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Investigating optimal conditions for obtaining medicinal extracts with the best functional properties from *Kleinia anteuphorbium* L.

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ABSTRACT

Kleinia anteuphorbium L. is a medicinal plant widely utilized in traditional medicine by the Souss-Massa population. This study aimed to investigate the best extraction conditions, including method and solvents, to achieve the highest polyphenol, flavonoid, antioxidant, and antibacterial activities. The polyphenol and flavonoid contents were quantified using spectrophotometric methods, while the antioxidant activity was assessed through Ferric Reducing Power (FRAP) and 2,2-diphenyl-picrylhydrazyl radical (DPPH) scavenging assays. The antibacterial activity was assessed against four pathogenic clinical isolates: *P. aeruginosa, E. coli, K. pneumonia,* and *S. aureus*. The phytochemical analysis revealed that all extracts of *Kleinia anteuphorbium* exhibited high polyphenol and flavonoid contents, along with significant antioxidant activity. Moreover, the extracts showed inhibitory effects against the tested bacteria strains. The GC-MS analysis revealed the existence of abundant bioactive compounds, with potential therapeutic activities. These findings suggest that *Kleinia anteuphorbium* has promising potential as a medicinal plant.

Keywords: antibacterials, polyphenols, flavonoids, phenolics, antioxidants, GC-MS.

INTRODUCTION

Secondary metabolites are diverse organic compounds synthesized by plants to interact with their environment (Khare et al., 2020; Lamers et al., 2020). For humans, these metabolites serve as a valuable source of bioactive compounds, with potential applications in the pharmaceutical industries or food industries, such as food additives or other applications (Clemensen et al., 2020; Kirubakari et al., 2019).

Aromatic and medicinal plants have been used for healthcare and nutritional benefits for thousands of years. Both ancient and modern societies worldwide continue to utilize extracts and compounds derived from these plants for various purposes, particularly for therapeutic applications. According to the World Health Organization, 80% of the global population still relies on medicinal plants as a major source of primary health care (World Health Organization, 2022). In recent years, medicinal plant research has garnered significant interest as potential sources of novel bioactive compounds (Chrysargyris et al., 2024). Numerous studies have focused on natural products, especially those derived from aromatic and medicinal plants, to identify the compounds with highly selective and specific biological activities based on their mechanism of action (Bernardini et al., 2018). Among the bioactive compounds of interest are phenolic compounds, which exhibit significant biological activity, including antioxidant,

antibacterial (Al-Tayawi et al., 2024), and antifungal activities (Raja and Sreenivasulu, 2015).

The Arganeraie biosphere in Morocco has a diverse and rich flora. In addition to the iconic Argan trees (*Agrania spinosa*), several endemic species are found within the Arganeraie biosphere. Among these species, the conducted research focused on *Kleinia anteuphorbium* L., an endemic medicinal plant belonging to the Asteraceae family and native to the southwest of Morocco. To the best of authors' knowledge, no comprehensive information on the antioxidant and antibacterial activities of various *K. anteuphorbium* L. extracts is available. Hence, this study aimed to: (i) evaluate the antioxidant and antibacterial activities of different extracts of *Kleinia anteuphorbium* L., (ii) compare the extracts obtained using different extraction methods, and solvents in terms of yield, total phenolic, total flavonoids, as well as their antioxidant and antibacterial proprieties, and (iii) study the chemical and structure profile of the extract with the best antioxidant and antibacterial activities using GC-MS.

MATERIALS AND METHODS

Plant material

The aerial parts of *Kleinia anteuphorbium* L., an endemic medicinal plant, were collected in May 2020 from the Souss-Massa region, Morocco, during its flowering phase. The identification was conducted by the Department of Botany and Plant Ecology at the Scientific Institute in Rabat, Morocco. A voucher specimen (No.1141118) has been deposited in the Herbarium of the same institute.

Extracts preparation

The extraction was performed using two distinct methods: maceration (ME), and ultrasound-assistance extraction (UAE), to assess the impact of the extraction method on the chemical composition of the extracts. Methanol, water, and methanol (70%) were employed as solvents. In both methods, 2.5 g of *Kleinia anteuphorbium* L. powder was rigorously extracted with 50 mL of each solvent. For ultrasound-assisted extraction, the flasks were placed in an ultrasound bath (Tanssonic Tl-H-15, Germany) for 45 min, and the bath was set to 25 kHz at 25 °C. For maceration, the flasks were placed in a dark place with magnetic stirring for 24 h. Subsequently, the extracts were filtered through Whatman paper No.1, concentrated under reduced pressure at 40 °C, and stored at 4 °C until further use. The extraction yields were calculated as the difference between the weights of the plant powder used and obtained crude extracts multiplied by 100.

