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ORIGINAL ARTICLE

Screening the *in vitro* Antimicrobial and Antioxidant Activities of Methanolic Extract of *Epiphyllum oxypetalum* (DC.) Haw. Leaf

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ABSTRACT – . *Epiphyllum oxypetalum* (*E. oxypetalum*) is traditionally used to cure liver infections, for wound healing, and to alleviate viral–related diseases. However, as the alcohol and aqueous solvents have low total phenolic and flavonoid content (TPC and TFC), antioxidant, and antibacterial capabilities, a different polarity would be more suitable. Thus, this study aimed to evaluate the methanolic extract of E. oxypetalum leaf. The chemical constituents were identified through TPC and TFC, as well as GC–MS analysis. The methanolic extract of E. oxypetalum leaf was evaluated for antimicrobial properties against five bacterial strains - *Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis*, and a fungal strain of *Candida albicans* using the disc diffusion method. The antioxidant activity of the methanolic extract of E. oxypetalum leaf was also assessed using the DPPH assay. The highest values of the TPC and TFC of the methanolic extract of E. oxypetalum leaf were 179.86 ± 0.17 mg GAE/g and 75.07 ± 0.17 mg QE/g, respectively. The GC–MS had 27 m/z peaks, indicating the presence of various bioactive compounds including phenolic, flavonoid, and fatty acids compounds. In the antimicrobial study, the zone of inhibition (ZOI) was between 2.8–3.5 mm for antibacterial activity and there was also a significant antifungal activity of 1.8 mm against *Candida albicans*. The IC50 DPPH assay value of methanolic extract of *E. oxypetalum* leaf was 14 µg/ml, indicating high antioxidant properties. This study provides evidence that the methanolic extract of *E. oxypetalum* leaf possesses significant antioxidant and antimicrobial properties which could be attributed to its diverse chemical constituents. These findings suggest the potential of *E. oxypetalum* leaf as a natural source of medicine, antimicrobial, and antioxidant properties.

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INTRODUCTION

The tropical medicinal plant of *Epiphyllum oxypetalum (E. oxypetalum)* has been used to treat liver infections, for wound healing, and to alleviate antiviral disorders [1–3]. Previous studies from Malaysia and China have demonstrated that medicinal plants contain a range of chemicals with recognized medicinal effects [2]. Alkaloids, flavonoids, phenolic substances, and tannins are the most common bioactive compounds that may be derived from plants and contribute to medicine, antibacterial and antioxidant properties [4]. According to Upendra et al. [5], the phylloclades from *E. oxypetalum* leaf extract contains specific active components and antibacterial action against Escherichia coli, Bacillus subtilis, Staphylococcus aureus and Klebsiella pneumoniae. However, the zone of inhibition (ZOI) against fungi such as Aspergillus terreus, Aspergillus niger, Rhizopus oryzae, or Candida albicans has not been reported [5]. In this work, the broad–spectrum antibacterial activity for *E. oxypetalum* leaf was identified and can be used to treat ailments in humans caused by bacteria [1].

Protecting the immune system against oxidative stress generated by free radicals is becoming more important. Antioxidants are compounds that are becoming more significant due to their capacity to prevent oxidative stress–related damage to the body. Using extracts of *E. oxypetalum* leaf, the hydrogen–donating characteristic of the hydroxyl groups in organic polyphenols is able to function as an antioxidant [6]. Moreover, natural antioxidants derived from the extract of *E. oxypetalum* leaf are vital for reducing or mitigating the adverse effects of oxidative stress [7]. One of the methods to assess the antioxidant capability from the extract of *E. oxypetalum* leaf is through the DPPH test. The extract of *E. oxypetalum* leaf and DPPH solution are able to form non–radicals when antioxidant compounds including hydrogen–donating groups (phenols and flavonoids) are available [8].

