and Bamigboye, 2020; Putri et al., 2013).

Antibacterial Testing with Agar Diffusion Method

The bacterial suspension with the amount of 10^8 – 10^9 cells/ml was put in a sterile petri dish containing Mueller Hinton (MH) agar which had been liquid, the dish was then shaken so that the bacteria were evenly distributed and waited until the agar medium solidified. Paper disc (She Leicher & Scguell) with a diameter of 6 mm which was dripped with 500 g/ml (ppm) extract, placed on the surface of the medium using sterile tweezer, the dish was incubated for 24 hours at 37^{o}C. Observation was made on the appearance of bright zone(s) around the paper disc during the 24-hour incubation then the Diameter of Inhibition Zone was measured (Pietrocola et al., 2018).

Antibacterial Testing with Direct Bioautography Method

The compounds in the Tithonia diversifolia plant extracts were separated using Thin Layer Chromatography (TLC) on silica gel 60 (merck) plates using suitable solvent mixtures. For petroleum ether fraction using N-Hexan : ethyl acetate = 7:3 (v/v) solvent, for chloroform fraction using N-Hexan : ethyl acetate = 3:7 (v/v) solvent, while for ethyl acetate fraction using chloroform : ethyl acetate = 2:3 (v/v) solvent. After the chromatography was dried, it was sprayed with bacterial suspension that had been grown for 24 hours in NB medium with a suspension amount of 10^8 – 10^9 cells/ml, then incubated for 24 hours in a closed vessel with wet cotton on the bottom. Furthermore, it was visualized by spraying the chromatogram using the bacterial staining reagent iodonitrotetrazolium chloride (5 mg/ml). The entire surface covered with bacteria would turn red. While the inhibition zone would remain colorless. The measurement of Rf value was carried out on the appearing bright zone(s) or inhibition zone(s) (Fathoni et al., 2021).

RESULTS AND DISCUSSION

The results of phytochemical screening on crude extracts of leaves, flowers, and roots of *Tithonia diversifolia* for petroleum ether, chloroform and ethyl acetate fractions are shown in the Table 1.

Table 1. Showing the Results of Phytochemical Screening on Petroleum Ether, Chloroform and Ethyl Acetate Fractions from Crude Extracts of *Tithonia diversifolia* Leaves, Flowers and Roots

Phytochemical Test	Petroleum ether			Chloroform			Ethyl acetate		
	Leaf	Flower	Root	Leaf	Flower	Root	Leaf	Flower	Root
Sterol and triterpenoid test									
Salkowski test	+	+	+	+	+	+	+	+	+
Saponin test									
Foam test	+	+	-	+	+	+	+	-	-
Tannin test									
$FeCl_3$ test	+	+	+	+	+	+	+	+	+
Lead acetate test	+	+	+	+	+	+	+	+	+
Flavonoid									
NaOH test	+	-	-	+	+	+	+	+	-
Shinoda test	+	+	-	+	+	+	+	+	+
Alkaloid									
Dragendroff reagent	-	-	-	+	+	+	+	+	+
Mayer reagent	-	-	-	+	+	+	+	+	+

Notes : (+) Indicates the presence of the tested compound, (-) indicates the absence of the tested compound.

The testing of antibacterial activity on petroleum ether, chloroform and ethyl acetate fractions from crude extracts of leaves, flowers and roots of *Tithonia diversifolia* by diffusion method used filter paper discs to determine the Diameter of Inhibitory Zone, which is characterized by the appearance of bright zone(s) around the filter paper disc (Kurama, 2020). The data concerning diameter measurement of inhibition zone in petroleum ether, chloroform and ethyl acetate fractions from the crude extracts of *Tithonia diversifolia* leaves, flowers and roots against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa* bacteria can be seen in the following Table 2.

Table 2. showing the average diameter of inhibition zone (± SE in cm) of petroleum ether, chloroform and ethyl acetate fractions from crude extract of *Tithonia diversifolia* leaves, flowers and roots

