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Chemical Constituents and Evaluation of Bioactivity of *Vernonia hymenolepis* A. Rich Root Extracts

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Abstract

The medicinal herb *Vernonia hymenolepis* has been used to cure a wide range of ailments such as cancer, gonorrhea, stomach ache, malaria, hepatitis, pneumonia, toothache, diarrhea, amoebiasis, typhoid, hypertension, and constipation by diverse societies in Ethiopia, Kenya, and Tanzania. However, its biological and phytochemical data is extremely insufficient. Thus, the aim of this research work was to study the chemical constituents and antimicrobial activity of the root part of this plant. The roots of plant were dried by air under shade and then successively extracted with chloroform and methanol. The chloroform root extract was subjected to silica gel column chromatographic separation which gave two compounds, namely; betulinic acid (1) and 2-hydroxy-3-(4-hydroxyphenyl)-2-propenoic acid (2). The structures of the isolated compounds were elucidated using ^{1}H and ^{13}C NMR spectroscopic techniques and by comparing with literature reports. The crude extracts were assayed *in vitro* employing disc diffusion technique against four bacterial strains (*Bacillus subtilis* ATCC11778, *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25922, and *Pseudomonas aeruginosa* ATCC27853) and one fungal strain (*Candida albicans* ATCC 10231). Significant antibacterial activity was demonstrated by the crude extracts; with the chloroform extract exhibiting greater activity against *Escherichia coli* (18 mm) when compared to gentamicin (25 mm). The methanol extract revealed the highest activity (13 mm) against *Candida albicans* with the reference drug, clotrimazole showing an inhibition zone of 12 mm. The antimicrobial activity exhibited by root extracts of *V. hymenolepis* corroborates its traditional use against bacterial diseases.

Keywords: Chemical constituents; Antimicrobial activity; *Vernonia hymenolepis*; Medicinal plant.

INTRODUCTION

Since ancient times, plants have served as the primary source of traditional medicinal remedies (R. U. and A. S. Alqahtani, 2022). According to the World Health Organization, 80% of the world's population uses different plant fractions and their active ingredients as traditional remedies (A. S. Alqahtani et al., 2022; Mrabti et al., 2022; Shahat et al., 2018). Research on medicinal plants is more focused than ever since they can benefit humanity in many ways. The potential these plants as medicines may stem from their bioactive phytochemical components, such as terpenoids, flavonoids, lignans, alkaloids, anthraquinone, etc., which are responsible for their physiological action (Fagbohun et al., 2012; Parekh et al., 2006).

Higher plants are a possible source of novel antibiotic drugs, according to the results of antimicrobial activity screening of plant products. In recent years, the prevalence of various types of resistances in human pathogenic microorganisms has increased, primarily as a result of the improper use of commercial antimicrobial medications that are frequently prescribed to treat infectious disorders. Because of this, scientists are now compelled to look for novel antimicrobial chemicals from a variety of sources, including medicinal plants (Khan et al., 2013).

Vernonia hymenolepis is an ethnomedicinally important plant that is used by many herbalists and communities in Tanzania for the treatment of several diseases such as diarrhea, stomachache, faint fever, diarrhea, spleen enlargement, amoebiasis, malaria, typhoid and constipation (Abebe, 2016; Onzago et al., 2014). The plant is utilized for treating pneumonia, hypertension, and diarrhea in infants by using various parts of it. Moreover, herbalists in Kenya widely use it for treating toothaches (Onzago et al., 2014).

Medicinal plants have been utilized by Ethiopians and have become crucial to traditional customs within the nation (Mesfin et al., 2009). *V. hymenolepis* 'Sooyyoma' (Afan Oromo) is commonly grown in diverse areas of Ethiopia. It is known traditional medicinal plant and its leaf part is utilized to treat cancer (Abebe, 2016) and gonorrhea (Bizuayehu & Garedew, 2018). Similarly, the

crumpled leaves are boiled with water and taken orally for the management of hepatitis (Yineger et al., 2019).

