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PHARMACOGNOSTIC INSIGHTS, PHYTOMETABOLITE ANALYSIS AND *IN-VITRO* ASSESSMENT OF ANTIOXIDANT, ANTI-DIABETIC AND ANTI-PARKINSON'S EFFECTS OF *KALANCHOE FEDTSCHENKOI* LEAVES

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Keywords:

Kalanchoe fedtschenkoi, Antioxidant activity, Antidiabetic, neurodegenerative diseases

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ABSTRACT: Background: *Kalanchoe fedtschenkoi* is a traditionally used medicinal plant reputed for its antioxidant, antidiabetic, and neuroprotective properties. However, detailed scientific validation of its pharmacognostic and bioactive potential is limited. **Objective:** This study aimed to evaluate the pharmacognostic characteristics, phytochemical profile, and *in-vitro* biological activities of *K. fedtschenkoi* leaves to support their therapeutic applications. **Methods:** Macroscopic, microscopic, and physicochemical analyses were performed to ensure plant authenticity and quality. Preliminary phytochemical screening identified flavonoids, alkaloids, tannins, glycosides, phenolic compounds, terpenoids, and carbohydrates. The ethanolic leaf extract was analyzed by High-Performance Thin Layer Chromatography (HPTLC) and Gas Chromatography–Mass Spectrometry (GC–MS), revealing 69 compounds, with 1,2,3,4-Cyclopentanetetrol (46.94%) as the major constituent. Antioxidant activity was assessed using the DPPH assay. Antidiabetic potential was evaluated *via* α-amylase and α-glucosidase inhibition assays, while acetylcholinesterase inhibition was performed to determine neuroprotective activity. **Results:** The extract showed moderate antioxidant activity (IC_{50} = 83.06 µg/mL) and dose-dependent inhibition of α-amylase (IC_{50} = 863.42 µg/mL) and α-glucosidase (IC_{50} = 973.46 µg/mL), indicating antidiabetic potential. Strong acetylcholinesterase inhibition (IC_{50} = 59.77 µg/mL, R^2 = 0.9917) suggested neuroprotective efficacy. **Conclusion:** *K. fedtschenkoi* leaves exhibit significant antioxidant, antidiabetic, and neuroprotective activities, supporting their traditional use. These findings provide a basis for future development of standardized herbal formulations and therapeutic applications in oxidative stress, diabetes, and neurodegenerative disorders such as Parkinson's disease.

INTRODUCTION:

Herbal Medicine [1, 2]: Herbal medicine (HM) continues to serve as a primary healthcare resource globally, especially in developing countries, due to its accessibility, safety, and cultural acceptance.

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India possesses a rich heritage of plant-based therapies; however, many medicinal plants remain insufficiently investigated for their pharmacological potential and bioactive constituents. The lack of standardization and limited scientific validation underscore a critical research gap.

Succulents [3, 4]: Succulent species, such as those in the genus Kalanchoe, are adapted to arid environments via specialized water-storage tissues. Beyond ecological significance, they are emerging as potential sources of bioactive compounds with

medicinal properties, yet systematic studies on their therapeutic effects are limited.

FIG. 1:

Diabetes Mellitus AND Parkinson's Disease [5, 6, 7, 8, 9]: Diabetes mellitus (DM) and Parkinson's disease (PD) remain significant global health challenges. DM involves chronic hyperglycemia leading to organ complications, while PD is characterized by progressive dopaminergic neuron loss and motor dysfunction. Conventional treatments often have side effects and limited disease-modifying potential, emphasizing the need for safer, plant-based alternatives with antioxidant, antidiabetic, and neuroprotective properties.

Study Objective: To address these gaps, the present study aims to evaluate the pharmacognostic characteristics, phytochemical profile, and *in-vitro* antioxidant, antidiabetic, and neuroprotective activities of *Kalanchoe fedtschenkoi* leaves, providing a scientific foundation for future therapeutic applications.

METERIALS AND METHODS:

Plant Collection: Fresh leaves of *Kalanchoe fedtschenkoi* were collected in February 2025 from Gobichettipalayam, Erode district, Tamil Nadu. The plant was botanically identified and authenticated by Dr. P. Radha, Research Officer (Botany), Siddha Medicinal Plants Garden, Mettur Dam. It belongs to the Crassulaceae family.

Pharmacognostical Study [10]:

Organoleptic Characters: Macroscopic studies the fresh leaves *Kalanchoe fedtschenkoi* of were collected and different organoleptic characters such as colour, odour, taste, size, shape, type were observed. These parameters are considered useful in the qualitative control of the crude drug.

Microscopy:

Transverse Section: Fresh specimens were cut into thin transverse section using a sharp blade and the sections were stained with 1% Safranin and 0.5% fast green. Transverse sections were observed at different magnifications under Trinocular Microscope (Magnus MX21iLED) and micro photographs captured using Magnus Pro 3.7 digital camera under bright field.

Fresh, mature leaves were cleaned and sectioned from the middle region between the midrib and margin. Samples were cleared using 10% NaOH, rinsed with water, and mounted in glycerin. Leaf constants like stomatal number, stomatal index, epidermal cell count, and palisade ratio were observed under a light microscope with a digital camera.

