Identification of Bioactive Compounds and Antimicrobial Activity Test of Methanol Extracts of Leaves and Flowers of Various Tabebuya Species (*Tabebuia* spp.)

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Abstracts: *Tabebuia* species in South America have many functions as traditional medicines, while *Tabebuia* species in Indonesia are useful for making the air clean and becoming ornamental plants. The aim of this study was to determine the content of bioactive compounds and antimicrobial activity of methanol extracts of leaves and flowers of various *Tabebuia* genus. The method to determine the content of bioactive compounds using GC-MS (Gas Chromatography-Mass Spectrometry) method while to determine antimicrobial activity using Kirby-Bauer method. Bioactive compounds found in all extracts were Siliconfett and Cyclotrisiloxane, hexamethyl. Methanol extract of *T. aurea* leaves at a concentration 6.25% had the highest mean ZOI against *C. albicans.* The methanol extract of *T. rosea leaves* at 100% concentration had the highest mean ZOI against *E. coli.* The methanol extract of *T. aurea* leaves at a concentration 25% has the highest mean ZOI against *S. aureus*.

Keywords: GC-MS, T. aurea, T. roseo-alba, T. Rosea, Antimicrobial Activity.

1. INTRODUCTION

For thousands of years, herbal remedies have been used as traditional medicine in Eastern Countries[1]. Nature is a sourceof new medicines to alleviate suffering and cure various diseases. According to World Health Organization, nearly 80% of the global population relies on herbal species for health care needs[2]. Plants used in traditional medicine come from the Bignoniaceae family, one of the plants from the Bignoniaceae family is *Tabebuia*[3].

Tabebuia is the largest genus of the Bignoniaceae family with about 100 species that grow in tropical and subtropical regions[4] [5]. Several studies have shown the benefits of bioactive compounds of *Tabebuia* genus members, such as lapachol, which has been used in cancer therapy[6]. Compounds contained in water, methanol, ethyl acetate, and hexane extracts of *T. impetiginosa* bark detected using GC-MS methods are o-guaiacol; Ethyl 4-ethoxybenzoate; 2,2,4-trimethyl-1,3-pentanedialdiisobutyrate[7]. *Tabebuia* species are widely used in tradicional medicine for the treatment of syphilis, malaria, skin infections, stomatch disorders, cancer, inflammation, pain, bacterial and fungal infections, anxiety, poor memory, irritability, depression, and for the treatment of diabetes, prostatitis, constipation, and allergies[8] [4].

The emergence of antimicrobial resistance is becoming the most issue in the treatment of infectious diseases, the impact of microbial resistance to most available antibiotics, in particular the emergence of multidrug-resistant bacteria is becoming a globalheaalth problem and threat. Antimicrobial resistance could lead to an increase in deaths from 1 million to 10 million by 2050[9]. Ethanol extract of *T. rosea* leaves showed antimicrobial activity against *Klebsiela pneumonia* with Zone of Inhibition (ZOI) around 9,9-16,0mm, while *Staphylococcus epidermidis* with ZOI around 8,4-13,8mm[10]. The ethanol exctract of *T. aurea* bark showed a Minimum Inhibition Concetration (MIC) of 12,5 and 25 mg/mL for *Staphylococcus aureus* and *Escherichia coli,* while for *Candida albicans,* a MIC of 25 mg/mL was obtaines[11].

In this study using plant specimens of *T. aurea, T. roseo-alba* and *T. rosea* these types of plants were chosen for

research because in Indonesia no one has examined these 3 types of plants, besides that there are differences in locations and plant organs to be used, plant organs that have been used by previous researchers are leaves, stems, bark, and seeds, while those used in this study are leaves and flowers. *Tabebuia* trees in Indonesia have not been widely studied for their scientific benefits and bioactive compound content, therefore it is necessary to conduct a study to prove and confirm the antimicrobial abilities of *Tabebuia* exracts. The data from this study can be used by the pharmaceutical industry, especially medicines made from herbal plants, besides that the dara from this study can also be used as support for other studies, especially related to the potential of *Tabebuia* as medicinal plant.

2. MATERIEL AND METHODS

2.1. Research Tools and Materials

The materials used in this study are flower and leaf organs of *Tabebuia aurea, Tabebuia roseo-alba*, and *Tabbeuia rosea* taken from Mulyorejo village, Mulyorejo sub-district, Surabaya city, East Java province, Indonesia. Chemicals to be used are Mueller Hinton agar, Mueller Hinton broth, Mannitol Salt Agar, Eosin Methylene Blue Agar, Potato Dextrose Agar, distilled water, chloramphenicol, *Escherichia coli, Staphylococcus aureus*, and *Candida albicans.*

The tools that will be used in this research are blender, jar, funnel, filter paper, analytical balance, vial bottle, GC-MS tool, autoclave, disposable petri dish, ose needle, tweezers, erlenmeyer tube, paper disc, caliper, spectrophotometer, incubator, and Laminar Air Flow (LAF).

2.2. Extraction

Extraction of leaves and flowers of various of *Tabebuia* species was carried out by maceration using the most widely used solvent, methanol. The collected samples were cleaned with running water, then the samples were dried at room temperature without direct sunlight. The leaf and flower samples were then pulverized into coarse powder using a blender. Simplisia powder was weighed to 50 grams, then soaked in 500mL of methanol solvent in a closed container at room temperature for 9 days and stirred every 24 hours. The macerate was filtered every 3 days using a funnel lined with filter paper, then the simplisia powder was re-macerated by adding a new solvent of the same type and amount. The filtrate from the filtration was evaporated so that the results were obtained in the form of thick methanol extracts of leaves and flowers of various of *Tabebuia* species [12].

2.3. Identification of Bioactive Compounds GC-MS Method

Bioactive compounds contained in an extract can be determined using gas chromatography (6580 Network GC System, Agilent) and mass spectrometry (Agilent 5975C VL MSD) equipped with HP5-MS capillary column of 30m length and 0.25µm film thickness (Agilent, ID 250 µm). The initial column temperature was adjusted to 100°C for 5 minutes and increased by 5°C per minute until the final temperature reached 320°C with a total run time 57 minutes. The volume of extract injected was 1µL with heium as the carrier gas at 1mL/min. The equipment used was an injector, ion source, and quadropole temperature with temperatures 300°C, 230°C, and 150°C, respectively. Mass spectrometry was detected using an ionization energy of 70eV, working in full scan acquisition mode between 50 and 600m/z at 2.66 scans/s[13].