		Bacteria			
		B. subtilis	***S. aureus***	***E. coli***	***P. auruginosa***
Leaf	Petroleum ether	1.78 ± 0.11	2.18 ± 0.17	0.71 ± 0.03	0.96 ± 0.07
	Chloroform	2.33 ± 0.12	2.56 ± 0.22	0.72 ± 0.03	1.41 ± 0.03
	Ethyl acetate	1.02 ± 0.02	1.33 ± 0.08	0.72 ± 0.03	0.74 ± 0.02
Flower	Petroleum ether	1.61 ± 0.20	1.59 ± 0.22	0.70 ± 0.05	0.85 ± 0.07
	Chloroform	1.28 ± 0.14	2.51 ± 0.20	0.72 ± 0.03	1.62 ± 0.03
	Ethyl acetate	2.34 ± 0.22	1.42 ± 0.08	0.72 ± 0.03	0.75 ± 0.02
Root	Petroleum ether	0.94 ± 0.06	0.84 ± 0.07	0.71 ± 0.03	0.68 ± 0.03
	Chloroform	1.35 ± 0.04	1.02 ± 0.20	0.71 ± 0.05	0.74 ± 0.02
	Ethyl acetate	0.76 ± 0.08	0.71 ± 0.12	0.71 ± 0.03	0.71 ± 0.03

To clarify the presence of the diameter of inhibition zone, which is marked by the appearance of a bright zone around the filter paper disc, it can be seen in the Figure 1.

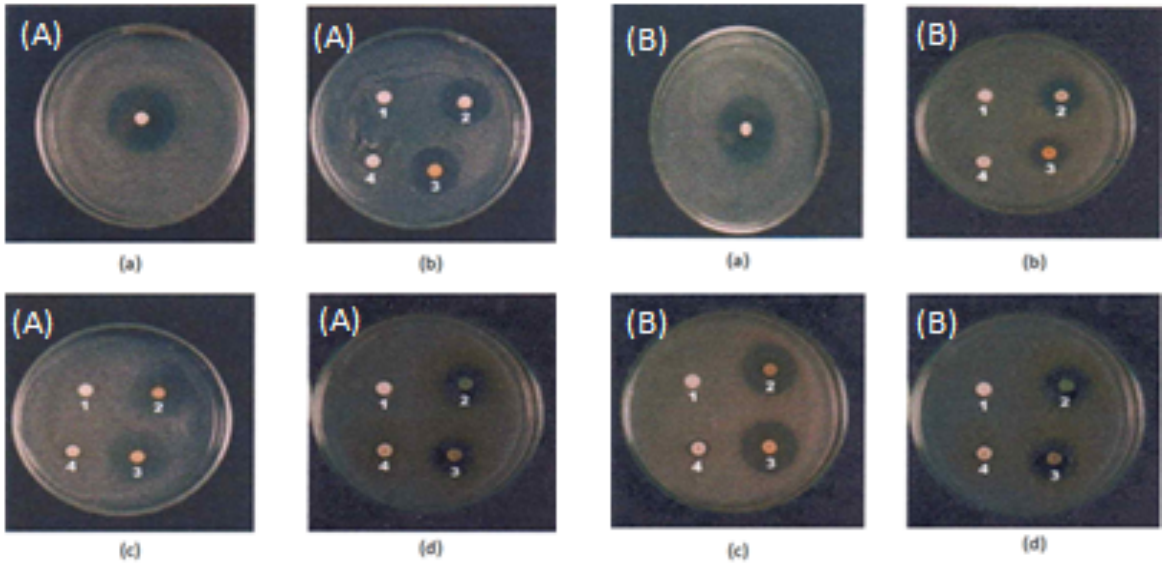


Figure 1. of Antibacterial Testing of Petroleum ether, Chloroform and Ethyl acetate Fractions from Leaf, Flower and Root extracts of *Tithonia diversifolia* against *B. subtilis* and *P. aeruginosa*.
Notes : (A) *B. subtilis* media, (B) *P. aeruginosa* media, (a) terramycin antibiotic control showing positive results with the appearance of bright zone, (b) Petroleum ether fraction, (c) Chloroform fraction, (d) Ethyl acetate fraction, (1) Reagent control – without samples or antibiotic, (2) Crude extract of the leaves, (3) Crude extract of the flowers and (4) Crude extract of the roots

The Figure 1. above shows that at the extract-dose used, namely 500 µg/ml (ppm), for petroleum ether, chloroform, and ethyl acetate fractions from the leaf, flower, and root extracts of *Tithonia diversifolia* there was inhibition of bacterial growth as indicated by the presence of bright zone(s) around the filter paper discs. This is in line with Salni et al. (2011) which states that for compounds whose activity is not known, crude extracts of 500 µg/ml to 1000 µg/ml are sufficient to test the inhibitory activity of bacterial growth.

