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Application of FTIR Spectroscopy and Chemometric to Differentiate *Azadirachta excelsa* (Jack.) Jacobs Leaves Extracts Based on Solvent Polarity and Assessment of Antibacterial Activity

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ABSTRACT. *Azadirachta excelsa* is a plant belonging to the same genus as the Indian neem (*Azadirachta indica*) which is expected to have similar biological activities. However, its active components and pharmacological effects are limited. The composition and quantity of these metabolites in *A. excelsa* may differ due to different polarities of extracting solvents, so selecting an effective extractive solvent with a high level of biological activity is important. Therefore, in this study, we examined differences in the metabolite finger printing using FTIR-based metabolomics, as well as evaluated their antibacterial activity against *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 8739). *A. excelsa* was extracted using chloroform, ethyl acetate, ethanol p.a., 70% and 50% ethanol. Extracts obtained were analyzed using FTIR and the inhibition zones were then determined. The results showed that principal component analysis (PCA) could distinguish each sample based on the extraction solvent. In this study, we found 50% and 70% ethanol extracts had similar metabolite compositions and concentrations based on their respective FTIR spectrum. The inhibition zone of *A. excelsa* extracts ranged from 12.37-17.20 mm and 13.88-15.89 mm against *S. aureus* and *E.coli,* respectively. The chloroform extract was more effective against both bacteria. Duncan's further test showed that chloroform extract reduced *E. coli* similarly to ethyl acetate but not *S. aureus*. While 50%, 70%, and ethanol p.a. extracts inhibited *S. aureus* and *E. coli* equally. Based on these results, the polarity of the extracting solvent had an important influence on the metabolite profile and antibacterial activity of *A. excelsa*.

Keywords: *Azadirachta excelsa*, antibacterial activity, metabolite fingerprinting, FTIR.

INTRODUCTION

Plants within the Meliacea family are typically characterized by the presence of various constituents, including limonoids, terpenoids, flavonoids, coumarins, chromones, lignin, and phenylpropanoids (Adfa, 2021; Yadav et al., 2015; Kakumu et al., 2014). These plants have also been reported to possess several bioactivities, such as antitumor, antioxidant, antidiabetic, anti-inflammatory, antibacterial, and antiviral properties (Hieu et al., 2022). Furthermore, previous reports have identified two essential Melicacea species around the main campus of Bengkulu University, including *Azadirachta indica* (nimba) and *A. excelsa* (kayu bawang). Leaves of *A. indica*, also known as nimba have been widely used in traditional Indonesian medicine for the treatment of various conditions, including dysentery, malaria, cancer, eczema, ringworm, acne, worms, blood sugar, gastrointestinal pain, and hunger stimulants (Seriasih, 2020). These medicinal effects can be attributed to the presence of quercetin and limonoids, including nimbanene, 6 desacetylnimbinene, nimbandiol, nimbolide, nimbiol, and nimbin, which possess antioxidant, anti-tumor, hepatoprotective, and antibacterial activity (Rahmani et al., 2018).

The ethanol extract of *A. indica* leaves has significant inhibitory effects on the growth of *Enterococcus faecalis*, causing enterococcal infection, with an inhibition diameter of 29 mm. Furthermore, it exhibits significant inhibition against *Candida albicans,* with an inhibition diameter of 7.1 mm, surpassing the activity of 2% NaOCl commonly used for germ eradication around the tooth root (Bohora et al., 2010). Porkhrel et al. (2015) stated that *A. indica* leaves extract had antibacterial efficacy against six different species of bacteria. A 10 mg/mL methanol extract of 70 µL can effectively inhibit the growth of *Staphylococcus aureus* and *Salmonella typhi* (22 and 20 mm diameter inhibition, respectively). The sample also showed moderate inhibition of *Proteus vulgaris* (19 mm) and *Pseudomonas aeruginosa* (17 mm), as well as weak inhibition of *Escherichia coli* (12 mm) and *Klebsiella pneumonia* (13 mm).

Compared to nimba (*A. indica*) leaves, young kayu bawang (*A. excelsa*) leaves are often used traditionally to treat various conditions, including high blood pressure, dysentery, and diarrhea (Noor Mohamad Zin et al., 2019). A phytochemical analysis also showed the presence of essential compounds, such as flavonoids (+++), tannins (++), triterpenes (++), steroids (++), and fatty acids (Sanjaya et al., 2019; Shafie et al., 2015). Despite the scientific evidence supporting the medicinal use of *A. indica*, there are limited studies on the pharmacological effects of *A. excelsa.* Considering the close botanical association between *A. indica* and *A. excelsa,* the species is expected to share similar pharmacological activity. Chemotaxonomy suggests that plants within the same genus are likely to possess similar chemical components and exhibit comparable activity. Therefore, this study was carried out to investigate the antimicrobial potential of plants belonging to the same genus as *A. indica,* particularly *A. excelsa* in the surrounding area of the Bengkulu University Campus.

Plant biological activity is intricately linked to the type and concentration of the constituent bioactive compounds. Extraction has been identified as the primary method for obtaining bioactive compounds from biomass materials. The extraction process is often carried out to extract several target components and maximize the biological activity within the extract. Several studies have shown that the method and selected solvent are essential in influencing extract yield and biological activity. Several solvents have been used to extract bioactive chemicals from plant materials, including methanol, ethanol, acetone, chloroform, ethyl acetate, and water. Due to the presence of a wide range of bioactive chemicals with varying polarity, solubility, and chemical properties in plant materials, the selection of a suitable solvent is dependent on the plant material and the compounds to be extracted (Stéphane et al., 2022; Truong et al., 2019).

Previous reports have shown that there is a growing interest in the use of metabolomics for assessing the effects of different extraction solvents on extracted metabolites. Fourier transform infrared (FTIR) spectroscopy is a well-established metabolite fingerprinting technology with high throughput, minimal sample handling, and the absence of reagents. The FTIR has been reported to effectively detect functional groups, making it suitable for analyzing samples with high specificity and reproducibility. This method follows a straightforward method, which requires minimal sample preparation. Furthermore, as a metabolite fingerprinting method, FTIR spectroscopy provides important chemical information, enabling the prompt and reproducible identification of significant changes in the metabolome (Rafi et al., 2021; Ribeiro Da Cunha et al., 2020).

