Original Article

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by IJRPS Journal

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GC-MS Analysis of Bioactive Phytochemicals in *Kalanchoe lanceolata* for Antimicrobial and Antidiabetic Activities

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Article History

Received on: 04 Feb 2024
Revised on: 11 Mar 2024
Accepted on: 15 Mar 2024

Keywords

Kalanchoe lanceolata,
GC-MS,
Bioactive phytochemicals,
Antidiabetic,
Antimicrobial

Abstract

The herb *Kalanchoe lanceolata*, which is widely used in traditional medicine, has showed promise in treating a range of ailments. Despite its widespread usage in traditional medicine, the phytochemical contents of *K. lanceolata* are not well studied, particularly in terms of its potential involvement in diabetes management. In this study, the objective was to identify the phytochemical constituents of *K. lanceolata* and determine how they can help with diabetes management. The whole plant methanolic, ethyl acetate, and petroleum ether extracts were analysed with GC-MS to identify bioactive components. In this study, 63 compounds were identified, 27 of which possess bioactive properties. Acorenone B, columbin, phytol, astaxanthin, β-sitosterol, 2,4-di-tert-butylphenol, and caryophyllene oxide are significant compounds found to be present in *K. lanceolata*. *In silico* molecular docking studies predicted the antidiabetic and antibacterial properties of phytochemical compounds. These compounds show potential as antibacterial, antifungal and antidiabetic agents. This study stresses the significance of *K. lanceolata* bioactive components and sheds light on the therapeutic potential in diabetes control.

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eISSN: 0975-7538
DOI: https://doi.org/10.26452/ijrps.v15i3.4678

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INTRODUCTION

Traditional remedies are widely used for the treatment of diabetes. Plant-based traditional medicine has been shown to be more broadly available, therapeutically efficacious, and have less side effects than contemporary medications [1]. Conventional drug therapy has its own set of limitations, and in many low- and middle-income countries, timely availability and cost remain issues [2]. The use of traditional medicine to treat diabetes and other conditions has gained popularity. To prevent wound infection and promote all aspects of wound healing, traditional medicine has put a great focus on "naturally" available products. Herbal medicines have demonstrated potential anti-diabetic properties and can be utilized as a complement or alternative to existing diabetes treatment procedures.

Traditional medicines require more study to determine the particular constituents and method of action for their antidiabetic benefits [3,4]. Kalanchoe has long been utilized in traditional medicine. Numerous Kalanchoe species have been used to heal wounds, inflammation, infections, and other ailments. Typically, the entire plant is employed, whether as juice or crude extracts [5].

The genus is known for its pharmacological properties, which include the potential to decrease inflammation, heal wounds, suppress tumour development, and relax muscles. The principal secondary metabolites found in kalanchoe extracts, flavonoids and bufadienolides, are responsible for these activities. *Kalanchoe pinnata* has been studied specifically for its anti-diabetic, anti-cancer, antibacterial, insecticidal, and anti-urolithiatic properties. Furthermore, *K. pinnata* shows potential in the treatment of stomach ulcers. More study is needed to examine the pharmacological properties of other Kalanchoe genus members that have received less attention.

The succulent plant *Kalanchoe lanceolata*, often known as "Mother of Thousands" or "*Bryophyllum lanceolatum*," belongs to the Crassulaceae family. Although there hasn't been as much study on *K. lanceolata* traditional usage in diabetes management as some other medicinal plants, it has been investigated for potential antidiabetic properties. The pharmacological properties of the plant have been identified, including antioxidant, antibacterial, and anti-urolithiatic action. Furthermore, a phytochemical analysis of *K. lanceolata* revealed the presence of a variety of compounds, including glycosides, alkaloids, triterpenes, and flavonoids [6]. These chemicals may be implicated in the potential of the plant towards anti-diabetic activities. More research is needed to fully understand *K. lanceolata* therapeutic potential and mechanisms of action in diabetes control [7].

Identifying and describing the phytochemical components of *K. lanceolata* requires sophisticated analytical approaches such as GC-MS [8]. Bioactive compounds may be promptly and accurately detected by GC-MS analysis. These chemicals have the potential to be employed as new therapeutics [9]. Computer-aided approaches such as molecular docking are utilized to predict the interaction between these medications and target proteins in order to resolve pharmacokinetic properties. Thus, the objective of this study is to gain insight into the bioactive components in *K. lanceolata* and utilize molecular docking to predict how these chemicals would interact with target proteins associated with microbial infections and diabetes.

MATERIALS AND METHODS

Plant material

Kalanchoe lanceolata (Forst.) Pers was collected from the Andhra Pradesh district of Chittoor in India. Plant collection, identification, and verification (Voucher number-0669). was done at the Department of Botany, S.V. University, Tirupati by Dr. K. Madhava Chetty.

Extract Preparation

To prepare the methanolic (M), ethyl acetate (EA), and petroleum ether (PE) extracts from *Kalanchoe lanceolata*, the entire plant had been selected, air-dried, and then crushed to a coarse powder with a crusher and pestle. Following that, a 300 g sample of coarse powder was cold macerated for a week using methanol, ethyl acetate and petroleum ether as solvents. Following soaking, the plant material was extracted from the solvent using muslin cloth, filtered through a funnel lined with filter paper, and the extract was allowed to evaporate at room temperature in a beaker without being disturbed. After being placed on a petri dish, the concentrated extract was stored in a desiccator until needed. This process ensured that the components of *K. lanceolata* were obtained for further analysis.

