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Exploring the Therapeutic Potential of Kalanchoe Pinnata: A Comprehensive Analysis of Bioactive Compounds and Pharmacological Activities

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Abstract

Natural products are vital in drug discovery for their bioactive compounds. This study evaluated *Kalanchoe pinnata*'s leaf extracts for antidiabetic, antimicrobial, antioxidant, and cytotoxic properties. GC-MS identified seven components in hexane and twenty in methanol extracts. The ethyl acetate extract had high phenolic (485.17 mg GAE/g) and flavonoid (40.25 mg QE/g) contents. Methanol extract showed strong antioxidant (IC50: 293.53 µg/mL) and anti-alpha glucosidase (IC50: 195.39 µg/mL) activity. Antimicrobial tests showed varied effectiveness, while chloroform and acetone extracts exhibited notable cytotoxicity. ADMET predictions assessed absorption and blood-brain barrier penetration, highlighting *K. pinnata*'s potential for drug development.

Keywords: ADMET; Antioxidant; Cytotoxicity; GC-MS; Phytochemical screening.

INTRODUCTION

Natural products, sourced from living organisms like plants, animals, and microorganisms, constitute a wide array of bioactive compounds. They play a crucial role in drug discovery due to their abundant availability and varied chemical compositions, serving as the primary source of many medicinal active ingredients (Harvey, 2008). Typically, they possess biological functions suitable for application in drug discovery and design (Zhang et al., 2013). The medicinal properties of plants arise from their constituents, which induce physiological responses in the human body (Olowa & Nuñeza, 2013). These constituents include alkaloids, essential oils, tannins, and resins. Kalanchoe pinnata (Linn.) Pers. is a plant primarily located in temperate and tropical areas worldwide. This tall perennial has thick, fleshy, scalloped leaves and hollow stems bearing pendulous bell-shaped flowers (Okwu & Nnamdi, 2011). It has long been recognized for its diverse pharmacological properties, often utilized in traditional medicine to address various significant human ailments (Rajsekhar et al., 2016). The compounds found in the leaves of K. pinnata comprise various substances such as flavonoids, alkaloids, triterpenes, glycosides, and steroids. They are known for their potential to reduce inflammation and regulate blood sugar levels, and are widely used in traditional remedies for diabetes management, kidney stones, and fighting infections (Ojewole, 2005).

Kidney stones, a prevalent urological condition, involve the development and occasional passage of clusters of crystals within the urinary tract (Singh & Saini, 2009). Compounds in K. pinnata hinder kidney stone formation and offer diverse benefits: quercetin reduces crystal deposition, phenolics dissolve stones, kampferol inhibits crystal formation, glycosides protect kidneys, and steroids aid in stone passage, collectively providing comprehensive management for kidney stones and related urinary issues (Nagpal & Sharma, 2020). Diabetes Mellitus (DM) represents a significant multifaceted chronic ailment contributing substantially to global morbidity (Arroyave et al., 2020). Traditional clinical approaches to managing DM have primarily revolved around administering oral hypoglycemic medications and insulin (George et al., 2019). Many of the therapy choices in current use are burdened by

various adverse effects. These encompass issues like weight gain and hypoglycemia associated with insulin, beta-cell exhaustion with secretagogues, lactic acidosis with sensitizers like metformin, and gastric disturbances with alpha-glucosidase inhibitors. Extracts from K. pinnata have shown efficacy in controlling blood sugar levels (Efanova et al., 1998; Kimmel & Inzucchi, 2005; Lalau & Race, 1999; Patil et al., 2013). Besides these, plant extracts also possess the ability to inhibit the growth of certain harmful microorganisms. They can hinder germ proliferation and their antimicrobial potency can be evaluated accordingly (Kalemba & Kunicka, 2005).