By looking at the inhibition zone diameter qualification above, it was obtained that the leaf samples that fell in the very strong category were shown in chloroform fraction against *B. subtilis* and *S. aureus*, as well as petroleum ether fraction against *S. aureus* bacteria. The flower samples that was included in the very strong category were shown in chloroform fraction against *S. aureus* and ethyl acetate fraction against *B. subtilis* bacteria. More than 150 of these secondary metabolites have been isolated from *T. diversifolia* (Zhao et al., 2012),

including sesquiterpenoids, most of the sesquiterpene lactones such as thyrotundin, tagitinin A, tagitinin C, diversifol and others that are cytotoxins (Karebba et al., 2019).

Table 3. Showing the Classification of Inhibition Zone Diameter of Petroleum ether, Chloroform and Ethyl acetate Fractions from the Crude Extracts of *Tithonia diversivolia* Leaves, Flowers and Roots.

		Bacteria			
		B. subtilis	***S. aureus***	***E. coli***	***P. auruginosa***
Leaf	Petroleum ether	Strong	Very strong	Moderate	Moderate
	Chloroform	Very strong	Very strong	Moderate	Strong
	Ethyl acetate	Moderate	Strong	Moderate	Moderate
Flower	Petroleum ether	Strong	Strong	Moderate	Moderate
	Chloroform	Strong	Very strong	Moderate	Strong
	Ethyl acetate	Very strong	Strong	Moderate	Moderate
Root	Petroleum ether	Moderate	Moderate	Moderate	Moderate
	Chloroform	Strong	Moderate	Moderate	Moderate
	Ethyl acetate	Moderate	Moderate	Moderate	Moderate

Notes : Inhibition Zone Diameter-based antibacterial classification was based on the categories of: Very strong (≥ 20 mm); Strong (11 - 20 mm); Moderate (6 – 10 mm); and Weak (≤ 5 mm) (Minarni and Yusnelti, 2020).

Previous research has shown that sesquiterpene lactones are responsible for antibacterial activity, sesquiterpene lactones inhibit the growth of bacteria, especially gram-positive bacteria, but are less effective against gram-negative bacteria. Sesquiterpene lactones act to disrupt microbial cell membranes, this dsruption is caused by polar groups of antimicrobial compounds disrupting phospholipid membranes (Facey et al., 2010; Luo et al., 2011; Ivanescu et al., 2015)

In all concentrations of extracts tested, the inhibitory power of the extract against the growth of the four types of bacteria was smaller than the inhibitory power of terramycin antibiotic. This was because the extract was still in the form of crude extract, which was a mixture of various types of compounds that not all of which were antibacterial, while the standard solution of terramycin antibiotic only contained a single compound.

Sesquiterpene lactones are included in the terpenoid group and are non-polar, so it was suspected that the petroleum ether and chloroform solvents used were able to lift the active compounds contained in the leaf and flower extracts of *T. diversifolia*. *T. diversifolia* flower extract for the ethyl acetate fraction gave a very strong effect against *B. subtilis* bacteria. According to Harbone (1993), ethyl acetate solvent can lift compounds that tend to be polar. This means that the

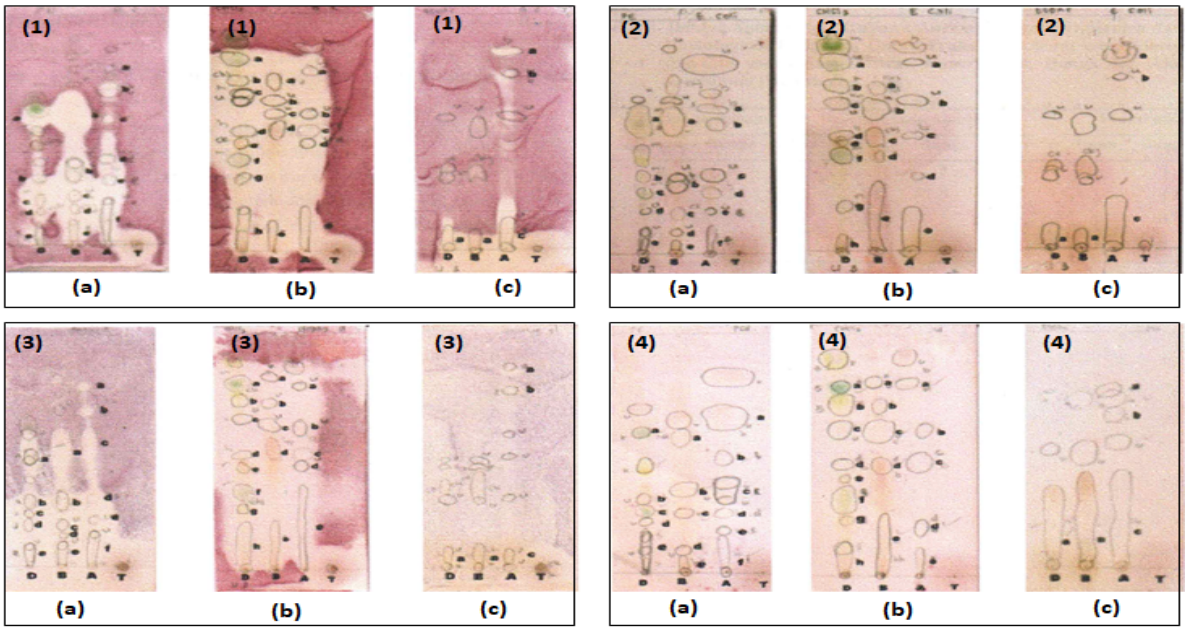
active compound lifted in the ethyl acetate fraction is more polar than the active compound lifted in chloroform.