Dandekar et al. [7] also reported the antioxidant activity of alcohol and aqueous extract of *E. oxypetalum* leaf *in vitro* utilizing free radical scavenging activities [9–10]. Their results demonstrated that the extract of *E. oxypetalum* leaf

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possessed substantial antioxidant activity [10]. However, the data for TPC, TFC, antioxidant, and antibacterial properties were unsatisfactory with the alcohol and aqueous extract of *E. oxypetalum* leaf. Therefore, the extract of *E. oxypetalum* leaf using methanol as an alternative solvent was evaluated to study the TPC, TFC, phytochemicals compounds, antioxidant and antimicrobial properties of *E. oxypetalum* leaf in this study. The correlation between the TPC, TFC, and antioxidant and antimicrobial potential activities may contribute to a cost–effective and safer alternative medicine in therapeutic applications.

MATERIAL AND METHODS

Chemicals

All chemicals used were analytical grade.1,1–Diphenyl–2–picryl–hydrazyl (DPPH), quercetin, gallic acid, ascorbic acid, methanol, sodium carbonate, aluminum chloride, and ciprofloxacin were obtained from Sigma Aldrich Malaysia. The plant *E. oxypetalum* leaf was collected from botanical gardens, in Ipoh, Perak, Malaysia.

Preparation *E. oxypetalum* Leaf for Extraction

The *E. oxypetalum* leaf was washed with distilled water and dried for a week at 40 ºC in the oven. To obtain the fine powder, the *E. oxypetalum* leaf was pulverized in a mechanical grinder. The dried *E. oxypetalum* leaf was then stored in an airtight container at 4 ºC until needed. 86.97 g of powdered *E. oxypetalum* leaf were extracted with methanol solvent (purity 96%) at room temperature using the maceration method. The liquid extract was filtered with filter paper after extraction and concentrated in a rotary evaporator to yield a dry residue.

GC–MS Analysis

A diluted methanolic extract of *E. oxypetalum* leaf was used to perform the GC–MS analysis. The methanolic extract of *E. oxypetalum* leaf was conducted in the GC–MS analysis to analyze the composition of the metabolites. The GC was accomplished with an Agilent 7890 instrument, while the MS was performed with a Joel Accu TOF GCV analyzer. With a flow rate of 1 ml/min, the inert gas helium (99.99 %) was utilized as the carrier gas. BP–20 (polyethylene glycol) column with 30 mm x 0.25 mm x 0.25 m was used. The 1 µl of the methanolic extract of *E. oxypetalum* leaf was injected into the equipment. The starting temperature was 100°C, the injector temperature was 250°C, and the temperature flow rate was 10°C/min throughout the procedure. The final temperature was set to 280 °C and the experiment ran for 5 minutes.

Mass Spectral Interpretation and Identification of Compounds

The methanolic extract of *E. oxypetalum* leaf was identified by using the National Institute of Standard and Technology (NIST) databases. The structure of the components in the methanolic extract of *E. oxypetalum* leaf was verified based on the molecular formula and molecular weight [9–10](Dandekar and Bhaskar, 2015).

Antioxidant Activities

DPPH free radical scavenging activity

22 mg of 2, 2–Diphenly 1–picryl hydrazyl (DPPH) solution was combined with 100 ml of methanol to form a solution. Separately, 2 mL DPPH solution, 100 ml of methanolic extract of *E. oxypetalum* leaf, and a standard ascorbic acid were added to the solution. A series of concentrations (16.5 to 1000 µg/ml) of methanolic extract of *E. oxypetalum* leaf were diluted in this analysis [11]. All of the solutions were incubated for 30 minutes at 37 °C, and the absorbance was measured at 490 nm using a UV spectrophotometer. The IC_{50} which is the sample concentration required to scavenge 50% of free radicals was estimated [6]. All test analyses were run in triplicates. Equation (1) was used to obtain the radical scavenging activity [11]:

$$\text{DPPH scavenging effect (\% inhibition)} = \frac{A_o - A_1}{A_o} \text{ x } 100 \quad (1)$$

with A_o = absorbance of the control (DPPH) and A_1 = absorbance (reference and sample)

Determination of total phenolic content (TPC)