The main objective of the research was to investigate the biological effects such as antidiabetic, antimicrobial, antioxidant, and cytotoxic properties of various extracts obtained from K. pinnata leaves, as well as to evaluate the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the compounds in these extracts. The in vivo lethality test has proven effective as an initial assessment of cytotoxic and antitumor agents (Ramachandran et al., 2011). The compounds verified via GC-MS were also assessed for their ability to reach the brain and be absorbed in the gastrointestinal tract using the SWISSADME tool. This model predicts two crucial properties of a compound: its ability to permeate the blood-brain barrier (BBB), indicating whether it can reach the brain, and its gastrointestinal (GI) absorption, determining if it can be effectively absorbed in the GI tract, which is vital for oral bioavailability.

The outcomes of this current study would provide fundamental insights into the most prospective plant extracts, serving as a foundation for developing novel therapeutic tools of significant importance.

MATERIALS AND METHODS

Study area

The plant part was collected from the Rupandehi district of Nepal, which was identified as *Kalanchoe pinnata* at the Department of Botany, Amrit Science Campus, Thamel, Kathmandu, 44600 (Supplementary Table 1).

Sample preparation

About 4 kg of the plant's leaves were collected, cleaned, and air-dried for a few days. They were then shade-dried, ground into a fine powder (almost 400 g), and stored at a low temperature for later use.

Extraction process

The ultrasonic extraction process began with 400 g of powdered plant material in a sterile, dry beaker. Hexane was added, stirred, and the beaker was immersed in an ultrasonic cleaner tub (30×15×20 cm) filled one-third with distilled water. After extraction, the contents were decanted, cooled, and filtered. The filtrate was then concentrated using a rota evaporator, resulting in an extract that was measured, dried, and stored in airtight vials. This process was repeated three times using various solvents (dichloromethane, chloroform, ethyl acetate, acetone, methanol, and water), with hexane added to the filtered residue before sonication.

Gas chromatography-mass spectroscopy

Before undergoing GC-MS analysis, the concentrated extract obtained from hexane and methanol via the rota-evaporator was dissolved in chloroform. GC-MS was utilized to separate and analyze the multicomponent mixtures.

Phytochemical screening

The phytochemical analysis of various extracts was done based on the protocol by Banu and Cathrine in 2015 (Banu & Cathrine, 2015).

Total phenolic content

The overall phenolic content of the plant extract was determined using a colorimetric technique called Folin-Ciocalteu, which relies on an oxidation-reduction process. The gallic acid concentration was used as a benchmark. Milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g of dry extract) were used to represent the sample's total phenolic content based on the gallic acid calibration curve. Initially, a stock solution was prepared with an extract concentration of 1000 µg/mL (ppm) by dissolving 1 milligram of extract in 1 mL of methanol. Subsequently, different concentrations of the extracts were prepared by serially diluting the stock solution, and their respective absorbance values were recorded (Balasundram et al., 2006).

The following Formula 1 was used to determine the sample's total phenolic content, which was represented as milligrams of gallic acid equivalent per gram.

$$C = cV / m \quad (1)$$

Where C is the total content of the phenolic compounds (mg/g) in gallic acid equivalent, c is the concentration of the gallic acid established from the calibration curve (µg/mL), V is the volume of the extract (mL), and m is the weight of the plant extract (µg).

Total flavonoid content

To quantify the total flavonoid content of the plant extract, an aluminum chloride colorimetric assay was performed (Chandra et al., 2014). Quercetin was used as the reference. Firstly, a stock solution, was prepared with an extract concentration of 1000 µg/mL (ppm) by dissolving 1 milligram of the extract in 1 mL of methanol. Following this, the stock solution was subjected to repeated dilution to produce a range of extract concentrations. The absorbance values of these diluted solutions were recorded.

Formula 2 was used to determine the sample's total flavonoid content, represented as milligrams of quercetin equivalents (QE) per gram of extract.

$$C = cV/m \quad (2)$$

Where C is the total flavonoid content (mg/g) in Quercetin equivalent (QE), c is the concentration of quercetin established from the calibration curve (µg/mL), V is the Volume of the extract (mL), and m is the weight of the plant extract (µg).

