Faculty of Natural and Applied Sciences Journal of Scientific Innovations
Print ISSN: 2814-0877 e-ISSN: 2814-0923
www.fnasjournals.com
Volume 3; Issue 3; September 2022; Page No. 1-10.

PHYTOCHEMICAL ANALYSIS AND ASSESSMENT OF THE ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF THE AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF LIFE PLANT (*KALANCHOE PINNATA* L.) IN THE NIGER DELTA, NIGERIA

***[1]Hamilton-Amachree, A., & [2]Uzoekwe, N. M.**
[1]Department of Chemistry, Faculty of Science, Federal University Otuoke, Bayelsa State, NIGERIA
[2]Department of Physical Sciences, Benson Idahosa University, Benin City, Edo State, NIGERIA

***Corresponding author**: hamiltonaa@fuotuoke.edu.ng

Abstract
This study investigates the antibacterial and antioxidant activity of the aqueous and ethanolic leaf extracts of *Kalanchoe pinnata* L. Phytochemical screening, conducted via standard techniques, yielded: alkaloids, flavonoids, phenolics, saponins, steroids and tannins in the aqueous and ethanolic leaf extracts. The Agar Well Diffusion Method was employed to screen the respective *k. pinnata* leaf (KPL) extracts for antibacterial activities against the test microorganisms: *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus.* The results revealed that the ethanolic leaf extract inhibited all the test organisms with zones of growth inhibition ranging between 10-22 mm as against 30 mm observed for gentamicin, the standard antibiotic. However, the aqueous leaf extracts were only active against *staphylococcus aureus*. The antioxidant activity based on DPPH assay showed the % inhibition ranges of 48.83 – 68.02 % for the ethanol KPL extract and 76.97 – 85. 52% for the reference antioxidant, Ascorbic Acid (AA). The antioxidant potential of KPL extract was comparable to that of AA as depicted by the IC_{50} values of 1.485 mg/mL and 1.038 mg/mL respectively. The macro elements determined in KPL extracts gave values: Ca (75.36 ± 0.17 mg/L), Mg (18.11 ± 0.18 mg/L), Na (6.14 ± 0.24 mg/L) and K (5.55 ± 0.022 mg/L). The results thus obtained indicate the antibacterial, antioxidant as well as nutritional potential of the KPL extracts. Consequently, the aqueous and ethanolic leaf extracts of this plant show ample prospects for widespread use in traditional medicine in the Niger Delta Region of Nigeria.

Keywords: Phytochemicals; antibacterial; antioxidant; elemental analysis and *Kalanchoe pinnata*

Introduction

Herbal medicines have been in use in managing, controlling and curing ailments since the stone age. Man has been largely reliant on plants for basic needs such as food and shelter. Many domestic herbs and spices that Africans have depended on for food and shelter are also packed with phytochemicals. These bioactive compounds occur naturally and are useful for preventing or combating diseases as a result of their antioxidant and antimicrobial properties (Lesley, 2004; Majaz et al., 2011). The plants reportedly rich in phytochemicals have become even more important given the worldwide health risk associated with the persistent resistance to synthetic antimicrobial drugs, thus revealing plant sources as viable and innovative alternatives (Tajudin & Ismail, 2022; De Araujo et al., 2018). *Kalanchoe pinnata* L. has been used over the years as a complementary alternative therapeutic remedy in Nigeria and other African countries. Previously known as *Bryophyllum pinnatum*, this plant is also commonly called cathedral bell, air plant, miracle plant or life plant, but it is locally called '*Beri*' (meaning 'ear plant' due its succulent leaves) by the ijaw-speaking people of Bayelsa State, Nigeria. *K. pinnata* which is a luxuriant perennial plant belonging to the family: Crassulaceae grows to approximately 1-meter-tall, has fleshy cylindrical stems and broad elliptical curve-shaped leaves with crimson crenate edges. This plant is natural in Madagascar and has now naturalized in subtropical and tropical areas of the globe (Al Snafi, 2013). Studies have reported its antileishmanial (Muzitano et al., 2006; Cavalcanti De Queiroz et al., 2014), hepatoprotective, antiulcer (Pal & Chaudhuri, 1991; Ilyas et al., 2016), antidiabetic, and antinociceptive properties (Ojewole, 2005; Forouzanfar & Hosseinzadeh, 2018). *K. pinnata* leaf extracts have also been employed in antiseptic and anti-inflammatory formulations and subsequently used in treating respiratory infections, sores, and lacerations in