There is no sufficient information related to the phytochemical investigation and antimicrobial activity of the plant. In addition to this, there are no further studies on the phytochemicals present in the root of this plant. To provide scientific support for the traditional use of *V. hymenolepis* in Ethiopia for the treatment of various illnesses, in this study, our objective was to study the chemical compounds and antimicrobial activity of the root part of the plant.

MATERIALS AND METHODS

Chemicals and Instrumentation

Solvents of analytical grade (chloroform, methanol, petroleum ether, and ethyl acetate) were used for successive extraction and column elution. Standard antibiotic drugs (gentamicin and clotrimazole), DMSO, Mueller-Hinton agar, potato dextrose agar and nutrient agar were utilized as a culture media during antimicrobial tests. A rotary evaporator was used to concentrate the extracts. UV light (254 nm and 365) was used for the detection of spots on TLC and oxalic acid impregnated silica gel (60-120 mesh) was used for column chromatography. Bruker avance 400 MHz spectrometer was employed for Nuclear Magnetic Resonance (NMR) analysis and tetramethylsilane was used as an internal standard and $CDCl_3$ and acetone-d_6 were used as solvents.

Plant Sample Collection

The roots of *V. hymenolepis* (Figure 1b) were collected from Jimma Arjo District, East Wollega Zone, Ethiopia. The plant was identified by a botanist at biology department, Jimma University. The collected root parts were cleaned, and shade dried at the Organic Chemistry laboratory, Jimma University. The dried roots were crushed to a suitable size using an electric grinder to enhance the extraction process.

(a) (b)

Figure 1. Picture of leaf (a) and root (b) parts of *V. hymenolepis* from Jimma Arjo, Ethiopia (photo taken by Alemu Geleta).

Extraction of Crude Extracts

Using the cold maceration method, approximately 600 g of ground root material was successively extracted in 3 L of chloroform and methanol twice for 24 hours each. The crude extracts were filtered with Whatmann No.1 filter paper and concentrated using a rotary evaporator at 40°C to yield 10 g and 16 g of chloroform and methanol extracts, respectively.

Isolation of Compounds

The chloroform extract (7 g) was meshed with an equal amount of silica gel and subjected to silica gel column chromatography eluting with an increasing gradient of petroleum ether: ethyl acetate: methanol to afford 28 fractions of 25 ml each. The fractions (F1-F8) were colorless and likely contained fat. Fractions 9-12 (petroleum ether/ethyl acetate, 3:2) formed a white precipitate and were subsequently washed with petroleum ether to obtain compound **1** (8 mg). Fractions (19- 24) were combined on the basis of their TLC profile and subjected to further purification via column chromatography, with gradient elution from chloroform through ethyl acetate to methanol, resulting in compound **2** (13 mg).

Antimicrobial activity

Antibacterial test

Using the disc diffusion method, the *in vitro* antibacterial properties of the *V. hymenolepis* root extracts were assessed against four bacterial strains: two grams negative strains (*Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853) and two grams positive strains (*Staphylococcus aureus* ATCC25923 and *Bacillus subtilis* ATCC11778). Nutrient agar slants,

which were kept at 40°C, were used to maintain stock cultures of bacteria. To obtain final stock concentrations of 200 mg/mL in DMSO, 200 mg of the test samples were dissolved to prepare the test solutions. Newly grown liquid cultures of the test pathogen solution with turbidity comparable to 0.5 McFarland were seeded on the Müeller-Hinton agar media plates. For the bacterial cells and agar to mix equally, the plates were gently shaken. After being individually soaked in the aforementioned stock solutions (samples and standards), sterile paper discs with a diameter of 6 mm were placed evenly spaced over the seeded plates. Control experiments were performed under similar conditions by using gentamicin as a positive control and DMSO as a negative control. After that, the plates were turned over and incubated for a full day at 37 °C. Following the incubation period, a clearance zone surrounding the disks on the plates was observed. Every experiment was run three times. To evaluate the antibacterial activity of each test sample, the mean inhibition zone was calculated. Comparisons were made between the inhibitory zones generated by plant extracts and those generated by commercial conventional antibiotics (Ogundare et al., 2006).

