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Phytochemical Profiling, Nutritional Evaluation and Antimicrobial Potency Assessment of *Kalanchoe pinnata* Leaf Extracts

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ABSTRACT

This study investigated the phytochemical composition, proximate nutritional content and antimicrobial potential of *Kalanchoe pinnata*, a plant commonly used in ethnomedicine. Selective solvent extraction based on polarity was employed: polar solvents (water, methanol, ethanol, propanone) were employed to extract polar phytochemicals, whereas non-polar solvents (n-hexane and trichloromethane) targeted non-polar constituents. Phytochemical screening followed standard methods, including: Mayer's test for alkaloids, Shinoda test for flavonoids, ferric chloride test for tannins and phenolics, foam test for saponins, Libermann – Burchard test for steroids and Salkowski test for terpenoids. Quantitative analysis was performed using a UV-Visible spectrophotometer and a HPLC system. Proximate analysis was conducted using AOAC methods. Antimicrobial assays were evaluated using aqueous, methanolic and ethanolic extracts. The results revealed significant levels of alkaloids (0.060 mg/g), flavonoids (0.140 mg/g), glycosides (0.055 mg/g), coumarins (60.0 µg), lignans (75.0 µg), steroids (0.065 mg/g) and phenolics (0.130 mg/g). Saponins, analyzed gravimetrically, recorded a high concentration of 52.4 mg/g. Proximate analysis showed: moisture (82.0%), ash (2.46%), crude protein (3.88%), crude fat (1.81 %), crude fiber (1.50%) and carbohydrates (8.19%). Antimicrobial assays demonstrated the strongest activity in ethanol extract, with the largest inhibition zones, lowest microbial inhibitory concentration and microbial bactericidal concentration against the tested bacterial strains.

KEYWORDS: Antimicrobial activity, *Kalanchoe pinnata*, phytochemicals, proximate analysis, zone of inhibition

INTRODUCTION

Kalanchoe pinnata (Lam.) Pers., usually known as the "Miracle Plant", "Leaf of Life" or "Cathedral Bells" is a succulent plant generally recognized for its healthy therapeutic potential. Originally native to Madagascar, it has acclimatized in tropical and subtropical

regions across the world, including Nigeria, India, the Caribbean and parts of South America. The plant flourishes in warm climates and is frequently found in home gardens and herbal medicine collections due to its outstanding resilience and wide range of medicinal uses [1].

Morphologically, *Kalanchoe pinnata* is a fleshy perennial herb that grows 1 -2 meters in height. Its leaves are fleshy; simple as well as ovate with serrated margins, often bearing minor plantlets along the edges that can detach and grow independently – a trait that contributes to its nickname "Mother of Thousands". The plant manufactures bell – shaped pendulous flowers, characteristically purplish – red, that hang in clusters from tall stalks.

Taxonomically, it belongs to the *Crassulaceae* family. In Nigeria, *Kalanchoe pinnata* is recognized by various native names across different ethnic groups. It is called "Odundun" in Yoruba, "Afarayin" in Hausa and "Umu-ama" or "Ewe abamoda" in Igbo, reflecting its extensive use and recognition among local communities.

Traditionally, the leaves of *K. pinnata* have been utilized in ethnomedicine for the management of numerous ailments including: wounds, ulcers, infections, hypertension, kidney stones, respiratory disorders and inflammatory conditions. The therapeutic potential of the plant is principally attributed to its rich phytochemical profile, including flavonoids, alkaloids, glycosides, steroids, tannins and saponins [2]. It also exhibits antimicrobial, anti-inflammatory, antioxidant, hepatoprotective and antihypertensive activities [3].

Given the worldwide resurgence in the use of herbal remedies and high demand for scientifically validated traditional medicines, it is vital to assess the phytochemical constituents, nutritional value as well as biological properties of such plants. The proximate analysis gives insights into the basic nutritional components such as moisture, ash, protein, fat, fiber and carbohydrate content, which are essential in assessing the dietary and therapeutic potential of the plant [4].