2.4. Antimicrobial Activity Test Disc Diffusion Method

The disc diffusion method is carried out by preparing Mannitol Salt Agar (MSA) media for *Staphylococcus aureus*, Eosin Methylene Blue Agar (EMB) media for *Escherichia coli*, and Potato Dextrose Agar (PDA) media for *Candida albicans* aseptically and poured into test tubes, then sterilized by autoclaving at 121 ° C for 15 minutes and allowed to stand in an inclined position until it solidifies. Pure microbial culture that will be used is inoculated on the solidified oblique agar and left for approximately 3 days to grow, after growing the microbial inoculum, the optical density (OD) value is measured using a spectrophotometer by mixing 1 ose of test microbes in 10 mL of Mueller-Hinton Broth (MHB) media, microbial OD measurements are measured at a

wavelength of 600nm for fungi and 625nm for bacteria. The microbes to be tested have an OD value of 0.1, if the OD value is <0.1 then 1 ose of recultured microbes is added but if the OD value is> 0.1 then a dilution is made using the formula M1.V1 = M2.V2. The antimicrobial activity of leaf and flower extracts of various types of *Tabebuia* was carried out using the standard Kirby-Bauer disc diffusion method (1996) which was carried out by comparing the results between standard antibiotics chloramphenicol (for *E. coli* and *S. aureus*) and (for *C. albicans*) as positive controls and DMSO as a negative control with concentrations of methanol extracts of leaves and flowers of various types of *Tabebuia* 100, 50, 25, 12.5 and 6.25% tested. Control solutions and methanol extracts of leaves and flowers of various types of *Tabebuia* concentrations of 100, 50, 25, 12.5 and 6.25% were injected on blank disc paper, disc paper that had been injected with extracts was then placed on a Petri dish containing Mueller-Hinton Agar (MHA) media which had previously been injected with 100µL of test microbes using the swab technique, the Petri dish was then incubated for 48 hours (for *C. albicans*) and 24 hours (for *E. coli* and *S. aureus*) at 37ºC after incubation a clear zone appears around the disc paper. The results of antimicrobial activity were carried out by calculating the diameter of the inhibition zone using a caliper, the measurement results of the Zone of Inhibition (ZOI) or the diameter of the inhibition zone were then classified according to Table 1 (Atwaa *et al.*, 2022)

Table 1. Antimicrobial properties based on ZOI value (Ouchari *et al.*, 2019)

ZOI value (mm)	Classifications
>20	Very strong
10-20	Strong
5-10	Moderate
<5	No Inhibition

3. RESULTS AND DISCUSSIONS

3.1. Identification of Bioactive Compounds GC-MS Method

Identification of bioactive compounds in methanol extracts of leaves and flowers of various types of tabebuia was carried out by GC-MS method. The analysis results in the form of chromatograms showing 37 peaks in the methanol extract of *T. aurea* leaves (Figure 1), 47 peaks in the methanol extract of *T. aurea* flowers (Figure 2), 61 peaks in the methanol extract of *T. roseo-alba* leaves (Figure 3), 21 peaks in the methanol extract of *T. roseo-alba* flowers (Figure 4), 36 peaks in the extract of *T. rosea* leaves (Figure 5), and 40 peaks in the methanol extract of *T. rosea* flowers (Figure 6). Based on these results, it can be seen that the methanol extract of *T. aurea* leaves contains 37 types of bioactive compounds detected, the methanol extract of *T. aurea* flowers contains 47 types of bioactive compounds detected, the methanol extract of *T. roseo-alba* leaves contains 61 types of bioactive compounds. methanol extract of *T. roseo-alba* flowers contains 21 types of bioactive compounds detected, methanol extract of *T. rosea* leaves contains 36 types of bioactive compounds detected, and methanol extract of *T. rosea* flowers contains 40 types of bioactive compounds detected The types of bioactive compounds are shown in table 2 to table 7.

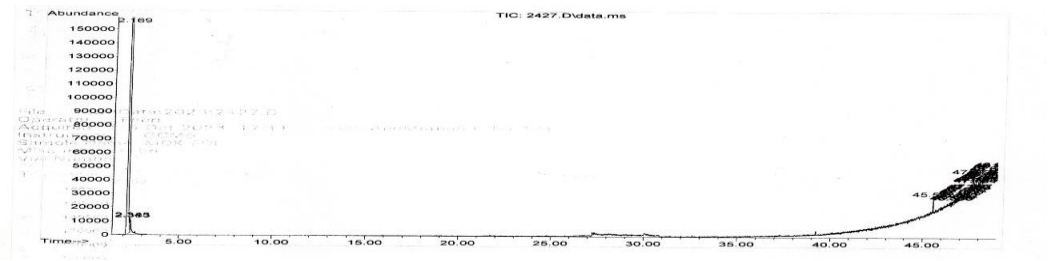

Figure 1. Chromatogram Profile of Methanol Extract of *T. Aurea* Leaves

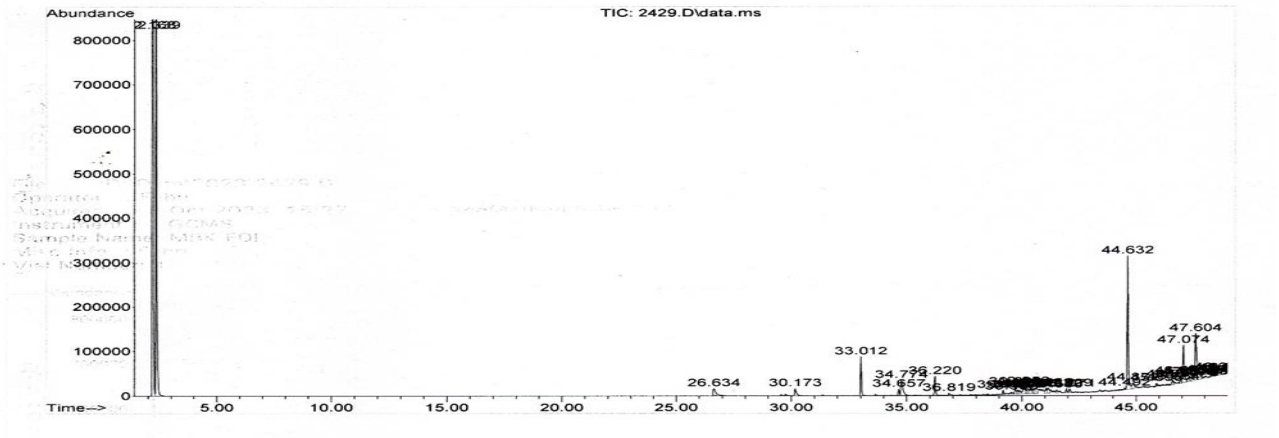

Figure 2. Chromatogram profile of methanol extract of *T. aurea* flowers.