DPPH free radical scavenging activity

The plant extracts were assessed for their ability to scavenge radicals using the spectrophotometric DPPH method (Sanna et al., 2012). The capacity to neutralize the DPPH radical was determined by observing the decrease in absorbance and calculated using the following Formula 3.

$$Radical\ scavenging\ (\%) = [(A0 - AS)/A0] \times 100 \quad (3)$$

Where A_0 is the absorbance of the control (DPPH solution + methanol) and A_S is the absorbance of the test sample.

The amount of an adequate sample needed to neutralize 50% of DPPH free radicals is known as the IC_{50} (50% inhibitory concentration) value. By graphing the extract concentration vs. the corresponding scavenging action, the inhibition curve and IC_{50} values were generated.

10 mg of each extract (chloroform, ethyl acetate, and methanol) were dissolved in 10 mL of methanol to create a stock solution of 1 mg/mL. This stock solution was then successively diluted to concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL, and 125 µg/mL. For each concentration, 50 µL of ascorbic acid was mixed with 150 µL of methanolic DPPH solution in a 96-well plate, totaling 200 µL. After incubation in the dark for 20 minutes, the absorbance at 520 nm was measured using a spectrophotometer, with methanol and DPPH as blanks.

Antimicrobial activity

Microbial growth inhibition was evaluated using the paper disc diffusion method, and the outcome was quantified as a zone of inhibition (ZOI) value. In this assay, one Gram-positive bacterium (*Bacillus subtilis:* ATCC 6051), one Gram-negative bacterium (*Escherichia coli*: ATCC 8739), and a fungus (*Candida albicans:* ATCC 2091) were utilized. The efficacy of various *K. pinnata* leaf extracts in inhibiting microbial growth at a constant concentration (5 µg/mL) was evaluated.

Screening and evaluation of antimicrobial activity

Initially, 100 µL of culture broth from each strain was spread onto Mueller Hinton agar plates and incubated at 37 °C for 15 minutes. After incubation, the plates were left overnight at the same temperature. The antibacterial effects of the samples on B. subtilis, E. coli, *and* C. albicans *strains were evaluated the next day. Kanamycin was the positive control, while DMSO was the negative control. The ZOI produced by each sample was measured using a scale.*

Alpha-amylase inhibition assay

The α-amylase inhibitory potential of plant extracts was assessed using the 3,5-dinitrosalicylic acid (DNSA) technique. *K. pinnata* leaf extract was diluted with 10% DMSO to create different concentrations. These mixtures were further combined with buffer and NaCl at pH 6.9. The extract and α-amylase solution were mixed in a 200 µL volume and incubated at 30 °C for 10 minutes. The Starch solution was added and left for 3 minutes before stopping the process with the DNSA reagent. After heating in a water bath, the sample was diluted with distilled water and absorbance at 540 nm was measured using a UV spectrophotometer.

The α-amylase inhibitory activity (%) was calculated using Formula 4.

$$\%\ \alpha - amylase\ inhibition = \frac{Abs100\%Control - AbsSample}{Abs100\%Control} \times 100 \quad (4)$$

Extract concentration was plotted against the percentage of α-amylase inhibition, and IC_{50} values were obtained from the resulting graph.

Brine shrimp lethality assay (BSLA)

The study employed a brine shrimp lethality bioassay to investigate the cytotoxicity of the plant extracts. Understanding the toxic effects of plant extracts is essential for safe treatment. The brine shrimp lethality test, utilizing *Artemia salina*, is a cost-effective and versatile method widely used to screen diverse chemical compounds for bioactivity (Waghulde et al., 2020).

Before beginning the process, artificial seawater was created by combining the components in the specified amounts as detailed in Supplementary Table 2. Brine shrimp eggs, approximately 50 mg in weight, were dispersed over simulated seawater collected in a beaker, covered with aluminum foil, with a few small pores created to facilitate heat and light conductivity. The beaker was left at room temperature for 48 hours, exposed to radiation from a 60-watt bulb. A stock solution of 2 mg extract was prepared by adding 2 mL of DMSO (dimethyl sulfoxide), resulting in a concentration of 1000 ppm (mg/mL).