Several studies have examined its phytochemical and antimicrobial potentials but lacked the evaluation of the minimum inhibitory and minimum bactericidal concentrations, besides these studies did not evaluate a comprehensive extraction of the plant bioactive compounds using a wide range of polar and non-polar solvents [5-8]. These limitations underscore the need for detailed extraction efficacy, and antimicrobial profiling that include both qualitative and quantitative parameters.

The aim of this study is to validate the traditional use of *Kalanchoe pinnata* and explore its potential for pharmacological and nutraceutical applications. The objective of the current study is to employ comprehensive phytochemical screening, quantitative analysis of bioactive compounds, proximate composition determination and antimicrobial evaluation

including minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and zone of inhibition studies of *Kalanchoe pinnata* leaf extracts.

MATERIALS AND METHODS

Qualitative analysis for selected phytochemicals from the leaves of *Kalanchoe pinnata*

Fresh green leaves of *Kalanchoe pinnata* were harvested from a farm in Owerri, Imo State, Nigeria, washed with distilled water to remove surface contaminants and blotted dry with paper towels. The leaves were then subjected to air-drying at room temperature (25 – 28 ^{o}C) in a well-ventilated space, away from direct sunlight for a period of 10 days to avert degradation of thermo liable bioactive compounds in the leaves. Afterwards, the dried leaves were ground into fine powder using a laboratory electric grinder and stored in airtight containers at room temperature pending analysis [9].

Extraction of phytochemicals

Approximately 5.0 g of the pulverized leaf material was extracted using different solvents depending on the phytochemical to be investigated. For saponins and tannins, 5.0 g of the powdered leaf material was weighed with an electronic balance and boiled with 50 mL of each of the following solvents: water, 95% ethanol and methanol. For flavonoid, phenolic compounds and alkaloids, about 5.0 g of the powdered leaf substance was boiled with 50 mL of each of the following solvents: methanol, 95% ethanol and acetone (propanone). While for terpenoids, 5.0 g of the powdered leaf material was extracted with of each of the following solvents: hexane, chloroform and methanol.

Extraction was performed in all of the above cases by boiling the plant leaf powder with 50 mL of the respective solvents for 30 minutes using a water bath at 60 ^{o}C, followed by filtration through Whatman No 1 filter paper. These afforded green filtrates that were stored in amber vials at 4 ^{o}C prior to phytochemical screening. In the case of steroids, 5.0 g of the powdered leaf material was subjected to 40 mL each of n–hexane, chloroform (tricholoromethane) and methanol. The samples were left to macerate for 48 hours at room temperature. After the maceration, the mixtures were filtered with Whatman No. 1 filter papers and the filtrates were concentrated under reduced pressure using a rotary evaporator at 38 ^{o}C to obtain the crude extracts. The extracts were also stored at 4 ^{o}C in airtight vials for subsequent phytochemical assays [10]. The results of the qualitative screening of the leaf extract are recorded in Table 1.

Alkaloids (Mayer's test)

Mayer's reagent was prepared by dissolving 1.40 g of mercury (II) chloride ($HgCl_2$) and 5.0 g of potassium iodide (KI) in 100 mL of water. The mixture was homogenized. Approximately 2.0 mL of Mayer's reagent was then added in each case to 2.0 mL of the methanol, ethanol and acetone leaf extract in three different test tubes at room temperature and the mixture left to stand for about 3 – 4 minutes. The formation of a creamy white or yellow precipitate confirms the presence of alkaloids [11].

Flavonoids (Shinoda test)

In a clean test tube, 2.0 mL in each case of methanol, ethanol, and acetone extract of the leaf material were mixed with 1.0 g of magnesium turnings in three different test tubes followed by the careful addition of 1 -2 drops of concentrated hydrochloric acid using a dropping pipette to each of the mixtures. The mixtures were then gently shaken. The appearance of a pink or red coloration confirms the presence of flavonoids [12].

Tannins (Ferric chloride test)

Approximately 5.0 g of iron (III) chloride was accurately weighed using an electronic balance and dissolved in 100 mL of distilled water. Exactly 1.0 mL of 5% iron (III) chloride solution was added into three different test tube containing 2.0 mL of ethanol, methanol and aqueous extract of the leaf material. A blue-black coloration confirmed the presence of hydrolysable tannins [13].