Antifungal test

To test the antifungal activity of extracts of the plant against *Candida albicans,* the disc diffusion technique was used. Potato dextrose agar (PDA) medium was added to Petri dishes (diameter 90 mm) and activated for 24 hours. Then 0.3 ml of *Candida albicans* inoculum were seeded on activated medium. A sterile filter discs (diameter 6 mm, Whatman Paper No.1) were impregnated with test extracts (200 mg/ml) which were dissolved in dimethyl sulfoxide (DMSO) and placed on the surface of plate containing the microorganism. Clotrimazole was used as standard antifungal drug (positive control) while the solvent DMSO was used as a negative control. The experiments were repeated three times. The zones of growth inhibition around the discs were measured using transparent ruler after 72 hours at 25°C for (Mathela & Joshi, 2008; Ogundare et al., 2006; Veljic et al., 2008).

RESULTS AND DISCUSSION

Yield of Extracts

Table 1. Physical properties of the crude extracts.

Solvents	Color of Extracts	Mass of Extracts (g)	Yield (%)
Chloroform	Dark red	10	1.67%
Methanol	Black	16	2.71%

The extraction yield is a measure that quantifies how well a solvent extracts specific constituents from the source material. The percentage yield of the crude extract in the respective solvent was recorded. The methanol yield was relatively high (Table 1). This finding is consistent with several reports from the literature showing that the yield of extracts is lower for nonpolar solvents (Sintayehu & Adane, 2020).

Characterization of Isolated Compounds

Compound **1** was obtained as a white powder with an R_f value of 0.62 in ethyl acetate: petroleum ether (3:7). The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) (Table 2) indicated signals at δ_H 0. 98 (3H, s, H-23), 0.82 (3H, s, H-24), 0.73 (3H, s, H-25), 0. 93 (3H, s, H-26), 0.96 (3H, s, H-27) and 1.69 (3H, s, H-30) for six methyl groups; two geminal olefinic protons at δ_H 4.73 (1H, brs, H-29a) and 4.60 (1H, brs, H-29b), and five methine protons at δ_H 3.25 (1H, m, H-18) and 3.15 (1H, m, H-19), 2.00 (1H, m, H-5), 2.00 (1H, m, H-9), 2.12 (1H, m, H-13), and one oxymethine proton at δ_H 3.20 (1H, m, H-3).

The ^{13}C NMR spectrum (Table 2) showed signals for 29 carbon atoms consistent with a downward shifted carbon signals at δ_C 175.5, indicative of carboxylic acid carbonyl carbon, which is assignable to C-28 and the other six quaternary carbons at δ_C 37.7 (C-4), 42.2 (C-8), 35.9 (C-10), 42.2 (C-14), 51.6 (C-17) and 145.7 (C-22); five methine carbons at δ_C 51.5 (C-5), 51.5 (C-9), 37.7 (C-13), 44.5 (C-18), 45.8 (C-19), one oxymethine carbon at δ_C 74.3 (C-3); and eleven methylene carbons at δ_C 37.7 (C-1), 25.8 (C-2), 20.8 (C-6), 34.1 (C-7), 23.3 (C-11), 25.8 (C-12), 32.5 (C-15), 33.6 (C-16), 29.6 (C-20), 34.0 (C-21) and 104.9 (C-21); 6 methyl carbons at δ_C 29.6 (C-23), 16.1 (C-24), 16.1 (C-25), 16.1 (C-26), 13.6 (C-27) and 24.9 (C-30). The carbon signals at δ_C 104.9 (C-21) and 145.7 (C-22) indicates the presence of olefinic carbons, as in lupeol. As a result, using the spectroscopic data above and comparing them to relevant literature (Bisoli et al., 2008; Egbubine et al., 2020; Shin et al., 2009), the structure of compound **1** was found to be betulinic acid or 3β-hydroxy-lup-20(29)-en-28-oic acid (Figure 2). This compound was previously isolated from *Feretia canthioides* stem bark (Egbubine et al., 2020) and *Vernonia guineensis* root (Collins et al., 2020). Betulinic acid is a naturally occurring pentacyclic lupine-type triterpenoid that has several biological activities such as inhibition of human immunodeficiency virus (HIV), anthelmintic, antibacterial, anti-inflammatory, antimalarial, anti-HSV-1 and anticancer activities (Moghaddam et al., 2012).

