Isolation of Antioxidant and Antibacterial Compound from *Solanum ferox* L Leaves

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Abstract. The sour eggplant plant (Solanum ferox L), a vegetable that is frequently used in cooking and is a member of the Solanaceae family, is also used as an antipyretic and to treat syphilis, body aches, lack of appetite, fever, itching, cuts, and bruising. This is due to the presence of several bioactive chemicals that have toxic, rheumatoid, antiviral, anticancer, antibacterial, and anti-asthma properties. The objective of this work was to separate antioxidant and antibacterial substances from Solanum ferox L. leaves. N-hexane and methanol were used to extract the leaves of Solanum ferox. The characterization of chemicals in the n-hexane extract was done while the antioxidant and antibacterial properties of both extracts were examined. UV-Vis spectra, FTIR, proton and carbon nuclear magnetic resonance (1H-NMR and 13C-NMR), and FTIR were used to characterize the compound). Using vitamin C as a reference and the DPPH (1,1-diphenyl-2-picrylhydrazyl) technique to measure free radicals, the antioxidant activity of the Solanum ferox plant extract was assessed. E. coli, S. aureus, and S. subtilis bacteria are used in an agar diffusion method test for antimicrobial activity. The solvent used to dissolve the sample served as the negative control while amoxsan served as the positive standard. One pure substance that has been isolated with success is stigmasterol, with the chemical formula $C_{29}H_{48}O$. The IC50 of Solanum ferox leaf n-hexane extract at 1218.5 ppm, which included not active, indicated that the IC50 of Solanum ferox leaf methanol extract at 268.5 ppm was ineffective. E. coli is inhibited by n-hexane extract in antibacterial tests at an inhibition value of 9.27 mm. Methanol extracts had an inhibitory potency of 7.82 mm against E. coli and 11.29 mm against S. aureus.

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1 Introduction

Solanum ferox is used by people as a food flavoring, sour eggplant belongs to the Solanaceae family and is one of the three main food crops [1]. Solanaceae has many uses and is one of the most valuable and varied vegetable families. Several Solanaceae plant species that have economic value have become important model systems in plant research in the field of biology to study plant development, fruit ripening, and food security [2]. Solanum ferox contains terpenoids, steroids, flavonoids, alkaloids, and phenolic compounds. Literature studies show that a decoction of the roots is used to treat syphilis, body aches, loss of appetite, fever, itching, wounds, and bruises, and as an antipyretic [3]. Solanum ferox L is used for coughs and asthma in Bangladesh, and it has anti-rheumatic, anti-asthmatic, antiviral, and anticancer properties in India.This shows that the Solanum ferox plant has various bioactive compounds which are anti-bacterial, anti-rheumatic, anti-asthmatic, antiviral, anti-cancer and toxic [4]. Alkaloids, phenolics, and flavonoids are often the compounds with the most potential to act as antioxidants. Alkaloid molecules stop the proliferation of cancer cells, whereas flavonoid and polyphenol compounds operate as antioxidants, antidiabetic, anticancer, antibacterial, and anti-inflammatory substances [5]. Apart from being a source of antioxidants, secondary metabolites such as flavonoids, alkaloids, and saponins also inhibit the growth of microorganisms such as fungi and bacteria which are known as antimicrobial compounds. Antimicrobial compounds have been widely used in food and pharmaceutical production. Compounds that have functional groups such as hydroxyl, carbonyl, and lactone have been widely reported to have antimicrobial, antipantiport, and antiallergic properties [5]. People frequently use Solanum ferox to cure a variety of illnesses, including syphilis, bodily discomfort, loss of appetite, fever, itching, wounds, bruising, and antipyretics. According to reports, Solanum ferox has pharmacological properties like antioxidant and antibacterial [6]. Streptococcus pyogenes (S.pyogenes) and Staphylococcus aureus (S. aureus). Biofilm-associated diseases caused by gram-positive bacteria include caries, gingivitis, periodontitis, endocarditis, and prostatitis. Coral-associated bacteria (CAB) have antibiofilm activity against biofilm formed by S. pyogenes [8]. S. aureus is also a putative pathogen involved in many oral diseases such as oral mucositis, periodontitis, peri-implantitis, endodontic infections, and even dental caries [9]. S. aureus was involved in giving the yellow pigmentation in teeth that are considered pathognomonic of dental caries. n-hexane, ethyl acetate, and methanol are just a few of the organic solvents that can be used to extract active chemicals from plants. Various active secondary metabolites have also been recovered from the n-hexane fraction. [7] and n-hexane is a decent solvent when applied to extract non-polar compounds because it has various advantages, namely being volatile, stable, and selective. In this study, the stigmasterol component in the n-hexane fraction of Solanum ferox leaves was isolated and characterized. The latest of this research is the isolation of antioxidant and antibacterial compounds in Solanum ferox leaves because the literature study carried out that the discovery of Solanum chemical compounds ferox has not yet been discovered.