The TPC of 96 % (v/v) methanolic extract of *E. oxypetalum* leaf was analyzed using the Folin–Ciocalteu methods. In a tube, 2.5 mL of 10% of Folin–Ciocalteu reagent was prepared to a total volume of 0.5 ml of the methanolic extract of *E. oxypetalum* leaf, and gallic acid (as standard, GA). The mixture was diluted in a series of concentrations at 60–200 mg/ml. An hour after mixing in the sodium carbonate solution (20 % w/v), the reaction mixture was placed in the dark for one hour to measure the absorbance at 320 nm (UV–1800 spectrophotometer, Shimadzu Japan, fitted with a quartz cell). Using a gallic acid calibration curve (60–200 mg/ml), the TPC was estimated and presented as gallic acid equivalents (GAE) (Eq. (2)) [8]:

$$\text{GAE, mg/g} = X \times \text{(v/m)} \quad (2)$$

with X = Concentration methanolic extract of *E. oxypetalum* leaf obtained from a standard curve (mg/g), M = Weight of methanolic extract of *E. oxypetalum* leaf extract used (mg), and V = Volume of methanolic extract of *E. oxypetalum* leaf used (ml)

Determination of total flavonoid content (TFC)

The TFC of 96 % (v/v) methanolic extract of *E. oxypetalum* leaf was calculated by using quercetin. A total volume of 0.5 ml of methanolic extract of *E. oxypetalum* leaf was treated with a solution of 2 % weighted average aluminum chloride (1 ml) and standard of quercetin standard, and incubated for an hour at room temperature. The mixture was diluted in a series of concentrations at 40–200 mg/ml. With a UV spectrophotometer, the absorbance was read at 365 nm. Using a quercetin calibration curve (40–200 mg/ml), the TFC was determined and expressed as quercetin equivalents (QE) (Eq. (3)) [8]:

$$\text{QE, mg/g} = X \times (\text{v/m}) \tag{3}$$

with X = Concentration methanolic extract of *E. oxypetalum* leaf obtained from a standard curve (mg/g), M = Weight of methanolic extract of *E. oxypetalum* leaf used (mg) and V = Volume of methanolic extract of *E. oxypetalum* leaf used (ml)

Screening of Antimicrobial Activity

The bacterial strains utilized in this experiment were received from the American Type Culture Collection (ATCC). It was discovered that *Candida albicans* (ATCC 25922), *Staphylococcus epidermidis* (ATCC 12228), *Klebsiella pneumonia* (ATCC 13883), *Escherichia coli* (ATCC 25922), and *Candida aureus* (ATCC 9144) were all pathogenic bacteria (ATCC 10231). It was necessary to maintain the cultures' viability by subculturing on agar plates regularly, and they were kept at 4°C before use. One swab inoculum of the test strain were added to the respective plates for each of the microorganisms using the spread plate method. Around 10 µL of the methanolic extract of *E. oxypetalum* leaf with different concentrations was impregnated onto a paper disc on all of the cultured medium. The extracts were diluted with different concentrations – 50, 100, 200, and 300 mg/mL. Each test was carried out three times, with ciprofloxacin (5 µg/disc) serving as a positive control in each case. The microtiter plates were then subsequently incubated for hours at 37 °C to complete the process [8][9–10]. Immersion in methanol was used as a negative control for the disc. Antimicrobial activity was determined by measuring the ZOI (including the disc diameter).

Statistical Analysis

One–way analysis of variance (ANOVA) was performed using the SPSS programme (version 16.0) to examine the data, which was represented as mean with standard deviation (STDEV). Then, the data were set with $p \leq 0.05$ as the significance limit.

RESULTS AND DISCUSSION

Determination of Percentage Yield

The methanolic extract of *E. oxypetalum* leaf had a recovery yield of 39.02 ± 0.39 %. In this work, the methanolic extract of *E. oxypetalum* leaf was dissolved in a less polar solvent (methanol solvent) relative. Upendra et al. [5] reported the percentage yield using petroleum ether, ethanol, and acetone to extract the *E. oxypetalum* leaf. Methanol solvent showed the highest recovery yield compared to petroleum ether (4.51 %), ethanol (7.204 %), and acetone (5.75 %) [5]. This data shows that the polarity of the methanol has a massive effect on the extraction yield of *E. oxypetalum* leaf. The discrepancy in the extraction yield of *E. oxypetalum* leaf, could be attributed to differences in the solubility of the different chemicals based on the polarity of the solvents utilized [12]. Furthermore, Kanadi et al. [11] demonstrated that the polarity index of solvents was related to the extraction yield of *E. oxypetalum* leaf in their studies. As a consequence, the discrepancy between the earlier and present findings in the extraction yield of *E. oxypetalum* leaf in the earlier and present study may be linked to the varied extraction solvents.