Table 2. ^{1}H and ^{13}C NMR data (400 MHz, $CDCl_3$) of Compound 1.

Position	^{1}H NMR (m)	^{13}C NMR	Position	^{1}H NMR (m)	^{13}C NMR
1	1.25 (m)	37.7	16	1.70 (m)	33.6
2	1.27 (m)	25.8	17		51.5
3	3.20 (m)	74.3	18	3.25 (m)	44.5
4		37.7	19	3.15 (m)	45.8
5	2.00 (m)	51.5	20		29.6
6	1.37 (m)	20.8	21	1.85(m)	34.0
7	1.55 (m)	34.1	22		145.7
8		42.2	23	0. 98 (s)	29.6
9	2.00 (m)	51.5	24	0.82 (s)	16.1
10		35.9	25	0.73 (s)	16.1
11	1.60 (m)	23.3	26	0. 93 (s)	16.1
12	1.60 (m)	25.8	27	0.96 (s)	13.6
13	2.12 (m)	37.7	28		175.5
14		42.2	29a 29b	4.73 (brs) 4.60 (brs)	104.9
15		32.5	30	1.69 (s)	20.8

Key: brs = broad singlet, m = multiplet and s = singlet

Compound **2** was isolated as a light yellow powder with an R_f value of 0.45 (30% methanol in chloroform). The ^{1}H NMR spectrum (400 MHz, Acetone-d_6) (Table 3) revealed only two sets of aromatic protons at δ_H 7.12 and 6.85, which were integrated for two protons each and assigned to (H-5 and H-9) and (H-6 and H-8), respectively. It also indicated a down field shifted olefinic proton at δ_H 7.53 (1H, s, H-3), due to its peri-position to the carbonyl group.