2 Experimental

2.1 Chemical and Materials

One unit rotary evaporator, macerator, mortar, distillation apparatus, chromatographic column, TLC plate, capillary tube, 254/366 nm ultraviolet lamp (Camag®), Fisher John Melting Point Apparatus (SMP 11-Stuart®), analytical balance, set of Microplate apparatus reader 96 well (Berthold), Shimadzu type 'Prestige 21 IR spectrophotometer, a UV-Vis 10S Genesis spectrophotometer, and a JOEL type ECA 500 NMR spectrophotometer with a frequency of 500 Hz for protons and 125 Hz for carbon, box sterile, Petri dishes, loop needles, vials, aluminum foils, Solanum ferox L leaves, n-hexane, ethyl acetate, methanol, silica gel 70-230 mesh, silica gel 60 GF254, silica gel TLC plate GF_{254}, nutrient agar (NA),

nutrient broth (NB), 2,2diphenyl-1-picrylhydrazyl (DPPH), vitamin C, Amoxsan® antibiotics, solvents for spectroscopic analysis (UV, IR, NMR) are $CDCl_3$, cotton, aluminum foil, E.coli, S.aureus, B.subtillis, aquades, and glassware commonly used in laboratories.

2.2 Characterization Methods

2.2.1 Chemical compound isolation from Solanum ferox

500 grams of dried Solanum ferox leaves were macerated and repeatedly ultrasonically sonicated in n-hexane until colorless. A rotary evaporator was used to evaporate the colorless solvent, producing a thick n-hexane extract weighing 14.29 grams. The maceration n-hexane residue (12.47 grams) was used to create the methanol extract.

2.2.2 Separation by VLC (Liquid Vacuum Chromatography)

Liquid vacuum chromatography was used to fractionate the extract in order to separate the components of the n-hexane extract. Silica gel 60GF254 was stacked in a liquid vacuum chromatography (3 cm in diameter and 20 cm in height) to a height of about 5 cm. In order to achieve the highest packing density, the column filling is done in a vacuum. The pre-adsorbed n-hexane extract that was to be fractionated was added to the column. Then, using n-hexane solvent (100 ml), the ratio of n-hexane-ethyl acetate (60 ml:40 ml) was used to elute the sample in a gradient fashion. The results of the separation were entered into numbered Erlemeyer, and the number of components in each fraction was then ascertained using a TLC test. Since fractions 1, 2, and 3 of the hexane extract gradient had the identical spot pattern, they were merged, resulting in 6 fractions.

2.2.3 Separation by column chromatography

Use of column chromatography was used to separate a 1.25-gram n-hexane extract. Making a silica gel slurry that serves as an absorbent by dissolving it in n-hexane and thoroughly stirring can be used to make the column. It is then gradually poured into the column. The column's silica gel is solidified, and the top shouldn't become dry. Column elution was carried out with various eluents that had increasing polarity, for F3 from the VLC extract n-hexane results starting from eluent n-hexane (100%), n-hexane: ethyl acetate (9:1), n-hexane: ethyl acetate (8:2), n-hexane: ethyl acetate (7:3), n-hexane: ethyl acetate (6:4), so on until it reaches ethyl acetate: methanol (1:1) with an increase of 10%. Before inserting the sample into the column, it is pre-absorbed, and bands are formed when the material is eluted with a solvent. The resulting fractionation results are stored in vials that have been assigned numbers. Column chromatography was used to separate the fractions, which confirmed the presence of crystals. To combine the fractions, they underwent a TLC test to find the identical Rf (Retardation Factor) value. The produced crystals were recrystallized using a solvent that dissolves in a hot state but not a cold state and the TLC test was performed on the recrystallized crystals.