Saponins (Foam test)

Approximately 10.0 mL of the ethanol, methanol and aqueous extract of the plant material were transferred into three different 100 mL graduated cylinder and shaken vigorously for 15 minutes. The persistence of froth or foam of height greater than 1.0 cm for a period of 10.0 minutes confirmed the presence of saponins [14].

Phenolic compounds (Ferric chloride test)

Approximately 1.0 g of iron (III) chloride ($FeCl_3$) was weighed out using an electronic balanced and then dissolved in 100 mL of distilled water.Exactly 1.0 mL of 1% of the prepared iron (III) chloride solution was added 2.0 mL of the methanol, ethanol and acetone leaf extract in three different test tube at room temperature. The appearance of blue, green or violet colorations confirmed the presence of phenolic compounds [15].

Terpenoids (Salkowski test)

Approximately 2.0 mL of concentrated tetraoxosulphate(VI) acid was carefully added in each case to three test tubes, each containing 2.0 mL of hexane, chloroform and methanol extracts of the leaves. Reddish – brown colorations at the interface confirmed the presence of terpenoids [16].

Steroids (Liebermann – Burchard Reaction)

Approximately 2.0 mL of acetic anhydride was added to three clean test tubes containing 2.0 mL each of hexane, chloroform and methanol. This was followed by careful addition of 1.0 mL of concentrated tetraoxosulphate (VI) acid along the side of each test tube without mixing the layers.The formation of a blue–green, violet or bluish ring confirmed the presence of steroids [17].

Determination of selected phytochemicals from the leaves of *Kalanchoe Pinnata.*

Extraction of phytochemicals for quantitative analysis

For alkaloids and glycosides, 5.0 g of the powdered leaf material was refluxed with 50 mL of 95% ethanol for 2 hours. The extract was filtered and the solvent was evaporated under reduced pressure. The green residue obtained was dissolved in distilled water and extracted with chloroform to separate alkaloids and glycosides. For flavonoids, 2.0 g of the powdered leaves were homogenized in 20 mL of 80% methanol containing 1% hydrochloric acid. The mixture was incubated at 25 ^{o}C for 24 hours, centrifuged and the supernatant was collected. The solvent was evaporated and the residue was dissolved in methanol for analysis.

For steroids, 5.0 g of the powdered leaf material were extracted with 50 mL of chloroform by refluxing for 2 hours. The extract was filtered and the solvent was evaporated. The residue was dissolved in acetic anhydride for analysis.

For coumarins, 5.0 g of powdered leaf material were extracted with 50 mL of methanol by refluxing for 2 hours. The extract was filtered and the solvent was evaporated. The residue was dissolved in sodium hydroxide solution for analysis.

For lignans, 5.0 g of the powdered leaf material were extracted with 50.0 mL of methanol: acetonitrile (1:1) mixture containing 0.1 % formic (methanoic) acid by refluxing for 2 hours. The extract was filtered and the solvent was evaporated. The residue was dissolved in methanol for analysis.

For tannins, powdered leaf material were extracted with 50 mL of distilled water by boiling for half an hour. The extract was filtered and the solvent was evaporated. The residue was dissolved in distilled water for analysis. For phenolics, 5.0 g of the powdered leaf

material were extracted with 50 mL of methanol by refluxing for 2 hours. The extract was filtered and the solvent was evaporated. The residue was dissolved in distilled water for analysis.

For saponins, 5.0 g of the powdered leaf material were extracted with 50 mL of 20% ethanol by heating to 55 ^{o}C for 4 hours. The extract was filtered and the solvent was evaporated. The residue was dissolved in distilled water for analysis [18]. The results of the quantitative analysis of the leaf extract are recorded in Table 2.

Determination of alkaloids

Alkaloids were quantified using the Keller-Kilani technique. Approximately 1.0 mL of Keller's reagent (acetic acid and $FeCl_3$) and 1.0mL of concentrated tetraoxosulphate (VI) acid were added to 1.0 mL of the plant extract in a test tube.The mixture was incubated for 10 minutes and the absorbance was measured at 530 nm using a UV-Vis spectrophotometer (model UV-1800, Japan). A brown color was observed at the interface which indicated the presence of alkaloids. The intensity of the color was proportional to the alkaloid concentration.10-100 µg of caffeine solutions was used to create a standard curve [19]. The alkaloid concentration from the leaf extract was determined using equation 1

$$\text{Alkaloid content (mg/g)} = \frac{C \times V}{M \times 1000} \quad (1)$$

Where C = concentration from standard curve (g/mL), V = final extract volume (mL), M = sample weight (g).