Powder Microscopy: About 0.5gm of the finely powdered sample was mounted in Glycerin at room temperature for 2 hrs and observed under 10X and 40X objective of bright field microscope (Meswox, India) for powder characteristics. Photomicrographs of diagnostic characters were captured using attached camera.

Physicochemical Analysis Study [11]: Physicochemical analysis of *Kalanchoe fedtschenkoi* leaf powder was carried out using reported methods. Following determinations were done.

- Loss on drying
- Total Ash
- Acid insoluble ash
- Water soluble ash
- Water soluble extractive
- Alcohol soluble extractive
- Swelling index
- Foaming index
- pH.

Extraction of Plant Material [12]: Fresh leaves of *Kalanchoe fedtschenkoi* were coarsely powdered, and 100 g of the material was extracted with

ethanol (60–80 °C) using a Soxhlet apparatus. The extract was then concentrated by simple distillation. The yield, color, and consistency were documented for subsequent phytochemical and pharmacological investigations.

Preliminary Phytochemical Analysis [13]: All plant materials underwent preliminary phytochemical screening using standard protocols to identify bioactive constituents. The study focused on major secondary metabolites, including alkaloids, flavonoids, tannins, saponins, glycosides, and phenolics. These compounds are linked to therapeutic activities such as antioxidant, antimicrobial, and anti-inflammatory effects. The findings provide a basis for further detailed phytochemical and *in-vitro* pharmacological investigations.

HPTLC [14, 15]: Various solvent systems were tested, and the optimal separation was achieved using ethyl acetate:toluene:formic acid (6:1:0.1). Ethanol extracts of *Kalanchoe fedtschenkoi* were applied on silica gel 60 F254 aluminium plates (10 × 10 cm) using a CAMAG ATS4 system. After development in a pre-saturated chamber, plates were dried and visualized under UV light at 254 nm and 366 nm using a CAMAG Visualizer. Densitometric scanning was performed with TLC Scanner 4, and Rf values were recorded using winCATS software. Post-derivatization was done with vanillin-sulphuric acid and heated at 105°C, followed by scanning at 575 nm to document the final fingerprint profiles.

(GCMS) [16, 17]: GC-MS was employed to analyze the ethanol extract of *Kalanchoe fedtschenkoi* to identify bioactive compounds such as alkaloids, flavonoids, glycosides, and terpenoids. This technique is widely used to quantify active constituents in herbal preparations applied in pharmaceuticals, cosmetics, and food products. The analysis was conducted using an AOC-20i autosampler and a GC-QP2010SE instrument. Key operating conditions included a column oven temperature of 50°C, injector temperature of 250°C, and split injection mode. The mobile phase flow rate was 1.20 mL/min, with a purge flow of 3.0 mL/min and a split ratio of 10:1. The mass spectrometer was set to scan mode (m/z 50–500), with an ion source temperature of 200°C and interface temperature of 250°C. The total run time was 35 minutes, and data acquisition was carried out using standardized parameters to ensure accurate compound detection and profiling.

***In-vitro* Antioxidant Study [18]:**

DPPH Radical Scavenging Activity: Prepared 0.1 mM of DPPH solution in methanol and add 100 µl of this solution to 300 µl of the solution of Sample at different concentration (500, 250, 100, 50, and 10 µg/ml). The mixtures have to be shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance has to be measured at 517 nm using a UV-VIS spectrophotometer. (Ascorbic acid can be used as the reference). Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical can be calculated by using the following formula

DPPH scavenging effect (% inhibition) = (Absorbance of control - Absorbance of reaction mixture) / Absorbance of control × 100

Pharmacological Study:

***In-vitro* Antidiabetic Activity:**

α-amylase Inhibitory Assay Method [19]: Different concentrations of sample (250 µg/mL - 1000 µg/mL) and Standard (250µg/mL -1000µg/mL) was make up to 100µl using 25mM phosphate buffer pH 6.9, containing 25µl of porcine α amylase at a concentration of 0.5 mg/ml were incubated at 25°C for 10 min. After pre- incubation, 25µl of 0.5% starch solution in 25mM phosphate buffer pH 6.9 was added. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 50µl of 96mM 3, 5 di-nitro-salicylic acid colour reagent. The micro plate was then incubated in a boiling water bath for 5 min and cooled to room temperature. Absorbance was measured at 540nm using a microplate reader (Erba, Lisascan).

Percentage of inhibition = Control-Test / Control × 100

Alpha Glucosidase Inhibitory Assay [20]: Different concentrations of sample such as 250 µg/mL – 1000 µg/mL from a stock concentration and make up to 100µl using 0.1M phosphate buffer pH 7.2, containing 25µl of α Glucosidase (SIGMA - ALDRICH, LOT- 0000221279) was incubated at

25°C for 10 min. After pre incubation, 1ml of 0.1 M phosphate buffer (pH 7.2) containing 37mM sucrose was added. Then the reaction mixture was incubated for 30 min at 37 °C and the reaction was stopped incubating in a boiling water bath for 2 minutes. A tube with phosphate buffer and enzyme was maintained as control. The tubes were added with 250µL of glucose reagent and incubated for 10 minutes followed by measuring absorbance at 510nm using a microplate reader (Erba, Lisascan).