2.2.4 Testing the results of separation with TLC

By dabbing the fractions obtained from column chromatography separation on the TLC plate using a capillary tube, they were put through the test. According to the vial number, each fraction was spotted on a numbered plate, and after being eluted with crude extract TLC, a satisfactory stain separation pattern was obtained. In the TLC elution process, the eluent is added to the chamber to provide a homogeneous pressure. Once the plate has been eluted to its top limit and dried, the plate is removed. A stain suppression reagent or an ultraviolet (UV) lamp can be used to see stains. The solvent is evaporated after combining the fractions with the same Rf value.

2.2.5 Characterization

Five mg of crystals were examined by NMR, two mg by UV spectroscopy at the UR FMIPA Biochemistry Laboratory, one mg by IR spectroscopy at the IR Laboratory, UR FMIPA, and two mg by 1H and 13C-NMR at the Institute of Technology in Bandung.

2.3 Antioxidant Test

The antioxidant activity of 0.0022 grams of n-hexane extract and 0.0024 grams of methanol was tested by the DPPH method (1,1-diphenyl-2-picryl hydroxyl) at a wavelength of 520 nm using a microplate reader. Each was dissolved in 2 mL of methanol to obtain a concentrated solution with a concentration of 1000 g/mL. Ascorbic acid 100 g/mL, obtained from a concentrated solution of 1000 g/mL ascorbic acid prepared by dissolving 2 mg of ascorbic acid in 2 mL of methanol, and 80 g/mL of DPPH solution prepared by dissolving 0.16 mg of DPPH in 2 mL methanol.

The microplate consists of 8 rows (A-H) and 12 columns (1-12), so the total number of wells on the microplate is 96 wells. A total of 100 L of sample mother liquor of 1000 g/mL and 100 g/mL of ascorbic acid were added to different wells in row A. In row B-H wells, 50 L of methanol was added. The sample from row A was pipetted as much as 50 L and put into row B. Samples from row B were pipetted as much as 50 L and put into row C, and so on until row F. In row F 50 L samples were pipetted and discarded. 80 g/mL DPPH solution was added as much as 80 L starting from row A to row G. Rows G (as control) and H (as blank). Then incubated for 30 minutes, analyzed with a microplate reader and the data is ready to be processed. The value of % inhibition [6], [7] is calculated by the following formula:

$$\% \text{ Inhibition} = ((A\_control - A\_sample))/A\_control \times 100) \quad (1)$$

Description: A control= Absorbance does not contain samples
A sampel = Absorbance sample

2.4 Antibacterial Test

NA (nutrient agar) was heated until it melted and cooled at 500 ^{o}C in a water bath, the tube was then homogenized, 1 mL of cultivated E. coli, S. aureus, and B. subtilis (OD600 nm0,1)[8] added, and the mixture was then placed into a petri plate. The test sample disc paper (concentration 5.7 mg/mL, 3.8 mg/mL, and 1.9 mg/mL) was dropped onto the agar medium after the media had set. Amoxsan, which had a concentration of 3.8 mg/mL, was employed as the positive control, and the sample's dissolving solvent served as the negative control. A 37 0C incubator was used to incubate petri dishes. After a 24-hour incubation period, the diameter of the zone that inhibited bacterial growth was assessed. Every treatment were carried out aseptically and repeated twice [6]–[10].

3 Result And Discussion

3.1 Solanum ferox leaves extract

500 grams of bitter eggplant (Solanum ferox) leaves were macerated three times for a total of 24 hours with n-hexane as the solvent to 14.29 grams of dark green extract are produced. The components included in n-hexane viscous extracts were subsequently identified using a TLC test with a solvent comparison of hexane and ethyl acetate (9:1).

3.2 Recrystallization and determination of melting point

In order to recrystallize, heated n-hexane solvent was used, and white needle crystals weighing 0.0161 g were produced. The Fisher-Johns instrument was used to determine the melting point of the crystals. The crystal's melting point is between 125 and 126 °C, while stigmasterol's typical melting point is between 127 and 128 °C.

3.2 Characterization

White crystals were characterized using UV spectroscopy at a wavelength of 200–400 nm. As shown in Figure. 1, the UV-Vis spectrum of an isolated compound has a peak at 271 nm. This spectrum feature was similar to the spectrum obtained by Lee et al. (2015), which had a peak at 254 nm. Stigmasterol gives a peak at a wavelength of 254 nm, indicating that the isolated compound is stigmasterol [11].