GC–MS Analysis of Phytoconstituents

Plants are the most important source for the discovery of novel chemicals with medical potential for the creation of pharmaceuticals. GCMS is one of the most popular method in chromatography technologies for the separation of phytocompounds in recent years [13]. The presence of diverse phytoconstituents components from the dissolved methanolic extract of *E. oxypetalum* leaf revealed 27 peaks overall with different retention times, as shown in Figure 1. The peak heights show the relative concentrations of components found in methanolic extract of *E. oxypetalum* leaf and were compared to the main library data. As shown in Table 1, 2–methoxy–4–vinylphenol, 2,6–dimethoxy–, dodecanoic acid, n–hexadecanoic acid, and lup–20(29)–en–3–one compounds were present in the methanolic extract of *E. oxypetalum* leaf. However, hexanoic acid, bicyclo[2.2.2]octane, morpholine, 9h–fluorene, furazano[3,4–e] [1,2,4]–triazolo [4,3–a] pyrazin–5–amine, cis–4–chlorocinnamic acid, 2,5–dimethoxyterephthalic acid, ethanone, tetradecanoic acid, desaspidinol, imidazole, indolo[3,2–b]quinoline, 2–butynone, 7–methylenebicyclo[4.2.0]octane, cycloheptane, hexanoic acid, 9–decenoic acid, cyclohexane–1,3–dione, 7–chlorocinchoninic acid, 5h–dibenzo[a,d]cyclohepten–5–one, –dihydro–, androsta–1,4,6–triene–3,17–dione and quinoline, 8–bromo– compounds were absent in the methanolic extract of *E. oxypetalum* leaf. The phytochemical GC–MS data study of methanolic *E. oxypetalum* leaf extract proved the

presence of phenolic, flavonoid, tannins, and fatty acids compounds [14][3]. The chemical ingredients in the methanolic extract of *E. oxypetalum* leaf were identified by comparing them to an internal spectral database based on >80 % similarity index [4][9–10].

The methanolic extract of *E. oxypetalum* leaf has a number of biological activities as well as industrial uses. For example, 2–methoxy–4–vinylphenol is known to have aromatic flavoring food, antioxidant and antimicrobial agent [15]; vanillyl alcohol is well–known to have food flavoring, antioxidant, anti–angiogenic, anti–inflammatory and anti–nociceptive activities activity [16–17]; dodecanoic acid, n–Hexadecanoic acid and lup–20(29)–en–3–one has antibacterial, anti–inflammatory, antioxidants and antifungal activity [18–20]. The *E. oxypetalum* leaf is frequently utilized as a popular alternative treatment in certain regions of China [21–22], Central America and Northern South America, South Africa, Guatemala to Venezuela, and Brazil [22]. This study also lends support to and establishes a solid scientific foundation that *E. oxypetalum* leaf might be a valuable source of a potential medication. Antibacterial and anti–inflammatory properties of methanolic extract of *E. oxypetalum* leaf may be applied as a safe and cost–effective herbal remedy for wound healing [2]. These findings encourage additional research to be conducted on extracts and the identification of specific active chemical components with medicinal effects [23].