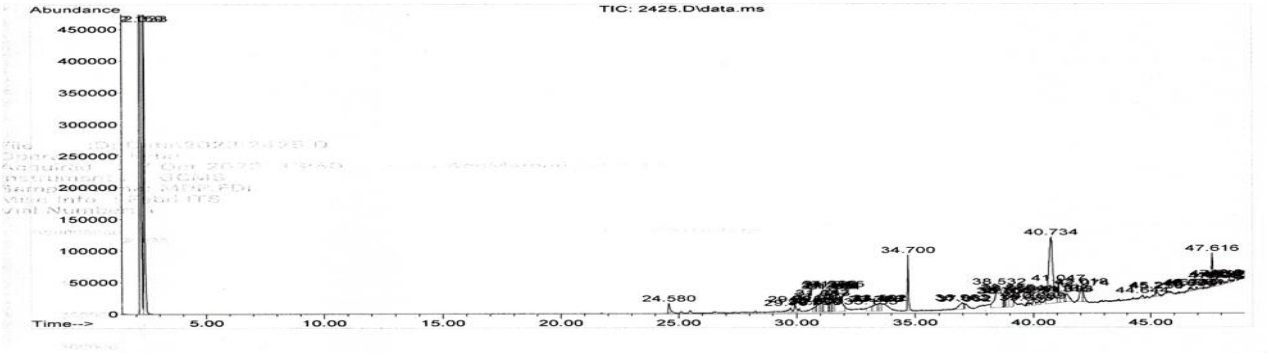

Figure 3. Chromatogram profile of methanol extract of *T. roseo-alba* leaves

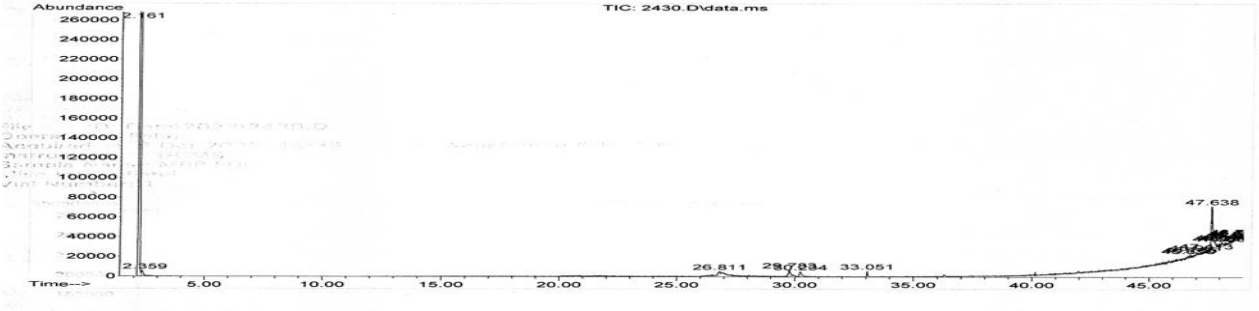

Figure 4. Chromatogram profile of methanol extract of *T. roseo-alba* flowers

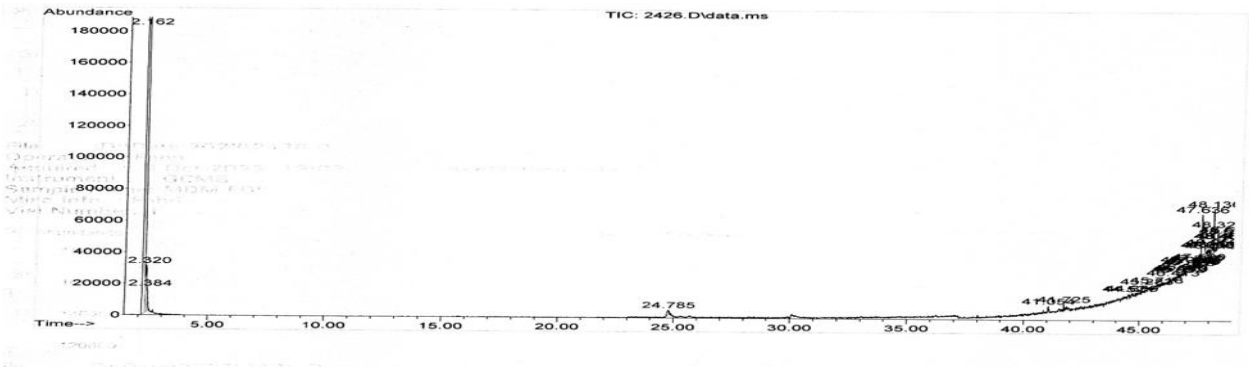

Figure 5. Chromatogram profile of methanol extract of T. rosea leaves

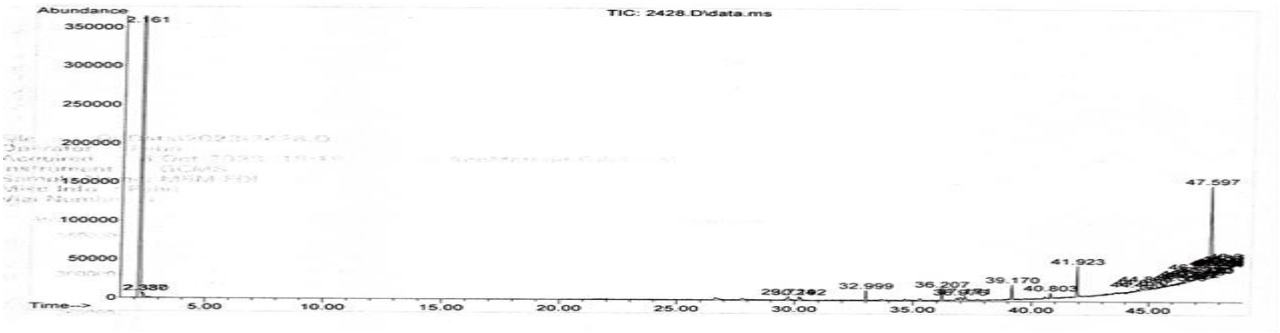

Figure 6. Chromatogram profile of methanol extract of *T. rosea* flowers

Based on figures 2 to 7, it can be seen that bioactive compounds were first detected in the first 2 minutes and then bioactive compounds were detected again at the 24th minute to the 48th minute. The X axis of the chromatogram profile shows the time the bioactive compound was detected by the GC-MS device, while the Y axis of the chromatogram profile shows the abundance of the bioactive compound in the methanol extract of the leaves and flowers of various of *Tabebuia* species. The highest abundance was found in the methanol extract of *T. aurea* flowers, namely 800000, while the lowest abundance was found in the methanol extract of *T. aurea* leaves, namely 150000.