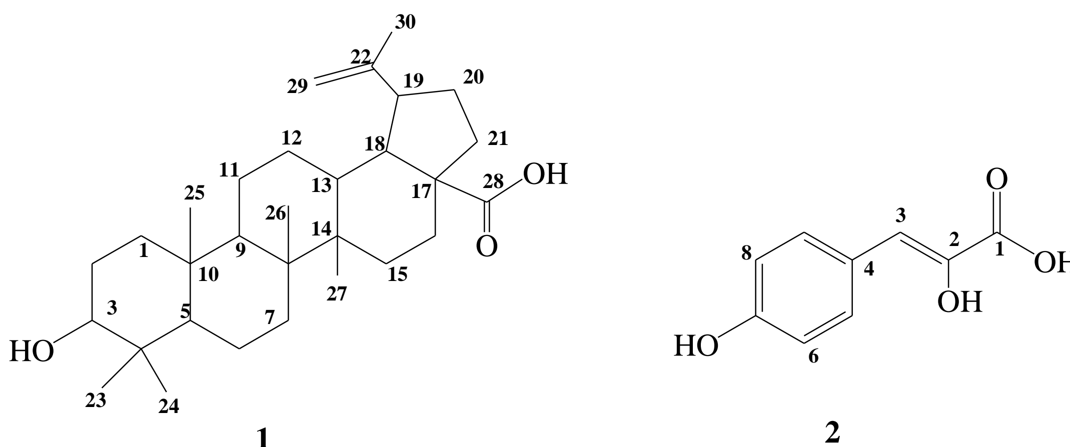

Figure 2. Chemical structures of compound **1** and **2.**

The ^{13}C NMR spectrum (Table 3) indicated the presence of seven signals for nine carbon atoms. The signal at δ_C 167.9 assignable to the carbonyl carbon (C-1), the signals at δ_C 155.5, and 129.9 correspond to C-4, and C-7, respectively and the intensified carbon signals (due to the symmetric effect) at 131.9 and 113.5 conforming two set of meta carbons (C-5 and C-9) and (C-6 and C-8), respectively. The carbon signals at δ_C 144.6, 118.1 and 128.2 were assigned to C-2, C-3 and C-4, respectively. Therefore, on the basis of the above spectroscopic data and a comparison of the data with the related literature (Ayoola et al., 2017; Somaia A. Al-Madhagy, Nada M. Mostafa, Fadia S. Youssef & Awad, Omayma A. Eldahshan, 2019), the structure of compound 2 was identified as 2-hydroxy-3-(4-hydroxyphenyl)-2-propenoic acid (Figure 2). This is the first report on the isolation of betulinic acid (1) and 2-hydroxy-3-(4-hydroxyphenyl) -2-propenoic acid (2) from *V. hymenolepis*.

Table 3. ^{1}H and ^{13}C NMR (400 MHz, Acetone-d_6) spectral data of compound 2.

Position	^{1}H NMR (m)	^{13}C NMR	DEPT-135
1	_	167.9	C
2	_	144.6	C
3	7.53 (s)	118.1	CH
4	_	128.2	C
5	7.12 (dd)	131.9	CH
6	6.85 (dd)	113.5	CH
7	_	153.5	C
8	6.85 (dd)	113.5	CH
9	7.12 (dd)	131.9	CH

Antimicrobial Activities

Using the disk diffusion method, the two crude extracts were evaluated for their *in vitro* antimicrobial activity against four bacterial strains and one fungal strain. The results were shown in Table 4 and indicated varying degrees of response.

Table 4. Zones of Inhibition (mm) for Bacterial and Fungal Growth.

Extracts and standards	Growth inhibition zone (mm)				
	Bacterial strains				Fungal strain
	B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***	***C. albicans***
Chloroform extract	14	17	18	11	NI
Methanol extract	13	12	14	13	13
Gentamicin	17	23	25	12	NT
Clotrimazole	NT	NT	NT	NT	12
DMSO	NI	NI	NI	NI	NI

Key: NI = no inhibition, NT = not tested and DMSO = dimethylsulfoxide.

The extracts exhibited significant antibacterial activity against all strains of both gram negative and gram positive bacteria, with inhibition zones ranging from 11-18 mm. The chloroform extract showed better antibacterial activities against *Bacillus subtilis*, *Staphylococcus aureus,* and *Escherichia coli* with inhibition zones of 14 mm, 17 mm and 18 mm, respectively, whereas the methanol extract displayed moderate activities. Both the chloroform and methanol extracts indicated substantial antibacterial activities against gram negative bacterium, *Pseudomonas aeruginosa* with zones of inhibition of 11 mm and 13 mm, respectively, which is comparable to that of the reference drug, gentamicin (12 mm). The observed activity may be due to the existence of phytochemical constituents such as flavonoids, triterpenes, and phenolics present in this plant (Mengome et al., 2010; Ndam et al., 2014). The methanol extract displayed the highest activity (13 mm) against *Candida albicans,* but the chloroform extract did not show any remarkable antifungal activity. These *in vitro* antimicrobial activities are in good agreement with those reported in a previous study conducted in Kenya (Onzago et al., 2014).

CONCLUSION

The chloroform root extract was subjected to silica gel column chromatography which yielded two compounds (betulinic acid and 2-hydroxy-3-(4-hydroxyphenyl)-2-propenoic acid). The extracts exhibited significant antibacterial activity against all gram positive and gram negative bacteria with inhibition zones ranging from 11 - 18 mm. The antimicrobial activity results agree with the outcomes of a previous study. Hence, these results support traditional use *V.hymenolepis* of in Ethiopia as well as its potential as the most abundant source of bioactive chemicals with antibacterial properties. Consistent with the previous research work, the extensive traditional use of this plant may be attributed to its terpenes and phenolic phytochemicals.