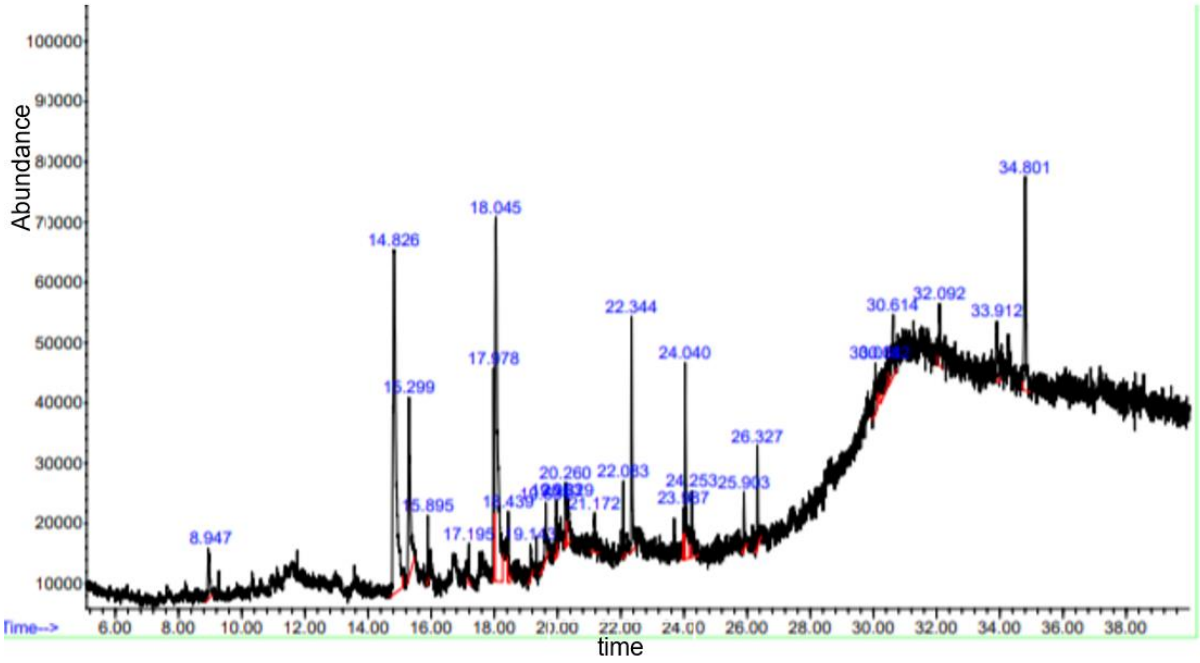

Figure 1. GC–MS chromatogram of methanolic extract of *E. oxypetalum* leaf.

Antioxidant Activity

The antioxidant activity of methanolic extract of *E. oxypetalum* leaf was defined using the DPPH free radical scavenging assay with ascorbic acids as a standard solution [6] (See Figure 2). Table 2 displays the mean percentage of DPPH free–radical scavenging activity for the methanolic extract of *E. oxypetalum* leaf at different extract concentrations. The amount of antioxidants present and their effectiveness are proportional to the extent of color change. The absorbance of the methanolic extract of *E. oxypetalum* leaf that decreased significantly shows the significant free radical scavenging activity [24–25]. Ascorbic acid as a benchmark has an IC_{50} value of 17 µg/ml. Lower radical scavenging activity of antioxidant capabilities of methanolic extract of *E. oxypetalum* leaf is indicated by a greater IC_{50} value. Also, the IC_{50} value of methanolic extract of *E. oxypetalum* leaf was 14 µg/ml showing higher antioxidant properties due to the higher value of TPC and TFC. It is also possible that the presence of phenolic, flavonoid, and fatty acids compounds in the methanolic extract of *E. oxypetalum* leaf contributed to the strong antioxidant activity [24–25]. Moreover, the lower radical scavenging activity resulted in higher antioxidant activity of the phenolic hydroxyl on the benzene ring [25]. In addition to that, Dandekar et al. [4] reported the IC_{50} for DPPH radical scavenging assay for alcohol extract of *oxypetalum* leaf was estimated to be around 500 to 1000 µg/ml, while the aqueous extract of *oxypetalum* leaf was insignificant. This suggests that the methanolic extract of *E. oxypetalum* leaf has a higher potential for antioxidant activity compared to the alcohol extract of *E. oxypetalum* leaf. Thus, making a methanolic extract of *E. oxypetalum* leaf may significantly contribute to traditional and therapeutic uses.

Table 1. Phytoconstituents identified in methanolic extract of *E. oxypetalum* leaf by GC–MS analysis.