Total polyphenolic content

The TPC of *Kleinia anteuphorbium L.* was quantified using the Folin-Cioculteu colorimetric method with minor modifications as described by Ben ElHadj Ali et al., (2020). In brief, 50 µL of the extracts were mixed with 450 µL of Folin-Cioculteu (10%), and after 5 min, 7.5% of saturated aqueous sodium carbonate was added, then the mixture was incubated in the dark at room temperature. Following a 2-hour incubation, absorbances were measured at 756 nm using a UV-Vis spectrophotometer (RoHs, UV-Viz spectrophotometer-1800PC). The TPC was determined using the gallic acid calibration curve, and results were expressed as milligrams of gallic acid equivalents (mg GAE) per 100 g of dry weight.

Total flavonoid content (TFC)

The TFC was measured using the aluminum chloride assay, as described by Kocira et al., (2018), with some modifications. Briefly, 250 µL of plant extracts were mixed with 250 µL of aluminum chloride solution (10%), and then the mixture was allowed to settle for 1 h at room temperature in the dark. The absorbances of the resulting solutions were measured at 450 nm using a UV-Vis spectrophotometer (RoHs, UV-Viz spectrophotometer-1800PC). A calibration curve was prepared using Rutin as a standard. TFC was expressed as mg of Rutin equivalent (RE) per 100 g dry weight.

Antioxidant activity

DPPH radical scavenging activity

The radical scavenging activity of extracts was evaluated using the DPPH radical assay with slight modifications (Nascimento et al., 2018). Firstly, 50 µL of samples or a standard were mixed with 950 µL of a methanolic solution of DPPH (63.4 µM) and then incubated for 30 min in the dark at room temperature. The absorbances

were read at 517 nm using a spectrophotometer (RoHs, UV-Viz spectrophotometer-1800PC). Ascorbic acid was used as the standard.

Ferric reducing antioxidant power

The FRAP method was conducted following the method described by YILDIRIM et al., (2001), with minor modifications. In test tubes, 250 µL of sample or standard solution was mixed with 125 µL of phosphate buffer and 125 µL of 1% aqueous potassium hexacyanoferrate solution. The mixture was incubated in a water bath at 50 °C for 20 minutes. After incubation, 125 µL of 10% trichloroacetic acid was added, and the reaction mixture was centrifuged for 10 minutes, then 125 µL of supernatant was mixed with 125 µL of water and 250 µL of freshly prepared 0.1% $FeCl_3$ solution. The absorbance was read at 700 nm using a UV-Vis spectrophotometer (RoHs, UV-Viz spectrophotometer-1800PC). The FRAP antioxidant capacity was calculated using a calibration curve ($y = 3.9988x + 0.3138$, $R^2 = 0.998$) obtained from the ascorbic acid standard.

Antibacterial activity

In this study, clinical strains of pathogenic and antibiotic-resistant, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumonia*, were selected due to their significant clinical relevance, rising rates of resistance, and impact on public health, which are critical for understanding and addressing contemporary challenges in infectious disease management. The antibacterial activity of *Kleinia anteuphorbium L.* was evaluated using the well-diffusion method described by Jaradat et al. (2022). In Petri plates containing the Luria-Bertani agar inoculated with 1 mL of the bacterial suspension, wells with a 5 mm diameter were aseptically drilled using a sterile plug drill. Subsequently, 20 µL of the extract solution (50 mg/ml) was introduced into the well. The plates were incubated at 37 °C for 24 h, and antibacterial activity was estimated by measuring the inhibition zone in millimeters.

Acetylation reaction

The acetylation process was carried out with minor modifications following the method published by Haida et al. (2020). Five milligrams of the methanolic extract were dissolved in one milliliter of acetic anhydride, and four drops of pyridine were added. After two hours of heating to 700 °C, the mixture was left to cool overnight. After cooling, 6 ml of distilled water was added to the mixture and stirred in an ice-water bath to hydrolyze it. The organic phase was then dried over anhydrous sodium sulfate, condensed under reduced pressure, and rinsed with a saturated solution of sodium hydrogen carbonate after the aqueous phase was extracted using 10 milliliters of chloroform.