Determination of flavonoids

Approximately 0.5 mL of the leaf extract was added to 2.8 mL of water in a test tube. This was followed by the addition of 0.1 mL of 10% $AlCl_3$ and 0.1 mL of 1.0 M potassium ethanoate. The mixture was incubated for 30 minutes at room temperature and absorbance was measured at 415 nm using a UV-Vis spectrophotometer.About 10-100 µg of quercetin solutions was used to prepare a standard curve. The concentration of flavonoid in the plant extract was calculated using equation 1 and the result was expressed in mg/g QE (milligram of quercetin equivalent per gram of sample) [20].

Determination of glycosides

Approximately 1.0 mL of the leaf extract was added to 2.0 mL of glacial acetic acid followed by the addition of 1 drop of 2% $FeCl_3$ in a test tube. Additionally, 1.0 mL of concentrated tetraoxosulphate (VI) acid was carefully added. The absorbance of the mixture was measured at 530 nm using a UV-Vis spectrophotometer. 10-100 µg of digitoxin solutions was used to

prepare a standard curve. The concentration of glycosides in the plant extract was calculated using equation 1 and the result was expressed in mg/g DE (milligram of digitoxin equivalent per gram of sample) [21].

Determination of steroids

Approximately 2.0 mL of acetic anhydride was added to 1.0 mL of the leaf extract, followed by slow stepwise addition of concentrated tetraoxosulphate (VI) acid. The mixture was incubated for 10 minutes and absorbance was measured at 520 nm using a UV-Vis spectrophotometer. 10-100 µg of cholesterol solutions was used to prepare a standard curve. The concentration of steroids in the plant extract was calculated using equation 1 and the result was expressed in mg/g CE (milligram of cholesterol equivalent per gram of sample) [22].

Determination of coumarins

Approximately 1.0 mL of 10% sodium hydroxide solution was mixed with 1.0 mL of the leaf extract. The measure was incubated for 10 minutes and the absorbance was measured at 345 nm using a UV-Vis spectrophotometer. 10-100 µg of coumarin solutions was used to prepare a standard curve. The concentration of coumarins in the plant extract was calculated using equation 1 and the result was expressed in µg/g [23].

Determination of lignans

Approximately 20 L of the leaf extract was injected into the HPLC system. The mobile phase employed was methanol: acetonitrile (1:1) mixture with 0.1 % formic acid. The wavelength of the UV detector was set at 280 nm. Secoisolariciresinol is used as a standard to calibrate the instrument. 1 – 10 g/mL of secoisolariciresinol was used to prepare the standard curve. The Lignans concentration was determined with equation 2 and expressed in µg/g [24].

$$\text{Lignan content (µg/g)} = \frac{\text{Peak area}}{\text{Slope}} \times \frac{V}{M} \quad (2)$$

Determination of tannins

Approximately 1.0 mL of the leaf extract was mixed with 1.0 mL of 1% $FeCl_3$ and the mixture was incubated for 10 minutes. The absorbance was then measured at 700 nm using a UV-Vis spectrophotometer. Approximately 10-100 µg of Tannic acid solutions was used to prepare a standard curve. The concentration of Tannins in the plant extract was calculated using equation 1 and the result was expressed in mg/g TAE (milligram of tannic acid equivalent per gram of sample) [25].

Determination of phenolics

Approximately 0.5 mL of the leaf extract was mixed with 2.5 mL 10% Folin-Ciocaiteu reagent. After a period of 5 minutes, 2.0 mL of 7.5% sodium carbonate was added. The mixture was incubated for 30minutes and absorbance was measured at 760 nm using a UV-Vis spectrophotometer. 10-100 µg of Gallic acid solutions was used to prepare a standard curve. The concentration of phenolics in the plant extract was calculated using equation 1 and the result was expressed in mg GAE/g (milligram of gallic acid equivalent per gram of sample) [26].