Toxicities of compounds were tested at 1, 0.5, 0.125, and 0.0625 ppm in sea-water solutions with 2 mL DMSO. In each test, ten nauplii were used, and survivors were counted after 24 hours. Three replications were performed for each concentration. A blank control was conducted using DMSO. The lethal concentration for 50% mortality (LC_{50}) after 24 hours of exposure, was determined using the probit method to measure the toxicity of the extract or fractions. LC_{50} values greater than 1000 ppm for plant extracts were considered inactive.

The mortality percentage was then computed using the following Formula 5.

$$\%\, Mortality = \frac{No.\ \text{of dead shrimps}}{\text{Total No. of shrimps}} \times 100 \quad (5)$$

ADMET Properties

In addition to conducting laboratory assays, the BOILED-Egg model was used for compounds verified through GC-MS analysis to determine their gastrointestinal absorption and blood-brain barrier (BBB) penetration capabilities (Dong et al., 2018). The canonical SMILES retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) for each compound were uploaded to the web server, and their properties were analyzed. Moreover, ADMET properties (absorption, distribution, metabolism, excretion, toxicity) were assessed using ADMET lab 2.0, and the toxicity class for each compound was identified with Protox 3.0 (https://tox.charite.de/protox3/).

RESULTS AND DISCUSSION

Gram yield

The ultrasonication technique was utilized to extract compounds from the *K. pinnata* plant. The resulting extracts using hexane, dichloromethane, chloroform, ethyl acetate, acetone, methanol, and water showed varying yields: 7.31 g, 9.08 g, 2.21 g, 0.21 g, 1.04 g, 10.34 g, and 14.50 g, respectively (Supplementary Table 3).

Qualitative analysis of phytochemicals

The extracts of *K. pinnata* were analyzed for their phytochemical contents, and the presence of these compounds was confirmed through observable color changes. Among the extracts, varying compositions of volatile oils, alkaloids, carbohydrates, phenolic compounds, tannins, flavonoids, terpenoids, quinones, reducing sugar, and saponins were identified (Supplementary Table 4).

The results of the phytochemical screening revealed that the polar extracts predominantly contained the highest concentrations of phytochemicals. Almost all solvent extracts tested positive for flavonoids and terpenoids. The absence of alkaloids in the extracts might be attributed to the method of extraction, which involved the use of an ultrasound sonicator followed by concentration using a rota-evaporator. This process could potentially lead to the decomposition of alkaloids due to heat and molecular vibration.

The literature often presents slightly different findings compared to the results obtained. Discrepancies can arise due to several factors, including differences in plant altitude, varying environmental conditions, the specific extraction method employed, the timing of sample collection, differences in laboratory setups, and variations in chemical grades used during analysis. Consequently, the outcomes of phytochemical screenings for a particular sample may vary from one study to another, even when targeting the same constituents.

GC-MS spectra analysis

Hexane and methanol extract of *K. pinnata* were used for the GC-MS analysis. The analysis revealed seven primary components in the composition of the *K. pinnata* hexane extract (Table 1) (Supplementary Fig. 1 to Supplementary Fig. 8) and 20 components in the methanol extract (Table 2) (Supplementary Figure 9 to Supplementary Figure 29). The compounds along with their PubChem CID and canonical SMILES are presented in Supplementary Table 5.

Table 1. Components present in the hexane extract according to GC-MS analysis.

S.N.	Name of compound	Retention time	Molecular formula	Area (%)
1.	2H-1,2,3,4-Tetrazole-2-ethanol, .alpha.-(chloromethyl)-5-phenyl-	8.228	$C_{10}H_{11}ClN_4O$	11.13
2.	N-benzyl-2-(5-phenyl-2H-tetrazol-2-yl)acetamide	8.506	$C_{16}H_{15}N_5O$	3.90
3.	2H-tetrazole-2-ethanole, 5-phenyl-	13.942	$C_9H_{10}N_4O$	27.44
4.	1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1.alpha.,4.alpha.,4a.alpha.,10a.alpha.)-	15.677	$C_{13}H_{20}N_2$	11.98
5.	Pentadecafluorooctanoicacid, dodec-2-en-1-yl ester	24.668	$C_{20}H_{23}F_{15}O_2$	29.36
6.	Octanoic acid, 4-methyl-,ethyl ester,(.+/-.)-	26.96	$C_{11}H_{22}O_2$	10.99
7.	Dodecane, 2-methyl-	28.00	$C_{13}H_{28}$	5.19

Table 2. Components present in the methanol extract according to GC-MS analysis.