Qualitative Analysis

Chemical tests on *K. lanceolata* were utilized to identify specific compounds. To confirm their presence, tests for mucilage, flavonoids, steroids, carbohydrates, alkaloids, tannins, and fats/oils were conducted.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was done utilizing a chromatograph equipment and a mass spectrophotometer (Agilent 8890 USA). The HP-5 MS fused silica column (30.0 m × 250 µm, 0.25 µm film thickness) was linked to a 5675C Inert MSD with a triple-axis detector. Helium served as the carrier gas, and the column velocity flow rate was adjusted to 1.0 ml/min. The interface temperature was set to 300 °C, the pressure to 11.367 psi, and the temperature of the ion source to 250 °C. A 1 µL injector in split mode with a split ratio of 1:50 and an injection temperature of 350°C was used, with a split vent out duration of 1.8 minutes. After five minutes at 36 °C, the column temperature increased to 150 °C at 4 °C/min rate, then to 350 °C at 20 °C/min rate, where it remained for two minutes. Elution took place in 35 minutes total. Each component relative percent amount was

Table 1 *Kalanchoe lanceolata* phytochemical identification using chemical tests on methanol, ethyl acetate, and petroleum ether extracts

Plant constituents /Chemical tests	Methanol	Ethyl acetate	Petroleum ether
1. Tests for Carbohydrates			
(a) Molisch's test	+	+	+
(b) Benedict's test	+	+	+
(c) Fehling's test	+	+	+
2. Test for Saponins	+	+	+
3. Test for Flavonoids			
(a) Lead acetate test	+	+	+
(b) Sodium hydroxide test	+	+	+
(c) Shinoda test	+	+	+
4. Tests for steroids			
(a) Liebermann-Burchard reaction	+	+	+
(b) Salkowski reaction	+	+	+
5. Test for Tannins and Phenolic compounds	+	+	-
Legal's Test for glycosides	+	+	+
6. Test for Triterpenoids	+	-	-
7. Test for Proteins			
(a) Biuret's test	+	+	+
(b) Millon's test	+	-	+
(c)Ninhydrin test for Amino acids	+	+	-
8. Test for Fats and Oils	+	+	+
9. Test for Gums	-	-	-

where, +ve means identified and –ve means not identified

computed by adding the average peak areas from every site. The system was operated and data was collected using the supplier's MS solution software.

Identification of compounds

Utilizing the National Institute of Standards and Technology (NIST) database, the compounds in question were identified. Retention indices were utilized to identify components, while mass spectra were interpreted using the NIST database, which contains more than 62,000 patterns of well-known compounds. The mass spectra of recognized components from the NIST collection were compared to those of unidentified components in the *K. lanceolata* fraction.

In silico analysis

The tools for Virtual Screening - AutoDock, AutoDock Vina, MGL Tools, Open Babel, and Biovia Discovery Studio – were used to conduct molecular docking studies. Protein Data Bank was used to extract 3D structures of target proteins related with Type 2 diabetes, alpha-glucosidase and alpha-amylase and microorganisms, such as, lanosterol 14α-demethylases from *Saccharomyces cerevisiae*, glucosamine 6-phosphate synthase from *Escherichia coli*, and tyrosyl-tRNA synthetase from *Staphylococcus aureus*. Ligands from GC-MS identified phytochemicals of *K. lanceolata* were subjected to energy minimization and molecular docking to predict potential therapeutic interactions.

RESULTS & DISCUSSION

Phytochemical Screening of *Kalanchoe lanceolata*

The phytochemical screening of the methanolic/ethyl acetate/petroleum ether extraction from *K. lanceolata* revealed the presence of many phytochemical compounds, as shown in Table 1. *K. lanceolata* methanolic extract included carbohydrates, tannins, steroids, mucilage, alkaloids, flavonoids, and fats/oils. These data give a concise summary of the many phytochemicals found in the *Kalanchoe lanceolata* methanolic extract. *Kalanchoe lanceolata* has medicinal applications. It has a long history of application in treating a range of conditions, including infections, respiratory and digestive issues, skin disorders, and pain management [8].

The plant contains a number of phytochemical components that contribute to its therapeutic properties, including fatty acids, polysaccharides, alkaloids, flavonoids, polyphenols, and iridoid glycosides. Research has shown that *K. lanceolata* contains antiulcerogenic, cytotoxic, wound-healing, antioxidant, and antibacterial activities [10]. In this study, we focused on the therapeutic potential and drug-like capabilities of the bioactive compounds in *Kalanchoe lanceolata*, identified particularly for their antidiabetic and antimicrobial properties.