Analysis of extracts by gas chromatography-mass spectrometry

The gas chromatography-mass spectrometry analysis of *Kleinia anteuphorbium L.* extract was performed at the National Institute of Agriculture Research. The employed instrument was a Bruker 456-GC, EVOQ TQ system that operates in electronic impact mode with an ionization energy of 70 eV and has an RX-5ms capillary column (30 m × 0.25 mm ID × 0.25 mm df). The injection and ion source temperatures were kept at 280 °C and 250 °C, respectively. the initial temperature was set at 65 °C for 10 minutes, then increased to 10°/min at 80 °C for 3 minutes, and finally by 2°/min at 300 °C for 5 minutes. Helium was used as a carrier gas with a constant flow rate of 1 ml/min. The volume of the injection solution was 15 ml. The compounds were identified by studying their mass spectrum and confirmed through the apparatus database.

Statistical analysis

Three duplicates of each experiment were carried out. The result is presented as the mean± standard deviation. IBM SPSS 22.0 version was used to perform the statistical analysis. The analysis of variance (ANOVA), and the Tukey test were used to compare all means at a 0.05 significance level.

RESULTS

The extraction yields

The extraction yields obtained ranged from 21.82 ± 0.5% to 13.7 ± 0.1%, as detailed in Table 1. For the maceration method, the highest extraction yield was obtained with the aqueous extract, followed by the methanol (70%) solution, while the methanolic extract yielded the lowest.

Table 1. Percentage extraction yields

Extraction methods	Yield (%)		
	ME	Methanol (70%)	WE
Maceration	$15.6^{a} \pm 0.2$	$17.36^{b} \pm 0.3$	$21.82^{c} \pm 0.5$
UAE	$13.7^{a} \pm 0.1$	$19.08^{b} \pm 0.13$	$20.78^{c} \pm 0.3$

Note: ME – methanol extract, methanol (70%) – methanol/water extract, WE – water extract.

For UAE, the highest yield was also obtained by aqueous extract, followed by aqueous methanol, and the lowest yield was obtained by methanolic extract. For both extraction methods, the aqueous extracts consistently exhibited the highest extraction yields. This could be attributed to the capacity of water to extract the polar substances present in the plant material, including carbohydrates and proteins (Chirinos et al., 2007).

Total polyphenols content

The total polyphenol contents (TPC) of *Kleinia anteuphorbium L.* extracts obtained by ultrasound assistance extraction (UAE) and maceration (ME) using different solvents are illustrated in Figure 1. For UAE extracts, the highest TPC was obtained by aqueous extract (787.92 ± 17.33 mg GAE/100 g DW), followed by aqueous methanol solution (686.72 ± 10.01 mg GAE/100 g DW), whereas the lowest TPC was obtained by methanolic extract (471.83 ± 4.04 mg GAE/100 g DW). For ME extracts, the highest TPC was recorded in aqueous extract (1013.09 ± 10.51 mg GAE/100 g DW), followed by aqueous methanol extract (821.63 ± 2.52 mg GAE/100 g DW), and the lowest TPC was obtained by methanolic extract (490.53 ± 11.48 mg GAE/100 g DW).

Total flavonoid content

Total flavonoid contents (TFC) in *Kleinia anteuphorbium L.* stem were evaluated, and the results are illustrated in Figure 2. Significant differences ($p < 0.05$) were observed in the flavonoid content of *Kleinia anteuphorbium L.* extracts. The highest TFC amounts were recorded in methanolic extracts with 170.16 ± 0.35 mg RE/100 g DW for maceration (ME) and 146.97 ± 0.2 mg RE/100 g DW for ultrasound-assisted extraction.

The antioxidant activity

Radical scavenging activity

The DPPH radical scavenging capacity of *Kleinia anteuphorbium L.* extracts is illustrated in Table 2. The results indicate that the scavenging