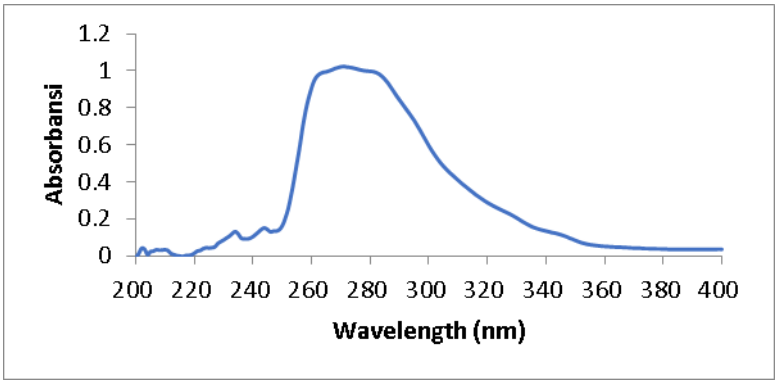


Fig 1. UV-Vis Cromatogram

This indicates that the chemical has no conjugated bonds; the carbonyl group is the cause of this, and the double bond is obscured by overlapping spectra. IR measurements that show absorption at wave number and 1666 cm-1 (compound) data that show the presence of a C=C double bond support this.

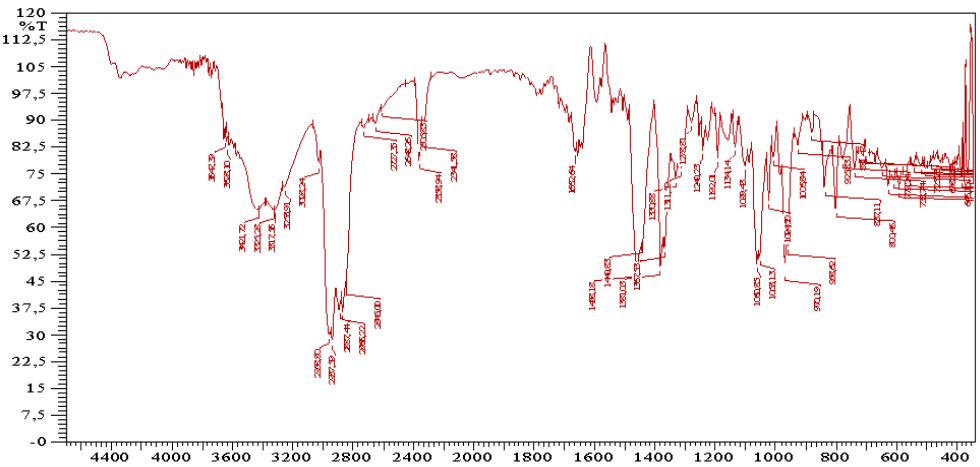


Fig.2 FT-IR Chromatogram

The IR spectrum (KBr) of the compound (Figure 2) reveals the presence of hydroxyl group (3421 cm^{-1}), C-H sp^3 (2958 cm^{-1}), olefinic group C=C sp^2 (1666 cm^{-1}) and C-O group (1052 cm^{-1}).

Based on FTIR results and compared with literature data, the isolated compound is stigmasterol [12].

The ^{1}H-NMR spectrum of the compound (Figure 4) reveals the presence of six sp^3 methyl signals consisting of two tertiary methyl signals that resonate at δ_H 0.67 (3H, s) and 1.00 (3H, s), three secondary methyls at δ_H 0.92 (3H, d, J = 5.9 Hz), 0.84 (3H, d, J = 6.4 Hz), and 0.82 (3H, d, J = 6.4 Hz), one primary methyl signal that resonates at δH 0.80 (3H, t, J = 6.0 Hz) suggesting characteristics of steroid group compounds [13]. Three olefinic methine signals resonating at δ_H 5.35 (1H, d, J = 5.1 Hz, H-6), 5.18 (1H, dd, J = 8.5; 15.1 Hz, H-22), and 5.00 (1H, dd, J = 8.6; 15.6 Hz, H-23), as well as one oxygenated methin signal at δ_H 3.52 (1H, m, H-3) suggest characteristics of 3-β stigmasterol compounds [13], [14].