% inhibition = Control-Test / Control × 100

Invitroanti-Parkinson's Activity [21]:

Acetylcholinesterase (AChE) Inhibition Assay: The assay involved reagents such as acetylthiocholine iodide (ATCI), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), sodium phosphate buffer (pH 8.0), enzyme solution, and the test sample. A modified version of the method developed by Ellman *et al.* (1961) was followed. Electric eel acetylcholinesterase was used as the enzyme, and ATCI served as the substrate. To perform the assay, 150 µL of 0.1 M sodium phosphate buffer, 10 µL of the test sample (dissolved in ethanol), and 20 µL of enzyme solution (0.09 units/mL) were mixed and incubated at 25°C for 15 minutes. Subsequently, 10 µL of DTNB (10 mM) was added, and the reaction was initiated by adding 10 µL of ATCI (14 mM). The hydrolysis of ATCI released thiocholine, which reacted with DTNB to produce a yellow-colored 5-thio-2-nitrobenzoate ion. The absorbance was measured at 410 nm after 10 minutes. Physostigmine, a known AChE inhibitor, was used as a positive control. The percentage inhibition of AChE activity was calculated using the formula

% Inhibition = 100 - [(Test OD / Control OD) × 100]

RESULTS AND DISCUSSION:

Organoleptic Characters: The physical parameters like state, nature, odor, taste, touch, flow, property, and appearance revealed as given in **Table 1.**

TABLE 1:

S. no.	Specification	Character
1	State	Solid
2	Nature	Fine
3	Odour	No characteristic odour
4	Touch	Smooth
5	Flow Property	Non-Free flowing
6	Appearance	Greyish green in colour
7	Taste	Bitter