S.N.	Name of compound	Retention time	Molecular formula	Area (%)
1.	2H-Tetrazole-2-ethanole, 5-phenyl-	9.542	$C_9H_{10}N_4O$	19.61
2.	Carbon dioxide	6.425	CO_2	9.51
3.	1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1.alpha.,4.alpha.,4a.alpha.,10a.alpha.)-	16.229	$C_{13}H_{20}N_2$	6.29
4.	1-bromo-2-phenyl-cyclopropanphosphonic acid, diethyl ester	15.281	$C_{13}H_{18}BrO_3P$	0.70
5.	4-Diethylaminophenyl isothiocyanate	18.255	$C_{11}H_{14}N_2S$	0.82
6.	Pentadecafluorooctanoic acid, undecyl ester	19.710	$C_{19}H_{23}F_{15}O_2$	0.79
7.	Oxirane, octyl-	21.876	$C_{10}H_{20}O$	0.76
8.	Methyl 6-methyloctanoate	22.244	$C_{10}H_{20}O_2$	0.89
9.	alpha-Methyl-alpha-[4-methylpentyl]oxiranmethanol	22.858	$C_{10}H_{20}O_2$	0.74
10.	Bacteriochlorophyll-c-stearyl	25.126	$C_{52}H_{72}MgN_4O_4$	1.71
11.	Levomenthol	24.284	$C_{10}H_{20}O$	1.68
12.	2-Isopropenyl-5-methyl-6-hepten-1-ol	25.028	$C_{11}H_{20}O$	0.67
13.	13-Borabicyclo[7.3.0]tridecane, 13-butoxy-, (Z)-or (E)-	25.439	$C_{16}H_{31}BO$	0.61
14.	8-Methylnonanoic acid, methyl ester	26.555	$C_{11}H_{22}O_2$	22.38
15.	Decanoic acid, silver (1+) salt	26.285	$C_{10}H_{19}AgO_2$	1.07
16.	1,2-Oxathiane, 6-dodecyl-,2,2-dioxide	27.204	$C_{16}H_{32}O_3S$	0.92
17.	Methyl-9,10-octadecadienoate	28.517	$C_{19}H_{34}O_2$	14.50
18.	Cyclododecyne	28.639	$C_{12}H_{20}$	9.65
19.	3,7-Dimethyl-6-nonen-1-ol	28.799	$C_{11}H_{22}O$	2.46
20.	Decanoic acid, methyl ester	28.930	$C_{11}H_{22}O_2$	3.73

Estimation of total phenolic and flavonoid content

The results indicated that the methanol extract of K. pinnata contains 182.87 mg GAE/g, while the ethyl acetate extract contains 485.17 mg GAE/g of phenolic content. These findings imply that phenolic compounds generally show higher solubility in polar organic solvents compared to non-polar ones. Additionally, ethyl acetate displayed a markedly higher total flavonoid concentration of 40.25 mg QE/g, in contrast to methanol, which showed a concentration of 19.05 mg QE/g (Table 3).

Table 3. Total phenolic and flavonoid content in ethyl acetate and methanolic extract of *K. pinnata* leaves.

Extract	Ethyl acetate	Methanol
Total phenolic content (mg GAE /g extract)	485.17	182.87
Total flavonoid content (mg QE/g extract)	40.25	19.05

Antioxidant activity

The chloroform extract of *K. pinnata* demonstrated an IC_{50} value of 2219.41 µg/mL, indicating relatively low antioxidant activity. In contrast, the ethyl acetate extract showed a much lower IC_{50} value of 365.22 µg/mL (Figure 1), suggesting stronger antioxidant properties. The methanol extract exhibited the highest antioxidant activity with an IC_{50} value of 293.53 µg/mL at 520 nm absorbance. These IC_{50} values reflect the varying effectiveness of the extracts in scavenging free radicals, with lower values indicating greater antioxidant potency.