No.	Retention Time (min)	Peak area (%)	Similarity (%)	Name of Compound	Molecular formula	Molecular weight ($g \cdot mol^{-1}$)	Nature of compound	Uses
1	14.827	21.16	95	2–Methoxy–4–vinylphenol	$C_9H_{10}O_2$	150.18	phenolic, flavonoid	flavoring food, antioxidant, antimicrobial agent
2	15.297	7.54	91	Vanillyl alcohol	$C_8H_{10}O_3$	154.16	phenolic, flavonoid, tannins	flavoring food, antioxidant, anti–angiogenic, anti–inflammatory, antimicrobial
3	17.976	3.19	94	dodecanoic acid	$C_{12}H_{24}O_2$	200.32	fatty acid	Antibacterial, anti–inflammatory, antioxidants
4	22.346	4.76	93	n–hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	fatty acid	Antibacterial, anti–inflammatory, antioxidants
5	34.803	7.86	96	lup–20(29)–en–3–one	$C_{30}H_{48}O$	424.70	fatty acid	Antibacterial, anti–fungal, antioxidants

Table 2. Percentage of inhibition (scavenging capacity) at different concentrations of methanolic extract of *E. oxypetalum* leaf and ascorbic acid. The results were done in triplicate, and a significant difference at $p \leq 0.05$; $n = 3$.

Concentration (µg/ml)	16.5	31.25	62.5	125	250	500	1000
Methanolic extract of *E. oxypetalum* leaf (%)	56.09 ±0.02	63.83±0.05	64.80±0.12	70.60±0.02	73.11±0.05	68.90±0.02	62.86±0.07
Ascorbic Acid (%)	49.32±0.10	70.66 ±0.12	74.47±0.12	73.82±0.08	73.31±0.14	75.18±0.03	72.99±0.02

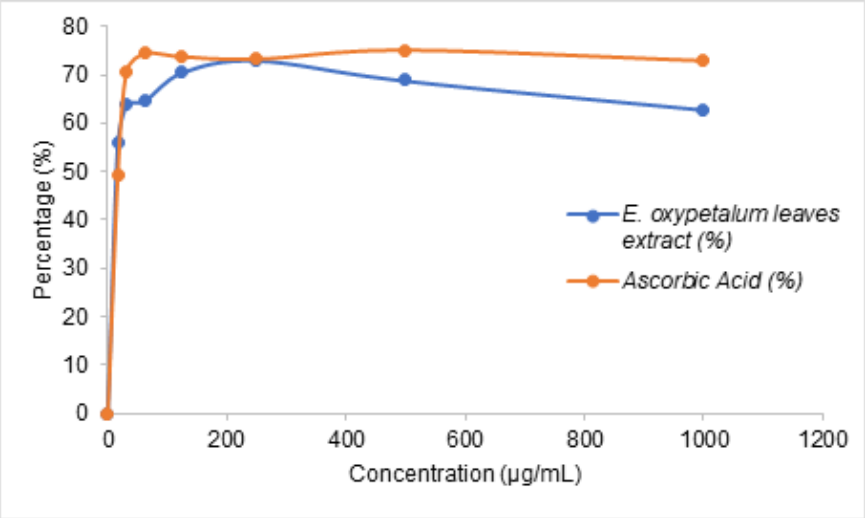

Figure 2. Graph of radical scavenging % between methanolic extract of *E. oxypetalum* leaf extract and ascorbic acid. Results are expressed as the mean of three independent experiments ± standard deviation. Ascorbic acid was used as standard.