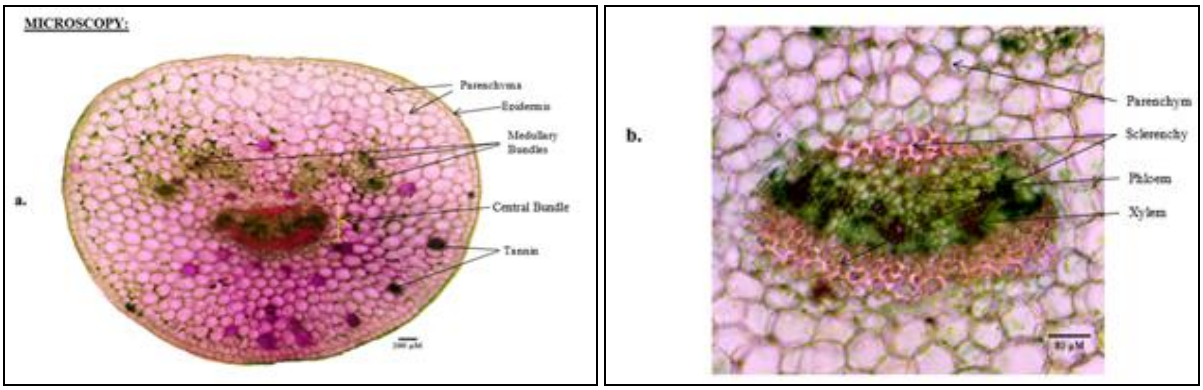


FIG. 2: PETIOLE: A. GROUND PLAN, B- VASCULAR BUNDLE

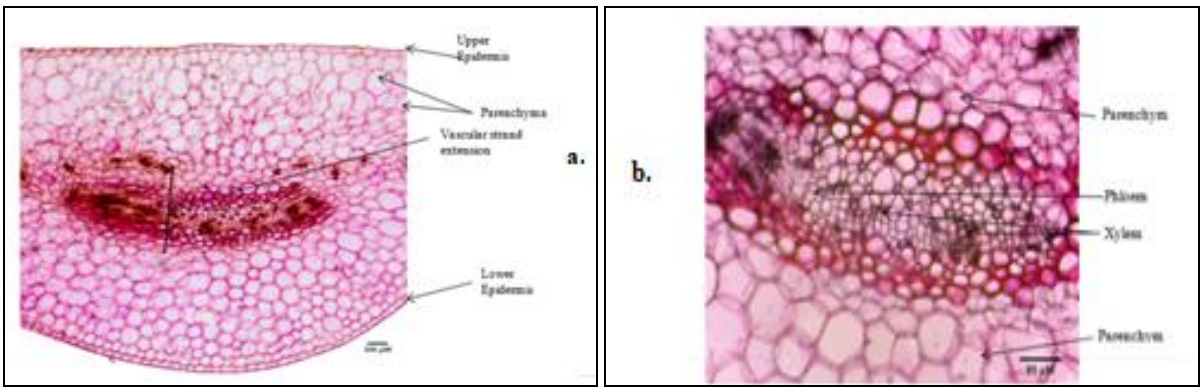


FIG. 3: MIDRIB: A. GROUND PLAN, B- VASCULAR BUNDLE

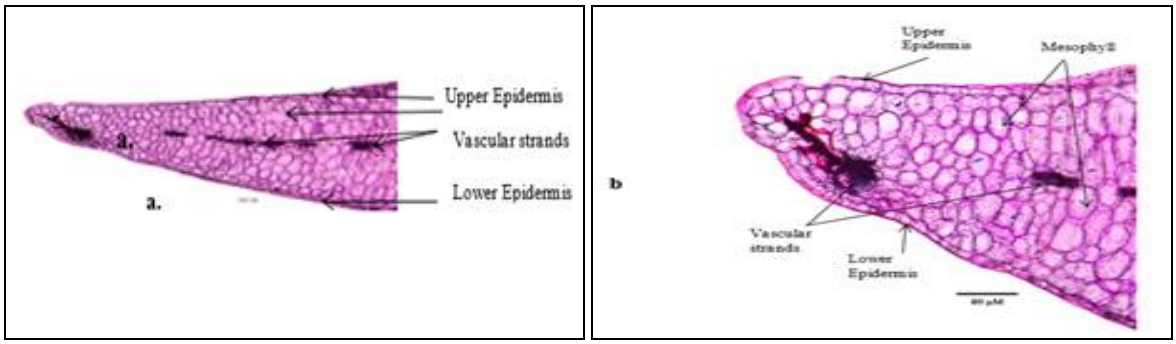


FIG. 4: LAMINA: A. GROUND PLAN, B- MARGIN PORTION ENLARGED

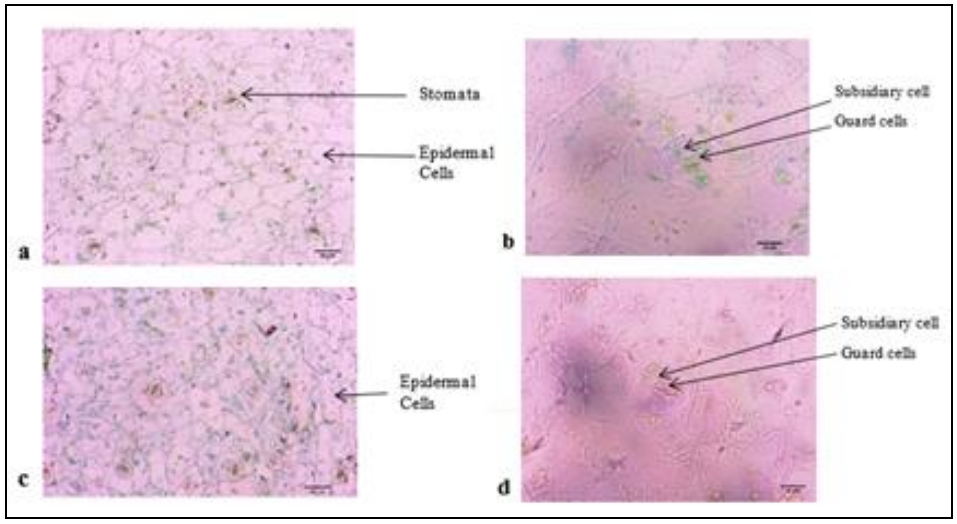


FIG. 5: EPIDERMIS: (A-B)-UPPER EPIDERMIS, (C-D): LOWER EPIDERMIS

Microscopy: Leaf anatomy showed regional variation with distinct ground plans in the petiole and midrib containing vascular bundles. The lamina displayed a typical structure with modified margins. The epidermis, differentiated into upper and lower layers, highlighted protective and regulatory roles.

Powder Microscopy:

FIG. 6: UPPER EPIDERMIS

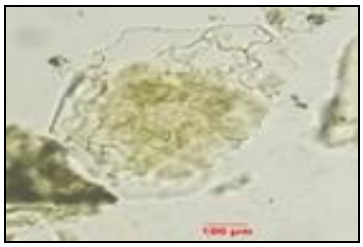


FIG. 7: LOWER EPIDERMIS WITH ANISOCYTICSTOMATA

FIG. 8: ISOLATED STOMATA

FIG. 9: EPIDERMIS WITH BROWNISH CONTENT

FIG. 10: PARENCHYMA CELLS

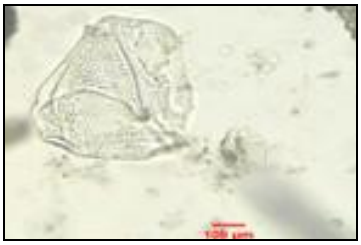


FIG. 11: PITTED PARENCHYMA

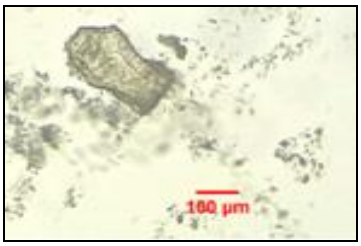


FIG. 12: STONE CELL

FIG. 13: SPIRAL VESSEL

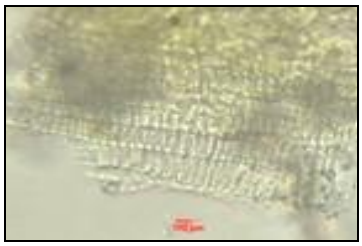


FIG. 14: GROUP OF ANNULAR VESSELS

FIG. 15: BROWNISH CONTENT

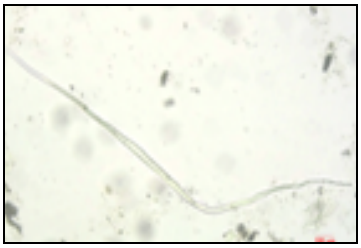


FIG. 16: FIBRE

Powder Microscopy: The following cellular characters were observed in the *K. fedtschenkoi*. Xylem vessels with spiral thickenings, pitted vessels, epidermal fragments and stomata etc.,