The FTIR spectrum is a complex dataset that offers detailed information on the character and identification of metabolites. Changes in band position and intensity within the FTIR spectrum correspond to alterations in the chemical composition of natural materials. Due to the substantial volume and intricacy of the data, visually detecting variations in metabolite numbers becomes challenging, thereby necessitating the use of chemometric analysis. The use of FTIR spectra along with chemometric methods, specifically principal component analysis (PCA), has been used in the categorizing plant extract using different solvent extraction. This method has also been applied to the analysis of extracts from *Momordica charantia* (Khatib et al., 2017), *Guazuma ulmifolia* (Rafi et al., 2020), and *Syzygium polyanthum* (Rohaeti et al., 2021). Based on the results, there are no reports on the effect of various polarity solvents on the extraction of bioactive compounds from *A. excelsa* and their biological activity. Therefore, this study aims to determine the influence of solvent polarity (chloroform, ethyl acetate, ethanol p.a., 70% ethanol, and 50% ethanol) on extraction yield and classify extract based on metabolite functional groups using FTIR data. The antibacterial activity of extracts obtained was further tested against *S. aureus* and *E. coli.*

EXPERIMENTAL SECTION

Materials and Reagents

Undamaged leaves, whether young and old were collected from Air Napal Village, Air Napal Subdistrict, North Bengkulu Regency. Furthermore, the plant specimens were identified at the Herbarium Bogoriense (BRIN), and the voucher specimens (MA0207-22) were stored in the organic chemistry laboratory at the Department of Chemistry, University of Bengkulu. The two types of test bacteria used included *S. aureus* (ATCC 6538) and *E. coli* (ATCC 8739). Ethanol, chloroform, ethyl acetate, acetone, methanol, dimethyl sulfoxide, Muller-Hinton Broth

(MHB), and Muller-Hinton Agar (MHA) were purchased from Merck (Darmstadt, Germany). Other materials used included distilled water, 96% ethanol (Brataco), clindamycin, chloramphenicol, Whatman No. 3 filter paper, and plastic wrap.

Instrumentation

FTIR spectra were recorded by platinum Attenuated Total Reflectance (FTIR-ATR) with a portable Bruker spectrometer (Alpha, Bruker Optics GmbH, Ettlingen, Germany) using a diamond crystal, and OPUS v7.5 software. The other instruments used in this study were a rotary evaporator (Heidolph), freeze dryer Power Dry LL1500 (Thermo Scientific), laminar airflow (Telster AV-100), incubator (Froilabo), autoclave (AIP), Petri dishes (Normax, outer diameter 100 mm x 20 mm), cork borer (6 mm), and other standard laboratory equipment. The equipment and supplies for antibacterial activity were sterilized before the procedures to prevent contamination with bacteria or other microorganisms.

Preparation of Sample

The samples were transported to the laboratory after the collection of fresh kayu bawang (*A. excelsa*) leaves from Air Napal Village. Subsequently, impurities were removed, and the samples were sliced into small pieces. Leaves were then air-dried for three weeks in an open atmosphere, then mashed with a blender and sieved through a 50-mesh sieve.

Plant Extraction

Based on recent studies (Esclapez et al., 2011; Liu et al., 2021), ultrasound-assisted extraction (UAE) at 35 KHz combined with cold maceration was used to extract *A. excelsa* leaves. A total of 10 g of simplisia was soaked in 100 mL (1:10 w:v) chloroform ($CHCl_3$) and ultrasonicated at 45°C for 30 minutes before maceration for 24 hours in quadruplicate. Other extracting solvents used included ethyl acetate (EtOAc), and various ethanol concentrations, including 50% (50% EtOH), 70% (70% EtOH), and absolute (EtOH p.a.) using a similar method to that of chloroform extraction. A total of 20 extracts were collected, and the solvent was evaporated using a rotary evaporator at 45-60°C, producing a thick paste ranging from brownish green to reddish brown. Subsequently, the thick extract was separated from the remaining water and solvent using a freeze dryer. The dried extract was then ready for further investigation.

FTIR Spectra Measurement

Approximately 50 mg of the sample was placed on the ATR crystal and forced down with the swivel press to provide optimal contact between the sample and the crystal. The ATR crystal was cleaned with acetone, ethanol, and lint-free wipes before each measurement Furthermore, all spectra were recorded in the range of 4000-600 cm^{-1}, with 32 scans per spectrum at a spectral resolution of 4 cm^{-1} in absorbance mode with the ATR module. The sample was then retrieved after the measurement, and the FTIR spectra were saved as a data point table.

Evaluation of Antibacterial Activity

The bacterium samples used in this study were provided by the IPB University Microbiology Laboratory, Indonesia. A bacterial suspension with standard turbidity of 0.5 McFarland was prepared according to Nurhasana et al. (2023). The well diffusion method was used to evaluate antibacterial activity, which included pouring 15 mL of MHA media containing a 2% suspension of test bacteria into a Petri dish. A total of 7 wells were then made in each Petri dish using a 6 mm diameter cork borer, and 20 μL of 50 mg/mL of each type of extract was added. Positive control, containing 0.25 mg/mL clindamycin and 0.25 mg/mL chloramphenicol, and negative control DMSO were added to each well, and incubated at 37°C for 24 hours. The calliper was used to measure the clear zone that developed around the samples in millimetres, and the testing procedure was confirmed in quadruplicate. Furthermore, according to Ponce et al. (2003), the average results of the bacterial growth inhibition zone were described as the sensitive response of bacteria to the addition of extract.

Data Analysis

Extraction yield and antibacterial activity were determined in quadruplicate and provided as mean ± standard deviation. Furthermore, statistical comparisons were carried out using SPSS (Version 21.0) and one-way analysis of variance (ANOVA), followed by the Duncan test, with significant differences established at a 95% confidence level ($p<0.05$). PCA was used to cluster *A.excelsa* extract based on the polarity of extracting solvent. PCA model was then developed using Unscrambler X version 10.1 software (Camo, Oslo, Norway) and included a preprocessing using baseline correction and standard normal variate (SNV).

RESULTS AND DISCUSSION

Extraction Yields

Leaves powder of *A. excelsa* was extracted using the UAE method and maceration with five solvents of varying polarities, including chloroform, ethyl acetate, ethanol p.a., 70% ethanol, and 50% ethanol. In the present study, leaves extract yields of *A. excelsa* varied between 4.39 ± 0.192 and 31.05 ± 0.981%, as shown in **Table 1**. The highest yield (31.05%) was obtained from extraction using 50% ethanol, followed by 70% ethanol, ethanol p.a., ethyl acetate, and chloroform. The results showed that the polarity of extracting solvent increased along with the extract's yield. Based on the ANOVA, extract yield was significantly influenced by the type of solvent, as determined by its polarity level (p-value <0.05), with a significance level of 5%. Duncan's test showed no significant difference between the chloroform and ethyl acetate extract. The polarity of metabolites present in *A. excelsa* ranged from semi-polar to polar.

Furthermore, the concentration and composition of extracted compounds depended on extracting solvent.

FTIR Spectra Interpretation and Classification of *A. excelsa* Leaves Extracts

Figure 1 shows the FTIR spectra of all extracts, while **Figure 2** presents the IR spectra representative of each *A. excelsa* leaves extract. FTIR spectra provided different profiles that described the signals produced by the vibration and rotation of metabolites collected from the sample, often known as fingerprint analysis. The results showed that the extract had almost identical spectrum patterns.

Similar spectrum patterns were observed in chloroform, and ethyl acetate extracts, as well as in ethanol p.a., 50% ethanol, and 70% ethanol extracts, with the only difference being an alteration in absorbance intensity. The similar pattern in each extract showed that metabolites extracted from *A.excelsa* leaves using five different solvents with differing polarities had the same composition but different concentration levels. A variation in concentration levels influenced biological activity, such as antibacterial activity.