Table 2. Identification results of bioactive compounds of methanol extract of *T. aurea* leaves by GC-MS method

Number	Retention time (minute)	Area (%)	Bioactive compound
1	2.169	99.18	Hydroxylamine
2	2.345	0.06	Silane, dioxymethyl
3	2.383	0.05	2-Diisopropylsilyloxypropane
4	45.555	0.03	6-Methyl-2-phenylindole
5	46.500	0.01	4'Methyl-2 phenylindole
6	46.566	0.03	5,5"-Diethynyl-2,2':6',2"-terpyridine
7	46.659	0.02	4'Methyl-2 phenylindole
8	46.822	0.01	Acetamide, N-[4-(trimethylsilyl)phenyl]-
9	46.871	0,01	5,5"-Diethynyl-2,2':6',2"-terpyridine
10	46.955	0.01	Acetic acid, 2-[bis(methylthio)methylene]-1-phenylhydrazude
11	47.027	0.03	Silikonfett
12	47.100	0.02	5,5"-Diethynyl-2,2':6',2"-terpyridine
13	47.230	0.03	Cyclotrisiloxane, hexamethyl
14	47.282	0.02	Acetamide, N-[4-(trimethylsilyl)phenyl]
15	47.399	0.01	Silikonfett
16	47.440	0.01	5,5"-Diethynyl-2,2':6',2"-terpyridine
17	47.524	0.01	Arsenous acid, tris(trimethylsilyl) ester
18	47.577	0.01	Silikonfett
19	47.679	0.13	6-Methyl-2-phenylindole
20	47.753	0.02	Guanidine, N''-[3,5-bis(trifluoromethyl)phenyl]-N,N,N',N'-tetramethyl
21	47.784	0.03	Silikonfett
22	47.846	0.01	5,5"-Diethynyl-2,2':6',2"-terpyridine
23	47.974	0.02	Acetamide, N-[4-(trimethylsilyl)phenyl]
24	48.028	0.01	Silane, trimethyl[4-1[(trimethylsilyl)oxy]ethenyl-2,2-d2}phenoxy}
25	48.088	0.04	5,5"-Diethynyl-2,2':6',2"-terpyridine
26	48.174	0.01	5,5"-Diethynyl-2,2':6',2"-terpyridine
27	48.290	0.03	5,5"-Diethynyl-2,2':6',2"-terpyridine
28	48.347	0.01	Silikonfett
29	48.407	0.01	Cyclotrisiloxane, hexamethyl

30	48.453	0.01	5,5”-Diethynyl-2,2’:6’,2”-terpyridine
31	48.556	0.01	Silikonfett
32	48.619	0.02	Acetamide,N-[4-(trimethylsilyl)phenyl]
33	48.646	0.01	Cyclotrisiloxane, hexamethyl
34	48.788	0.04	2-Ethylacridine
35	48.848	0.01	6-Methyl-2-phenylindole
36	48.902	0.01	5,5”-Diethynyl-2,2’:6’,2”-terpyridine
37	48.931	0.03	1,1,1,3,5,5,5-Heptamethyltrisiloxane

Table 3. Identification results of bioactive compounds of methanol extract of *T. aurea* flowers by GC-MS method

Number	Retention time (minute)	Area (%)	Bioactive compound
1	2.168	81.54	Hydroxylamine
2	2.339	7.30	Pirolidine, 1-[8-(3-octyloxiranyl)-1-oxooctyl]
3	26.634	0.29	Hexadecanoic acid, methyl ester
4	30.173	0.23	Methyl stearate
5	33.012	0.73	Tricosane
6	34.657	0.12	Heneicosane
7	34.774	0.54	Hexanodecanoic acid, bis(2-ethylhexyl) ester
8	36.220	0.38	Pentacosane
9	36.819	0.03	Methyl 20-methyl-heneicosane
10	39.179	0.08	Eicosane, 9-octyl
11	39.533	0.05	Phthalic acid, bis(7-methyloctyl) ester
12	39.698	0.17	Tetracosanoic aid, methyl ester
13	39.808	0.08	Phthlic acid, nonyl 4-octyl ester
14	39.894	0.11	Ribitol, pentaacetate
15	40.056	0.27	Mono(2-ethylhexyl)phthalate
16	40.164	0.09	Phthalic acid, bis(7-methyloctyl) ester
17	40.252	0.13	Phthalic acid, bis(7-methyloctyl) ester
18	40.346	0.15	Phthalic acid, bis(7-methyloctyl) ester
19	40.521	0.11	Benzaldehyde, 4-methoxy-3(8-quinolinyloxymethyl)
20	40.623	0.21	Phthalic acid, bis(7-methyloctyl) ester
21	41.043	0.14	4-methoxy-2,6-dimethylbenzyl acetate
22	41.119	0.17	1,2-Benzenedicarboxylic acid, dinonyl ester
23	41.506	0.08	2H-Benzo[4,5]thiazolo[2,3-C][1,2,4] triazole-3-thione
24	41.929	0.09	2H-Benzo[4,5]thiazolo[2,3-C][1,2,4] triazole-3-thione
25	42.071	0.11	Silane, chlorodiethylheptyloxy
26	44.492	0.04	2-Ethylacridine
27	44.632	2.80	Nonacos-1-ene
28	44.872	0.23	Dodecahydropyrido[1,2-b]isoquinolin-6-one
29	45.898	0.06	2-Ethylacridine
30	46.597	0.13	Cyclotrisiloxane, hexamethyl
31	46.654	0.04	N-methyl-1-adamantaneacetamine
32	46.955	0.14	1-(3-Methylphenyl)-1h-indole
33	46.997	0.04	2-Ethylacridine
34	47.074	0.96	1-docosanol
35	47.298	0.13	Cyclotrisiloxane, hexamethyl
36	47.349	0.04	4’Methyl-2-phenylindole
37	47.378	0.09	Methyltris(trimethylsiloxy)silane
38	47.604	1.72	Stigmast-5-en-3-ol
39	47.883	0.02	2-Ethylacrdine
40	48.057	0.08	Cyclotrisiloxane, hexamethyl
41	48.276	0.03	6-Methyl-2-phenylindole
42	48.325	0.05	Cyclotrisiloxane, hexamethyl
43	48.417	0.01	Tetrasiloxane, decamethyl
44	48.686	0.14	Cyclotrisiloxane, hexamethyl
45	48.801	0.01	1,4-Bis(trimethylsilyl)benzene
46	48.847	0.01	2-Ethylacridine
47	48.937	0.04	2-Methyl-7-phenylindole