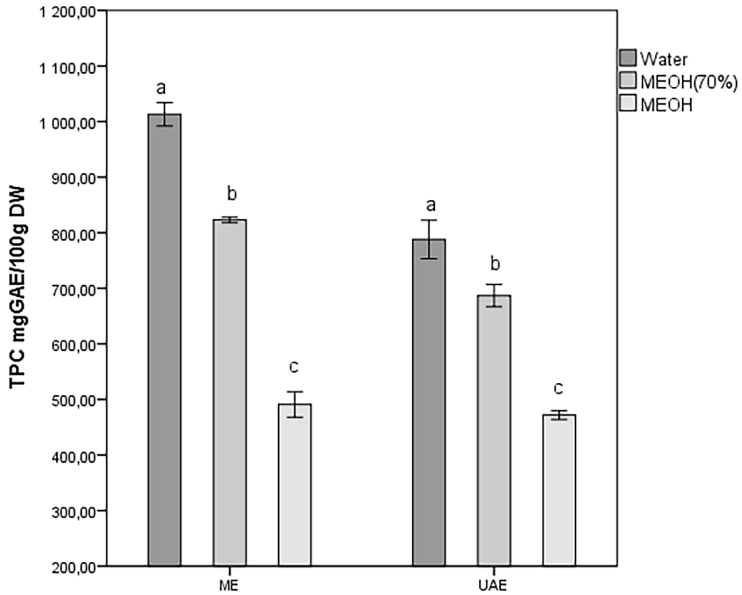

Figure 1. Total phenolic content (TPC) of *Kleinia anteuphorbium L.* extracts. Values are expressed as mean ± standard deviation. Letters (a, b, and c) within the same group (ME, UEA) indicate significance at $p < 0.05$

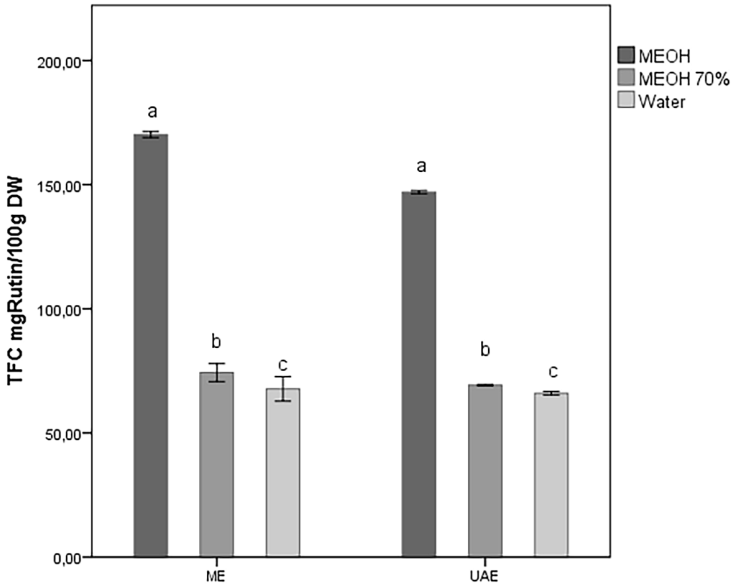

Figure 2. Total flavonoid content of different extracts of *Kleinia anteuphorbium L.* values are expressed as mean ± standard deviation. Letters (a, b, and c) within the same group (ME, UEA) indicate a significant difference ($p < 0.05$)

capacity of the extracts varies depending on the solvent and the extraction method employed. Statistically significant differences ($p<0.05$) were observed in the antioxidant activity among the different extracts. The aqueous methanolic extract showed the highest DPPH scavenging capacity, irrespective of the extraction method, followed by methanol extract, and the lowest radical scavenging capacity was obtained with aqueous extracts. Nevertheless, ascorbic acid showed higher radical scavenging capacity than all the tested extracts (IC50 = 0.074 mg/ml).

FRAP analysis

Ferric reducing antioxidant power of *Kleinia anteuphorbium* L. extracts, is presented in Table 2. The methanolic extracts of *Kleinia anteuphorbium* obtained by maceration and UAE exhibited higher antioxidant activity, followed by aqueous methanol extracts, and the lowest antioxidant activity was recorded by aqueous extracts. Overall, methanol proved to be the most effective solvent for extracting compounds with the highest ferric-reducing power among the solvents tested.

Antibacterial activity

On the basis of the classification established by Moreira et al., (2005), inhibition zones were classified as sensitive (9–14 mm) or very sensitive (15–19 mm). All tested extracts showed notable antibacterial activity against *Pseudomonas aeruginosa*, with the aqueous methanol extract obtained via ultrasound-assisted extraction exhibiting the highest efficacy, producing an inhibition zone of 19 ± 0.1 mm. For *Escherichia coli,* the most effective extract was the aqueous extract obtained by maceration, with an inhibition zone of 12 ± 0.04 mm. In the case of *Klebsiella*

Table 2. Antioxidant activity of different extracts from *Kleinia anteuphorbium L.* determined by DPPH and FRAP assays

Extracts	Maceration		UAE	
	DPPH (IC_{50} mg/ml)	FRAP (mgAAE /100g DW)	DPPH (IC_{50} mg/ml)	FRAP (mgAAE /100g DW)
MeOH	$0.38^{b} \pm 0.02$	$850.79^{c} \pm 1.7$	$0.364^{b} \pm 0.03$	$734.87^{c} \pm 0.8$
MeOH (70%)	$0.308^{a} \pm 0.03$	$380.29^{b} \pm 4.5$	$0.261^{a} \pm 0.06$	$346.26^{b} \pm 0.5$
Water	ND	$338.69^{a} \pm 1.8$	ND	$330.01^{a} \pm 0.9$

Note: ND: not detected. All values are presented as the mean ± standard deviation of triplicates. Subscript letters within the same column indicate a significant difference ($p < 0.05$) difference according to the Tukey test of means.

pneumonia, the aqueous methanol extract was the only effective extract, with an inhibition zone of 15 ± 0.05 mm. None of the tested extracts effectively inhibited the growth of *Staphylococcus aureus* (Table 3).