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Guyana (El Abdellaoui et al. 2010) In Southern Nigeria, the juice extracted from the fresh succulent leaves of this plant is applied to the umbilical cord of infants to aid the healing process. Although various extraction methods have been reportedly employed in obtaining the leaf extracts of *K. pinnata* for medicinal preparations (Bhavsar et al., 2018; Kendeson et al., 2019), fresh leaf juice remains the preferred extract for ethnomedicinal formulations in Bayelsa State. Based on the foregoing information, the need, therefore, arises to explore the efficacy of other extracts obtained by solvent extraction for possible widespread use in traditional medicine in this region. This study is therefore aimed at investigating the antibacterial and antioxidant properties of solvent extracts of the leaves of *Kalanchoe pinnata* L. randomly harvested from the backyard gardens of Otuoke, a suburb situated in the Niger Delta region of Nigeria. This study consequently reports the qualitative phytochemical analysis, the antioxidant and antibacterial activities as well as the essential macro elemental analysis of the aqueous and ethanolic extracts of *Kalanchoe pinnata* leaves.

Fig 1: *Kalanchoe pinnata* plant

Materials and Methods

Sample collection and pretreatment

Kalanchoe pinnata was harvested randomly from some random backyard gardens in Otuoke and subsequently authenticated by a taxonomist in the Biology Department of the University. The leaves (2kg) were cleaned with distilled water and dried in a food dehydrator (Bosch 5238157) set at 45°C for five days. An electric blender (Silver Crest SC 1589) was used to pulverize the dried leaf samples, which were then preserved in airtight sterile Ziploc bags in readiness for the extraction procedures.

Preparation of the extracts and phytochemical Screening

The standard methods for extraction and analyses of phytochemicals reported by Harborne (1984), Sofowora (1993), Trease and Evans (1996) were employed with slight modifications. The powdered leaf samples (200g) of *Kalanchoe pinnata* L. (Life plant) were extracted in distilled water (500 mL) and 75% ethanol (500 mL) respectively. The resultant filtrates were next concentrated at 45^{o}C using a rotary evaporator (Buchi 850V). and subsequently screened for the phytochemicals: Anthocyanins, Anthraquinones, Alkaloids, Flavonoids, Phenolics, Saponins, Steroids, Tannins and Terpenoids.

Qualitative analysis of phytochemicals

A stock solution was constituted by dissolving 1g of each of the solvent-free crude extracts in 100 mL of the respective extracting solvents (distilled water and 75 % ethanol) and then exposed to phytochemical screening as described below.

Test for Flavonoids

To test tubes containing 2 mL of each of the respective stock solutions, three drops of weak ammonia solution were introduced. The detection of a yellowish-coloured solution which became colourless upon adding a few drops of dilute H_2SO_4 was a clear indication of the presence of flavonoids.

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Test for Alkaloids

To test tubes containing 2 mL of each of the respective stock solutions, 2 mL of Wagner's Reagent was added. The detection of a reddish-brown precipitate confirmed the presence of alkaloids. The preparation of Wagner's reagent required the dissolving of 2g of iodine and 6g of potassium iodide in 100 mL of water.

Test for Steroids

To test tubes containing 2 mL of each of the respective stock solutions, 2 mL of ethanoic anhydride and 1mL of dilute Sulphuric acid were added respectively. The change in the colour of the solutions from violet to green confirmed the presence of Steroids.