Determination of saponins

Approximately 5.0 g of *Kalanchoe pinnata* powdered leaf materials were extracted using 150 mL of 20% ethanol in a Soxhlet apparatus for 6 hours. The extract was filtered and concentrated to approximately 20 mL using a rotary evaporator at 60^{o}C. The concentrated extract was then transferred into a separatory funnel and partitioned with 10 mL of diethyl ether. The ether layer was discarded to remove non-saponin impurities. The aqueous layer was then extracted three times with 30 mL portions of n-butanol. The combined n-butanol extracts were washed twice with 5 mL of 5% sodium chloride solution. The final n-butanol layer was evaporated to dryness in a pre-weighed beaker in an oven at 60^{o}C. After drying, the saponin residue was weighed [27].

W_1 = Weight of empty beaker

W_2 = Weight of beaker + saponin residue

W_3 = Weight of plant sample

$W_4 = W_2 – W_1$ (weight of saponin residue)

$W_5 = W_4$ x 1000 (weight of saponin residue in mg)

The weight of saponin in mg/g was obtained by dividing the weight of saponin in mg (W_5) by the weight of the dried sample (5 g).

Proximate analysis (Nutritional composition) of *Kalanchoe pinnata l*eaves

Sample collection and preparation

Fresh *Kalanchoe pinnata* leaves were collected from a pesticide-free farm in Owerri, Imo state, Nigeria. The leaves were washed, air-dried at room temperature (25^{o}C) for a period of 10 days and ground into powder using an electric grinder. The powder was stored in air-tight containers at room temperature (25 ± 1 ^{o}C) [28].

Proximate analysis

All analysis were conducted in triplicate following AOAC (2019) methods [29] and results were recorded in Table 3.

Moisture content

Approximately 5.0 g of fresh *Kalanchoe pinnata* leaves was accurately weighed using an electronic balance and placed in a crucible and the weight of the crucible and its content was determined. The sample was the dried in an oven at 105ºC for 5 hours, thereafter; it was cooled in a desiccator to room temperature and weighed again [30]. The percentage moisture content in the fresh leaf sample was estimated with equation 3.

$$\% \text{ Moisture} = \frac{W_1 - W_2}{W_1} \times \frac{100}{1} \quad (3)$$

W_1 = initial weight of fresh sample + Crucible before drying

W_2 = final weight of dried sample + Crucible

Ash content

Approximately 5.0 g of the powdered sample was accurately weighed out using an electronic balance and placed in a crucible. Thereafter, it was incinerated in a muffle furnace at 600 ºC for 6 hours. The residue obtained was cooled to room temperature in a desiccator and weighed [31]. The percentage ash content was estimated with equation 4.

$$\% \text{ Ash} = \frac{W_2}{W_1} \times \frac{100}{1} \quad (4)$$

W_2 = Weight of sample before ashing

W_1 = Weight of residue (ash) after ignition at 600 ºC

Crude Fat

Approximately 5.0 g of the dried powder was accurately weighed using an electronic balance and was placed in Soxhlet apparatus. It was subsequently extracted for 5 hours using 200 mL of analytical grade diethyl ether. Thereafter, the solvent was evaporated using a rotary evaporator and the fat content weighed [32]. The percentage crude fat content was estimated with equation 5.

$$\% \text{ Crude Fat} = \frac{W_2}{W_1} \times \frac{100}{1} \quad (5)$$

W_2 = weight of extracted fat

W_1 = Original weight of sample

Crude Fiber

Approximately 2.0 g of defatted sample (sample after Soxhlet extraction) was weighed accurately into a 500 mL beaker. Exactly 200 mL of 1.25% tetraoxosulphate(VI) acid was added to the sample and the mixture was gently boiled for a period of 30 minutes, stirring occasionally. After which the mixture was filtered using a muslin cloth.The residue obtained was washed with distilled water to make it acid free. The washed and dried residue was then transferred into a clean 500 mL beaker followed by the addition of 200 mL of 1.25% aqueous sodium hydroxide. Subsequently, the mixture was boiled for another 30 minutes. On cooling, the mixture was filtered again and the residue was washed with hot water, followed by alcohol and propanone. The residue was then dried in an oven at 105 ^{o}C and weighed. After which, the dried residue was ignited in a muffle furnace at 600 ^{o}C for about 4 hours. The resulting ash was cooled in a desiccator and weighed [33]. The percentage ash content of the sample was estimated with Equation 6.