Table 4. Identification results of bioactive compounds of methanol extract of *T. roseo-alba* leaves by GC-MS method

Number	Retention time (minute)	Area (%)	Bioactive compound
1	2.159	78.47	Hydroxylamine
2	2.333	5.51	Methane, trichloro
3	24.580	0.17	Neophytadiene
4	29.751	0.12	2-Norbornanone,1,7-dimethyl-7-(4-methyl-3-pentenyl)-,(-)stereoisomer
5	29.925	0.19	Hexadecyl pentyl ether
6	30.750	0.08	(-)-Selina-4.alpha.,11-diol
7	30.807	0.07	.beta.-Eudesmol
8	30.838	0.03	Arenarone
9	30.941	0.17	beta.-Eudesmol
10	31.007	0.16	2-Indolinol, 1-acetyl-3-,methyl
11	31.087	0.19	2,5,5,8a-Tetramethyl-octahydronaphthalene-1-methanol(bicyclofarnesol)
12	31.113	0.09	9-Bromomethylanthracene
13	31.306	0.86	A'-Neogammacer-22(29)-ene
14	31.348	0.15	Pyrrolo[3,2,1-jk]carbazole
15	31.398	0.40	A'-Neogammacer-22(29)-ene
16	31.454	0.13	3-Fluoro-5,7-dimethylquinol-2(1H)-one
17	31.483	0.23	beta.-Eudesmol
18	31.591	0.36	2-Naphthalenemethanol, decahydroalpha.,.alpha.,4a-trimethyl-8-methylene-,[2R-(2.alpha.,4a.alpha.,8a.beta.)]
19	31.705	1.38	3-Fluoro-5,7-dimethylquinol-2(1H)-one
20	33.133	0.21	Hahnfett
21	33.323	0.25	Neophytadiene
22	33.396	0.07	Citronellyl 3-methylbutanoate
23	33.457	0.12	1-octadecanol
24	33.502	0.14	Phytyl palmitate
25	34.700	0.66	Diisooctyl adipate
26	36.962	0.28	Ambrein
27	37.032	0.03	Trichotheca-9,12-diene-4,15-diol,(4.alpha.,11.beta.)
28	37.062	0.05	Methyl ionone
29	28.532	1.47	Lupan-3-ol, (3.beta.)
30	38.704	0.12	beta.-iso-Methyl ionone
31	38.756	0.12	13(16),14-Labdien-8-ol
32	38.803	0.12	Cheloviolene D
33	38.885	0.35	1H-pyrrole-2,5-dione,1-(4-fluorophenyl)
34	38.966	0.44	A'-Neogammacer-22(29)-ene
35	39.688	0.03	Spiro[benzothiazole-2(3H),4'-[4H]pyran],2',3',5',6'-tetrahydro
36	39.766	0.05	Acetic acid, bicyclo[2.2.1]hept-2-en-7-ylidene
37	39.991	0.18	1,4-Benzenedicarboxylic acid, 1,4-bis(2-ethylexyl)ester
38	40.239	0.08	Eicosyl nonyl ether
39	40.734	3.14	7.alpha.,8.alpha.-Epoxyfernan-25-o
40	41.047	0.32	Supraene
41	41.198	0.31	3-Hydroxy-3(5-phenyl-1,3,4-oxadiazol-2-yl)-1,3-dihydro-2H-indol-2-one
42	41.315	0.09	Squalene
43	41.393	0.29	Ergosta-8,25-dien-3-one, 14,24-dimethyl
44	42.018	0.41	Trimethyl-ethyl-butyl-hexahydro-indene
45	42.074	0.26	Trimethyl-ethyl-butyl-hexahydro-indene
46	44.644	0.09	Benzo[h]quinoline, 2,4-dimethyl
47	45.219	0.07	1H-indole, 1-methyl-2-phenyl
48	45.250	0.11	Cyclotrisiloxane, hexamethyl
49	46.636	0.04	Cyclotrisiloxane, hexamethyl
50	46.661	0.06	Ginsenol
51	46.736	0.04	2-Ethylacridine

52	46.954	0.05	2-amino-3-carboxy-6-(3-indolyl)pyridine
53	47.616	0.63	gamma.-Sitosterol
54	47.749	0.03	2-Ethylacridine
55	47.813	0.21	2-Ethylacridine
56	48.063	0.07	2-Ethylacridine
57	48.328	0.07	4'Methyl-2-phenylindole
58	48.404	0.04	2-Amino-3,5-dicyano-6-ethoxy-4-(2-hydroxypyrid-3-yl)pyridine
59	48.512	0.02	2-Ethylacridine
60	48.627	0.07	Cyclotrisiloxane, hexamethyl
61	48.701	0.01	Cyclotrisiloxane, hexamethyl

Table 5. Identification results of bioactive compounds of methanol extract of *T. roseo-alba* flowers by GC-MS method

Number	Retention time (minute)	Area (%)	Bioactive compound
1	2.161	98.60	Hydroxylamine
2	2.359	0.07	Silane, dimethoxymethyl
3	26.811	0.05	Decanoic acid, methyl ester
4	29.783	0.11	7-Hexadecanoic acid, methyl ester, (Z)
5	30.234	0.09	Octadecanoic acid, methyl ester
6	33.051	0.05	Pentacosane
7	46.650	0.02	Cyclotrisiloxane, hexamethyl
8	46.735	0.01	Silikonfett
9	46.991	0.03	2-Ethylacridine
10	47.638	0.01	Silikonfett
11	47.968	0.75	Silikonfett
12	47.968	0.09	1H-indole, 1-methyl-2-phenyl
13	48.007	0.05	Cyclotrisiloxane, hexamethyl
14	48.219	0.03	Cyclotrisiloxane, hexamethyl
15	48.270	0.01	Acetamide, N-[4-(trimethylsilyl)phenyl]
16	48.482	0.05	1,3-dimethyl-4-azaphenanthrene
17	48.528	0.01	1,3-dimethyl-4-azaphenanthrene
18	48.570	0.02	2-Ethylacridine
19	48.647	0.02	Tetrasiloxane, decamethyl
20	48.675	0.02	Tetrasiloxane, decamethyl
21	48.711	0.01	Cyclotrisiloxane, hexamethyl