The 13C-NMR spectrum of the compound (Figure 4) reveals the presence of 29 carbon signals consisting of six methyl, ten methylene, eleven methine and two quaternary carbon signals that suggest steroid class compounds [13]. The presence of six methyl signals resonating at δc 12.40 (C-18), 19.56 (C-19), 21.24 (C-21), 21.30 (C-26), 19.14 (C-27), and 12.20 (C-29), one oxymetin signal at δc 71.98 (C-3), three methine olefinics at δc 121.87 (C-6), 138.46 (C-22), 129.43 (C-23), as well as one singlet olefinic carbon signal at δc 140.91 (C-5) suggest characteristics of stigmasterol (Figure 5) compounds [13], [14]. Such functionality is calculated as two of the total six degrees of unsaturation. The remaining four degrees of unsaturation, correspond to the basic framework of steroid stigmastan [14].

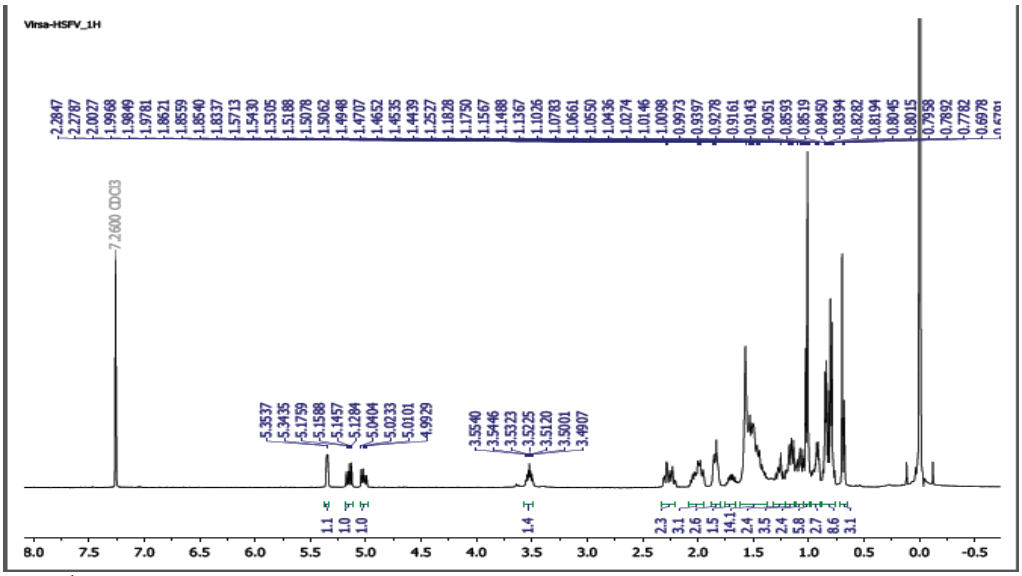


Fig. 3 ^{1}H NMR Spectrum

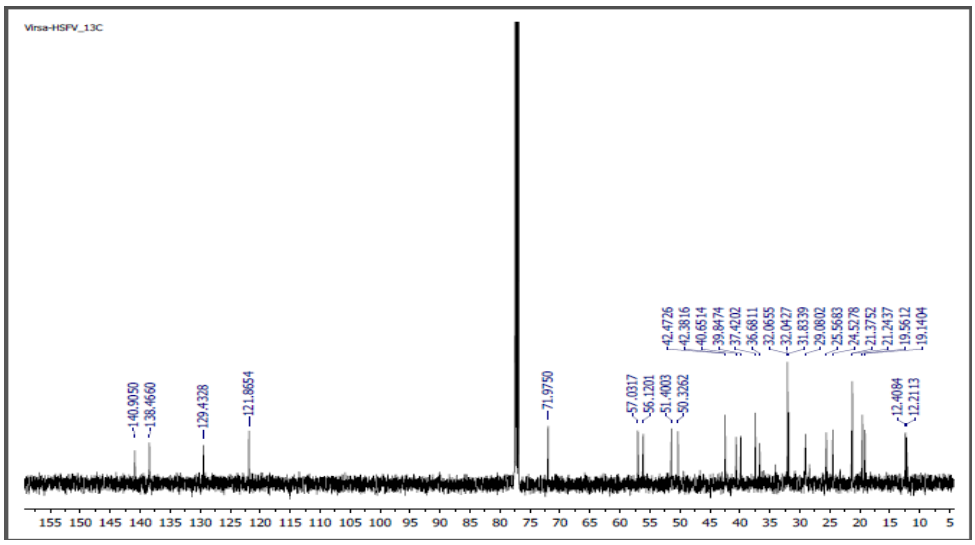


Fig. 4 ^{13}C NMR Spectrum

Comparison of compound spectroscopic data with stigmasterol compounds [14] it reveals that the two compounds have a very high degree of compatibility, thus the compounds are identified as stigmasterol (**Table 1**).