Test for Anthraquinones

To conical flasks containing 2mL of each of the respective stock solutions, 20 mL of benzene was added and manually agitated for 5 min and the solutions were filtered. The filtrates were subsequently swirled with 5 mL of 10 % NH_3 solution. The detection of a pink or red or violet colour in the ammonical lower phase indicates the presence of free Anthraquinones.

Test for Saponins

To the test tubes containing 5 mL of each of the respective stock solutions, 2 mL of water was added, agitated vigorously and observed for a persistent frothing which signifies that Saponins are present.

Test for Anthocyanins

To test tubes containing 2 mL of each of the respective stock solutions, 2 mL of dilute HCl was introduced. Pale pink-coloured solutions depict the presence of anthocyanins.

Test for Tannins

To test tubes containing 2 mL of each of the respective stock solutions, 2 mL of iron (III) chloride solution was added. The detection of a brownish-green solution depicts the presence of tannins.

Test for Terpenoids (Salkowski Test)

To test tubes containing 5 mL of each of the respective stock solutions, 2 mL of chloroform and 3 mL of concentrated Sulphuric acid were introduced respectively. The manifestation of a reddish-brown layer at the interface depicts the existence of Terpenoids.

Determination of Macro Elements of *Kalanchoe pinnata* leaf extracts

A number of essential macro elements were quantitatively determined in the samples using standard analytical procedures (AOAC, 2005). The leaf samples were first ashed using a muffle furnace (SX-5-12), then dissolved in 1mL nitric acid, transferred into a 100 mL volumetric flask, diluted and subsequently made up to the mark using double distilled water (Suresh & Hui-Fen, 2012) The determination of the elements were done using spectrophotometric methods: Flame emission spectrophotometer and Atomic Absorption spectrophotometer (Taylor, 1999)

Assessment of the Antibacterial Activities of *Kalanchoe pinnata* leaf (KPL) extracts

Standard and clinically isolated strains of bacteria: *Bacillus subtilis, Escherichia coli Pseudomonas aeruginosa and Staphylococcus aureus* were obtained from the Federal Medical Centre, Yenagoa, Bayelsa State. These selected microbes were used to determine the antibacterial efficacy of the leaf extracts of *Kalanchoe pinnata* L. using the Agar Well Diffusion Method (Valgas et al., 2007)

Antioxidant Activities of *Kalanchoe pinnata* leaf (KPL) extracts using DPPH Assay

The assessment of the antioxidant activities of the ethanol based *K. pinnata* leaf (KPL) extracts and the standard antioxidant, Ascorbic Acid (AA) was analyzed using DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay (Brand-William et al., 1995; Amarowicz & Pegg, 2019).

The DPPH radical scavenging activity was derived using the relation shown below

$$\%\ \text{DPPH radical scavenging activity} = \frac{(Ao - A1)}{Ao} \times \mathbf{100}$$

Ao = the absorbance of the control

A_1 = the absorbance of the plant extract/reference antioxidant

The % Inhibition or % DPPH scavenging radical activity was plotted against concentration and the IC_{50} was calculated using the AAT Bioquest online calculator (AAT Bioquest, 2022). The determinations were carried out in triplicates for each concentration, and the data obtained were expressed as an average of replicate measurements ± Standard Deviation (SD).

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Results

Qualitative phytochemical analysis of *Kalanchoe pinnata* leaf (KPL) extracts

The phytochemical constituents of the aqueous and ethanolic leaf extracts of *Kalanchoe pinnata* were investigated for the presence of the bioactive compounds: alkaloids, flavonoids, phenolics, tannins, saponins, steroids, anthraquinones, anthocyanins and terpenoids. The results as presented in Table 1 revealed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, and steroids in the water and 75% ethanol extracts. On the other hand, anthraquinones, anthocyanins and terpenoids were absent in both extracts.