Among the three plant parts tested, namely the leaves, flowers and roots of *T. diversifolia*, it seemed that the root part had the weakest inhibition compared to the others. It was suspected that the content of active compound, namely sesquiterpene lactones, in the roots was relatively low, because in general this compound is widely found in the leaves and flower crowns and is often present in a mixture form with other compounds. This is in line with research conducted by Kyslychenko et al. (2017), in which the results of analysis related to the content of sesquiterpene lactones in Hosta plantaginea plant showed that the highest content of such compound was found in the leaves, followed by flowers and the lowest was in the roots.

Gram-positive bacteria are more susceptible to antibacterial compounds than gram-negative bacteria, the mechanism of antibacterial action is due to loss of cell permeability (Karsha and Bhagya, 2010). Gram-positive bacteria have relatively weaker activity compared to the gram-negative bacteria due to the presence of an outer membrane which provides the bacterial surface with a strong hydrophilic barrier against hydrophobic chemicals (Nakamura and Akikazu, 2002).

The results of Rf determination of antibacterial compounds using the direct bioautography method are shown in the Figure 2. The number of spots produced for each fraction tested for leaf, flower and stem extracts of *T. diversifolia* is shown in the Table 4.

Based on the table above, the number of spots from TLC results showed that chloroform fraction from leaf extract has the highest number of spots, and if compared to other fractions tested, namely on leaf, flower and root extracts, it showed that chloroform fraction produced a lot of antibacterial spots. This indicated that the compounds which acr as antibacterial contained in *T. diversifolia* were dissolved in chloroform solvent.



Figur 2. of Silica Gel 60 TLC Plate (Merck), the Results of Bioautography against *B. subtilis, E. coli, S. aureus* and *P. aureginosa* Bacteria

Notes : (1) *B. subtilis*, (2) *E. coli*, (3) *S. aureus*, (4) *P. aureginosa*, (a) Petroleum ether fraction, (b) Chloroform fraction, (c) Ethyl acetate, extract spot : (D) Leaf, (B) Flower, (A) Root, (T) terramycin antibiotic 50 mg/ml control.
Mobile phase : Petroleum ether fraction is N-Hexan : Ethyl acetate = 7:3 (v/v)
Chloroform fraction is N-Hexan : ethyl acetate = 3:7 (v/v)
Ethyl acetate fraction is Chloroform : ethyl acetate = 2:3 (v/v)

Table 4. Showing the Number of Antibacterial Compound Spots Obtained from Direct Bioautography Test on Petroleum Ether, Chloroform and Ethyl Acetate Fractions from Leaf, Flower and Root Extracts of *Tithonia diversifolia*.

		Bacteria			
		B. subtilis	***S. aureus***	***E. coli***	***P. auruginosa***
Leaf	Petroleum ether	5	5	5	5
	Chloroform	8	8	8	8
	Ethyl acetate	1	1	1	1
Flower	Petroleum ether	5	5	5	5
	Chloroform	5	5	5	5
	Ethyl acetate	1	1	1	1
Root	Petroleum ether	6	6	6	6
	Chloroform	5	5	5	5
	Ethyl acetate	3	3	3	3

CONCLUSIONS

Based on the results of the study, it could be concluded that the crude extracts of *Tithonia diversifolia* leaves, flowers and roots for petroleum ether, chloroform and ethyl acetate fractions contained antibacterial compounds that could inhibit the growth of the tested bacteria, with better inhibitory activity for the chloroform fraction than the petroleum ether and ethyl acetate

fractions. The results of bioautography test showed that the inhibition was good on gram-positive bacteria with the highest number of spots in the chloroform fraction for crude leaf extract samples. Chloroform fraction This crude leaf extract has the potential as an antibacterial, especially for gram-positive bacteria. The next development process is through isolation of the contained active compounds.