The Folin–Ciocalteu (F–C) technique was measured the TPC in methanolic extract of *E. oxypetalum* leaf using gallic acid as the standard. The calibration curve was constructed utilizing absorbance readings acquired at various concentrations of gallic acid. The TPC of the extracts was estimated using the regression equation of the calibration curve ($y=0.0014x+0.0691$; $R^2 = 0.9805$) and represented as gallic acid equivalents (GAE) (mg/g) (Figure 3(a)). When compared to Sunaja Devi et al. [26] research, the TPC value of methanolic extract of *E. oxypetalum* leaf was found to be the highest at 179.86 ±0.17 mg GAE/g, indicating the highest TPC value ever recorded. This is following the study of Upendra et al. [5], where the presence of phenolic and flavonoid contents of methanolic extract of *E. oxypetalum* leaf was discovered. TPC of methanolic extract of *E. oxypetalum* leaf samples was determined to be 19.09 ±0.08 µg/0.6 mL in another work [26]. The antioxidant properties of any plant are hypothesized to be directly proportional to its TPC content. Phenolic compounds are capable of behaving as hydrogen donors, reducing agents, and scavengers of free radicals. The methanolic extract of *E. oxypetalum* leaf contained an abundant quantity of phenolic and flavonoid compounds that may have a vital role in antioxidant activities [6]. In this study, TFC of methanolic extract of *E. oxypetalum* leaf was estimated using the

calibration curve's regression equation ($y=0.0045x + 0.1749$; $R^2 = 0.9992$) (Figure 3(b)) and expressed as quercetin equivalents (QE) (mg/g). The highest concentration of TFC for the methanolic extract of *E. oxypetalum* leaf was 75.07± 0.17 mg QE/g. In prior research by Sunaja Devi et al. [26], the data found that the TFC of methanolic extracts of *E. oxypetalum* leaf was 8.728 ± 0.02 g/mL, which is much lower than this study's value. The polarity of the methanol used for extraction affected the concentration of phenols and flavonoids in the final product [6].

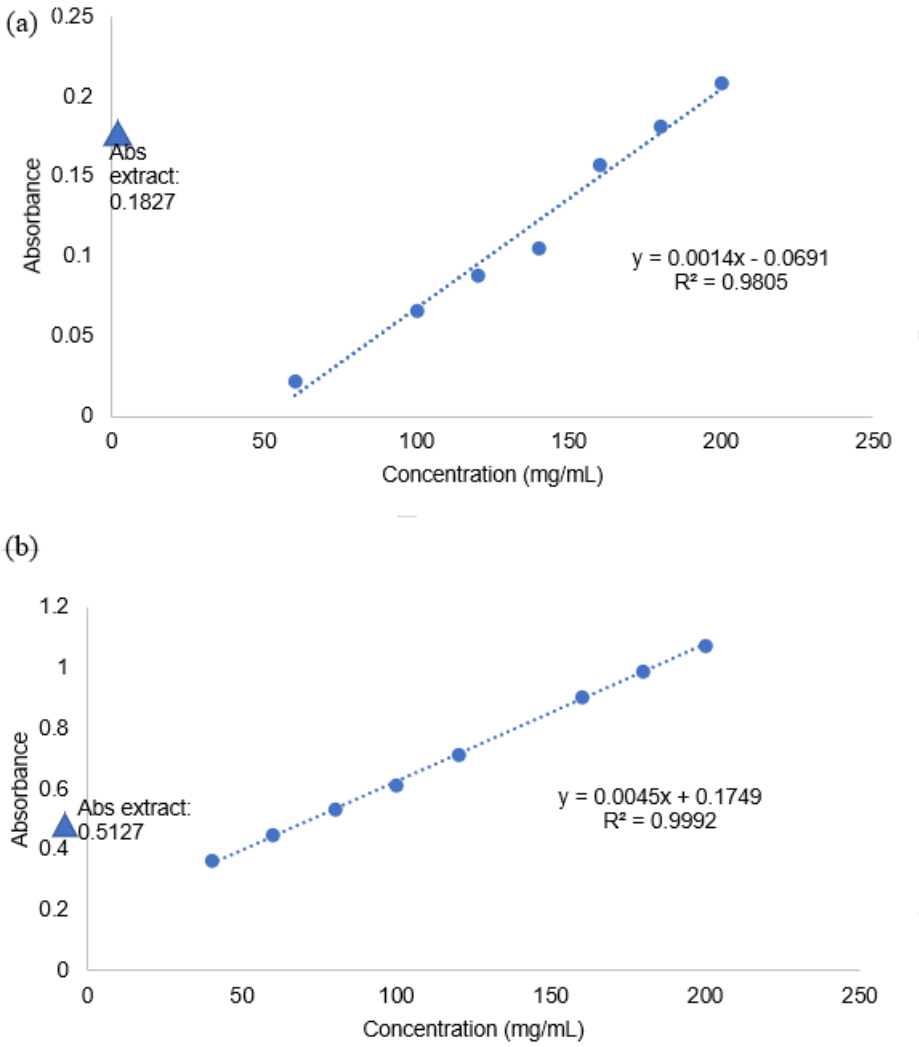

Figure 3. Calibration curve of (a) gallic acid and (b) quercetin. The results were done in triplicate, and a significant difference at $p \leq 0.05$. The result represents the mean ± standard deviation.