Table 1: Phytochemical constituents of *Kalanchoe pinnata* leaf (KPL) extracts

Test	Aqueous leaf extract	Ethanolic leaf extract
Flavonoids	+	+
Phenolics	+	+
Alkaloids	+	+
Steroids	+	+
Anthraquinones	-	-
Saponins	+	+
Anthocyanins	-	-
Tannins	+	+
Terpenoids	-	-

Legend: Presence (+), Absence (-)

Antibacterial Activity of *Kalanchoe pinnata* leaf (KPL) extracts

The results as presented in Table 2 and Figure 2 indicate the zones of growth inhibition which were assessed using the Agar Well Diffusion (AWD) method for *K. pinnata* leaf extracts against the test organisms: *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.* The growth inhibition for the ethanolic and aqueous extracts varied significantly ($P<0.05$) for the various test organisms. However, the ethanolic extracts based on the fresh leaf (FL) samples showed relatively higher zones of growth inhibition for all the bacteria tested. The results revealed that the inhibition zones recorded were 13, 14, 20 and 22 mm for *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli* respectively. Similarly, the zones of growth inhibition for the ethanolic extract based on the dry leaf (DL) of *K. pinnata* were 10,10,12 and 18 mm for *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli* respectively. On the other hand, the aqueous KPL extracts of the plant were only effective in controlling *staphylococcus aureus* having shown zones of growth inhibition of 16 mm for aqueous DL extracts and 8 mm for the aqueous FL extracts. The ethanolic extracts is comparable ($P > 0.05$) to the inhibition zone of 30 mm recorded for gentamicin antibiotic, used as the control.

Table 2:Antibacterial Activity of *Kalanchoe pinnata* leaf (KPL) extracts Zone of growth inhibition expressed in millimeter (mm)

Organism	Strain type (+/-)	Ethanol extract (DL)	Ethanol extract (FL)	Aqueous Extract DL	Aqueous Extract FL	STD GEN
Staphylococcus aureus	+	10	14	16	8	29
Bacillus subtilis	+	10	13	NI	NI	25
Pseudomonas aeruginosa	-	12	20	NI	NI	28
Escherichia coli	-	18	22	NI	NI	30

Legend: DL = Dry Leaves, FL = Fresh leaves, NI= No Inhibition, STD = Standard, GEN = Gentamicin.

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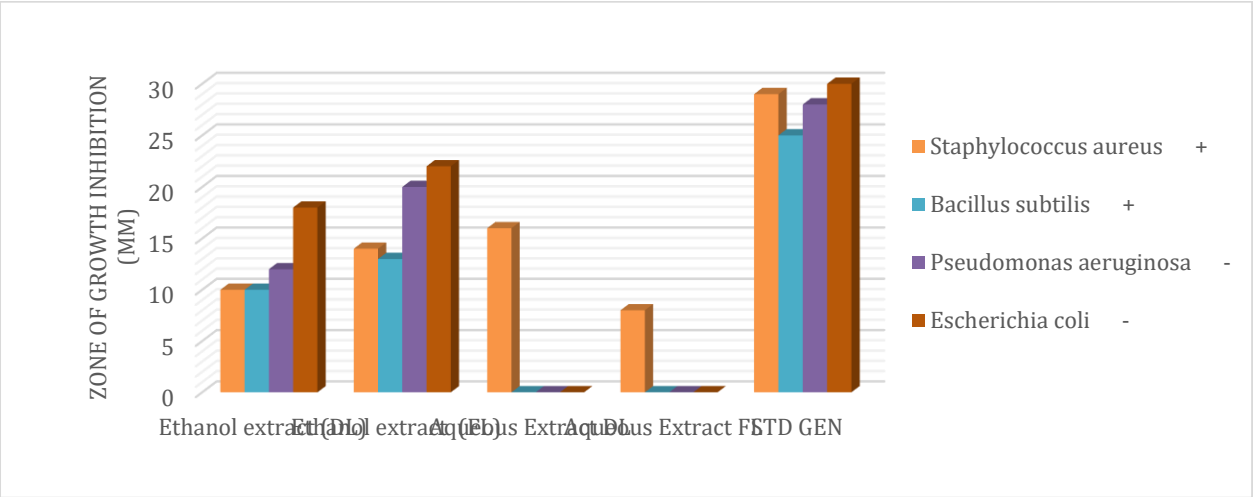