REFERENCES

Ahmed, S. O. and Bamigboye Samson. O. (2020.) Preliminary Phytochemical screening of bioactive chemicals in sunflower (*Tithonia diversifolia*) roots. *International Journal on Integrated Education, 3*(12), 184-192. DOI: 10.31149/ijie.v3i12.942.

Alaofin, Sefunmi, and Onifade Anthony Kayode. (2019). **A**nti-bacterial Activity of *Tithonia diversifolia*, *Secamore afzelli* and *Jaundea pinnata* Against Plasmid-bearing Multiple Antibiotics Resistant Bacteria from Different Water Sources in Akure, Nigeria. *Advances in Bioscience and Bioengineering, 7*(2), 22-26. doi: 10.11648/j.abb.20190702.12**.**

Dlamini, B. S., Chen, C.-R., Shyu, D. J. H., and Chang, C.-I. (2020). Flavonoids from *Tithonia diversifolia* and their Antioxidant and Antibacterial Activity. *Chemistry of Natural Compounds*, *56*(5), 906–908. doi:10.1007/s10600-020-03182-0

Douglas, Kemboi and Janet Jeruto. (2016). hytochemistry and Antimicrobial Activity of Extracts from Medicinal Plants *Tithonia diversifolia* and *Olea Africana*. *British Journal of Pharmaceutical Research*, *12*(3), 1-7. DOI: 10.9734/BJPR/2016/26566.

Fathoni, A., S. Hudiyono, E Budianto, A H Cahyana, and A Agusta. (2021). Metabolite Detection and Antibacterial Activity of Fungal Endophytic Extracts Isolated from Brotowali (*Tinospora crispa*) Plants using TLC-Bioautography Assay. *IOP Conf. Series: Materials Science and Engineering, 1011*(1), 1-10. doi:10.1088/1757-899X/1011/1/012041

Facey, PC, Pascoe, KO, Porter, RB, & Jones, A. (1999). Investigation Of Plants Used In Jamaican Folk Medicine For Anti-Bacterial

Activity. *Journal of Pharmacy and Pharmacology*, *51*(12), 1455-1460. https://doi.org/10.1211/0022357991777119.

Gutierrez, R.M., Baculi, R., Pastor, N., Puma-at, T., Balangcod, T., (2013). Antibacterial potential of some medicinal plants of the Cordillera Region, Philippines. *Indian J. Tradit. Knowl*. 12(4), 630–637.

Gutierrez, R., Balladan, K. and Patacsil, M. (2015). The antibacterial property of *Tithonia diversifolia* extract from Baguio-Benguetareas in the Philippines in response to exposure to vehicular traffic. *Asian Journal of Microbiology, Biotechnology and Environmental sciences*, *17*(1), 43-52.

Harborne, J.B. and H.Baxter. (1993). *Phytocemical Dictionary : A Handbook of Bioactive Compounds from Plants*. Taylor and Francis. London, 399-637.

Ivanescu, Bianca, Anca Miron, Andreia Corciova. (2015). Sesquiterpene Lactones from Artemisia Genus: Biological Activities and Methods of Analysis. *Journal of Analytical Methods in Chemistry*, *2015*(21), 1-21 Article ID 247685. https://doi.org/10.1155/2015/247685.

Karsha, Pavithra Vani, and O Bhagya Lakshmi. (2010). Antibacterial Activity of Black pepper (*Piper nigrum* Linn.) With Special Reference To Its Mode of Action on Bacteria. *Indian journal Of Natural Products and Resources*, *1*(2), 213-215.

Kerebba, N., A.O. Oyedeji, R. Byamukama, S.K. Kuria, O.O. Oyedeji. (2019). Pesticidal activity of *Tithonia diversifolia* (Hemsl.) A. Gray and *Tephrosia vogelii* (Hook f.); phytochemical isolation and characterization: A review. *South African Journal of Botany*, *121*, 366-376. https://doi.org/10.1016/j.sajb.2018.11.024.