GC-MS analysis results

The analysis results of the Methanolic extract of *Kleina anteuphorbium L.* by GC-MS are illustrated in Table 4 and Figure 3. The GC-MS analysis of the methanolic extracts of *Kleina anteuphorbium L.* revealed the presence of 38 compounds. The major compounds were 1-Decene,3,4-dimethyl with a peak area of 56.63%, Germanicol, and Lupeol with a peak area of 11.36% and 8.31, respectively. Also, the analysis revealed the presence of two alkaloids, 1H-Pyrazol,5-(t-butyl)-3-phenyl- and 2-Pyrrolidinone,5-(cyclohexylmethyl)-, However, these compounds are present in modest quantities with the peak area of 0.28% and 0.065% respectively. Also, Propyl gallate (0.32%), was identified in modest quantity.

DISCUSSION

Plants produce an enormous variety of secondary metabolites with strong biological activities. The presence of these compounds in high levels in plant extracts indicates the potential of medicinal plants. Due to the diversity and chemical properties of these compounds, no specific protocol exists that can be applied to all medicinal plants.

The results indicated that the use of different solvents and extraction methods influences extract yields, which can be attributed to the nature of the secondary metabolites being extracted. Among the solvents tested, the water extract showed a significant difference ($p < 0.05$) compared to other solvents. The highest yield was recorded in the water extract, while the methanolic extract yielded the lowest amount. This difference may be due to the capacity of water to extract the polar substance present in the plant under study, including carbohydrates and proteins. These findings are consistent with those reported by Dirar

Table 3. Antibacterial activity of *Kleinia anteuphorbium* L. expressed as zones of inhibition (mm)

Extracts	*Pseudomonas aeruginosa*	*Escherichia coli*	*Klebsiella pneumonia*	*Staphylococcus aureus*
Mac-MeOH	18 ± 0.03^{c}	7 ± 0.03^{a}	15 ± 0.05^{c}	9 ± 0.2^{c}
Mac-MeOH (70%)	12 ± 0.05^{a}	10 ± 0.03^{b}	8 ± 0.02^{b}	7 ± 0.6^{b}
Mac-Water	15 ± 0.05^{b}	12 ± 0.04^{c}	6 ± 0.7^{a}	6 ± 0.5^{a}
UAE -MeOH	19 ± 0.1^{c}	8 ± 0.07^{b}	7 ± 0.9^{a}	8 ± 0.4^{b}
UAE-MeOH (70%)	15 ± 0.02^{b}	8 ± 0.8^{b}	7 ± 0.31^{a}	5 ± 0.9^{a}
UAE-Water	13 ± 0.01^{a}	6 ± 0.2^{a}	8 ± 03^{b}	5 ± 015^{a}

Note: all values are presented as the mean± SD of triplicates. Subscript letters within the same column indicate a significant ($p < 0.05$) difference according to the Tukey test of means. MeOH: Methanol; UAE: ultrasound-assisted extraction; MAC: Maceration.

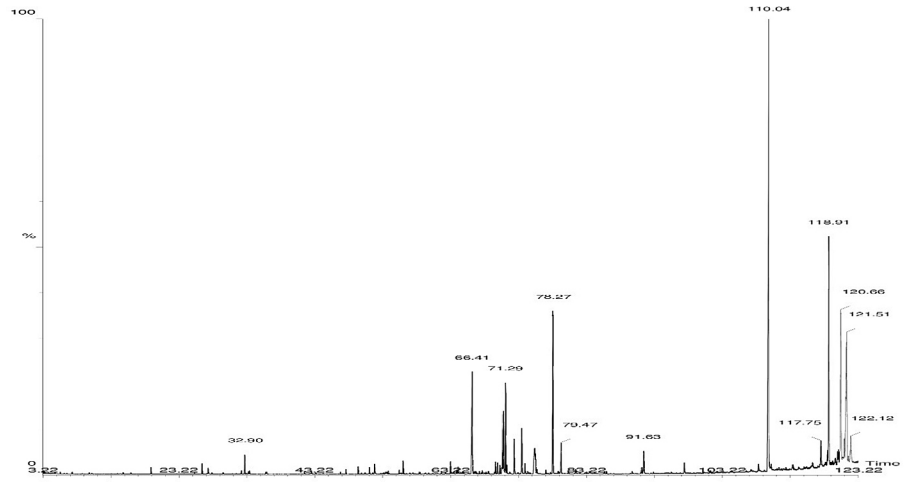

Figure 3. GC-MS chromatogram of the acetylated derivative of Methanolic extract of *Kleinia anteuphorbium* L.