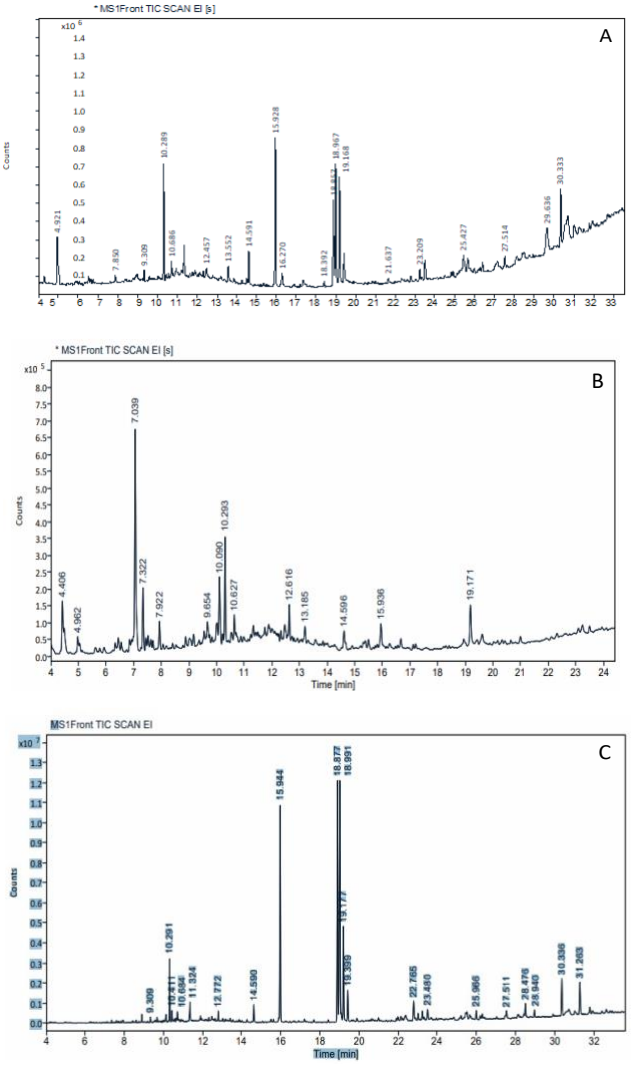


Figure 1 Chromatograms of *Kalanchoe lanceolata* extracts using GC-MS: (A) Methanol, (B) Ethyl Acetate, (C) Petroleum Ether

GC-MS Identification of Bioactive Compounds

As illustrated in Figure 1 and Table 2, the GC-MS analysis of *K. lanceolata* solvent extracts in MeOH(CH_3OH), EtOAc ($C_4H_8O_2$), and petroleum ether revealed a diverse variety of bioactive compounds. While Table 2 provided detailed information on retention time, molecular formula, molecular weight of notable bioactive compounds such as caryophyllene, 2,4-Di-tert-butylphenol, caryophyllene oxide, 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one, methyl ester of hexadecanoic acid, astaxanthin, β- and γ-sitosterol, columbin, phytol, spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-.and Acorenone B, the chromatograms in Figure 1 provide insights on their distribution.

The bioactive compounds found by GC-MS in *Kalanchoe lanceolata* have a wide range of pharmacological effects, as indicated in Table 2. These compounds exhibit antibacterial, antifungal, anti-inflammatory, and anticancer properties [8]. Furthermore, Nascimento *et al.* (2023) report that they exhibit antibacterial and antifungal properties, analgesic and anti-inflammatory effects, antinociceptive and antioxidant capacities, and anti-diabetic properties [10]. This study illustrates the flexibility of these compounds in treating a variety of health-related conditions and highlights their therapeutic potential [11].

Research has been conducted on the antidiabetic characteristics of the phytochemicals β-Longipinene, Caryophyllene, Caryophyllene oxide, Columbin, Retinol, Phytol, Spirost-8-en-11-one, Astaxanthin, β-Sitosterol, γ-Sitosterol, 2,4-Di-tert-butylphenol, Hexadecane, Heptadecane, and 2-Pentadecanone (Table 2). Numerous phytochemicals found in natural sources have demonstrated possible anti-diabetic properties [12]. Furthermore, a number of phytochemicals have been shown to have direct, particular antidiabetic effects, including alkaloids, flavonoids, terpenoids, saponins, and organic acids [13]. Certain plant metabolites, such as those from Cyperus species plants, have been studied for their potential to prevent diabetes [14].

The phytochemicals identified with antimicrobial qualities included 2,4-Di-tert-butylphenol, hexadecane, heptadecane, 2-Pentadecanone, dodecanoic acid, methyl ester, methyl tetradecanoate, hexadecanoic acid, methyl ester, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) (Table 2). Numerous pathogens, such as *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*,

Table 2 Bioactive compounds in GC-MS identified in methanol, ethyl acetate, or petroleum ether extracts of *Kalanchoe lanceolata*