The identification of the functional groups in all leaves extracts of *A. excelsa* is presented in **Table 2**. The hydroxyl functional group was detected by absorption at a wavenumber around 3400-3200 cm^{-1} in all extracts. Ethanol p.a. extract exhibited the highest absorbance value, while the lowest was found in chloroform extract. The absorption of the carbonyl functional group (C=O) was detected at 1740-1690 cm^{-1} and C-O absorption was within the wavenumber of 1300-1000 cm^{-1}. The results showed that ethyl acetate extract had the highest absorbance value, with 70% ethanol showing the lowest.

The absorption of aromatic groups was detected at 1600-1500 cm^{-1} (C=C aromatic), supported by the absorption of C-H aromatic (stretch) at wave numbers 3150-3050 cm^{-1} and 900-690 cm^{-1}. The ethanol p.a. extract exhibited the greatest absorption of aromatic groups, while chloroform showed the lowest. C=C alkenes absorption was observed at 1680-1610 cm^{-1}, which was supported by C-H alkenes (stretch) at 3010-3000 cm^{-1} and 1000-650 cm^{-1} (bend). For C-H alkane absorption, the chloroform extract had the highest intensity, followed by ethyl acetate, and 70% ethanol extract had the lowest intensity in the 3000-2850 cm^{-1} wave number region (stretch) and 1340-1470 cm^{-1} region (bend) (Pavia et al., 2013).

The FTIR spectra data observed and analyzed from five types of *A. excelsa* extracts was in the form of 414 wave number and 20 absorbance data, causing the classification of *A. excelsa* leaves extracts based on only IR spectra problematic. To classify the data, chemometric analysis was required to differentiate each extract. The chemometric analysis used in this study was PCA, which was carried out to classify the samples based on their solvent polarity for extraction. PCA conducted exploratory analysis on FTIR spectrum data in the 4,000-600 cm^{-1} range. The most critical stage before chemometric bi-linear modeling was spectral data preprocessing. Furthermore, the baseline correction and SNV were used as a preprocessing step. Preprocessing could remove noise and other interferences from the spectrum data, thereby improving the accuracy of the grouping results without reducing analytical information. In spectroscopy data analysis, baseline correction was often the first preprocessing step. Baseline correction procedures were typically carried out to correct low-frequency noise, but high-frequency baseline correction procedures and smoothing methods could be required to reduce high-frequency changes and increase signal-to-noise ratio (S/N) (Pierce et al., 2012). The SNV transformation was introduced by Barnes et al. (1993) to reduce the multiplicative effects of scattering and particle size as well as differences in the global intensities of the signals. Principle components 1 (PC1) and PC2, which explained 93% and 3% of the total variance, respectively, were used to construct the Principle Components Analysis (PCA) score plot shown in **Figure 3**. The score plot explained 96% of the variance, and each extract was assigned to a group.

The correlation between closely related groups suggested a higher degree of similarity in the concentration and composition of extracted metabolites, as showed by similarities and differences in the samples' FTIR spectra (Rafi et al., 2021; Rohman et al., 2021). Based on the results, extracted metabolite concentration did not differ significantly between the 50% and 70% ethanol extracts.

Table 1. Extraction yields of kayu bawang (*A. excelsa*) leaves extracts

No.	Extracting Solvent	Extraction Yields (%)
1	$CHCl_3$	4.39 ± 0.192 a
2	EtOAc	4.65 ± 0.218 a
3	EtOH p.a.	22.71 ± 0.281 b
4	70% EtOH	29.32 ± 0.908 c
5	50% EtOH	31.05 ± 0.981 d

*Note: The values reported are the mean ± SD of quadruplicate assays for each sample. Mean ± SD within each extract in the same column followed by different letters show significant differences at $p < 0.05$

Table 2. The identified functional groups in kayu bawang (*A. excelsa*) leaves extracts

Wavenumber (cm^{-1})	Functional Groups
3400-3200	O–H Alcohol, phenols
3000-2850	C–H Alkanes (stretch)
3010-3000	C–H Alkenes (stretch)
3150-3050	C–H Aromatic rings (stretch)
1740-1690	C=O
1680-1610	C=C Alkenes
1600-1500	C=C Aromatic rings
1340-1470	C–H Alkanes (bend)
1300-1000	C–O
1000-650	C–H Alkenes (bend)
900-690	C–H aromatic

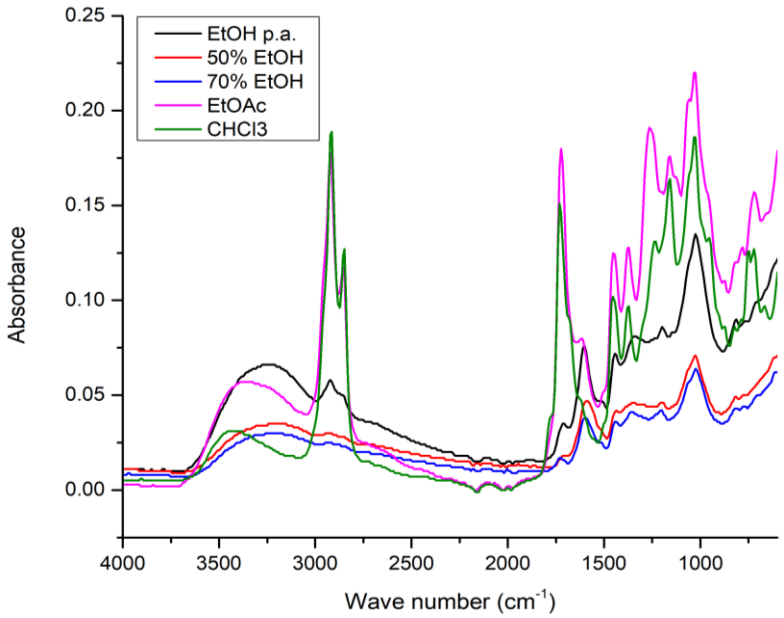

Figure 1. Representatives FTIR spectrum of kayu bawang (*A. excelsa*) leaves extracts