Fig 2: Antibacterial Activity of *Kalanchoe pinnata* leaf (KPL)

Antioxidant Activity of Ethanolic extract of *Kalanchoe pinnata* leaf (KPL)

The antioxidant potential of the ethanolic KPL extract was assessed in this study using DPPH, a purple-coloured organic nitrogen compound composed of stable free radicals. The results as presented in Table 3 and Figure 3. indicated that the DPPH assay, based on the loss of DPPH* after reacting with the plant extract was recorded as % Inhibition. This scavenging activity was largely dependent on the specified concentrations, ranging from 0.1mg/mL - 0.5mg/mL for the KPL leaf extracts and the reference antioxidant, Ascorbic Acid. The percentage (%) inhibition reported for the KPL extract ranged between 48.83 - 68.02% whereas for the standard antioxidant: AA, the % Inhibition ranged between 76.97%-85.52%. as shown in Table 3.

Table 3: Antioxidant Activity of Ethanolic extract of *Kalanchoe pinnata* leaf (KPL)

Sample	Concentration (mg/mL)	Absorbance 518 nm	% Inhibition mean ± SD
Ethanolic leaf Extract	0.1	0.780	48.83±0.166
	0.2	0.752	50.52±0.112
	0.3	0.650	57.11±0.140
	0.4	0.621	59.14±0.114
	0.5	0.486	68.02±0.132
Ascorbic acid	0.1	0.350	76.97±0.122
	0.2	0.321	78.88±0.101
	0.3	0.293	80.72±0.083
	0.4	0.284	81.31±0.154
	0.5	0.220	85.52±0.161
Ethanol		1.520	

Legend: SD (Standard Deviation)

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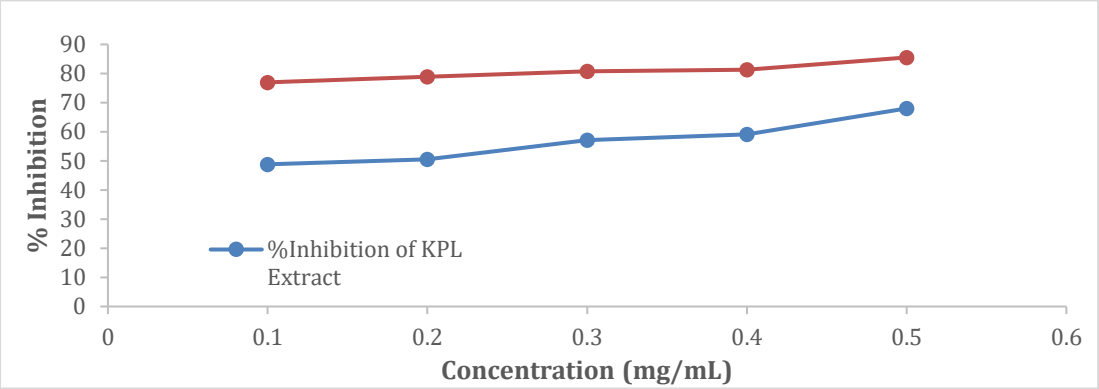

Fig 3: Percentage inhibition of *Kalanchoe pinnata* leaf (KPL) Extract and Ascorbic Acid via DPPH Assay

Fig.4: IC_{50} of Ethanolic Extract of *Kalanchoe pinnata* Leaf (KPL) and Ascorbic Acid (AA)

The IC_{50} values calculated for KPL extract and AA presented in Fig. 4 were 1.485mg/mL and 1.038 mg/mL respectively (AAT Bioquest, 2022).

Table 4: Elemental Analysis of *Kalanchoe pinnata* leaf extract.