S.No.	Name of the Molecule	Solvent	Retention Time	Molecular Weight (g/mol)	Bioactivity
1.	2-Methoxy-4-vinylphenol $C_9H_{10}O_2$	M	7.851	150.17	antioxidant, anti-inflammatory, and potentially antimicrobial
2.	Caryophyllene $C_{15}H_{24}$	M/PE	9.307	204.35	anti-inflammatory, anticancer, antioxidant and potentially analgesic
3.	β-Longipinene $C_{15}H_{24}$	M	9.307	204.35	antioxidant, anti-inflammatory, and potentially antimicrobial
4.	β-Acorenol $C_{15}H_{26}O$	M	9.307	222.37	antioxidant and anti-inflammatory
5.	Caryophyllene oxide $C_{15}H_{24}O$	M/PE	11.320	220.35	anticancer, analgesic, anti-inflammatory, antimicrobial and potentially antimicrobial
6.	Acorenone B $C_{15}H_{24}O$	M	11.320	220.35	antioxidant and potential anti-inflammatory
7.	Cedran-diol, 8S,13- $C_{15}H_{26}O_2$	M	11.320	238.37	potential antioxidant and anti-inflammatory
8.	Columbin $C_{20}H_{22}O_6$	M	16.271	358.4	antioxidant and potential anti-inflammatory, antidiabetic
9.	Retinol $C_{20}H_{30}O$	M	18.396	286.5	roles in vision, immune function, and skin health.
10.	Phytol $C_{20}H_{40}O$	M	19.165	296.5	antimicrobial, antioxidant and potential anti-inflammatory
11.	Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)- $C_{27}H_{40}O_4$	M	21.634	428.6	antibacterial, potential anti-inflammatory and antioxidant
12.	Astaxanthin $C_{40}H_{52}O_4$	M	25.429	596.8	potent antioxidant with potential anti-inflammatory
13.	β-Sitosterol $C_{29}H_{50}O$	M	29.642	414.7	anti-inflammatory, antioxidant, and potential cholesterol-lowering effects.

Table 2 Bioactive compounds in GC-MS identified in methanol, ethyl acetate, or petroleum ether extracts (Continued)

S.No.	Name of the Molecule	Solvent	Retention Time	Molecular Weight (g/mol)	Bioactivity
14.	γ-Sitosterol $C_{29}H_{50}O$	M	29.642	414.7	anti-inflammatory, antioxidant, and potential cholesterol-lowering effects, antidiabetic.
15.	Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5β)- $C_{29}H_{50}O$	M	27.517	430.7	various bioactivities.
16.	Squalene $C_{30}H_{50}$	M	30.330	410.7	antioxidant, potential anti-inflammatory.
17.	2,4-Di-tert-butylphenol: $C_{14}H_{22}O$	EA/PE	10.288	206.32	antioxidant, anti-inflammatory, anticancer and antimicrobial, antifungal.
18.	Hexadecane, 2,6,10,14-tetramethyl $C_{20}H_{42}$	EA	9.651	282.5	antimicrobial and anti-inflammatory.
19.	Heptadecane, 2,6,10,15-tetramethyl $C_{21}H_{44}$	EA	12.614	296.6	antimicrobial and anti-inflammatory.
20.	2-Pentadecanone, 6,10,14-trimethyl: $C_{18}H_{36}O$	EA/PE	14.595	268.5	antimicrobial and anti-inflammatory.
21.	Dodecanoic acid, methyl ester $C_{13}H_{26}O_2$	PE	10.413	214.34	antimicrobial and anti-inflammatory.
22.	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro 4,4,7a-trimethyl- $C_{11}H_{16}O_2$	PE	10.682	180.24	diverse pharmacological activities – analgesic, antidiabetic, antibacterial, antifungal.
23.	Methyl tetradecanoate $C_{15}H_{30}O_2$	PE	12.770	242.4	Antimicrobial.
24.	Hexadecanoic acid, methyl ester $C_{17}H_{34}O_2$	PE	15.946	270.5	antimicrobial and anti-inflammatory.
25.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester $C_{19}H_{34}O_2$	PE	18.878	294.5	anti-inflammatory.
26.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- $C_{19}H_{32}O_2$	PE	18.990	292.5	antimicrobial, anti-inflammatory.
27.	1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1,4a $C_{26}H_{34}O$	PE	23.478	362.5	diverse pharmacological activities.