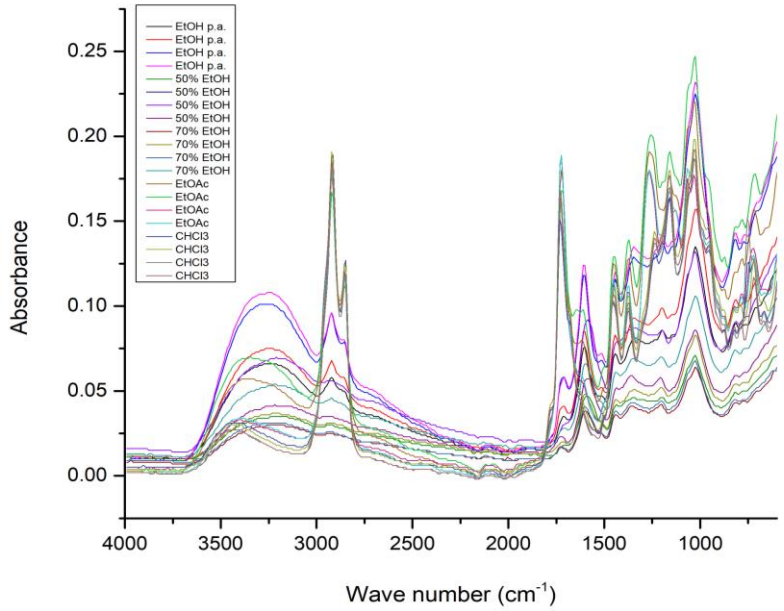

Figure 2. FTIR spectra of kayu bawang (*A. excelsa*) leaves extracts

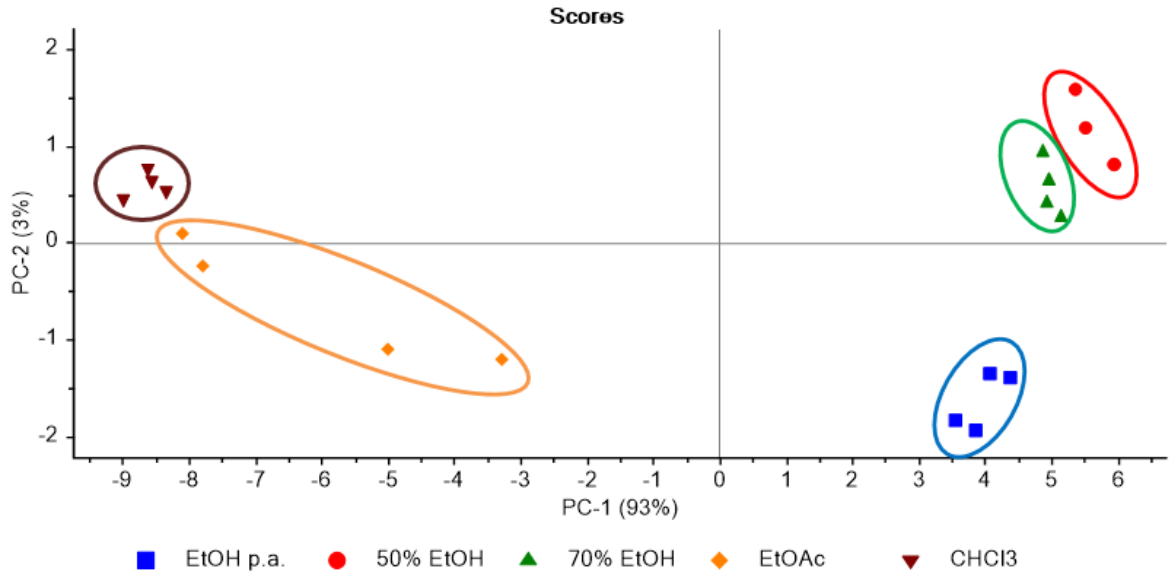

Figure 3. PCA score plot FTIR spectra of kayu bawang (*A. excelsa*) leaves extracts with various solvents polarity ($CHCl_3$, EtOAc, EtOH p.a., 70% EtOH, and 50% EtOH)

Antibacterial Activity of *A. excelsa* Leaves Extracts

Antibacterial activity of five different *A. excelsa* leaves extracts was tested in vitro against two types of bacteria that caused infectious diseases, namely *S. aureus* (ATCC 6538) for Gram-positive bacteria and *E. coli* (ATCC 8739) for Gram-negative bacteria. At a concentration of 50 mg/mL, all five extracts of *A. excelsa* leaves could suppress the growth of the test bacteria (**Figures 4, 5**). Clindamycin (0.25 mg/mL) was used as a positive control for *S. aureus* inhibition and chloramphenicol (0.25 mg/mL) for *E. coli* inhibition, while DMSO served as a negative control. The width of the inhibition zone of the five *A. excelsa* leaves extracts against *S. aureus* and *E. coli* growth ranged from 12.37 ± 0.21 mm to 17.20 ± 0.4 mm and 13.88 ± 0.25 mm to 15.89 ± 0.2 mm, respectively, as shown in **Figures 6** and **7**. According to Ponce et al. (2003), the sensitivity response of bacterial to extract addition could be described based on these observations. *S. aureus* and *E.coli* were shown to be very sensitive to chloroform and ethyl acetate extract, as well as sensitive category to 70% ethanol, ethanol p.a., and 50% ethanol extracts. The inhibitory activity of the five extracts against the growth of the two bacteria followed a similar pattern. Furthermore, the sample with the lowest polarity (chloroform extract) showed the highest inhibitory activity against both test bacteria, followed by ethyl acetate, 70% ethanol, ethanol p.a., and 50% ethanol extracts. ANOVA results showed that the type of extract based on solvent polarity significantly effected on antibacterial activity at the 5% significance level (p-value 0.05). According to Duncan's additional test, the inhibitory potential of ethanol p.a, 70%, and 50% ethanol extracts against *S. aureus* was not significantly different, while the inhibitory potential against *E. coli* was not significantly different between ethyl acetate, ethanol p.a, as well as 70% and 50% ethanol extracts. The chloroform extract inhibited *E. coli* growth similarly to ethyl acetate, ethanol p.a, and 70% ethanol extracts, but significantly differently when inhibiting *S. aureus*. These results could be attributed to the chemical ingredients responsible for their antibacterial action. The 50% ethanol extract with the highest extraction yield had the lowest percentage inhibition, showing that the polar metabolites in *A.excelsa* leaves did not contribute positively to its antibacterial activity or antagonism effects that occurred between metabolites (Caesar & Cech, 2019). In line with the results of Benisheikh et al. (2019), the chloroform extract of *A. indica* leaves exhibited the greatest inhibitory effect against *S. aureus* and *E. coli* when compared to the n-hexane, ethyl acetate, and methanol extracts.

Scientific investigations into the inhibitory effects of *A. excelsa* against enteric pathogens remained limited, despite its historical use in treating of diarrhea and dysentery. A study on antibacterial activity of an ethanol extract of *A. excelsa* leaves against *Shigella sonnei*, *Salmonella typhirium*, and *E. coli* (Raja Yahya et al., 2013) had been previously carried out. The results showed that the ethanol extract could mildly inhibit *S. sonnei* with a minimum inhibitory concentration (MIC) of 6.25 mg/mL and a minimum bactericidal concentration (MBC) of 200 mg/mL. However, no such effect was observed on *E. coli* and *S. typhirium*. The results of this study showed that 5 types of *A. excelsa* extracts inhibited *E. coli* and *S. aureus* with a moderate degree of inhibition, which differed from previous reports.