Tabel 1. Ratio NMR data with Stigmasterol (Katja, 2021).

No	Crystal		Stigmasterol	
	^{13}C NMR δc (mult)	^{1}H NMR δH (Integral, mult, J=Hz)	^{13}C NMR δc (mult)	^{1}H NMR δH (Integral, mult, J=Hz)
1	'37.40'	'1.08 (1H, m)'	'37.4'	'1.08 (1H, m)'
		'1.84 (1H, m)'		'1.84 (1H, m)'
2	'31.80	'1.49 (1H, m)'	'31.8'	'1.49 (1H, m)'
		'1.81 (1H, m)'		'1.81 (1H, m)'
3	'71.98'	'3.52 (1H, m)'	'72.0'	'3.52 (1H, m)'
4	'42.47'	'2.28 (1H, dd, 2.0;5.2)'	'42.5'	'2.28 (1H, dd, 2.0;5.2) 2.30 (1H, dd, 2.0;5.2)'
5	'140.91'	-	'140.9'	-
6	'121.87'	'5.35 (1H, d, 5.1)'	'121.9'	'5.35 (1H, d, 5,1)'
7	'32.06'	'1.54 (1H, m) 1.96 (1H, m)'	32.1	s1.54 (1H, m) 1.96 (1H, m)'
8	'32.04'	'1.46(1H, m)'	'32.0'	'1.46(1H, m)'
9	'50.33'	'0.94 (1H, m)'	'50.3'	'0.94 (1H, m)'
10	'36.68'	-	'36.7'	-
11	'21.37'	'1.46 (1H, m) 1.49 (1H, m)'	'21.3'	'1.46 (1H, m) 1.49 (1H, m)'
12	'39.85'	'1.15 (1H, m) 1.95 (1H, m)'	'39.9'	'1.15 (1H, m) 1.95 (1H, m)'
13	'42.38'	-	'42.5'	-
14	'57.03'	'1.03 (1H, m)'	'56.9'	'1.03 (1H, m)'
15	'24.53'	'1.07 (1H, m) 1.56 (1H, m)'	'24.5'	'1.07 (1H, m) 1.56 (1H, m)'
16	'29.08'	'1.26 (1H, m) 1.67 (1H, m)'	'28.4'	'1.26 (1H, m) 1.67 (1H, m)'
17	56.12	1.13 (1H, m)	56.1	1.13 (1H, m)
18	'12.40'	'0.67 (3H, s)'	'12.1'	'0.67 (3H, s)'

19	‘19.56’	‘1.00 (3H, s)’	‘19.5’	‘1.00 (3H, s)’
20	‘40.65’	‘2.02 (1H, m)’	‘40.7’	‘2.02 (1H, m)’
21	‘21.24’	‘0.92 (3H, d, 5.9)’	‘21.2’	‘0.92 (3H, d, 5,9)’
22	‘138.46’	‘5.18 (1H, dd 8.5;15.1’	‘138.5’	‘5.18 (1H, 8,5;15,1’
23	‘129.43’	5.00 (1H, dd, 8,6; 15.6)	‘129.5’	5.00 (1H, dd, 8.6; 15.6)
24	‘51.40’	‘1.53 (1H, m)’	‘51.4’’	‘1.53 (1H, m)’
25	‘31.80’	‘1.45 (1H, m)’	‘31.8’	‘1.45 (1H, m)’
26	‘21.30’	‘0.84 (3H, d, 6,4)’	‘21.3’	‘0.84 (3H, d, 6.4)’
27	‘19.14’	‘0.82 (3H, d, 6,4)’	‘19.1’	‘0.82 (3H, d, 6.4)’
28	‘25.57’	‘1.51 (1H, t, 3,2)’	‘25.6’	‘1.51 (1H, t, 3.2)’
29	‘12.20’	‘0.80 (3H, t, 6,0)’	‘12.2’	‘0.80 (3H, t, 6.0)’