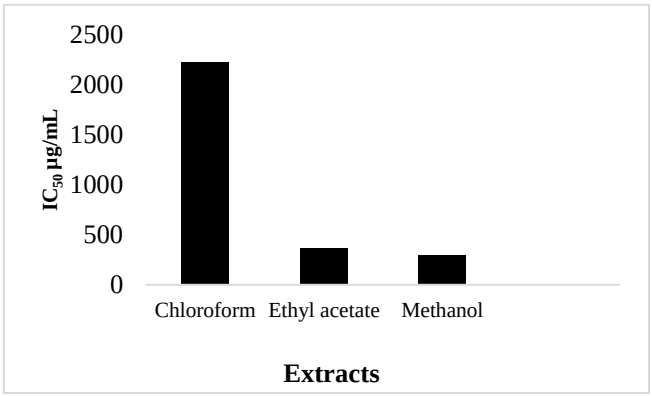

Figure 1. IC_{50} values of different extracts (chloroform, ethyl acetate, and methanol) of *K. pinnata*.

Antimicrobial activity

The antimicrobial activity of various extracts was measured by the diameter of the inhibition zone (ZOI) in centimeters, compared to the negative control (DMSO). For *Bacillus subtilis*, the hexane, dichloromethane (DCM), chloroform, and acetone extracts showed inhibition zones ranging from 0.70 to 0.85 cm, indicating moderate antimicrobial activity. The ethyl acetate, methanol, and aqueous extracts displayed smaller inhibition zones of 0.50 cm. In the case of *Escherichia coli*, both the hexane and methanol extracts had the largest inhibition zones at 0.80 cm, while the other extracts ranged between 0.60 and 0.70 cm. For *Candida albicans*, the hexane, DCM, and acetone extracts exhibited the largest inhibition zones of 0.75 cm, while the ethyl acetate and aqueous extracts had smaller inhibition zones of 0.60 and 0.50 cm, respectively.

Overall, the extracts varied in their antimicrobial efficacy across different microbes. The effectiveness of extracts varied with the type of microbe. For example, hexane and acetone extracts showed higher inhibition against *Candida albicans* compared to other extracts, suggesting that certain extracts might be more effective against specific microbes.

Table 4. Antimicrobial activity is shown by the different extracts in diameter (cm).

Microbes	Negative control DMSO	Hexane extract	DCM extract	Chloroform extract	Ethyl acetate extract	Acetone extract	Methanol extract	Aqueous extract
Bacillus subtilis (ATCC 6051)	0	0.85	0.80	0.85	0.7	0.85	0.50	0.50
Escherichia coli (ATCC 8739)	0	0.65	0.80	0.70	0.65	0.60	0.80	0.70
Candida albicans (ATCC 2091)	0	0.70	0.75	0.60	0.60	0.75	0.70	0.50

Alpha-amylase inhibition assay

In the alpha-amylase inhibition assay, *K. pinnata* acetone extract displayed an IC_{50} of 285.71 µg/mL, while ethyl acetate extract showed 504.21 µg/mL, and methanol extract exhibited a value of 195.39 µg/mL (Figure 2). Comparatively, among these three extracts, the methanol extract of *K. pinnata* showed higher α-amylase inhibition activity.