The diameter of the bacterial growth inhibition zone after the application of the treatments was smaller compared to the positive controls of 0.25 mg/mL clindamycin and chloramphenicol, while the

negative control (DMSO) showed no growth inhibitory activity (0.0 ± 0.00) mm. **Figures 6** and **7** showed that the negative control (DMSO) did not affect *S. aureus* or *E. coli* growth. In this study, the positive control 0.25 mg/mL clindamycin had an average inhibitory zone diameter of 31.18 ± 1.49 mm. Clindamycin was a semisynthetic antibiotic developed from lincomycin and could be used topically to treat various infections caused by susceptible microorganisms as well as acne vulgaris topically. This antibiotic is effective against anaerobic bacterial, most gram-positive aerobic cocci bacterial, gram-positive and gram-negative bacilli, and some protozoa. Clindamycin also could inhibit the translocation of tRNA from the 50S ribosomal subunit (Singh et al., 2021). At a dosage of 0.25 mg/mL, the positive control chloramphenicol inhibited the growth of *E. coli*, leading to an average inhibition zone diameter of 19.77 ± 1.61 mm and confirming that the *E. coli* strains used for the test were not resistant to the treatment. Chloramphenicol was an antimicrobial agent with a broad spectrum of activity, including gram-positive bacteria, gram-negative bacteria, and anaerobes. Previous studies have shown its ability to bind to the elongating ribosome and prevent the transfer of the ternary complex aminoacylated-tRNA and EF-Tu, GTP to the A-site (Singhal et al., 2023). Despite the extensive use of antibiotics in medicine, the search for antimicrobial substances derived from plants was still continuous due to the need to develop better and safer drugs for combating bacterial and fungal infections. This was due to their biodegradable nature and relative safety to humans and non-target organisms in the environment. Plant extract that inhibited pathogenic bacteria without causing harm to the host could have promising applications in medicine.

A. excelsa was a member of the Meliaceae family, famous for its abundance of limonoid compounds. Previous studies had also shown meliaceous limonoids antiprotozoal, antimicrobial, and antineoplastic properties (Fuentes et al., 2015). While considerable studies had been conducted on various taxa of Meliaceae in terms of biological activity, information regarding their antibacterial and antifungal activity was limited, except for *A. indica*. The inhibitory effects of *A. indica* seed and leaves extract on fungi, such as *Candida albicans*, *C. tropicalis*, and *Neisseria gonorrhoeae* showed promising effects (Talwar et al., 1997). Other studies had shown that ethanol extract of *A. indica* leaves could inhibit the growth of *E. faecalis*, *S. aureus*, *S. typhi*, *P. vulgaris*, *P. aeruginosa*, *E. coli*, and *K. pneumonia* (Bohora et al., 2010; Pokhrel et al., 2015). 6-Homodesacetylnimbin and gedunin were isolated compounds from *A. indica* that had been shown to suppress the growth of *S. epidermidis* and *E. coli*, respectively (Lu et al., 2019). A previous study also effectively isolated 6-deacetylnimbin, the same limonoid as *A. indica*, from the seed kernel of *A. excelsa* (Adfa et al., 2023).

In line with the phytochemical test results, flavonoids (+++), tannins (++), triterpenes (++), steroids (++), and fatty acids were present in leaves of *A. excelsa* (Sanjaya et al., 2019; Shafie et al., 2015). The results showed that these compounds probably inhibited the proliferation of test bacterial in part. Plant secondary metabolites, such as polyphenols (flavonoids, tannins, coumarins, and quinones), phenolics, alkaloids, terpenoids, lectins, and polypeptides, are responsible for the antibacterial action exhibited by plants. Their antibacterial methods include breakdown of microbial cell membranes, and inhibition of cell metabolism. Plant antimicrobial substances hinder the formation of bacterial capsules, and weaken harmful bacteria via regulating quorum sensing (Ginovyan et al., 2017).

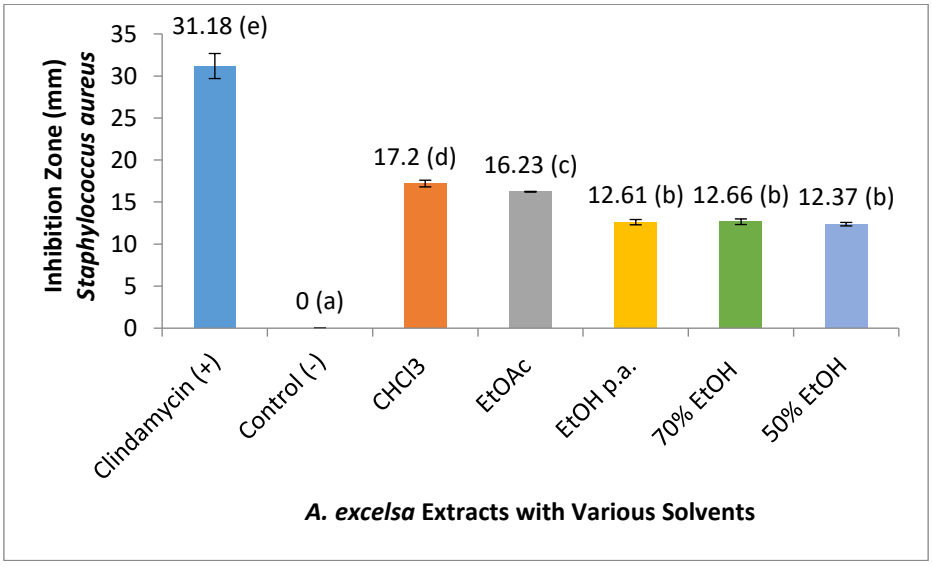

Figure 4. Antibacterial activity of kayu bawang (*A. excelsa*) leaves extracts ($CHCl_3$, EtOAc, EtOH p.a., 70% EtOH, 50% EtOH) against *S. aureus*

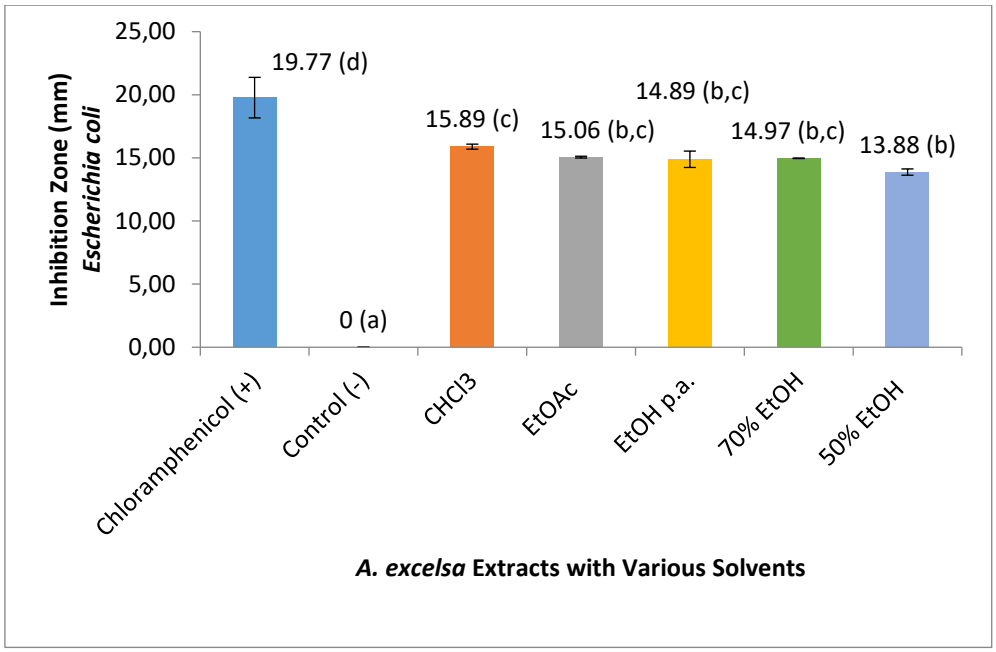

Figure 5. Antibacterial activity of kayu bawang (*A. excelsa*) leaves extracts ($CHCl_3$, EtOAc, EtOH p.a., 70% EtOH, and 50% EtOH) against *E. coli*