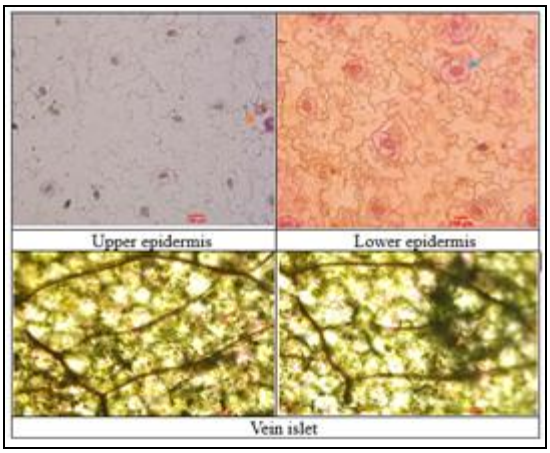


FIG. 17: UPPER EPIDERMIS, LOWER EPIDERMIS, VEIN ISLET

Quantitative Microscopy: All leaves are Amphistomatic in nature. Anisocyticstomata are seen on both surfaces.

TABLE 2: QUANTITATIVE MICROSCOPY OF *KALANCHOE FEDTSCHENKOI* LEAVES

Parameter	*Kalanchoe fedtschenkoi*	
	Upper	Lower
Stomatal number	30-40	30-40
Epidermal number	180-210	190-210
Stomatal Index	14.28-16	13.63-16
Vein islet	1	
Vein termination	0	
Palisade ratio	55-65/cell	

Extraction:

TABLE 3: PERCENTAGE YIELD OF TOTAL EXTRACT

S. no.	Extract/fraction	Percentage yield (%w/w)	Colour	Consistency
1.	Ethanolic extract	5.324	Cherry red colour	Semisolid (Ethanolic extract)

Phytochemical Screening:

TABLE 4: PRELIMINARY PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF *KALANCHOE FEDTSCHENKOI* LEAVES

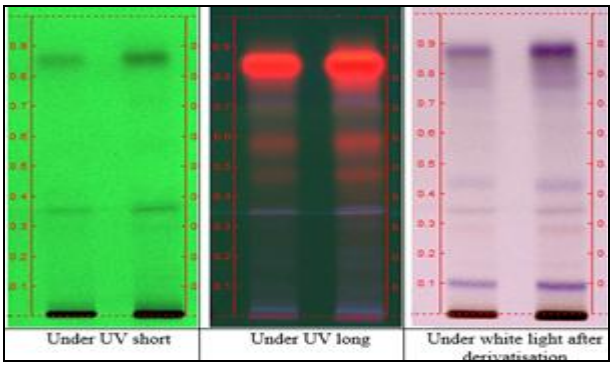
Ethanolic extract of (*Kalanchoe fedtschenkoi* Leaf)	
Tests	**Result**
Saponins	-
Tannins	+
Phenols	+
Terpenoids	+
Alkaloids	+
Flavanoids	+
Steroids	-
Glycosides	+
Carbohydrates	+
Quinones	-
Proteins	-

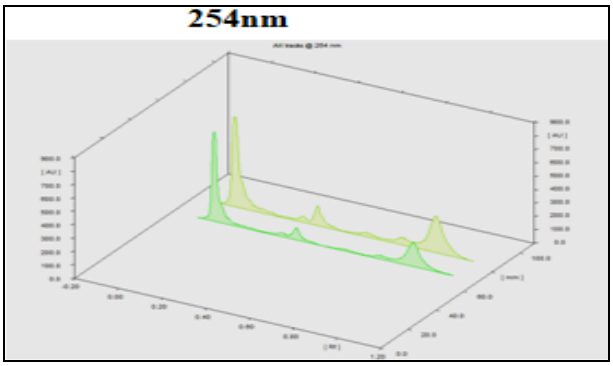
Phytochemical Analysis:

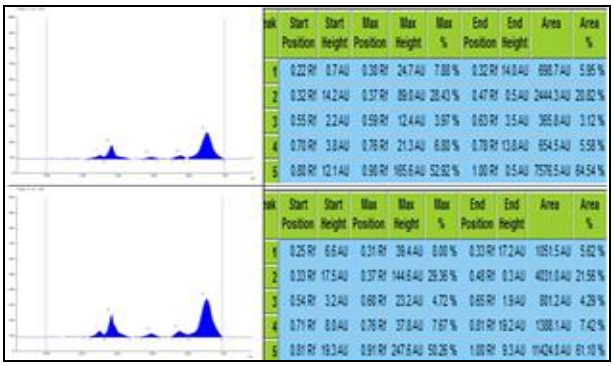
High Performance Thin Layer Chromatographic (HPTLC) Profile of *Kalonchoe fedstchenkoi* Leaves:

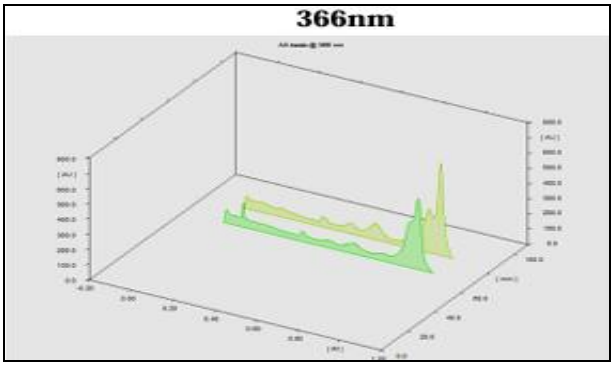
Ethanolic Extract:

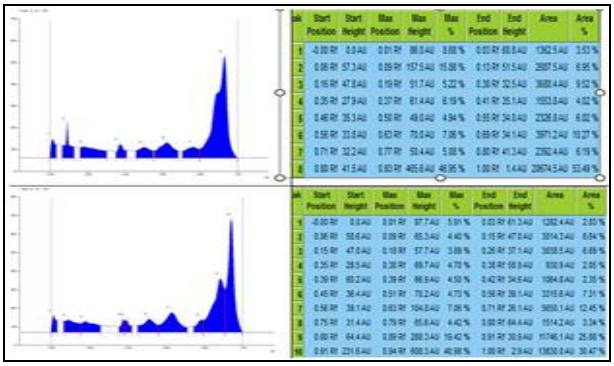
- Solvent system: Ethyl acetate: Toluene: Formic acid (6:1:0.1)
- Volume applied; Track 1- 5 µl: Track 2 – 7 µl

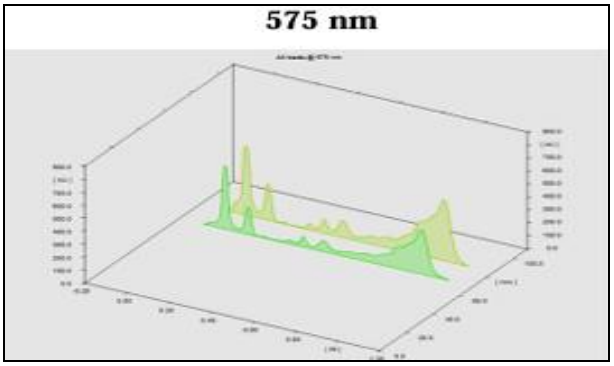


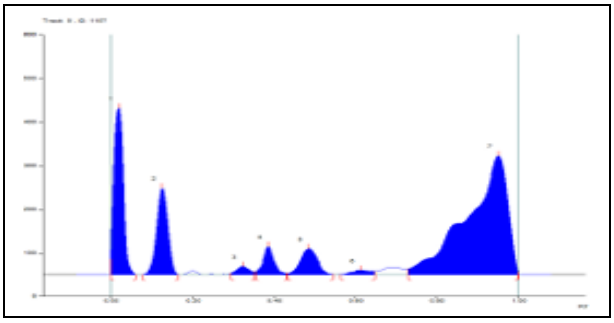




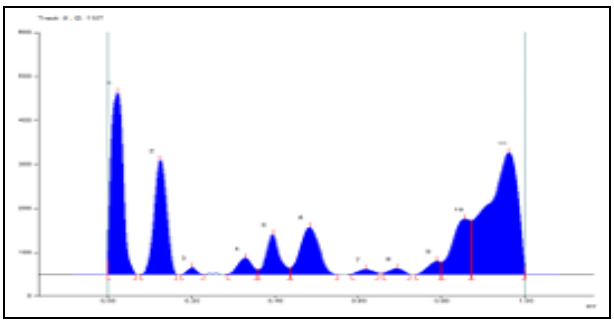








ak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	35.7 AU	0.02 Rf	383.3 AU	37.93 %	0.06 Rf	0.5 AU	6479.3 AU	19.93 %
2	0.08 Rf	0.1 AU	0.13 Rf	199.0 AU	19.70 %	0.17 Rf	0.2 AU	3711.3 AU	11.42 %
3	0.29 Rf	3.5 AU	0.32 Rf	19.5 AU	1.93 %	0.35 Rf	6.2 AU	419.9 AU	1.29 %
4	0.35 Rf	6.2 AU	0.39 Rf	65.3 AU	6.46 %	0.43 Rf	2.8 AU	1226.1 AU	3.77 %
5	0.44 Rf	2.8 AU	0.49 Rf	59.7 AU	5.91 %	0.55 Rf	0.7 AU	1741.8 AU	5.36 %
6	0.56 Rf	0.5 AU	0.62 Rf	10.3 AU	1.02 %	0.65 Rf	7.3 AU	330.2 AU	1.02 %
7	0.73 Rf	13.0 AU	0.95 Rf	273.3 AU	27.05 %	1.00 Rf	6.7 AU	18594.0 AU	57.21 %