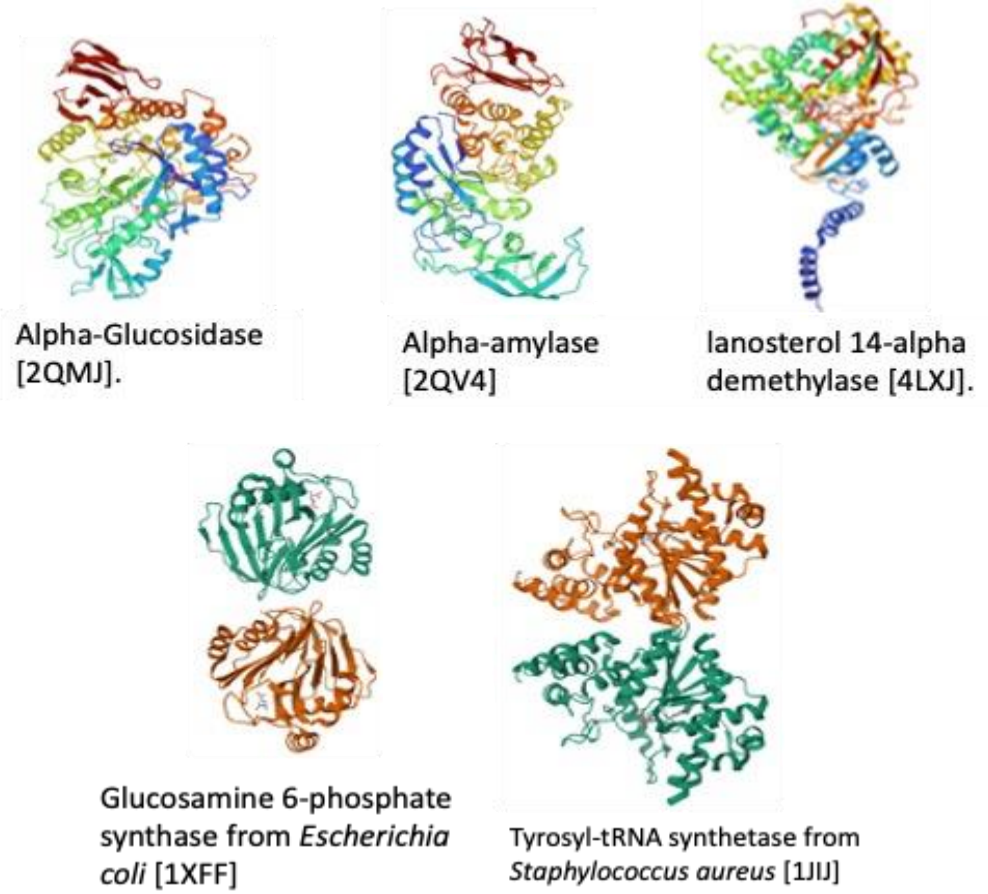


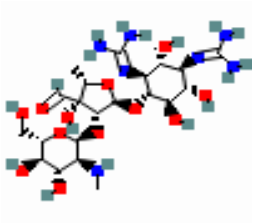
Figure 2 Target proteins with three-dimensional structures, alpha-glucosidase and alpha-amylase for Type 2 diabetes, tyrosyl-tRNA synthetase from *Staphylococcus aureus* and glucosamine 6-phosphate synthase from *Escherichia coli* for anti-bacterial, and lanosterol lanosterol 14-alpha demethylase from *Saccharomyces cerevisiae* for anti-fungal inhibition.

Salmonella typhimurium, *Shigella flexneri*, *Candida parapsilopsis* and *Candida albicans*, have been reported to be susceptible to the antibacterial activity of these phytochemicals. These compounds' antibacterial activity was evaluated using methods including disk diffusion and broth microdilution. Phytochemical screening and analytical methods, such as liquid chromatography and spectrophotometry, were employed to identify the presence of these phytochemicals in plant extracts. Positive correlations between specific chemicals and specific bacteria were observed during the examination of the link between chemical composition and antibacterial effect. These studies show phytochemicals' potential as natural antibacterial agents and advocate their use in the development of treatments and antimicrobial products [15,16].

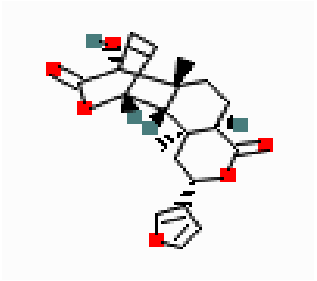
Research on the antimicrobial properties of bioactive compounds were obtained from *Kalanchoe lanceolata*. *Kalanchoe fedtschenkoi* leaves were revealed to have antimicrobial properties against fungi, bacteria, and both Gram-positive and Gram-negative bacteria[17]. Mejía-Méndez et al., (2023) found that the aqueous extract of *Kalanchoe blossfeldiana* had antibacterial properties against several bacterial strains, namely methicillin-resistant *Staphylococcus aureus* [18]. Furthermore, *Ficus benghalensis* L. fruit and leaf polar extracts have demonstrated antimicrobial activity against bacterial and fungal strains [19]. These findings suggest that *K. lanceolata* bioactive compounds may be able to inhibit the growth of fungus and bacteria.

Molecular Docking

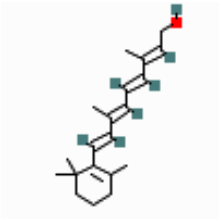
Phytochemicals from *K. lanceolata* identified by GC-MS analysis were also subjected to target protein molecular docking. Figure 2 shows the PDB codes for the target proteins, which included Alpha-amylase (2QV4) and Alpha-glucosidase



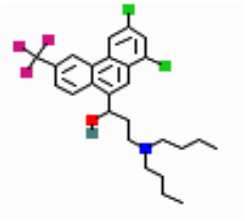
Streptomycin (Standard Drug)



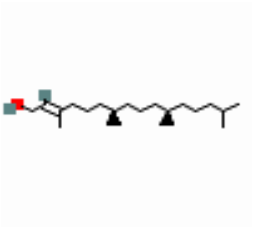
Columbin



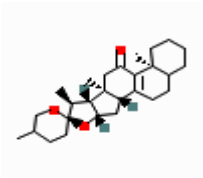
Retinol



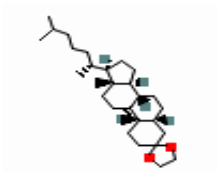
Phenanthrene, 1,2,3,4,4a,9,10,10 aoctahydro-1,1,4 a-trimethy



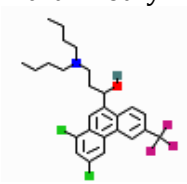
Phytol



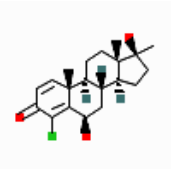
Spirost-8-en-11-one, 3- hydroxy-, (3β,5α,14β,20β,22β,25R)-