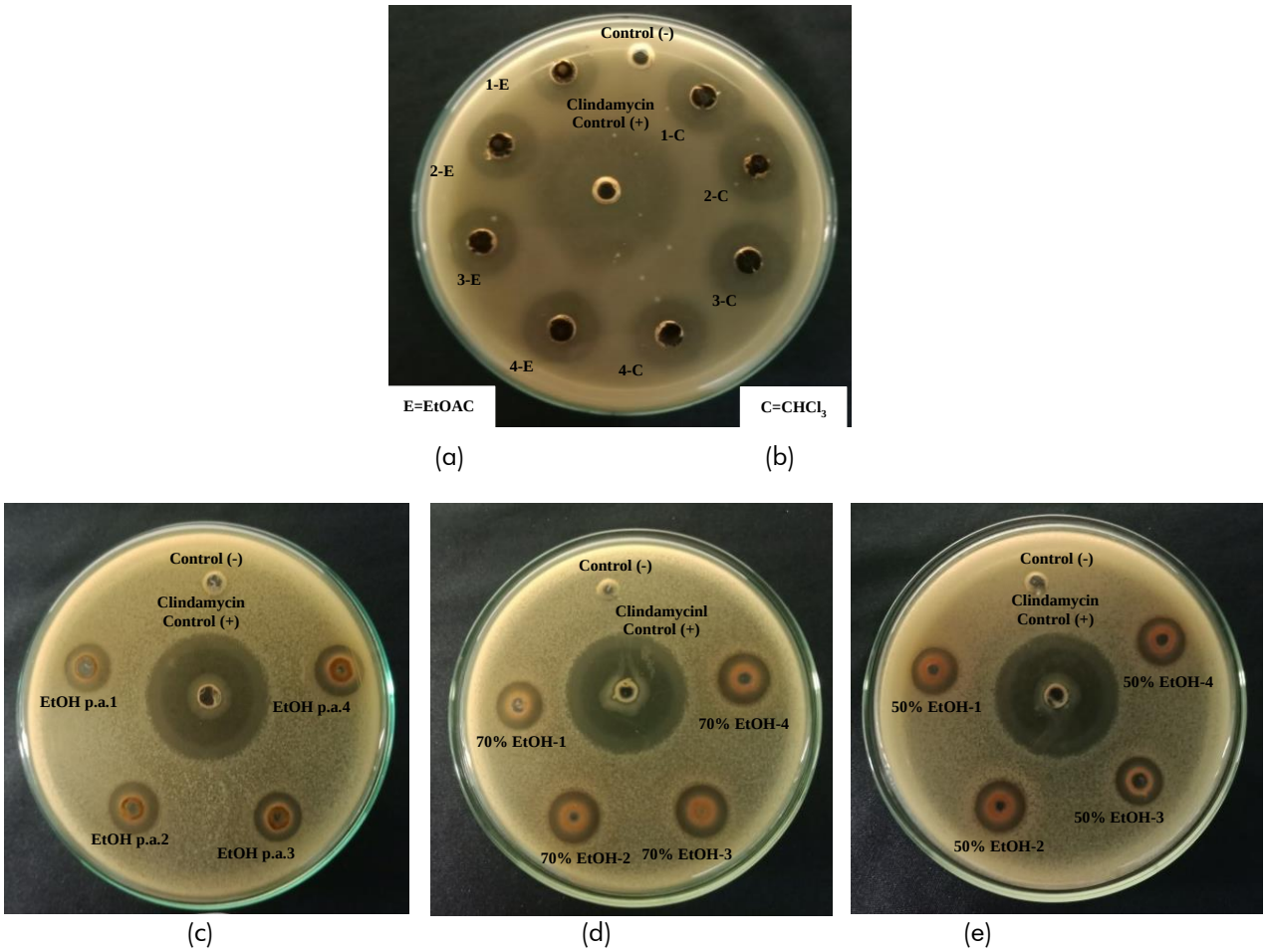

Figure 6. Diameter of the growth inhibition zone of *S. aureus* after application of kayu bawang (*A. excelsa*) leaves extracts: (a) $CHCl_3$ (b) EtOAc (c) EtOH p.a. (d) 70% EtOH, and (e) 50% EtOH extracts