Table 6. Identification results of bioactive compounds of methanol extract of *T. rosea* leaves by GC-MS method

Number	Retention time (minute)	Area (%)	Bioactive compound
1	2.162	98.48	Hydroxylamine
2	2.320	0.27	4-(Acetoxy)-3-methyl-1-butanol
3	2.384	0.07	Dimethylsilanol
4	24.785	0.03	Neophytadiene
5	41.045	0.03	14.alpha.-Cheilanth-12-enic methyl ester
6	41.725	0.01	Silicic acid, diethyl bis(trimethylsilyl)ester
7	44.521	0.02	Siliconfett
8	44.659	0.03	Tris(tert-butyldimethylsilyloxy)arsane
9	45.283	0.04	N-Methyl-1-adamantaneacetamide
10	45.718	0.02	1H-indole, 1-methyl-2-phenyl
11	46.413	0.01	6-Methyl-2-phenylindole
12	46.632	0.02	Acetic acid, 2-[bis(methylthio)methylene]-1-phenylhidrazide
13	46.690	0.01	3H-indole, 2-methyl-3-phenyl
14	46.770	0.01	2-Ethylacridine
15	46.871	0.01	1,4-Bis(trimethylsilyl)benzene
16	47.009	0.06	2-Ethylacridine
17	47.070	0.02	Acetamide, N-[4-(trimethylsilyl)phenyl]
18	47.164	0.02	2-Ethylacridine

19	47.248	0.01	2-Ethylacridine
20	47.292	0.01	3,4-di(4-trimethylsiloxyphenyl)hexane
21	47.328	0.01	1,3-dimethyl-4-azaphenanthrene
22	47.373	0.02	1,3-dimethyl-4-azaphenanthrene
23	47.440	0.02	Arsenous acid, tris(trimethylsilyl)ester
24	47.520	0.01	5,5'-Diethynyl-2,2':6'2"-terpyridine
25	47.636	0.44	Silikonfett
26	47.858	0.22	2-Ethylacridine
27	47.910	0.5	6-Methyl-2-phenylindole
28	48.013	0.01	Silikonfett
29	48.056	0.03	Cyclotrisiloxane, hexamethyl
30	48.136	0.06	Acetamide, N-[4-(trimethylsilyl)phenyl]
31	48.172	0.00	Silikonfett
32	48.320	0.10	Methyltris(trimethylsiloxy)silane
33	48.406	0.01	Cyclotrisiloxane, hexamethyl
34	48.488	0.02	Acetamide, N-[4-(trimethylsilyl)phenyl]
35	48.628	0.03	2-Methyl-7-phenylindole
36	48.670	0.02	Cyclotrisiloxane, hexamethyl

Table 7. Identification results of bioactive compounds of methanol extract of *T. rosea* flowers by GC-MS method

Number	Retention time (minute)	Area (%)	Bioactive compound
1	2.161	96.27	Hydroxylamine
2	2.337	0.04	1-Phenyl-2-triisopropylsilyloxy-propene
3	2.380	0.07	Thiodiglycol
4	29.724	0.05	2-Nonenal,8-oxo
5	30.192	0.04	Methy-24-methylhexacosanoate
6	32.999	0.11	Eicosane, 10-methyl
7	36.207	0.12	Tricosane
8	36.976	0.07	2-p-Nitrophenyl-oxadiazol-1,3,4-one-5
9	37.171	0.08	Bis(2-Ethylhexyl)phthalate
10	39.170	0.16	Tetracosane
11	40.803	0.05	Sebasic acid, 2,2-dichloroethyl nonyl ester
12	41.923	0.31	Eicosane
13	44.490	0.04	Silikonfett
14	44.670	0.05	4'Methyl-2-phenylindole
15	44.865	0.11	1,2-bis(Trimethylsilyl)benzene
16	46.300	0.02	Cyclotrisiloxane, hexamethyl
17	46.452	0.02	2-Ethylacridine
18	46.614	0.06	2-Ethylacridine
19	46.719	0.03	2-Ethylacridine
20	46.842	0.02	Acetamide, N-[4-(trimethylsilyl)phenyl]
21	46.966	0.13	2-Ethylacridine
22	47.056	0.01	6-Methyl-2-phenylindole
23	47.083	0.01	2-Ethylacridine
24	47.320	0.04	Cyclotrisiloxane, hexamethyl
25	47.420	0.01	Silane, 1,4-phenylenebis[trimethyl
26	47.450	0.01	6-Methyl-2-phenylindole
27	47.597	1.61	gamma-Sitosterol
28	47.839	0.03	6-Methyl-2-phenylindole
29	47.881	0.07	Cyclotrisiloxane, hexamethyl
30	47.967	0.01	Silikonfett
31	47.995	0.01	Tetrasiloxane, decamethyl
32	48.048	0.05	Silikonfett
33	48.096	0.02	Cyclotrisiloxane, hexamethyl
34	48.149	0.02	2-Methyl-7-phenylindole
35	48.335	0.09	2-Ethylacridine
36	48.406	0.01	Cyclotrisiloxane, hexamethyl
37	48.503	0.01	Silikonfett
38	48.461	0.11	1H-isoindole-1,3(2H)-dithione, 2-ethyl

39	48.820	0.02	2-Ethylacridine
40	48.863	0.02	1,1,1,3,5,5,5-Heptamethyltrisiloxane