Antimicrobial Activity

Antibacterial activity of the methanolic extract of *E. oxypetalum* leaf against *Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae, Escherichia coli*, and *Candida albicans* is shown in Table 3. The extracts were shown to have antibacterial activity against both gram–positive and gram–negative bacteria, with a ZOI ranging from 1.5–3.6 mm and ciprofloxacin (5 µg/disc) as a positive control bacteria activity. *Staphylococcus aureus, Staphylococcus epidermidis,* and *Candida albicans* are gram–positive bacteria while *Klebsiella pneumoniae* and *Escherichia coli* are gram–negative bacteria. The methanolic extract of *E. oxypetalum* leaf was most effective against *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli,* and *Klebsiella pneumonia* at 300 mg/ml, with a ZOI ranging from 2.8–3.5 mm. However, it was not effective against *Staphylococcus epidermidis* with a ZOI lower than 3.7 mm. In addition to that, the methanolic extract of *E. oxypetalum* leaf exhibited impressive antifungal activity against *Candida albicans*, with a ZOI of 1.8 mm at 300 mg/ml (Table 3). These findings imply that the methanol of *E. oxypetalum* leaf extract might be a source of broad–spectrum antimicrobial activity. The high number of phenolic and flavonoids in the methanolic extract of *E. oxypetalum* leaf extract may be accountable for its antibacterial action [6][8][24–25]. Huang et al. [18] also demonstrated that fatty acids exhibited patterns of inhibition against the bacterial species. However, the fatty acids were less effective against *Candida albicans*. These results indicate that the antimicrobial activity of fatty acids from

dodecanoic acid, n–hexadecanoic acid, and lup–20(29)–en–3–one from the methanolic extract of *E. oxypetalum* leaf can influence the microbial ecology.

Table 3. Antibacterial activity of methanolic extract of *E. oxypetalum* leaf.

Test sample	Concentration	ZOI (mm)				
		SA	SE	KP	EC	CA
Methanol	–	N/A	N/A	N/A	N/A	N/A
Ciprofloxacin	5 µg/disc	2.8	3.7	3.4	3.0	1.7
Methanolic extract of *E. oxypetalum* leaf	50 mg/ml	2.4	3.5	3.2	3.0	1.5
	100 mg/ml	2.6	3.5	3.2	3.0	1.5
	200 mg/ml	2.7	3.6	3.2	3.1	1.7
	300 mg/ml	2.8	N/A	3.5	3.3	1.8

SA = *Staphylococcus aureus*, EC = *Escherichia coli*, SE = *Staphylococcus epidermidis*, KP = *Klebsiella pneumonia*, CA = *Candida albicans*, N/A = Not applicable. The results were done in triplicate, and a significant difference at $p \leq 0.05$; n = 3.

CONCLUSION

The compounds present in the methanolic extract of *E. oxypetalum* leaf have biological activities that contribute to the plant's therapeutic use. The methanolic extract of *E. oxypetalum* leaf shows great results compared to alcohol and aqueos extract of *E. oxypetalum* leaf in terms of antioxidant and antimicrobial activities. Additionally, the GC–MS analysis revealed that the methanolic of *E. oxypetalum* leaf extract contained phenolic, flavonoid compounds and fatty acids that important for antioxidant and antimicrobial activity. The findings of this investigation revealed that the methanolic extract of *E. oxypetalum* leaf may be a natural antioxidant and antimicrobial agent due to its high value of TPC and TFC. Further research on the *in vivo* antioxidant and antibacterial activities for illness recovery should be conducted to strengthen their usage in a variety of therapeutic practices.

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