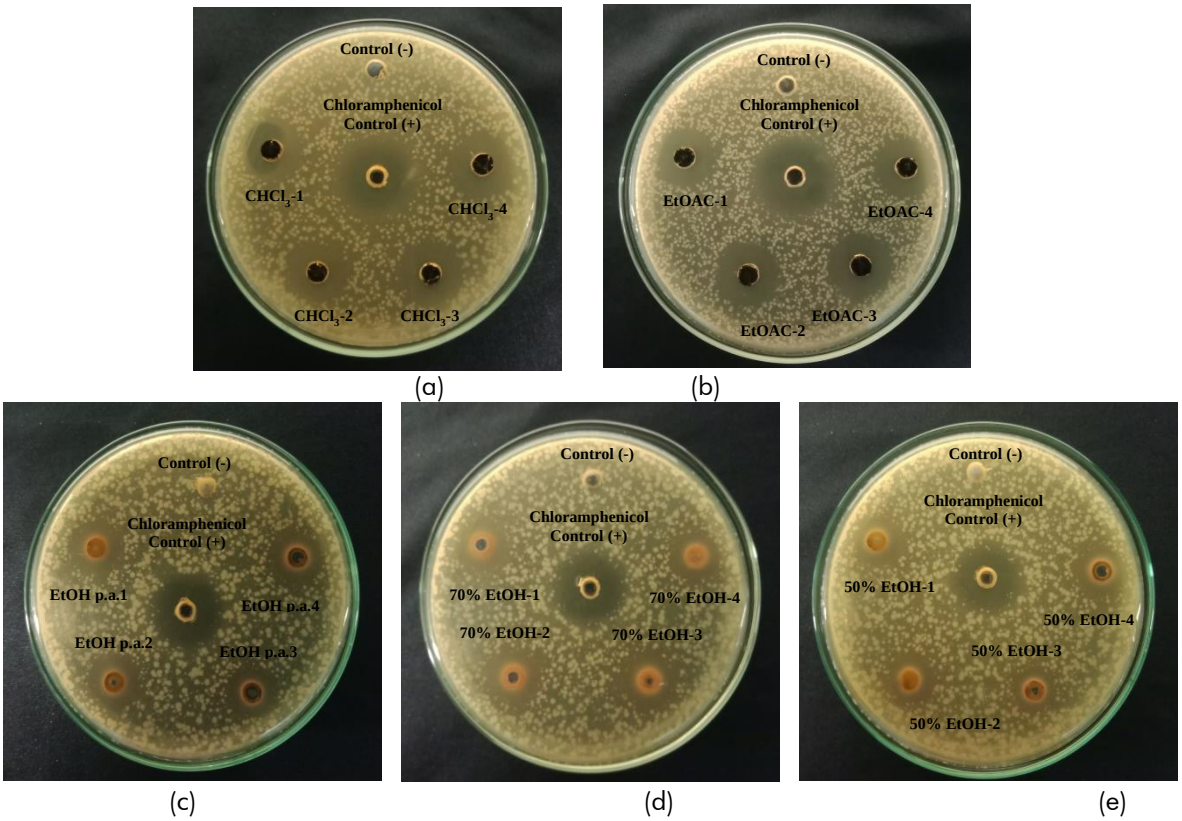

Figure 7. Diameter of the growth inhibition zone of *E. coli* after application of kayu bawang (*A. excelsa*) leaves extracts: (a) $CHCl_3$ (b) EtOAc (c) EtOH p.a. (d) 70% EtOH, and (e) 50% EtOH extracts

CONCLUSIONS

In conclusion, variations in solvent polarity influenced the FTIR spectra, extraction yields, and antibacterial activity of *A. excelsa* leaves extracts. Furthermore, the chloroform extract had the highest antibacterial activity among all extract against *S. aureus* and *E. coli*. The FTIR spectra of each sample showed similar patterns but could be grouped by PCA with a total variance of approximately 96% for PC-1 and PC-2. Based on the FTIR data, O-H, C-H alkane, C-H alkene, C-H aromatic ring, C=O, C=C alkene, C=C aromatic, and C-O were the functional groups of metabolites in *A. excelsa* extracts and could contribute to their antibacterial activity. The next step is to isolate the leading chemicals and determine their chemical structure. The results showed that the use of *A. excelsa* extracts was effective in suppressing harmful microbial species and could be used in therapeutic formulations in the future.

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REFERENCES

Adfa, M. (2021). *Potensi metabolit sekunder tumbuhan lokal sebagai biopestisida*. UNIB Press. Retrieved from http://repository.unib.ac.id/id/eprint/22945%0Ahttp://repository.unib.ac.id/22945/1/pdf

Adfa, M., Wiradimafan, K., Pratama, R. F., Sanjaya, A., Triawan, D. A., Yudha S., S., Ninomiya, M., Rafi, M., & Koketsu, M. (2023). Anti-termite activity of *Azadirachta excelsa* seed kernel and its isolated compound against *Coptotermes curvignathus*. *Journal of the Korean Wood Science and Technology*, *51*(3), 157–172. https://doi.org/10.5658/WOOD.2023.51.3.157

Barnes, R. J., Dhanoa, M. S., & Lister, S. J. (1993). Correction to the description of standard normal variate (SNV) and de-trend (DT) transformations in practical spectroscopy with applications in food and beverage analysis—2nd Edition. *Journal of Near Infrared Spectroscopy*, *1*(3), 185–186. https://doi.org/10.1255/jnirs.21

Benisheikh, A. A. G., Muhammad, F. M., Kelluri, H., Aliyu, Z. M., Mallam, U. B., & Jibrin, M. W. (2019). Phytochemical extraction and antimicrobial studies on crude leaf extract of *Azadirachta indica* (neem) in semi-arid region of Borno state, Nigeria. *International Journal of Research & Review*, *6*(12), 516–522. Retrieved from www.ijrrjournal.com

Bohora, A., Hegde, V., & Kokate, S. (2010). Comparison of the antibacterial efficiency of neem leaf extract and 2% sodium hypochlorite

against *E. faecalis*, *C. albicans* and mixed culture - an in vitro study. *Endodontology*, *22*(1), 10–14. https://doi.org/10.4103/0970-7212.351972

Caesar, L. K., & Cech, N. B. (2019). Synergy and antagonism in natural product extracts: When 1 + 1 does not equal 2. *Natural Product Reports*, *36*(6), 869–888. https://doi.org/10.1039/c9np00011a

Esclapez, M. D., García-Pérez, J. V, Mulet, A., & Cárcel, J. A. (2011). Ultrasonic assisted extraction of natural products. *Food Engineering Reviews*, *3*(2), 108–120. https://doi.org/10.1039/C9NP00011A

Fuentes, R. G., Toume, K., Arai, M. A., Sadhu, S. K., Ahmed, F., & Ishibashi, M. (2015). Limonoids with Wnt signal inhibitory activity isolated from the fruits of *Azadirachta excelsa*. *Phytochemistry Letters*, *11*, 280–285. https://doi.org/10.1016/j.phytol.2015.01.015

Ginovyan, M., Petrosyan, M., & Trchounian, A. (2017). Antimicrobial activity of some plant materials used in Armenian traditional medicine. *BMC Complementary and Alternative Medicine*, *17*(50), 1–9. https://doi.org/10.1186/s12906-017-1573-y

Hieu, T. T., Thuy, P. T., & Duc, D. X. (2022). Isolation and bioactivities of limonoids from meliaceae family: A review. *Current Organic Chemistry*, *26*(14), 1359–1430. https://doi.org/10.2174/1385272827666221017141255

Kakumu, A., Ninomiya, M., Efdi, M., Adfa, M., Hayashi, M., Tanaka, K., & Koketsu, M. (2014). Phytochemical analysis and antileukemic activity of polyphenolic constituents of *Toona sinensis*. *Bioorganic and Medicinal Chemistry Letters*, *24*(17), 4286–4290. https://doi.org/10.1016/j.bmcl.2014.07.022

Khatib, A., Perumal, V., Ahmed, Q. U., Uzir, B. F., Abas, F., & Murugesu, S. (2017). Characterization of antioxidant activity of *Momordica charantia* fruit by infrared-based fingerprinting. *Analytical Letters*, *50*(12), 1977–1991. https://doi.org/10.1080/00032719.2016.1261877

Liu, Y., Sun, W., Li, B., Shang, N., Wang, Y., Lv, W., Li, D., & Wang, L. (2021). Value-added application of *Platycodon grandiflorus* (Jacq.) A.DC. roots (PGR) by ultrasound-assisted extraction (UAE) process to improve physicochemical quality, structural characteristics and functional properties. *Food Chemistry*, *363*, 130354. https://doi.org/10.1016/j.foodchem.2021.130354

Lu, X. F., Lin, P. C., Zi, J. C., & Fan, X. N. (2019). Limonoids from seeds of *Azadirachta indica* and their antibacterial activity. *Zhongguo Zhongyao Zazhi= China Journal of Chinese Materia Medica*, *44*(22), 4864–4873. https://doi.org/10.19540/j.cnki.cjcmm.20190813.202

Noor Mohamad Zin, N. S., Hashim, N., Samsulrizal, N., & Azmi, N. S. ain. (2019). The protective effect of *Azadirachta excelsa* leaves extract and quercetin treatment on the learning and memory impairments in relation with insulin and amylin levels in the brain of streptozotocin-induced diabetic rats. *Journal of King Saud University - Science*, *31*(3), 299–307. https://doi.org/10.1016/j.jksus.2018.05.017

Nurhasana, D., Febriansyah, R., Avidlyandi, A., Yudha S., S., Banon, C., Oktiarni, D., & Adfa, M. (2023). In-vitro antibacterial activity of *Excoecaria cochinchinensis* Lour's methanol extract and its combination with commercial drugs. *Jurnal Ilmu Kefarmasian Indonesia*, *21*(2), 179. https://doi.org/10.35814/jifi.v21i2.1383

Pavia, D. L., Lampman, G. M., Kriz, G. S., & Vyvyan, J. R. (2013). *Introduction to spectroscopy* (Fifth Ed.). Cengage Learning, USA.