Kurama, G., Maarisit, W., Karundeng, E., & Potalangi, N. (2020). Uji Aktivitas Antibakteri Ekstrak Etanol Daun Benalu Langsat (*Dendropthoe sp*) Terhadap Bakteri *Klebsiella Pneumoniae*. *Biofarmasetikal Tropis*, *3*(2), 27-33. DOI: https://doi.org/10.55724/j.biofar.trop.v3i2.281.

Kyslychenko, O.A., Protska V.V., Zhuravel I.O. (2017). Determination of sesquiterpene lactones quantitative content in *Hosta plantaginea* Tratt. rhizomes with roots, leaves and

flowers. *The 8th International Conference on Pharmaceutical Sciences and Pharmacy Practice*. 80-81.

Luo, Xuan, Yan Jiang, Frank R. Fronczek, Cuiwu Lin, Ernest B. Izevbigie and Ken S. Lee. (2011). Isolation and structure determination of a sesquiterpene lactone (vernodalinol) from *Vernonia amygdalina* extracts. *Pharmaceutical Biology*, *49*(5), 464-470. DOI: 10.3109/13880209.2010.52342.

Maregesi, S.M., Pieters, L., Ngassapa, O.D., Apers, S., Vingerhoets, R., Cos, P., Berghe, D.A.V., Vlietinck, A.J., (2008). Screening of some Tanzanian medicinal plants from Bunda district for antibacterial, antifungal and antiviral activities. *J. Ethnopharmacol*. *119*(1)*,* 58–66. doi: 10.1016/j.jep.2008.05.033

Minarni dan Yusnelti. (2020). Test the Activity of Vegetable Oils from Shorea sumatrana Sym. Against Bacteria *Salmonella sp*. and *Bacteria Escherichia coli*. *Jurnal Akta Kimia Indonesia*, *13*, 60–64. DOI:http://dx.doi.org/10.20956/ica.v13i2.10526.

Nakamura, Soichiro and Akikazu Hatanaka. (2002). Green-Leaf-Derived C6-Aroma Compounds with Potent Antibacterial Action That Act on Both Gram-Negative and Gram-Positive Bacteria. *Journal of Agricultural and Food Chemistry*, *50*(26), 7639-7644. DOI: 10.1021/jf025808c.

Olayinka, B. U.; Raiyemo, D. A. & Obukohwo, E. (2015). Phytochemical and proximate composition of *Tithonia diversifolia* (Hemsl.) A. Gray. *Ann. Food Sci. Technol*, *16* (1), 195-200.

Panjaitan, R. S., & Warganegara, F. M. (2018). Aktivitas Antibakteri Ekstrak Lipid Sargassum polycistum Terhadap Bacillus cereus dan Staphylococcus aureus. *EduChemia (Jurnal Kimia dan Pendidikan), 3*(1), 29-39. DOI: http://dx.doi.org/10.30870/educhemia.v3i1.1752,

Pietrocola G, Ceci M, Preda F, Poggio C, Colombo M. (2018). Evaluation of the antibacterial activity of a new ozonized olive oil against oral and periodontal pathogens. *J Clin Exp Dent*, *10*(11), e1103-8.http://www.medicinaoral.com/odo/volumenes/v10i11/jcedv10i11p1103.

Putri, Widyana Sagita, Warditiani, N. K., Larasanty, L. P. F. (2013). Skrining Fitokimia Ekstrak Etil Asetat Kulit Buah Manggis (*Garcinia*

mangostana L.). *Jurnal Farmasi Udayana*, *2*(4), 56-60.

Salni, Marisa H, Mukti RW. (2011). Isolasi Senyawa Antibakteri dari Daun Jengkol *Pithecolobium lobatum* Benth dan Penentuan Nilai KHM-nya. *Jurnal Penelitian Sains*, *14*(1), 14109-4138.

Tagne, A. M., Marino, F. and Cosentino, M. (2018). *Tithonia diversifolia* (Hemsl.) A. Gray as a medicinal plant: a comprehensive review of its ethnopharmacology, phytochemistry, pharmacotoxicology and clinical relevance. *Journal of Ethnopharmacology, 220*(28), 94-116. https://doi.org/10.1016/j.jep.2018.03.025.

Zhao, ui-Jun, Zhong-Xin Xi, Wan-Sheng Chen, Xia Li, Lei Sun, Lian-Na Sun. (2012). Chemical constituents from *Tithonia diversifolia* and their chemotaxonomic significance. *Biochemical Systematics and Ecology, 44*, 250-254. https://doi.org/10.1016/j.bse.2012.06.019.