Data Availability: All the data used to support the findings of this study are available in the manuscript.

Disclosure: This manuscript was extracted from MSc thesis of the Jimma University institutional repository.

Conflicts of Interest: The authors declare that there are no conflicts of interest.

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REFERENCES

Abebe, W. (2016). An Overview of Ethiopian Traditional Medicinal Plants Used for Cancer Treatment. *European Journal of Medicinal Plants*, *14*(4), 1–16. https://doi.org/10.9734/EJMP/2016/25670

Alqahtani, A. S., Ullah, R., & Shahat, A. A. (2022). Bioactive Constituents and Toxicological Evaluation of Selected Antidiabetic Medicinal Plants of Saudi Arabia. *Evidence-Based Complementary and Alternative Medicine*, *2022*. https://doi.org/10.1155/2022/7123521

Alqahtani, R. U. and A. S. (2022). GC-MS Analysis, Heavy Metals, Biological, and Toxicological Evaluation of Reseda muricata and *Marrubium vulgare* Methanol Extracts. *Evidence-Based Complementary and Alternative Medicine*, *2022*. https://doi.org/https://doi.org/10.1155/2022/2284328

Ayoola, M. D., Balogun, J. O., Famuyiwa, F. G., Yeboah, S. O., & Famuyiwa, S. O. (2017). Isolation and characterization of anti-diabetic extract of the root bark of *Uvaria afzelii*. *South African Journal of Botany*, *112*, 527–532. https://doi.org/10.1016/j.sajb.2017.05.006

Bisoli, E., Garcez, W. S., Hamerski, L., Tieppo, C., & Garcez, F. R. (2008). Bioactive pentacyclic triterpenes from the stems of *combretum laxum*. *Molecules*, *13*(11), 2717–2728. https://doi.org/10.3390/molecules13112717

Bizuayehu, B., & Garedew, B. (2018). A review on the ethnobotanical study of medicinal plants used for the treatment of gonorrhea disease in Ethiopia. *Indian Journal of Natural Products and Resources*, *9*(3), 183–193.

Collins, S., Wouamba, N., Mouthé, G., Lenta, B. N., Sewald, N., & Fogué, S. (2020). Vernoguinamide : A new ceramide and other compounds from the root of *Vernonia guineensis* Benth . and their chemophenetic significance. *Biochemical Systematics and Ecology*, *88*(2), 103988. https://doi.org/10.1016/j.bse.2019.103988

Egbubine, C. O., Adeyemi, M. M., & Habila, J. D. (2020). Isolation and characterization of betulinic acid from the stem bark of *Feretia canthioides* Hiern and its antimalarial potential. *Bulletin of the National Research Centre*, *44*(49). https://doi.org/https://doi.org/10.1186/s42269-020-00302-2

Fagbohun, E. D., Ekiti, A., & Lawal, O. (2012). The proximate , mineral and phytochemical analysis of the leaves of *Ocimum gratissimum* L ., *Melanthera scandens* A . and *Leea guineensis* L . and their medicinal value . *International Journal of Applied Biology and Pharmaceutical Technology*, *3*, 15–22.

Khan, U. A., Rahman, H., Niaz, Z., Qasim, M., Khan, J., Tayyaba, & Rehman, B. (2013). Antibacterial activity of some medicinal plants against selected human pathogenic bacteria. *European Journal of Microbiology and Immunology*, *3*(4), 272–274. https://doi.org/10.1556/eujmi.3.2013.4.6

Mathela, C. S., & Joshi, N. (2008). Antimicrobial activity of Nepeta isolates. *Natural Product Communications*, *3*(6), 945–950. https://doi.org/10.1177/1934578x0800300625

Mengome, L., Akue, J. P., Souza, A., Raymond, G., & Tchoua, F. (2010). In vitro activities of plant extracts on human Loa loa isolates and cytotoxicity for eukaryotic cells. *Parasitology Research*, *107*(3), 643–650. https://doi.org/10.1007/s00436-010-1910-2