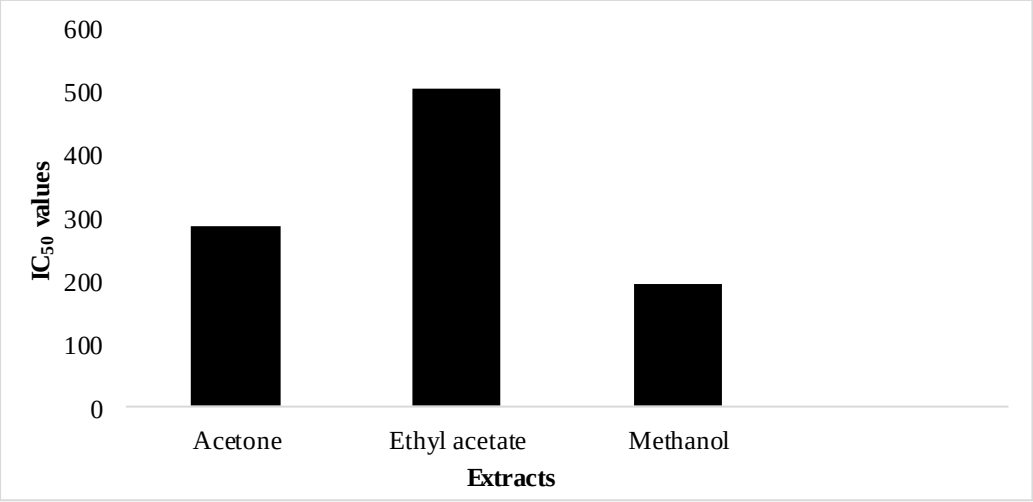

Figure 2. IC_{50} values of different extracts for α-amylase inhibition.

Cytotoxicity activity

After the freshly hatched live nauplii were exposed to concentrations of 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, and 0.0625 mg/mL, respectively, the LC_{50} value of chloroform, acetone, and methanol extracts was computed (Table 5). It was found that the concentration of the extract directly correlated with the level of lethality (Supplementary Table 6 to Supplementary Table 8). The highest and lowest numbers of s brine shrimp larvae perished at doses of 1 mg/mL and 0.0625 mg/mL, respectively.

Table 5. Lethality concentration of 3 different extracts of *K. Pinnata.*

Extracts	LC_{50} (mg/mL)
Chloroform	0.18
Acetone	0.17
Methanol	0.29

Among the three extracts, the chloroform extract exhibited a high mortality rate of 96.67%, while the methanol extract showed the lowest mortality rate of 56.66% at the extract concentration of 0.5 mg/mL. The LC_{50} values for the chloroform, acetone, and methanol extracts of *K. pinnata* were determined to be 0.181

mg/mL, 0.175 mg/mL, and 0.29 mg/mL, respectively (Table 5). As a result, extracts were observed to be pharmacologically significant and harmful to *Artemia salina* (brine shrimp) larvae.

The cytotoxic properties of these extracts may be attributed to the presence of alkaloids, tannins, and flavonoids (Waghulde et al., 2020). The significant lethality observed in various plant extracts towards brine shrimp implies the existence of potent cytotoxic compounds, highlighting the need for additional investigation.

ADMET Analysis

According to the BOILED-Egg model, the majority of molecules were concentrated within the yellow and white regions, indicating they possess balanced WLOGP and TPSA values. Out of the 27 compounds, 3 were found to be outside of the range as seen in the BOILED-Egg model. Molecule 9 was located at the lower end of the WLOGP scale (below -1), indicating it has low lipophilicity. As a PGP+ substrate, it might be actively transported out of cells, which could impact its bioavailability and penetration. Therefore, its low lipophilicity and the possibility of being actively transported out of cells make it an outlier compared to the other molecules. Such status implies that it may have limited absorption or distribution characteristics that are favorable for central nervous system (CNS)-targeted therapies.

The other two outliers, Molecule 1 and Molecule 2, were positioned towards the higher end of the TPSA scale (around 100), indicating a larger polar surface area (Figure 3). This correlates with lower membrane permeability, which can affect their ability to be absorbed in the intestine and penetrate the BBB. Their high TPSA values make them outliers, as they fall outside the optimal range for HIA and BBB penetration. All the compounds were found to fall into Class 4 toxicity, indicating they have low toxicity but can be detrimental if given in high doses according to the ProTox3.0 server. This classification suggests that these compounds are relatively safe and pose a low risk of causing significant harm at typical exposure levels.