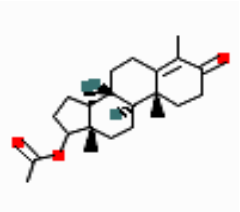
Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5β)-



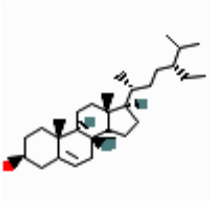
Phenanthren Phenanthrenemethanol, 1,2,3,4,4a,9,10,10 aoctahydro-1,4a emethanol,



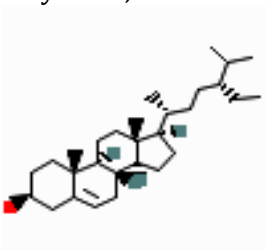
Androsta-1,4-dien-3-one, 6,17-dihydroxy-, (6β,17β)-



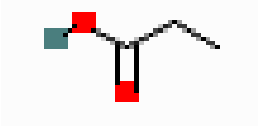
4-Methylandrost-4-en-17- ol-3-one acetate



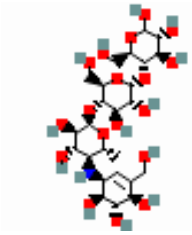
β-Sitosterol



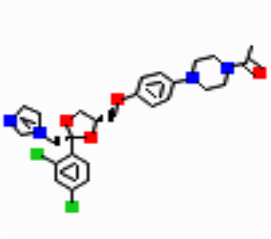
γ-Sitosterol



Propanoic acid, 2-(3- acetoxy-4,4,14-trimethylandrost-8-en-1



Acarbose (Standard Drug)



Ketoconazole (Standard Drug)

Figure 3 Twelve phytocompounds chosen for molecular docking tests with their two-dimensional structures. Three recognized standards (streptomycin, ketoconazole, and acarbose) used in the study are also shown

(2QMJ) for Type 2 diabetes, *Saccharomyces cerevisiae* lanosterol 14-alpha demethylase (4LXJ) for antifungal, *Staphylococcus aureus* Tyrosyl-tRNA synthetase (1JIJ) and *Escherichia coli* Glucosamine 6-phosphate synthase (1XFF) for antibacterial. It was feasible to find twelve bioactive compounds that are highly specific to target proteins. Initially, data on the 2D structures of bioactive compounds were gathered from the PubChem database. Figure 3 shows the structures of the bioactive compounds that were retrieved. These compounds were chosen using Lipinski's five-parameter method, which included log P, molecular weight, number of acceptors, and number of donors of hydrogen bonds (Table 3). The Lipinski metrics are critical indicators of drug-likeness, ensuring that potential treatment candidates have favourable physicochemical properties. These parameters are used to evaluate the compounds, such as Retinol, Spirost-8-en-11-one, Columbin, and others, for their medicinal potential. Predicting the drugs' oral bioavailability and overall pharmacological

Table 3 Lipinski's parameters and toxicity profiling of all the identified bioactive compounds from methanolic/ethyl acetate/petroleum ether extracts of *Kalanchoe lanceolata*

Bioactive compounds	Retention Time	Molecular weight	HBA	HBD	LogP
Columbin	16.271 min	358.4	6	1	2.20
Retinol	18.396 min	286.5	1	1	5.51
Phenanthrene, 1,2,3,4,4a,9,10,10aoctahydro-1,1,4a-trimethy	18.396 min	270.5	5	3	2.16
Phytol	19.165 min	296.5	1	1	4.85
Spirost-8-en-11-one,3-hydroxy-, (3β,5α,14β,20β,22β,25R)-	21.634 min	428.6	4	1	4.13
Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5β)-	23.210 min	430.7	1	0	4.70
1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10aoctahydro-1,4a	23.478 min	286.5	5	1	4.96
Androsta-1,4-dien-3-one, 6,17-dihydroxy-, (6β,17β)-	25.429 min	302.4	3	2	2.75
4-Methylandrost-4-en-17- ol-3-one acetate	29.642 min	344.5	3	0	3.27
β-Sitosterol	29.642 min	414.7	1	1	5.05
Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-1	29.642 min	430.6	4	1	3.62
γ-Sitosterol	29.642 min	414.7	1	1	5.07

MW (molecular weight ≤ 500); HBA (hydrogen bond acceptor ≤ 10); HBD (hydrogen bond donor ≤ 5); LogP ≤ 5; MR {Molar refractivity (40–130)}; LV vio (Lipinski's violations).

viability necessitates using Lipinski principles. The molecular docking study compared the bioactive compounds obtained from *K. lanceolata* to conventional controls such as acarbose (for diabetes), ketoconazole (for antifungal), and streptomycin (for antibacterial).