$$\% \text{ Crude fibre} = \frac{W_2 - W_3}{W_1} \times \frac{100}{1} \quad (6)$$

W_2 = weight of residue after drying

W_3 = weight of ash after ignition

W_1 = original sample weight

Crude Protein (Kjeldahl method)

Approximately 1.0 g of dried powder was weighed accurately into a Kjeldahl digestion flask. 2.0 g of concentrated tetraoxosulphate (VI) acid (98%) was added into the flask followed by the addition of exactly 1.0 g of a catalyst mixture (10:1 potassium tetraoxosulphate (VI) and copper (II) tetraoxosulphate (VI) pentahydrate). The mixture was gently heated for 2 hours until it became clear. After cooling the mixture, 50 mL of 40% aqueous sodium hydroxide was added to neutralize the acid. The resulting mixture was distilled to liberate ammonia gas into a receiving flask containing 25 mL of 4% boric acid and mixed indicator. The ammonia – boric acid complex was titrated with standardized 0.1 M hydrochloric acid until color changed from green to pink [34]. The percentage nitrogen content of the mixture was estimated with equation 7.

$$\% \text{ Nitrogen} = \frac{(VHCl \times NHCl \times 1.4007)}{\text{Weight of sample (g)}} \times \frac{100}{1} \quad (7)$$

VHCl = Volume of HCl used (mL)

NHCl = Normality of HCl

1.4007 = Equivalent weight factor to convert 1.0 mL of HCl to % N per gram sample.

% Crude Protein = % Nitrogen x 6.25

Carbohydrate content

% Carbohydrate = 100 - (% moisture + % ash + % protein + % fat + % fibre) [35].

***In vitro* determination of minimum inhibitory concentration and minimum bactericidal concentration of the leaf extracts against selected bacterial strains**

Fresh leaves of *Kalanchoe pinnata* were harvested, washed and air-dried. The dried leaves were powdered, sieved with a sieve shaker of 60 mesh size and subjected to extraction using three solvents: Ethanol (70%), methanol (70%) and distilled water. Each extraction was performed by macerating 10 g of powdered leaf material in 100 mL of solvent for 72 hours at room temperature, followed by filtration and evaporation to obtain crude samples [36].

Microbial strains

The following bacterial strains were obtained from the pathological section, Federal Medical Teaching Hospital Owerri, Nigeria: two Gram- positive bacteria namely: *Staphylococcus aureus* and *Bacillus subtillis* as well as two Gram-negative bacteria: *Escherichia coli* and *Pseudomona aeruginosa* [37]. The microbes were further identified at the Microbiology Department, Federal University of Technology, Owerri, Imo State, Nigeria.

Antimicrobial susceptibility test

Minimum Inhibitory Concentration

The MIC was determined using broth micro dilution method as per the clinical and laboratory standards institute (CLSI) guidelines. Two-fold serial dilutions of each extract were prepared in 96 – well micro-titer plates, starting from 512 mg/mL to 4.0 mg/mL. Inoculum of each bacterial strain was adjusted to 0.5 McFarland standard and added to each well. Plates were incubated at 37^{o}C for 24 hours. The MIC was defined as the lowest concentration of extract that inhibited visible bacterial growth [38]. The results of the MIC of the solvent extracts of the leaves is given in Table 4.

Minimum Bactericidal Concentration

The MBC was determined by sub culturing the content from each well showing no visible growth onto Mueller-Hinton agar plates and incubating at 37 ^{o}C for 24 hours. The MBC was

the lowest concentration of extract that resulted in no bacterial growth on the agar plate [39]. The MBC of the solvent extracts of the leaves is given in Table 4.

Positive control

Ampicillin was used as a positive control at concentrations ranging from 521 mg/mL to 4.0 mg/mL [40].