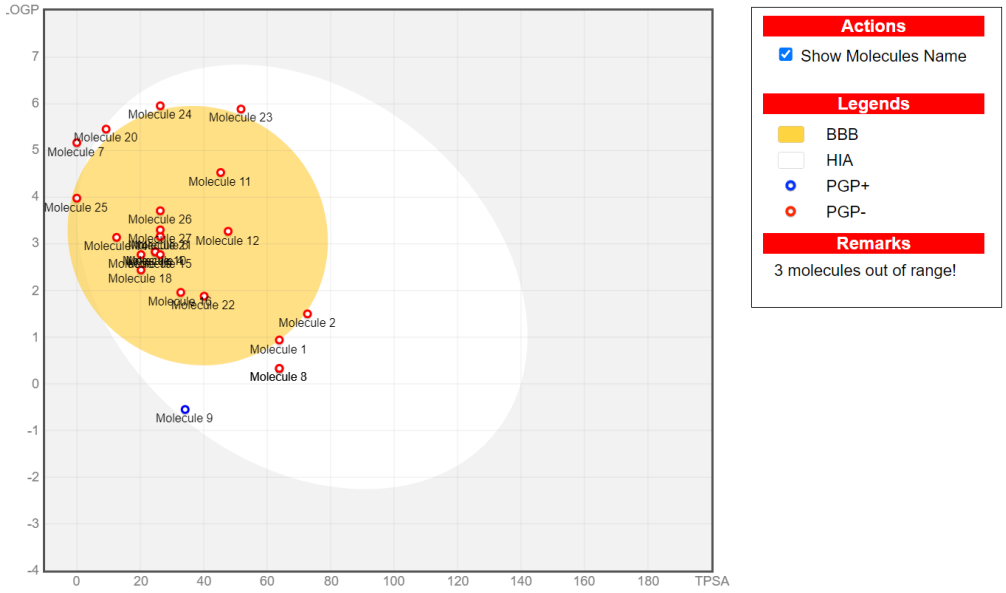

Figure 3. BOILED-Egg model for the identified 27 compounds.

Similarly, the ADMET properties of molecules extracted from hexane and methanol were analyzed. All molecules generally adhered to Lipinski's Rule of Five, indicating good drug-like properties. The majority of compounds exhibited excellent Caco-2 permeability and were classified as CYP3A4 substrates and inhibitors, suggesting effective absorption and metabolism. However, there was variability in their carcinogenic potential, ranging from non-carcinogenic to carcinogenic (Supplementary Table 9). Clearance rates were predominantly excellent, though a few molecules (6 out of 27) showed poor clearance, which could impact their overall efficacy and safety profiles. Molecule 5 was rejected by Lipinski's rule, indicating poor drug-like qualities, although it had excellent permeability and clearance. Molecule 9, while passing Lipinski's rule and showing excellent permeability, suffered from poor clearance, which potentially impacted its effectiveness. Molecule 17 was rejected by Lipinski's rule and had both poor permeability and clearance, suggesting significant issues with absorption and excretion. These factors highlighted potential challenges for these molecules in terms of drug development and effectiveness.

CONCLUSIONS

This study comprehensively evaluates the biological activities and phytochemical composition of *Kalanchoe pinnata* leaf extracts, highlighting their potential as therapeutic agents. The phytochemical analysis confirmed the presence of a diverse array of bioactive compounds, including flavonoids, phenolics, and terpenoids, across various extracts. Notably, the methanol and ethyl acetate extracts exhibited substantial antioxidant activity, with IC_{50} values indicating significant radical scavenging potential. The ethyl acetate extract, in particular, demonstrated the highest phenolic and flavonoid contents, correlating with its potent antioxidant effects. The alpha-amylase inhibition assay revealed that the methanol extract had superior α-amylase inhibitory activity, suggesting its potential for diabetes management. The brine shrimp lethality assay underscored significant toxic effects in the chloroform and acetone extracts, highlighting the need for further investigation into their mechanisms and safety. ADMET analysis showed that *K. pinnata* extracts generally exhibited favorable absorption and distribution profiles, though toxicity predictions indicated potential risks depending on dosage and exposure. The results emphasized the need for further research to balance therapeutic benefits with safety. Overall, *K. pinnata* demonstrated considerable promise for antioxidant, antimicrobial, and antidiabetic applications, with future studies needed to refine safety and efficacy while considering ADMET factors.

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