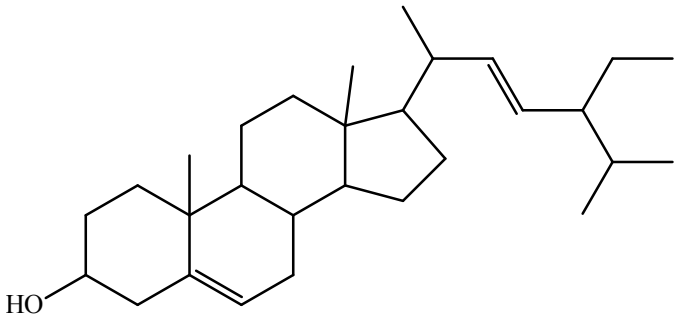


Figure 5. Stigmasterol

Antioxidant Test

Antioxidant activity for n-hexane extract of *S.ferox* leaves using DPPH (1,1-diphenyl-2-picrylhydrazil) and ascorbic acid (vitamin C) methods.

Table 1. Antioxidant activity of n-hexane and methanol extract of *S.ferox* leaves using the DPPH method using a microplate reader.

Table 2. Antioxidant test of n-hexane and methanol extracts

Sample	**IC_{50} (ppm)**
n- hexane extract	1218.5
Methanol extract	268.5
Ascorbic acid	7.2

In this study, IC_{50} values from a plant extract are obtained using the DPPH method. The sample's IC_{50} value indicates how much of it may inhibit 50% of free radicals. The antioxidant activity of a substance is classified as very active if its IC_{50} value is less than 50 ppm, active if it is between 50 and 100 ppm, moderate if it is between 101 and 250 ppm, weak if it is between 251 and 500 ppm, and not active if IC_{50} values is greater than 500 ppm (Jun et., al, 2003). Based on the results of the study of the IC_{50} of *Solanum ferox* leaf n-hexane extract at 1218.5 ppm, including not active, the IC_{50} of *Solanum ferox* leaf methanol extract at 268.5 ppm was weak [15].

Antimicrobial Test

Antibacterial activity tests were carried out on n-hexane and methanol extracts with concentrations of 1.9 g/mL, 3.8 g/mL, and 5.7 g/mL were performed on *Escherichia coli*,

Staphylococcus aureus, and *Bacillus subtilis* bacteria. Anti-bacterial activity test of the total extract of n-hexane and methanol showed the diameter of the inhibition zone against *E.coli* bacteria while *S.aureus* and *B.subtilis* were unable to inhibit bacterial growth.

To ascertain whether n-hexane and methanol extracts had any antibacterial activity, an antibacterial activity test was carried out. The agar diffusion method is employed. The test sample diffuses straight into the agar medium containing the test microorganisms with an OD value of 0.1 according to the theory behind this procedure. The creation of a clear zone surrounding the test sample revealed the presence of antibacterial activity. The test samples had values of 1.9 g/mL, 3.8 g/mL, and 5.7 g/mL. The test sample was dissolved in a solvent, which served as a negative control, while the antibiotic Amoxicillin served as a positive control for bacteria.

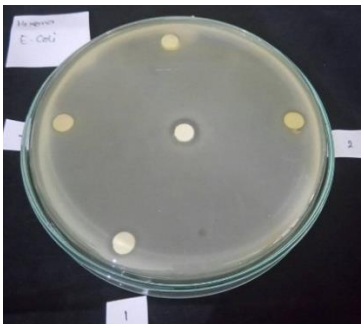
Table 3. Antibacterial test of n-hexane and methanol extracts

Concentration (µg/mL)	Clear zone diameter (mm)		
	E.coli	*S.aureus*	*B.subtilis*
Hexan extracts			
1.9	7.80	-	-
3.8	9.27	-	-
5.7	12.93	-	-
Methanol extracts			
1.9	7.72	-	-
3.8	7.82	-	-
5.7	11.67	-	11.29
Amoxicillin (3,8 µg/disk)	19.97	11.80	17.73
Negative control	-	-	-

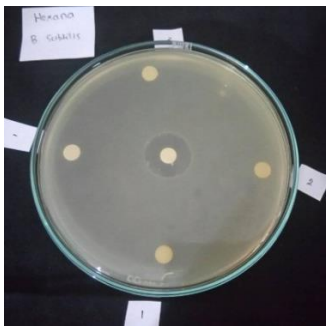
On disc paper, 10 L of the test sample was dripped using a micropipette with various concentrations of 1.9 g/mL, 3.8 g/mL, and 5.7 g/mL, then waited for it to dry. The disc paper that has been dripped with the test sample is placed on an agar medium (Nutrient Agar) which has solidified for bacteria. The purpose of drying this solution is so that the solvent evaporates and the sample is absorbed on the paper disc so that the sample is expected to have antibacterial activity (not the actual solvent) . This preliminary research was conducted to see the ability of each test sample to kill or inhibit the test bacteria.