Zone of Inhibition

The agar well diffused method was employed to assess the antimicrobial activity qualitatively. Wells of 6 mm diameter were punched into Mueller-Hinton agar plates inoculated with the test bacteria. Each well was filled with 100 µL of the extract (at 100 mg/mL concentrations), and the plates were incubated at 37 °C for 24 hours. The zones of inhibition were measured in millimeters [41].

RESULTS AND DISCUSSION

Table 1: The qualitative phytochemical screening of K. *Pinnata*

Phytochemicals	A.E	E.E	M.E	P.E	H.E	C.E
Saponins	+++	++	+	-	-	-
Tannins	+++	++	+	-	-	-
Alkaloids	-	++	+++	+	-	-
Flavonoids	-	++	+++	+	-	-
Phenolics	-	++	+++	+	-	-
Terpenoids	-	-	+	-	+++	++
Steroids	-	-	+	-	++	+++

+++ = Highly present, ++ = moderately present, + = present in low amount

A.E = aqueous extract, **E.E** = ethanol extract, **M.E** = methanol extract, **P.E** = propanone extract,**H.E** = hexane extract,**C.E** = chloroform extract

Table 2: Quantitative phytochemical screening of K. *pinnata*

Phytochemicals	Triplicate concentrations (mg/µg/g)	Mean concentration	Unit
Alkaloids	0.059	0.060	mg/g
	0.060		
	0.061		
Flavonoids	0.139	0.140	mg QE/g
	0.141		
	0.140		

Glycosides	0.054 0.055 0.054	0.055	mg DE/g
Steroids	0.066 0.064 0.065	0.065	mg CE/g
Coumarins	74 76 75	75	µg/g
Lignans	59 60 61	60	µg/g
Tannins	0.064 0.066 0.064	0.065	mg TAE/g
Phenolics	0.129 0.130 0.130	0.130	mg GAE/g

Values are expressed as mean ± standard deviation (n = 3)

Table 3: Proximate analysis of *K. pinnata*leaf extracts

Parameters	Analysis 1 (%)	Analysis 2 (%)	Analysis 3 (%)	Mean % ±S.D
Moisture	82.30	81.60	82.20	82.07 ± 0.25
Ash	2.44	2.50	2.43	2.46 ± 0.04
Crude protein	3.85	3.92	3.88	3.88 ± 0.03
Crude fat	1.80	1.84	1.80	1.81 ± 0.03
Crude fiber	1.50	1.54	1.59	1.58 ± 0.03
Carbohydrate	7.95	8.36	8.25	8.19 ± 0.22

Values are expressed as mean ± standard deviation (n = 3)

Table 4: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration of the extracts of K. *pinnata*

Bacterial Strain	Solvent	MIC (mg/mL)	MBC (mg/mL)
S. aureus	Ethanol	15	63
	Methanol	31	126
	Distilled water	63	255
B. subtillis	Ethanol	7	31

	Methanol	15	63
	Distilled water	30	127
E. coli	Ethanol	62	258
	Methanol	125	514
	Distilled water	254	1025
P. aeruginosa	Ethanol	33	125
	Methanol	65	252
	Distilled water	130	516

Table 5: Zone of inhibition (ZOI) of the extracts of *K. pinnata*

Bacterial Strain	Solvent	ZOI (mm)
S. aureus	Ethanol	21
	Methanol	19
	Distilled water	12
B. subtillis	Ethanol	22
	Methanol	21
	Distilled water	15
E. coli	Ethanol	17
	Methanol	13
	Distilled water	10
P. aeruginosa	Ethanol	17
	Methanol	15
	Distilled water	14

The result of the qualitative phytochemical screening of solvent extracts of *K. pinnata* is shown in Table 1. Phytochemical screening of the solvent extracts of *K. pinnata* revealed the presence of several bioactive compounds which include: saponins, tannins, alkaloids, glycosides, flavonoids, phenolics, terpenoids and steroids. Saponins and tannins were extracted using water, ethanol and methanol. Alkaloids, flavonoids and phenolics were extracted using methanol, ethanol and propanone (acetone). Terpenoids and steroids were extracted using hexane, chloroform and methanol. The choice of solvents played a vital role in extracting different classes of phytochemicals. Polar solvents like water, ethanol and

methanol were effective in extracting hydrophilic compounds such as saponins, tannins and phenolics, while non-polar solvents like hexane and chloroform were suitable for extracting lipophilic compounds like terpenoids and steroids.