Table 4. GC-MS analysis of methanolic extract of *K. anteuphorbium* L.

Number of peaks	RT (min)	Name of the compound	Formula	Area %
1	3.029	Hydroxy(4-benzyloxy-3-chlorophenyl) acetic acid, methyl ester	C16H15ClO4	0.04513022
2	3.334	1,1-Dimethyl-3-chloroprocaine	C18H24O12	0.03888999
3	5.684	3-Dioxolane, 2-(3-methoxypropyl) -2-methyl-	C8H16O3	0.03859369
4	16.104	Hexadecaneperoxoic acid, 1,1-dimethyl-3- [(1- oxohexadecyl) oxy]propyl ester	C37H12O5	0.05958141
5	25.548	psi.,.psi.-Carotene, 3,3',4,4'-tetradehydro-1',2'- dihydro-1-hydroxy-1'-methoxy	C41H58O2	0.04426615
6	26.603	2-Carene	C10H16	4.21847778
7	27.363	13-Tetradecynoic acid, methyl ester	C15H26O2	0.0399789
8	27.533	5-Caranol, trans,trans-(+)-	C10H18O	0.29130878
9	29.774	3-Methyl-2-furoic acid	C6H6O3	0.07596032
10	32.365	N-(5-Oxo-tetrahydro-furan-2-ylmethyl)-acetamide	C7H12NO3	0.14615896
11	32.90	1,2,3-Propanetriol, 1-acetate	C5H10O4	0.64005234
12	33.621	L-Proline, 1-acetyl-, methyl ester	C8H13NO3	0.11870707
13	36.057	2-Pyrrolidinone, 5-(cyclohexylmethyl)-	C11H19NO	0.06516075
14	41.709	Decane, 2,9-dimethyl-	C12H26	0.08748102
15	42.689	Phenol, 2,4-bis(1,1-dimethyl ethyl)-	C14H22O	0.17240586
16	51.273	1H-Pyrazole, 5-(t-butyl)-3-phenyl-	C13H16N2	0.27878963
17	52.048	1H-Inden-1-one, 2,3-dihydro-3,3-dimethyl-	C11H12O	0.44169782
18	58.646	Dodecane, 5,8-diethyl-	C16H34	0.09595268
19	60.006	Hexadecanoic acid, 1-(hydroxymethyl)-1,2- ethanediol ester	C35H68O5	0.04189866
20	62.477	5,8,11-Heptadecatriynoic acid, methyl ester	C18H24O2	0.10136452
21	63.658	Triarachine	C63H122O6	0.07087737
22	64.928	Dodecanoic acid, methyl ester	C13H26O2	0.06014442
23	66.40	1-Decene, 3,4-dimethyl-	C12H24	56.6268689
24	67.899	Cytidine, 5-methyl-	C10H15N3O5	1.1237832
25	68.210	Undecanoic acid, ethyl ester	C13H26O2	0.04676296
26	71.29	D-Glucose, 2,3,4,5,6-pentaacetate	C16H22O11	3.35939602
27	71.476	Propyl gallate	C10H12O5	0.32016001
28	73.707	Scyllo-inositol, hexaacetate	-	2.03956076
29	78.27	Isolongifolene, 4,5,9,10-dihydro-	C15H20	6.57197263
30	79.099	(+)-N-Acetylmuramic acid	C11H19NO8	0.08786301
31	91.339	Phthalic acid, di(2-propyl pentyl) ester	C24H38O4	0.2276002
32	93.104	Dodecanoic acid, methyl ester	C13H26O2	1.1327615
33	106.765	2-Piperidinecarboxylic acid, 1-acetyl-, ethyl ester	-	0.11189898
34	109.49	Astaxanthin	C40H52O4	0.06496507
35	114.268	Stearic acid, 3-(octadecyl oxy)propyl ester	C39H78O3	0.03819147
36	117.74	1,1,6-trimethyl-3-methylene-2-(3,6,9,13- tetramethyl-6-ethenyl-10,14-dimethylenepentadec-4-enyl) cyclohexane	C33H56	1.4106716
37	118.91	Germanicol	C30H50O	11.3560312
38	120.66	Lupeol	C30H50O	8.30863412

et al., (2019). It is important to note that extraction yield can be affected by many other factors, including the chemical nature of the phytochemicals, the extraction method employed (Zeroual et al., 2021), the particle size of the sample, the geographical origin of the plant (Ait Bouzid et al., 2023), and the presence of interfering compounds (Jan et al., 2021).