Element	*Kalanchoe pinnata* crude extract. (mg/L) Mean ± SD
Na	6.14 ± 0.24mg/L
K	5.55 ± 0.022 mg/L
Ca	75.36 ± 0.17 mg/L
Mg	18.11 ± 0.18 mg/L

Key: SD (Standard Deviation)

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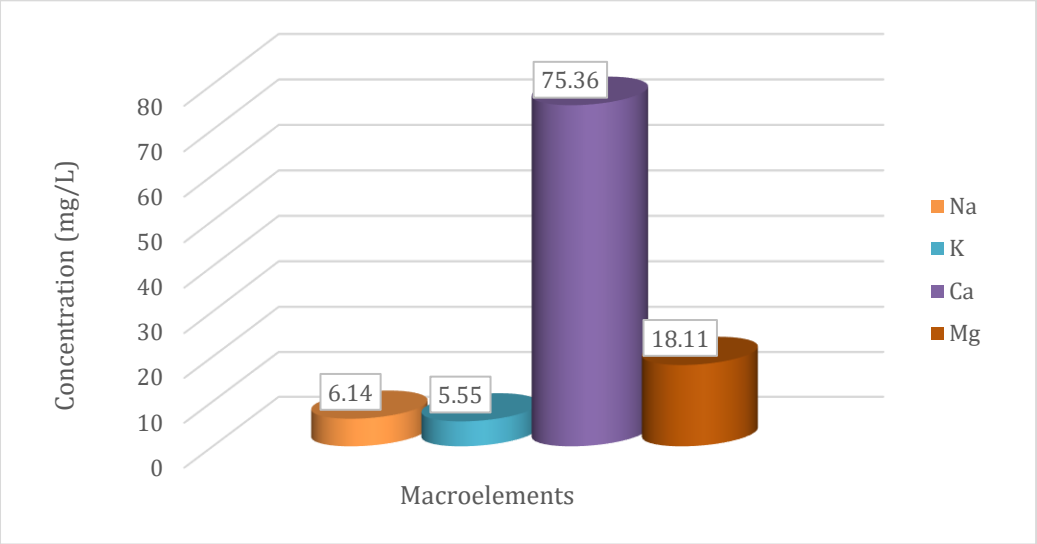

Fig 5: Elemental Analysis of *Kalanchoe pinnata* leaf (KPL) extract.

The macro elements determined in KPL extracts as presented in Table 4 and Fig. 5 gave values: Ca (75.36 ± 0.17 mg/L), Mg (18.11 ± 0.18 mg/L), Na (6.14 ± 0.24 mg/L) and K (5.55 ± 0.022 mg/L).

Discussion

Phytochemical components of *Kalanchoe pinnata* leaf (KPL) extracts

The results obtained in this study showed similar phytochemical profiles for the water and ethanol KPL extracts. This lends credence to the fact that the successful analysis of bioactive compounds depends to a large extent on the solvents employed in the course of the extraction process (Ncube et al., 2008; Abubakar et al., 2020). According to Chowdhury et al. (2011), the aqueous extracts of *K. pinnata* leaves indicated the presence of alkaloids, glycosides steroids, saponins, and tannins. Biswas et al. (2011) in a related study, reported that alkaloids, glycosides, steroids, gums, flavonoids, saponins reducing sugars and tannins were detected in the ethanolic extracts of *K. pinnata* leaves. Thus, the phytochemicals detected in the present study indicate that the Generally Regarded as Safe (GRAS) polar solvent, ethanol and water successfully extracted and preserved the target bioactive compounds in the leaf matrix of the plant under investigation. Consequently, the therapeutic worth of medicinal plant extracts is reported to lie largely in the presence of these vital phytochemicals. Studies have shown that these bioactive compounds possess important properties such as antioxidant activity, antimicrobial activity, anti-parasitic agents, anti-cancer, analgesic, antispasmodic, anti-inflammatory and other medicinal as well as physiological properties (Sofowora, 1993; Kpomah et al., 2008; Gafar and Itodo, 2011; Majaz et al., 2011; Yadav & Agarwala, 2011)