S. aureus and *B. subtilis*, two gram-positive bacteria, and *E. coli*, a Gram-negative bacterium, served as the test organisms. The three microorganisms were selected because they are frequently utilized in biological activity tests, are kept on hand in laboratories, and, in general, are the root of a number of diseases and infect humans.

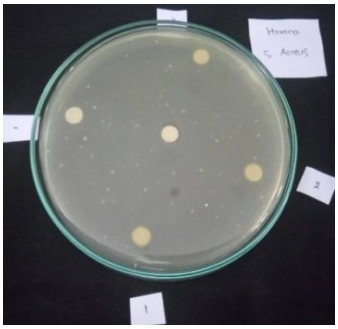
The test for bacterial resistance of the diameter was visible in n-hexane extract of the inhibition zone against *E.coli*. The diameter of the inhibition zone for *S. aureus* and *B.subtilis* was not able to inhibit the growth of bacteria. This is because the n-hexane extract dissolves volatile oil compounds, and saturated and unsaturated fats so that it can damage the outer membrane which is composed of proteins, lipoproteins, phospholipids, lipopolysaccharides, and porins so that they can interact and damage the peptidoglycan on the wall. Gram-negative bacterial cells.



E.coli



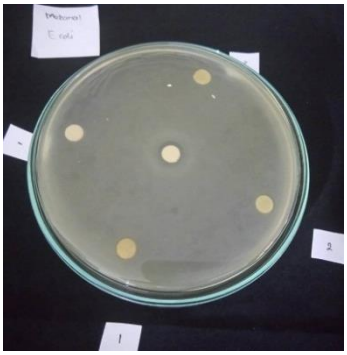
S.aureus



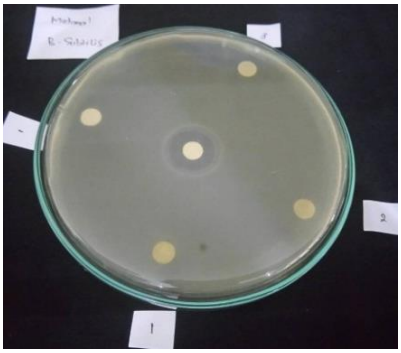
B.subtillis

Fig 6. Antibacterial activity test of n-hexane extract

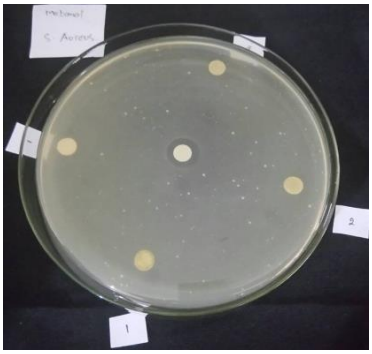
The activity test of methanol extract can inhibit the growth of *E.coli* and *B.subtilis* at a concentration of 5.7 g/mL. This is because methanol is a universal solvent that can dissolve almost most of the compound components contained in *Solanum ferox* leaves and when extraction uses only two solvents (hexane, and methanol), it does not use semi-polar solvents so that semi-polar compounds dissolve in polar solvents. Methanol extract can still inhibit *E.coli* bacteria.



E.coli



S.aureus



B.subtilis

Fig. 7 Bacterial activity test of methanol extract

4 Conclusion

The stigmasterol ($C_{29}H_{48}O$) was obtained from the third fraction of the n-hexane extract with a melting point of 125-126 ^{0}C.The IC_{50} value of methanol extract showed that it was able to inhibit DPPH free radicals at a concentration of 268.5661 g/mL. The n-hexane extract had no antioxidant activity. The inhibition zone value of n-hexane extract against *E.coli* was at a concentration of 3.8 g/mL of 9.27 mm and was unable to inhibit the growth of *S.aureus* and *B.subtilis* bacteria. The results of the bacterial activity test on methanol extract against *E.coli* bacteria at a concentration of 3.8 g/mL of 7.82 and *B. subtillis* at a concentration of 5.7 g/mL of 11.29 mm.

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