Based on table 2, it can be observed that the highest bioactive compounds are 6-methyl-2-phenylindole (0.13%), 5,5''-Diethynyl-2,2':6',2''-terpyridine (0.04%), and 2-Ethylacridine (0.04%). Based on table 3, it can be observed that the highest bioactive compounds are Nonacos-1-ene (2.80%), Stigmast-5-en-3-ol (1.72%), and 1-Docosanol (0.96%). Based on table 4, it can be observed that the highest bioactive compounds are 7.alpha.,8.alpha.-Epoxyfernan-25-o (3.14%), Lupan-3-ol, (3.beta.) (1.47%), and 3-Fluoro-5,7-dimethylquinol-2(1H)-one (1.38%). Based on table 5, it can be observed that the highest bioactive compounds are Siliconfett (0.75%), 7-Hexadecanoic acid, methyl ester, (Z) (0.11%), and Octadecanoic acid, methyl ester and 1H-indole, 1-methyl-2-phenyl (0.09%). Based on table 6, it can be observed that the highest bioactive compounds are Siliconfett (0.44%), Methyltris(trimethylsiloxy)silane (0.10%), and Acetamide, N-[4-(trimethylsilyl)phenyl] and 2-Ethylacridine (0.06%). Based on table 7, it can be observed that the highest bioactive compounds are gamma-Sitosterol (1.61%), Eicosane (0.31%), and Tetracosane (0.16%). The bioactive compounds found in all extracts were siliconfett and Cyclotrisiloxane, hexamethyl. Retention time is the time it takes for the solute to pass through the chromatographic column, which is calculated from sample injection until the bioactive compound is detected, with units of minutes. Percentage area is the peak area for the bioactive compound divided by the total area in the chromatogram, in units of percent. The wider and higher outer area indicates that the greater the concentration of bioactive compounds in the extract.

3.2. Antimicrobial Activity Test Disc Diffusion Method

The antimicrobial activity test of methanol extracts of leaves and flowers of various of *Tabebuia* species was carried out by disc diffusion method. The test results are in the form of bar charts of the average ZOI against 3 types of Microbes, namely *Candida albicans, Escherichia coli,* and *Staphylococcus aureus.* In this study, chloramphenicol (for *Escherichia coli* and *Staphylococcus aureus*) and nystatin (for *Candida albicans*) were used as a positive controls and DMSO (Dimethyl Sulfoxide) as a negative control. The results in the form of the average diameter of ZOI against 3 types of microbes are presented in the form of bar diagrams in Figure 7 to Figure 9.