Antimicrobial Activity of *Kalanchoe pinnata* leaf (KPL) extracts

The sensitivity of the various KPL extracts against the selected test organisms was done using the well-established Agar Well Diffusion method and the zones of inhibition were a function of the efficacy of the relative potency of the extracts. The antibacterial activity observed is likely due to the presence of the biologically active phytochemicals: alkaloids, tannins flavonoids phenolics, saponins and steroids in the extracts considered in this study. The ethanolic extracts gave the highest inhibitory activity against the test organisms. According to a study by Loi et al. (2020) on the effect of extraction solvents on *Kalanchoe pinnata* antimicrobial activity against several pathogens, the methanolic leaf extracts revealed the highest activity. Thus, alcoholic solvents exhibit better extractive and preservative effects on phytochemicals leading to enhanced antimicrobial activity.

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Antioxidant Activities of *Kalanchoe pinnata* leaf (KPL) extract

Antioxidants are reported to have a profound effect on DPPH radical due to their ability to donate hydrogen or transfer electrons. The use of DPPH free radical scavenging is an established mechanism designed for the screening of the antioxidant activity of plant extracts. Hence the harmful role of free radicals in several diseases including cancer is prevented by the radical scavenging activities of plant antioxidants. (Kasote et al., 2015; Rahman et al., 2015). Consequently, the results revealed that the free radical scavenging activity of KPL extracts is comparable to the reference antioxidant, Ascorbic acid as depicted by the respective IC_{50} values (Fig 4).

Determination of Macro Elements in *Kalanchoe pinnata* leaf Extract

The macro elements: Sodium(Na), Potassium (K), Calcium (Ca) and Magnesium (Mg) determined in the KPL extract essentially play multiple roles within the human body including the fact that they work together with vitamins and initiate hormone production as well as speed up metabolic processes (Pittas et al., 2007; Khalid et al., 2014). These essential elements also aid in the development of bone and teeth and are components of ATP, DNA, RNA and cell membranes (Mensah et al., 2008). The levels of Ca (75.36 ± 0.17 mg/L) and Mg (18.11 ± 0.18 mg/L) in the KPL extract as presented in the results revealed that this plant is a viable source of these vital minerals. The results are in line with the normal serum levels ranges of 90-105 mg/L and 18-30 mg/L reported for calcium and magnesium respectively. On the other hand, the levels of potassium and sodium in the KPL extract are well below normal serum levels of 136-145 mmol/L and 3.5-5 mmol/L reported for sodium and potassium respectively (Kratz et al., 2004).

Conclusion

The aqueous and ethanolic leaf extracts of *Kalanchoe pinnata* is reported to have a significant number of phytochemicals except for Anthraquinones, Anthocyanins and Terpenoids. This phytochemical profile is comparable to results reported for the widely used fresh leaf juice extracts and further confirms that the eco-friendly solvent extractions employed in this study had no significant effect on the bioactive compounds present in the leaves of *K. pinnata* growing abundantly in the backyard gardens in Bayelsa State, Nigeria. However, the antimicrobial activity of the aqueous extracts (based on the dry and fresh leaves) was significantly less effective against the tested organisms except for *staphylococcus aureus.* The study also revealed that the antioxidant activity of the biologically active phytochemicals detected in the ethanolic leaf extract was comparable to the activity recorded for Ascorbic Acid which served as the reference antioxidant.

Recommendations

The extraction optimization of the phytochemicals in the leaf extracts as well as other parts of *Kalanchoe pinnata* should be explored.

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Cite this article as:
Hamilton-Amachree, A., & Uzoekwe, N. M.(2022). Phytochemical analysis and assessment of the antibacterial and antioxidant activities of the aqueous and ethanolic leaf extracts of life plant (*kalanchoe pinnata* l.) in the Niger Delta Nigeria. *FNAS Journal of Scientific Innovations*, *3*(3), 1-10.

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