ak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	32.7 AU	0.03 Rf	413.8 AU	29.88 %	0.07 Rf	0.3 AU	8437.3 AU	21.70 %
2	0.08 Rf	0.6 AU	0.13 Rf	260.4 AU	18.80 %	0.17 Rf	0.1 AU	5265.9 AU	13.54 %
3	0.18 Rf	0.0 AU	0.20 Rf	15.9 AU	1.14 %	0.23 Rf	0.1 AU	228.5 AU	0.59 %
4	0.29 Rf	1.3 AU	0.33 Rf	37.4 AU	2.70 %	0.36 Rf	10.9 AU	878.2 AU	2.26 %
5	0.36 Rf	11.0 AU	0.40 Rf	91.4 AU	6.60 %	0.44 Rf	14.0 AU	1955.5 AU	5.03 %
6	0.44 Rf	14.4 AU	0.49 Rf	107.6 AU	7.77 %	0.55 Rf	0.1 AU	3330.5 AU	8.57 %
7	0.59 Rf	1.2 AU	0.62 Rf	11.8 AU	0.85 %	0.65 Rf	4.1 AU	281.8 AU	0.72 %
8	0.66 Rf	3.6 AU	0.69 Rf	13.7 AU	0.99 %	0.73 Rf	0.1 AU	318.4 AU	0.82 %
9	0.74 Rf	0.1 AU	0.79 Rf	30.0 AU	2.17 %	0.80 Rf	28.2 AU	655.9 AU	1.69 %
10	0.80 Rf	28.4 AU	0.86 Rf	126.1 AU	9.11 %	0.87 Rf	22.9 AU	3815.6 AU	9.81 %
11	0.88 Rf	123.0 AU	0.96 Rf	276.8 AU	19.99 %	1.00 Rf	17.5 AU	13713.3 AU	35.27 %

FIG. 18: UNDER UV-SHORT, LONG, WHITE LIGHT AFTER DERIVATISATION

GCMS:

TABLE 5: GCMS REPORT

Peak	Rt Time	Area	Height	A/H	Molecular wt & formula	Name
1	4.181	18678	3632	5.14	84-C4H4O2	2(3H)-Furanone
2	4.282	12005	2536	4.73	98-C5H6O2	2-Cyclopenten-1-one, 2-hydroxy-
3	4.606	209691	28464	7.37	68-C3O2	1,2-Propadiene-1,3-dione
4	4.939	40855	6340	6.44	110-C6H6O2	2-Furancarboxaldehyde, 5-methyl-
5	5.161	18854	4031	4.68	146-C5H6O5	2-Oxopentanedioic acid
6	5.247	22912	5554	4.13	94-C6H6O	Phenol
7	5.334	25016	5035	4.97	154-C5H10N6	Tetrazolo[1,5-b]1,2,4-triazine, 5,6,7,8-tetrahydro-6,7-dimethyl-
8	5.442	14563	2229	6.53	56-C3H4O	Propargyl alcohol
9	5.82	21602	4525	4.77	128-C6H8O3	3,4-Dimethyldihydrofuran-2,5-dione
10	5.942	3393	1246	2.72	68-C3O2	1,2-Propadiene-1,3-dione
11	6.162	17532	3714	4.72	87-F3NO	Trifluoroamine oxide
12	6.279	9876	2543	3.88	227-C15H33N	1-Tridecanamine, N,N-dimethyl-
13	6.915	29526	5166	5.72	168-C7H8N2O3	3-Acetylthymine
14	7.125	10963	1648	6.65	56-C3H4O	Propargyl alcohol
15	7.272	11597	2955	3.92	160-C8H16OS	tert-Butyl cyclopropylmethyl sulfoxide
16	7.493	4779	1051	4.55	56-C2H4N2	Aminoacetonitrile
17	7.775	9353	1167	8.01	56-C3H4O	Propargyl alcohol
18	7.878	5307	1598	3.32	85-C4H7NO	Oxazole, 4,5-dihydro-2-methyl-
19	7.979	8922	2127	4.19	102-C5H10S	1-(Methylthio)-2-butene
20	8.117	7914	2177	3.64	128-C6H8O3	2-Propenoic acid, oxiranylmethyl ester
21	8.172	15105	2499	6.04	158-C12H14	1-Phenyl-hexa-1,2-diene
22	8.361	10311	1954	5.28	138-C8H7FO	Ethanone, 1-(4-fluorophenyl)-
23	8.454	18568	4651	3.99	382-C20H18N2O6	Cyclobutane-1,1-dicarboxamide, N,N'-di-benzoyloxy-
24	8.608	8005	1354	5.91	320-C11H10F6O4	2,2,3,3,4,4-Hexafluoro-1,5-pentyl diacrylate
25	8.849	102086	23274	4.39	144-C6H8O4	Ethyl hydrogen fumarate
26	9.063	21664	5105	4.24	82-C5H6O	2-Cyclopenten-1-one
27	9.174	30327	5422	5.59	167-C8H9NO3	1,2-Benzenediol, mono(methylcarbamate)
28	9.323	12121	2723	4.45	302-C14H22O7	2-Propenoic acid, oxybis(2,1-ethanediyloxy-2,1-ethanediyl) ester
29	9.471	42825	10647	4.02	120-C8H8O	4-Vinylphenol
30	9.656	51041	10313	4.95	126-C6H6O3	5-Hydroxymethylfurfural
31	9.806	4248	1394	3.05	300-C15H24O6	2-{2-[2-(Acryloyloxy)-1-methylethoxy]-1-methylethoxy}-1-methylethyl acrylate
32	9.887	3185	902	3.53	112-C5H8N2O	4-Morpholinecarbonitrile
33	10.217	4817	1309	3.68	103-C4H9NO2	Propane, 2-methyl-1-nitro-
34	10.298	12913	3861	3.34	190-C8H14O5	Butanedioic acid, hydroxy-, diethyl ester
35	10.518	396996	76485	5.19	134-C4H6O5	Malic Acid
36	10.705	164057	22752	7.21	134-C4H6O5	Malic Acid
37	10.875	36518	6484	5.63	112-C6H8O2	Vinyl crotonate
38	11.042	18975	3706	5.12	74-C4H10O	1-Butanol
39	11.143	128153	24599	5.21	148-C8H4O3	Phthalic anhydride
40	11.537	12920	1263	10.23	98-C5H6O2	2-Propenoic acid, ethenyl ester
41	11.856	16843	3649	4.62	112-C6H8O2	3,4-Dihydro-6-methyl-2H-pyran-2-one
42	12.234	77559	9974	7.78	126-C6H6O3	1,2,3-Benzenetriol
43	12.869	10070	1885	5.34	242-C13H22O4	Oxalic acid, cyclobutylheptyl ester
44	13.168	65553	6604	9.93	118-C5H10OS	s-Ethyl thiopropionate
45	13.644	9922	1581	6.28	70-C4H6O	3-Butyn-2-ol
46	13.89	152031	20129	7.55	254-C14H26N2O2	5-Amino-1,3,3-trimethylcyclohexanemethylamine, N,N'-bis(acetyl)- (stereoisomer 1)
47	15.443	13683	3956	3.46	214-C13H26O2	Undecanoic acid, 2-methyl-, methyl ester
48	15.542	3914	985	3.97	56-C3H4O	Propargyl alcohol