Pierce, K. M., Kehimkar, B., Marney, L. C., Hoggard, J. C., & Synovec, R. E. (2012). Review of chemometric analysis techniques for comprehensive two dimensional separations data. *Journal of Chromatography A*, *1255*, 3–11. https://doi.org/10.1016/j.chroma.2012.05.050

Pokhrel, B., Rijal, S., Raut, S., & Pandeya, A. (2015). Investigations of antioxidant and antibacterial activity of leaf extracts of *Azadirachta indica*. *African Journal of Biotechnology*, *14*(46), 3159–3163. https://doi.org/10.5897/ajb2015.14811

Ponce, A. G., Fritz, R., Del Valle, C., & Roura, S. I. (2003). Antimicrobial activity of essential oils on the native microflora of organic swiss chard. *LWT-Food Science and Technology*, *36*(7), 679–684. https://doi.org/10.1016/S0023-6438(03)00088-4

Rafi, M., Meitary, N., Septaningsih, D. A., & Bintang, M. (2020). Phytochemical profile and antioxidant activity of *Guazuma ulmifolia* leaves extracts using different solvent extraction. *Indonesian Journal of Pharmacy*, *31*(3), 171–180. https://doi.org/10.22146/ijp.598

Rafi, M., Rismayani, W., Sugiarti, R. M., Syafitri, U. D., Wahyuni, W. T., & Rohaeti, E. (2021). FTIR-based fingerprinting combined with chemometrics for discrimination of *Sonchus arvensis* leaves extracts of various extracting solvents and the correlation with its antioxidant activity. *Indonesian Journal of Pharmacy*, *32*(2), 132–140. https://doi.org/10.22146/ijp.755

Rahmani, A., Almatroudi, A., Alrumaihi, F. ., & Khan, A. (2018). Pharmacological and therapeutic

potential of neem (*Azadirachta indica*). *Pharmacognosy Reviews*, *12*(24), 250–255. https://doi.org/10.4103/phrev.phrev_8_18

Raja Yahya, M. F. Z., Ismail, N. B., & Kamaruddin, M. S. (2013). *Azadirachta excelsa* ethanolic leaf extract and its effect against some enteric pathogens. *The Open Conference Proceedings Journal-Proceeding of the ICNP 2013*, *4*, 162. https://doi.org/10.2174/2210289201304010162

Ribeiro Da Cunha, B., Fonseca, L. P., & Calado, C. R. C. (2020). Metabolic fingerprinting with fourier-transform infrared (FTIR) spectroscopy: towards a high-throughput screening assay for antibiotic discovery and mechanism-of-action elucidation. *Metabolites*, *10*(4), 145. https://doi.org/10.3390/metabo10040145

Rohaeti, E., Karunina, F., & Rafi, M. (2021). FTIR-based fingerprinting and chemometrics for rapid investigation of antioxidant activity from *Syzygium polyanthum* extracts. *Indonesian Journal of Chemistry*, *21*(1), 128–136. https://doi.org/10.22146/ijc.54577

Rohman, A., Ikhtiarini, A. N., Setyaningsih, W., Rafi, M., Aminah, N. S., Insanu, M., Irnawati, I., & Santosa, D. (2021). The use of chemometrics for classification of Sidaguri (*Sida rhombifolia*) based on FTIR spectra and antiradical activities. *Indonesian Journal of Chemistry*, *21*(6), 1568–1576. https://doi.org/10.22146/ijc.64360

Sanjaya, A., Adfa, M., Pardede, A., Gustian, I., Ninomiya, M., Kusnanda, A. J., & Koketsu, M. (2019). Isolation and structure elucidation of steroid from methanol extract of Sentang (*Azadirachta excelsa* (Jack.) Jacobs) stem. *Proceeding of The 1st International Conference on Chemistry, Pharmacy and Medical Sciences (ICCPM), Universitas Bengkulu, 27– 28 November 2018*, 1–4. UNIB Press.

Seriasih, W. (2020). Tinjauan daun mimba (intaran) dari sisi mitologi dan usadha Bali. *Jurnal IKA*, *18*(1), 99–103.

Shafie, N. 'Izzati, Samsulrizal, N., Sopian, N. A., Rajion, M. A., Meng, G. Y., Ajat, M. M. M., & Ahmad, H. (2015). Qualitative phytochemical screening and GC-MS profiling of *Azadirachta excelsa* leaf extract. *Malaysian Applied Biology*, *44*(3), 87–92.

Singh, S. P., Qureshi, A., & Hassan, W. (2021). Mechanisms of action by antimicrobial agents: A review. *McGill Journal of Medicine*, *19*(4), 1–10. https://doi.org/10.26443/mjm.v19i1.217

Singhal, M., Agrawal, M., Bhavna, K., Sethiya, N. K., Bhargava, S., Gondkar, K. S., Joshi, K., Rana, V. S., Sahoo, J., & Arora, M. K. (2023). Chloramphenicol and tetracycline (broad spectrum antibiotics). In *Antibiotics-Therapeutic Spectrum and Limitations* (pp. 155–165). Academic Press. https://doi.org/ https://doi.org/10.1016/B978-0-323-95388-7.00019-X

Stéphane, F. F. Y., Jules, B. K. J., Batiha, G. E., Ali, I., & Bruno, L. N. (2022). Extraction of bioactive compounds from medicinal plants and herbs. In *Natural Medicinal Plants*, *11*,13. Retrieved from https://www.intechopen.com/ books/ advanced-biometric-technologies/ liveness-detection-in-biometrics

Talwar, G. P., Raghuvanshi, P., Misra, R., Mukherjee, S., & Shah, S. (1997). Plant immunomodulators for termination of unwanted pregnancy and for contraception and reproductive health. *Immunology and Cell Biology*, *75*(2), 190–192. https://doi.org/10.1038/icb.1997.27

Truong, D. H., Nguyen, D. H., Ta, N. T. A., Bui, A. V., Do, T. H., & Nguyen, H. C. (2019). Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of *Severinia buxifolia*. *Journal of Food Quality*, *2019*, 1-9. https://doi.org/ 10.1155/2019/8178294

Yadav, R., Pednekar, A., Avalaskar, A., Rathi, M., & Rewachandani, Y. (2015). A comprehensive review on meliaceae family. *World Journal of Pharmaceutical Sciences*, *3*(8), 1572–1577. Retrieved from https://wjpsonline.com/index.php/wjps/article/view/comprehensive-review-meliaceae-family