Mesfin, F., Demissew, S., & Teklehaymanot, T. (2009). An ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, *5*, 28. https://doi.org/10.1186/1746-4269-5-28

Moghaddam, M. G., Ahmad, F. B. H., & Samzadeh-kermani, A. (2012). Biological Activity of Betulinic Acid : A Review. *Pharmacology & Pharmacy*, *3*, 119–123. https://doi.org/http://dx.doi.org/10.4236/pp.2012.32018

Mrabti, H. N., Doudach, L., Mekkaoui, M., Khalil, Z., Harraqui, K., Fozia, F., Naceiri Mrabti, N., El-Shazly, M., Alotaibi, A., Ullah, R., Faouzi, M. E. A., & Bouyahya, A. (2022). Profile of Medicinal Plants Traditionally Used for the Treatment of Skin Burns. *Evidence-Based Complementary and Alternative Medicine*, *2022*. https://doi.org/10.1155/2022/3436665

Ndam, L. M., Mih, A. M., Fongod, A. G. N., & Tening, A. S. (2014). Phytochemical screening of the bioactive compounds in twenty (20) Cameroonian medicinal plants. *International Journal of Current Microbiology and Applied Sciences*, *3*(12), 1–12.

Ogundare, A. O., Adetuyi, F. C., & Akinyosoye, F. A. (2006). Antimicrobial activities of *Vernonia tenoreana*. *African Journal of Biotechnology*, *5*(18), 1663–1668.

Onzago, R. O., Kiama, S. G., Mbaria, J. M., Gakuya, D. W., & Nduhiu, J. G. (2014). Evaluation of antimicrobial activity and toxicity of *Vernonia hymenolepis* (A . Rich) traditionally used for toothache in Kenya. *The Journal of Phytopharmacology*, *3*(1), 22–28.

Parekh, J., Karathia, N., & Chanda, S. (2006). Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegata* L . bark. *African Journal of Biomedical Research*, *9*, 53–57.

Shahat, A. A., Ullah, R., Alqahtani, A. S., Alsaid, M. S., Husseiny, H. A., & Al Meanazel, O. T. R. (2018). Hepatoprotective Effect of *Eriobotrya japonica* Leaf Extract and Its Various Fractions against Carbon Tetra Chloride Induced Hepatotoxicity in Rats. *Evidence-Based Complementary and Alternative Medicine*, *2018*. https://doi.org/10.1155/2018/3782768

Shin, S. J., Park, C. El, Baek, N. I., Chung, I. S., & Park, C. H. (2009). Betulinic and oleanolic acids isolated from *Forsythia suspensa* Vahl inhibit urease activity of Helicobacter pylori. *Biotechnology and Bioprocess Engineering*, *14*(2), 140–145. https://doi.org/10.1007/s12257-008-0272-4

Sintayehu, N., & Adane, L. (2020). Phytochemical screening and isolation of fatty acid and fatty acid esters of triterpene from root extract of *Vernonia auriculifera* grown in Sidama zone , Southern Ethiopia. *Journal of Pharmacognosy and Phytochemistry*, *9*(1), 1702–1710.

Somaia A. Al-Madhagy, Nada M. Mostafa, Fadia S. Youssef, G. E. A., & Awad, Omayma A. Eldahshan, A. N. B. S. (2019). Isolation and Structure Elucidation of Compounds from *Coccinia grandis* Leaves Extract. *Egyptian Journal of Chemistry*, *62*(10), 1869–1877. https://doi.org/10.21608/ejchem.2019.10925.1700

Veljic, M., Tarbuk, M., Marin, P. D., Ciric, A., Sokovic, M., & Marin, M. (2008). Antimicrobial activity of methanol extracts of mosses from Serbia. *Pharmaceutical Biology*, *46*(12), 871–875. https://doi.org/10.1080/13880200802367502

Yineger, H., Kelbessa, E., Bekele, T., & Lulekal, E. (2019). Plants used in traditional management of human ailments at Bale Mountains National Park , Southeastern Ethiopia. *Journal of Medicinal Plants Research*, *8*(11), 1–22.