Molecular docking was utilized to evaluate the potential antimicrobial and antidiabetic effects of GC-MS analysed bioactive compounds from *K. lanceolata* (Figure 3 and Table 3). Numerous studies have looked at the link between bioactive compounds and diabetes-related proteins including alpha-glucosidase and alpha-amylase [20]. These enzymes have critical roles in glucose metabolism, making them promising targets for antidiabetic drug development [21]. Lupin protein hydrolysates show promise as alpha-amylase and alpha-glucosidase inhibitors, indicating their potential use in diabetes control [22]. Furthermore, it has been shown that natural chemicals derived from food, such as bioactive peptides, alkaloids, and phenolic compounds, can be utilized to treat Type 2 diabetes. These compounds have moderate pharmacokinetic profiles, low toxicity, and the capacity to inhibit alpha-amylase and alpha-glucosidase while also altering insulin receptors and incretin hormone [23]. *Eugenia jambolana's* bioactive component FIIc, also known as α-HSA, has been shown to improve insulin signaling, glucose metabolism, and reduce inflammation in rats with diabetes [24].

Binding Analysis and Inhibitory Effects

When target proteins and ligands were tested for binding, different patterns emerged. Figure 4 shows the docking results for the selected bioactive chemicals. Tables 4 and 5 present docking data demonstrating the inhibitory effects of *K. lanceolata* chemicals on target proteins. The lowest binding energy values and the number of hydrogen bonds produced are shown. Notably, Columbin inhibited multiple enzymes, but the

Table 4 The binding energies of drug-like compounds from *Kalanchoe lanceolata* for target proteins, including lanosterol 14-alpha demethylase, tyrosyl-tRNA synthetase, glucosamine 6-phosphate synthase, alpha-amylase, and alpha-glucosidase

Compound Name	Alpha-amylase	Alpha-glucosidase	Glucosamine 6-phosphate synthase from *Escherichia coli*	Tyrosyl-tRNA synthetase from *Staphylococcus aureus*	lanosterol 14-alpha demethylase from *Saccharomyces cerevisiae*
Columbin	-7.5	-7.5	-8.1	-	-8.6
Retinol	-7.5	-7.3	-	-	-8.9
Phenanthrene,1,2,3,4,4a, 9,10,10 aoctahydro-1,1, 4a-trimethy	-7.2	-7.2	-6.9	-	-8.8
Phytol	-7.7	-	-	-	-11.2
Spirost-8-en-11-one,3-hydroxy-, (3β,5α,14β,20β,22β,25R)-	-	-7.6	-7.3	-7.9	-
Cholestan-3-one, cyclic 1,2- ethanediyl aetal, (5β)-	-8.7	-8.7	-	-8	-10.5
1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10 aoctahydro-1,4a	-7.3	-7.3	-	-7.4	-8.5
Androsta-1,4-dien-3-one, 6,17-dihydroxy-, (6β,17β)-	-	-7.2	-	-7.4	-8.8
4-Methylandrost-4-en-17-ol-3-one acetate	-7.7	-7.7	-6.8	-8.3	-8.8
β-Sitosterol	-8.9	-9	-	-8	-10
Propanoic acid, 2-(3-acetoxy-4, 4,14- trimethylandrost-8-en-1	-7.2	-6.9	-	-	-9.4
γ-Sitosterol	-8.8	-8.7	-	-8.1	-9.8
Standard 1(Acarbose)	-7.3	-	-	-	-
Standard 2(Acarbose)	-	-6.3	-	-	-
Stardard 3(streptomycin)	-	-	-6.7	-	-
Standard 4 (streptomycin)	-	-	-	-8.4	-
Standard 5 (Ketoconoazole)	-	-	-	-	-9.9

effects of other medicines differed. Standard controls (acarbose, streptomycin, ketoconazole) were included to allow for comparison and shed light on the inhibitory effects and hydrogen bond interactions of *K. lanceolata* chemicals with specific target proteins.

Compounds with high binding affinities to target proteins have been identified using molecular docking techniques (Figure 4; Tables 4 and 5), indicating potential therapeutic medications. The docking studies in the literature provide valuable information on the molecular mechanisms behind various drugs' antibacterial and antidiabetic

Table 5 The hydrogen-bond interactions of compounds derived from *Kalanchoe lanceolata* with specific target protein enzymes.

Compound Name	Alpha-amylase	Alpha-glucosidase	Glucosamine 6-phosphate synthase from *Escherichia coli*	Tyrosyl-tRNA synthetase from *Staphylococcus aureus*	lanosterol 14-alpha demethylase from *Saccharomyces cerevisiae*
Columbin	5	7	10	-	8
Retinol	13	9	-	-	7
Phenanthrene,1,2,3,4,4a, 9,10,10 aoctahydro-1,1, 4a-trimethy	5	5	6	-	8
Phytol	10	-	-	-	13
Spirost-8-en-11-one,3-hydroxy-, (3β,5α,14β,20β,22β,25R)-	-	6	7	9	
Cholestan-3-one, cyclic 1,2- ethanediyl aetal, (5β)-	12	10	-	12	8
1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10 aoctahydro-1,4a	7	7	-	11	9
Androsta-1,4-dien-3-one, 6,17-dihydroxy-, (6β,17β)-	-	3	-	11	10
4-Methylandrost-4-en-17-ol-3-one acetate	9	10	10	18	9
β-Sitosterol	9	9	-	18	13
Propanoic acid, 2-(3-acetoxy-4, 4,14- trimethylandrost-8-en-1	8	9	-	14	9
γ-Sitosterol	10	9	-	18	8
Standard 1(Acarbose)	9	-	-	-	-
Standard 2(Acarbose)	-	7	-	-	-
Stardard 3(streptomycin)	-	-	7	-	-
Standard 4 (streptomycin)	-	-	-	8	-
Standard 5 (Ketoconoazole)	-	-	-	-	9