Plant-derived polyphenols and flavonoids have been reported to possess many pharmaceutical effects. Polyphenols have been found to affect the production of anti-inflammatory

and pro-inflammatory cytokines, which can reduce the incidence of many inflammatory diseases, such as Rheumatoid arthritis (Ferraz et al., 2020). Furthermore, studies have shown that polyphenols could be a supplementary treatment for diabetes mellitus because they can modulate carbohydrate and lipid metabolism, insulin resistance as well as alleviate oxidative stress and inflammatory processes (Dirar et al., 2019; Zeroual et al., 2021). According to the obtained results, the polarity of the extraction solvent affected the value of the TPC, i.e. Increase in the solvent polarity increases the yield of total phenolic content. TPC content can be ranked as water extract > aqueous methanolic extract > methanol extract. The present results are in line with those reported by Nawaz et al., (2020), who registered that the amount of TPC increases depending on the polarity of the solvent. Also, Ait Bouzid et al., (2022) while investigating the phytochemical composition of *Ziziphlus lotus* L. found that the extraction solvent significantly influenced the TPC content, which is in agreement with the present finding. Likewise, the addition of water to methanol enhances TPC. It has been shown that polyphenols are extracted in high amounts by the more polar solvents (Alara et al., 2021). In addition, Vural et al., (2020) found that adding water to organic solvent enhances TPC compared to the use of absolute solvents.

The TFC assay showed that the aqueous extracts exhibited the lowest TFC, which may be attributed to the low solubility of flavonoids in water (De Luna et al., 2020). numerous studies have found the use of both water and organic solvents enhances the TFC (Ngo et al., 2017). However, in the current study, methanol was the most efficient solvent for extracting flavonoids from *Kleinia anteuphorbium* L. The flavonoid content obtained in this study was higher than that obtained by Metrouh-Amir et al. (2015), for *M. pubesens* a plant from the same family (Asteraceae), which had TFC values ranging from 0.93 g QE/100DW to 0.47 g QE/100DW.

Antioxidants are well recognized for their ability to neutralize free radicals, which are implicated in numerous diseases such as oxidative stress, cancers, inflammatory disorders, diabetes, etc. (Kang and Yang, 2020). Free radicals result from an imbalance of oxidative stress, potentially leading to several human diseases (Sies and Jones, 2020), such as inflammation disorders. Several reports have shown that oxidative stress induces the expression of genes responsible for initiating inflammatory intermediaries, as well as cyclooxygenase, and lipoxygenase enzymes (Ngo et al., 2017; De Luna et al., 2020). This highlights the interrelationship between inflammation and antioxidants. In the present study, the antioxidant activity of *Kleinia anteuphorbium* L. was determined using DPPH and FRAP assays. The diversity of antioxidants and their distinct properties necessitates employing multiple methodologies to evaluate the antioxidant ability of plant extracts. The two techniques used in the present study had different mechanisms: the FRAP assay measures the capacity to reduce ferric ion (Fe^{3+}) to ferrous (Fe^{2+}), while the DPPH assay evaluates the total antioxidant capacity of compounds capable of donating hydrogen atoms. The results showed that the extraction conditions significantly influence the antioxidant ability of the extracts. In this analysis, *Kleinia anteuphorbium* L extracts exhibited antioxidant capacities with IC50 values of 0.261–0.380 mg/ml and 330–850 mg AAE/100 g DW in the DPPH and FRAP assays, respectively. Methanol and methanol (70%) extracts showed higher antioxidant activity, whereas aqueous extracts showed modest activity. This variation is possibly due to the capacity of organic solvents to extract strong antioxidant agents, such as flavonoids. These results align with previous studies in the literature, reporting higher antioxidant activity when methanol was used as an extraction solvent. Ezez et Tefera, (2021) reported that the methanol extract of ginger had the highest antioxidant activity among various extracts. Moreover, other factors such as the extraction method, can also influence the antioxidant activity. López-Perea et al. (2019) found that the extraction conditions or procedures influence antioxidant efficacy. Additionally, domestication could also be a factor that can affect antioxidant activity, Sakar et al. (2023) found that domestication affects the antioxidant activity of *Rosmarinus officinalis* essential oil.