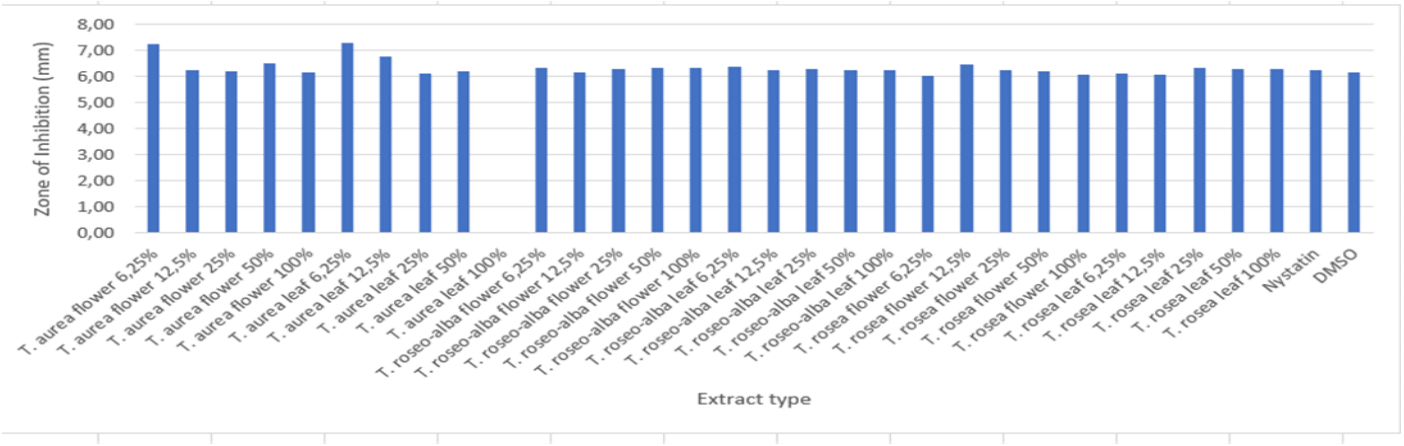

Figure 7. Mean diameter of ZOI against *Candida albicans*

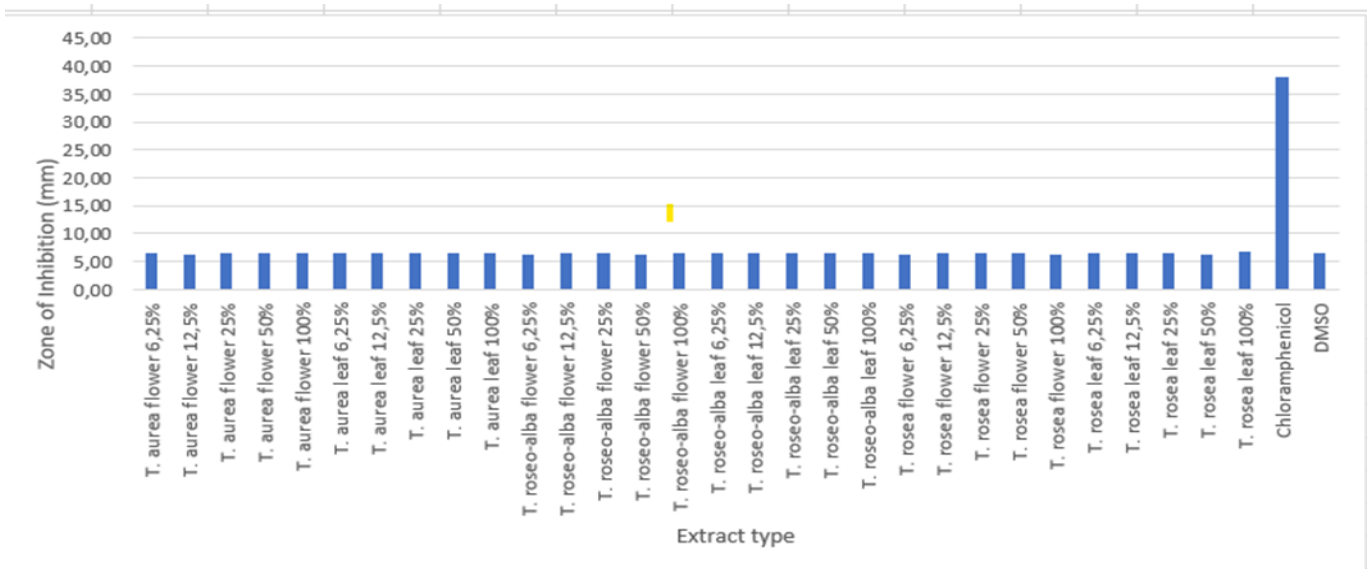

Figure 8. Mean diameter of ZOI against *Escherichia coli*

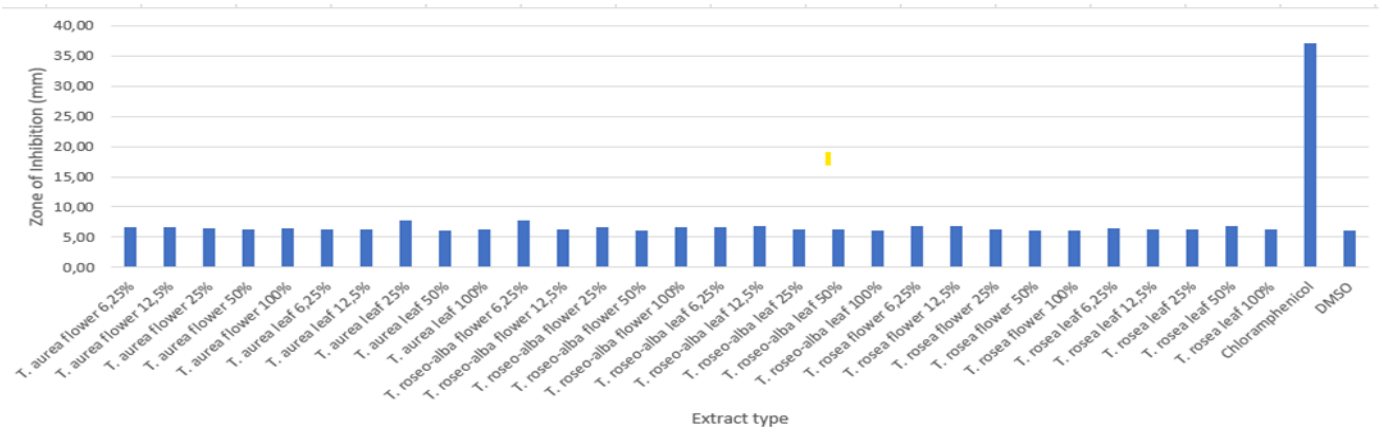

Figure 9. Mean diameter of ZOI against *Staphylococcus aureus*

Based on Figure 8, it can be observed that the methanol extract of *T. aurea* flowers and leaves at a concentration of 6.25% and methanol extract of *T. aurea* leaves at a concentration of 12.5% have the highest mean ZOI against *C. albicans* incubated for 48 hours, namely 7.25; 7.28; and 6.75mm. The highest mean ZOI against C. albicans incubated for 24 hours was 100% concentration of *T. aurea* leaf methanol extract, which was 7.28mm.

Based on Figure 9, it can be observed that 100% concentration of *T. rosea* leaf methanol extract, 25% concentration of *T. roseo-alba* flower methanol extract, and 6.25% concentration of *T. roseo-alba* leaf methanol extract have the highest mean ZOI against *E. coli* incubated for 24 hours, namely 6.67; 6.65; and 6.62mm. The highest mean ZOI against *E. coli* incubated for 24 hours was the methanol extract of *T. rosea* leaves at 100% concentration, which was 6.67mm.

Based on Figure 10, it can be observed that the methanol extract of T. aurea leaves at 25% concentration, methanol extract of T. roseo-alba flowers at 6.25% concentration, and methanol extract of T. rosea flowers at 12.5% concentration have the highest mean ZOI against S. aureus incubated for 24 hours, namely 7.82; 7.82; and 6.78mm. The highest mean ZOI against S. aureus incubated for 24 hours was the 25% concentration of T. aurea leaf methanol extract, which was 7.83mm.

CONCLUSIONS

Methanol extract of *T. aurea* leaves contains 37 types of bioactive compounds, methanol extract of *T. aurea* flowers contains 47 types of bioactive compounds, methanol extract of *T. roseo-alba* leaves contains 61 types of bioactive compounds, methanol extract of *T, roseo-alba* flowers contains 21 types of bioactive compounds, methanol extract of *T. rosea* leaves contains 36 types of bioactive compounds, and methanol extract of *T. rosea* flowers contains 40 types of bioactive compounds. The methanol extract of *T. aurea* leaves containing the highest bioactive compounds were 6-methyl-2-phenylindole (0.13%), 5,5''-Diethynyl-2,2':6',2''-terpyridine (0.04%), and 2-Ethylacridine (0.04%). The methanol extract of *T. aurea* flowers contained the highest bioactive compounds of Nonacos-1-ene (2.80%), Stigmast-5-en-3-ol (1.72%), and 1-Docosanol (0.96%). The methanol extract of *T. roseo-alba* leaves containing the highest bioactive compounds were 7.alpha.,8.alpha.-Epoxyfernan-25-o (3.14%), Lupan-3-ol, (3.beta.) (1.47%), and 3-Fluoro-5,7-dimethylquinol-2(1H)-one (1.38%). The methanol extract of *T. roseo-alba* flowers contained the highest bioactive compounds of Siliconfett (0.75%), 7-Hexadecanoic acid, methyl ester, (Z) (0.11%), and Octadecanoic acid, methyl ester and 1H-indole, 1-methyl-2-phenyl (0.09%). The methanol extract of *T. rosea* leaves containing the highest bioactive compounds were Siliconfett (0.44%), Methyltris(trimethylsiloxy)silane (0.10%), and Acetamide, N-[4-(trimethylsilyl)phenyl] and 2-Ethylacridine (0.06%). The methanol extract of *T. rosea* flowers containing the highest bioactive compounds were gamma-Sitosterol (1.61%), Eicosane (0.31%), and Tetracosane (0.16%).

The methanol extract of *T. aurea* leaves at a concentration of 6.25% has the highest average ZOI against *C. albicans* incubated for 48 hours, namely 7.283mm. The methanol extract of *T. rosea* leaves at a concentration of 100% has the highest average ZOI against *E. coli* incubated for 24 hours, which is 6.67mm. The methanol extract of *T. aurea* leaves at a concentration of 25% has the highest average ZOI against *S. aureus* incubated for 24 hours, which is 7.83mm.

CONCLUSIONS

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