The result of the quantitative phytochemical analysis is shown in Table 2. Quantitative phytochemical analysis revealed the amount of a wide range of phytochemicals. In addition to the bioactive compounds, coumarins and lignans were also determined.

The phytochemicals offer a wide range of potential health benefits. Saponins are known for their immune–boosting and cholesterol lowering properties. The substantial yield of saponins extracted supports the plants potential pharmacological value.

Tannins exhibit strong antioxidant and antimicrobial activities. Alkaloids provide analgesic, antimalarial and anticancer benefits.

Flavonoids contribute to antioxidant, anti-inflammatory and cardio-vascular effects. Phenolics are potent antioxidants with anti-inflammatory and anticancer properties. Terpenoids possess anti-inflammatory, antimicrobial and anticancer activities.While glycosides, steroids, coumarins and lignans have demonstrated potentials in treating various health conditions including: heart disease, blood clot prevention and hormone– related cancers.

Table 3 reveals the result of the proximate analysis of the leaves of *K. pinnata*. The high moisture content (approximately 82%) of the herb suggests that the leaves are highly perishable and require proper drying or preservation for storage. Crude protein and carbohydrate levels indicate that *K. pinnata* offers some nutritional value, although it is not a protein–rich plant. Low fat and fiber contents are consistent with its use in herbal infusions and poultices. The ash content is an indication of the total mineral matter and confirms the presence of inorganic elements. These results support traditional claims of *K. pinnata* as a medicinal and nutritional supplement.

Table 4 reveals the results of minimum inhibitory concentration and minimum bactericidal concentration of the leaf solvent extracts of *K. pinnata*. The results indicate that *Kalanchoe pinnata* leaf extracts possess significant antimicrobial activity against both Gram-positive and Gram-negative bacteria. Ethanol extracts exhibited the lowest MIC and MBC values, suggesting higher antimicrobial potency compared to methanol and distilled water extracts. This is consistent with previous studies that reported ethanol extracts of *K.pinnata* showing strong antimicrobial effects against various pathogens. This observed antimicrobial activity may be attributed to the presence of bioactive compounds such as flavonoids, alkaloids and tannins, which are known to possess antimicrobial properties. The positive

control, ampicillin, demonstrated expected antimicrobial activity with MIC and MBC values consistent with literature reports confirming the reliability of the experimental methods employed.

Ethanol extracts also exhibited highest zones of inhibition (Table 5), indicating superior antimicrobial efficacy. These findings support the potential use of *Kalanchoe pinnata* leaf extracts as natural antimicrobial agents, which could be further explored for therapeutic applications.

CONCLUSION

This study revealed that *Kalanchoe pinnata* contains a variety of phytochemicals with pharmacological relevance and moderate nutritional value. The ethanol extract showed the most significant antimicrobial activity, suggesting that ethanol is a highly effective solvent for isolating bioactive compounds. These findings validate the traditional medicinal use of *K.pinnata*, particularly for treating infections and inflammations. The levels of flavonoids and phenolics imply potential antioxidant benefits, whereas the substantial saponin content may contribute to immunomodulatory effects. Despite the promising results, the study was limited by the number of tested microorganisms and lack of advanced compound characterization. Phytochemical composition may vary with geographic location, harvest season and plant maturity. Therefore, further research is recommended to isolate and identify the individual bioactive compounds using techniques such as: LC – MS, GC – MS and NMR.

Broader antimicrobial testing against fungal and drug resistant strains is essential to determine the full spectrum of activity.

Additionally, *in vivo* studies, toxicological assessments and pharmacokinetic evaluations should be conducted to determine therapeutic safety and efficacy. Standardizing extraction methods will also enhance consistency and enable scale – up for pharmaceutical applications. Overall, *K. pinnata* shows great promise as a source of natural bioactive agents for potential use in phytotherapeutic and nutraceutical products.

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