The substances were tested for their capacity to inhibit the following enzymes: lanosterol 14-alpha demethylase from *Saccharomyces cerevisiae*, tyrosyl-tRNA synthetase from *Staphylococcus aureus*, glucose 6-phosphate synthase from *Escherichia coli*, and alpha-glucosidase and alpha-amylase for

effects [25]. Compounds such as Columbin, Retinol, and Spirost-8-en-11-one, among others, meet Lipinski's criteria, suggesting their potential therapeutic value [5]. The toxicity profile and Lipinski's criteria give valuable information on the drug-likeness of the found bioactive compounds [26]. The prospective drug candidates with physicochemical properties consistent with Lipinski's criteria have a higher oral bioavailability and overall pharmacological viability. These results provide the framework for future experimental validation, aid in the identification of

potential lead compounds for therapeutic development, and have important implications for the treatment of infectious illnesses and diabetes.

Implications for Drug Development and Future Directions

This research of bioactive compounds identified in *K. lanceolata* emphasizes their possible utility in to treat infectious illnesses and diabetes and the development of pharmaceuticals. Molecular docking investigations suggest that these compounds have promising antibacterial, antifungal and anti-diabetic properties, with a focus on key enzymes. Although meeting the criterion for drug-likeness boosts their potential for drug development, limitations such as the necessity for experimental validation and reliance on *in silico* evaluations highlight the need for more research. Future direction emphasizes the necessity of prioritizing extensive *in vitro* and *in vivo* investigations. These studies will benefit patients with infectious diseases and diabetes by validating pharmaceutical activities, discovering underlying mechanisms, and investigating possible synergistic effects.

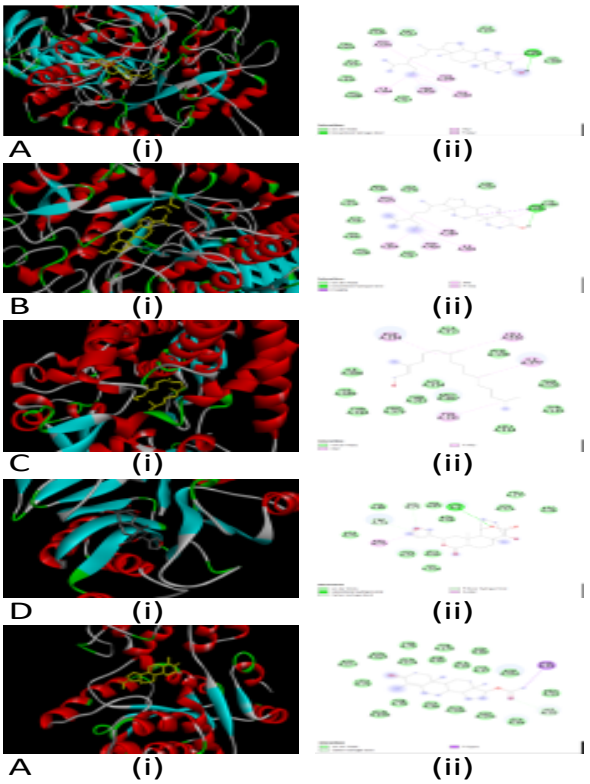


Figure 4 The selected phytochemicals with the highest docking scores for two- and three-dimensional (2-D) image interactions of: (A) β-Sitosterol with the target protein Alpha Amylase. (B)β-Sitosterol with the target protein Alpha Glucosidase. (C) Phytol with the target protein Lanosterol 14-alpha demethylase. (D) Columbin with the target protein Glucosamine 6-phosphate synthase. (E) 4-Methylandrost-4-en-17-ol-3-one acetate with the target protein Tyrosyl-tRNA synthetase.

CONCLUSION

In conclusion, the study indicates that *Kalanchoe lanceolata* has pharmacological value since it has been able to identify a number of bioactive compounds with antimicrobial, anticancer, and antidiabetic properties. The phytochemical components that contribute to these activities were identified *via* GC-MS analysis, underlining *K. lanceolata* potential as a source of therapeutic medicines. The findings also emphasize the potential of plants in treating infections and regulating diabetes, with compounds such as retinol and columbin exhibiting promising antibacterial and antidiabetic properties. More research is needed to determine the specific mechanisms of action of these compounds and their potential therapeutic use in the treatment of infections and diabetes.

ACKNOWLEDGEMENT

LL acknowledges National Fellowship for Higher Education of ST Students (NFST), Ministry of Tribal Affairs, Government of India, BG acknowledges University Grants Commission for JRF & SRF fellowship. AS acknowledges funding by CAS, DST-PURSE-II, UPE-FAR and DST-FIST and providing facilities to carry out the work.

Ethical Approval

No ethical approval was necessary for this study.

Author Contribution

All authors made substantial contributions to the conception, design, acquisition, analysis, or interpretation of data for the work. They were involved in drafting the manuscript or revising it critically for important intellectual content. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work, ensuring its accuracy and integrity.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

Funding Support

The authors declare that they have no funding for this study.

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