The variation in the antioxidant activity may be related to the quality of polyphenols extracted by each solvent, as shown in the results. However, the proficiency of *Kleinia anteuphorbium* L. extracts to neutralize DPPH radicals was superior compared to the antioxidant activity of *Matricaria Pubescens,* another medicinal plant from the Asteraceae family, which exhibited a significantly higher IC_{50} value of 4.14 mg/mL (Metrouh-Amir et al., 2015).

Medicinal plants are rich in a wide variety of bioactive compounds, including polyphenols, alkaloids, and terpenoids, which have antibiotic properties. Treatment with medicinal has been proposed as a potential solution to treat bacterial infections (Chiavari-Frederico et al., 2020). The present study investigated the antibacterial activity of *Kleinia anteuphorbium* L. crude extracts against Gram-positive and Gram-negative bacterial strains. The results revealed significant differences ($p < 0.05$) in the inhibition of bacterial growth among the tested bacteria. As reported in several studies, these variations in bacterial sensitivity may be attributed to the effect of extraction parameters, such as Temperature, pH, Extraction time, and Solvent polarity on the bioactive compound composition, consequently affecting bacterial susceptibility (Ezez and Tefera, 2021; Li et al., 2022; López-Perea et al., 2019). According to the literature, Gram-positive bacteria are generally more sensitive than Gram-negative bacteria due to differences in cell wall structure and composition (Bilal and Hossain, 2019; Sun et al., 2022). However, In the present study, *Kleinia anteuphorbium L.* extracts showed antibacterial activity against Gram-negative bacteria (*P. aeruginosa, E. coli,* and *K. pneumonia*). In contrast, the Gram-positive bacteria (*S. aureus*) were resistant to all the tested extracts. These findings align with those of Barral-Martinez et al., (2022), who reported the resistance of *S. aureus* to the extract of *Chamaemelum nobile* and *Arnica montana,* (both from the Asteraceae family), while *P. aeruginosa* was found to be the most sensitive strain. Conversely, Surco et al., (2022) reported that *Senecio nutans* extracts (Asteraceae) exhibited antibacterial activity against Gram-positive bacteria but not against Gram-negative bacteria. Those differences observed in the antibacterial activity among plant species may attributed to differences in their phytochemical composition and the efficiency of the extraction conditions in isolating bioactive compounds from plant material.

Gas chromatography-mass spectrometry (GC-MS) is a highly effective analytical method commonly employed for identifying and quantifying secondary metabolites (Olivia et al., 2021). The analysis of the methanolic extract of *K. anteuphorbium* L. showed that the extract obtained with methanol was composed of a diversity of chemical compounds, in total 38 compounds were identified. These compounds belong to different classes, including esters, alcohols, terpenes, hydrocarbons, and other compounds such as triterpenes and alkaloids. On the basis of the data, some of the compounds identified by GC-MS exhibited biological activities. It has been proven that they process pharmacological activities that may contribute to the healing potential of *K. anteuphorbium*. Lupeol and Germanicol are triterpenoids that exhibit anti-inflammatory, antioxidant, and anticancer properties (Srisawat et al., 2020). In particular, Lupeol is known for its potential to inhibit tumor growth and prevent oxidative stress (Park et al., 2023). Phenol,2,4-bis (1,1-dimethylethyl), is known for its antioxidant properties. Terpenes such as 2-Carene and isolongifolene have been found to have anti-inflammatory, antimicrobial, and anticancer activities. Astaxanthin a powerful antioxidant, is also known for its anti-inflammatory effects. Rather et al., (2021) reported that crude Astaxanthin extracts have antibacterial activity. In turn, 2-Pyrrolidinone has demonstrated potential antioxidant and anti-cancer activity (Sebastian and Anto, 2021).

CONCLUSIONS

The results showed that the extraction conditions significantly influenced the phytochemical composition, antioxidant activity, and antibacterial activity of *Kleinia anteuphorbium*. Methanol and aqueous methanol were the most effective solvents for enhancing the flavonoid content, antioxidant, and antibacterial activities, while water extracts had the highest polyphenol content. GC-MS analysis identified 38 compounds, with potential bioactivities. The identification of the chemical composition and the optimal extraction conditions bridge a critical gap in understanding the plant's functional properties. The obtained results successfully highlight the potential of *Kleinia anteuphorbium* as a natural source of antioxidants and antibacterial compounds. Furthermore, additional studies are required to fully understand the potential of *Kleinia anteuphorbium extract* and advance its application in the pharmaceutical industry.

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