49	15.724	8848	1083	8.17	56-C3H4O	Propargyl alcohol
50	15.892	11896	1627	7.31	142-C4H6N4O2	Propionic acid, 3-tetrazol-1-yl-
51	15.998	9268	1990	4.66	112-C6H8O2	Vinyl crotonate
52	16.604	6180	1354	4.56	70-C4H6O	3-Butyn-2-ol
53	17.14	5748	1103	5.21	56-C3H4O	Propargyl alcohol
54	18.157	1917882	71756	26.73	134-C5H10O4	1,2,3,4-Cyclopentanetetrol, (1.alpha.,2.beta.,3.beta.,4.alpha.)-
55	18.583	3691	1351	2.73	116-C6H12O2	Formic acid, neopentyl ester
56	20.27	47597	14192	3.35	172-C10H20O2	n-Decanoic acid
57	25.701	14984	4151	3.61	207-C15H13N	Benzonitrile, m-phenethyl-
58	28.969	25983	7320	3.55	88-C5H12O	Amylene hydrate
59	30.733	5072	921	5.51	208-C11H16N2O2	Neostigmine
60	31.267	3784	1034	3.66	237-C12H25B2NO2	Caprolactone oxime, (NB)-O-[(diethylboryloxy)(ethyl)boryl]-
61	32.4	7280	1107	6.58	253-C14H7NO4	1,4-anthracenedione, 6-nitro-
62	32.592	6960	1640	4.24	374-C24H22O4	Phthalic acid, di(2,3-dimethylphenyl) ester
63	32.717	13557	1479	9.17	282-C18H18O3	Thebaol, O-ethyl-
64	32.9	17704	1887	9.38	250-C11H10F4O2	2-Fluoro-5-trifluoromethylbenzoic acid, propyl ester
65	33.05	6484	1440	4.5	479-C32H33NO3	isoquinoline, 1,2,3,4-tetrahydro-6-methoxy-2-methyl-7-(phenylmethoxy)-1-[[4-(phenylmethoxy)phenyl]methyl]-
66	33.178	6973	1789	3.9	282-C20H14N2	6,13-Dihydrodibenzo(b,i)phenazine
67	33.345	6915	1171	5.91	420-C14H4Cl4F4O2	2-Fluoro-3-trifluoromethylbenzoic acid, 2,3,4,6-tetrachlorophenyl ester
68	34.506	4699	1083	4.34	282-C20H14N2	6,13-Dihydrodibenzo(b,i)phenazine
69	34.73	6713	1531	4.38	340-C24H20O2	9-Phenanthrenemethyl 2,6-dimethylbenzoate

Antioxidant Assay:

TABLE 6: DPPH RADICAL SCAVENGING ACTIVITY

S. no.	Tested sample concentration (µg/ml)	Percentage Inhibition (%)
1	Ascorbic acid	84.5961±0.1536
2	500 µg/ml	78.7322±0.4326
3	250 µg/ml	78.0320±0.2261
4	100 µg/ml	76.9192±0.3234
5	50 µg/ml	75.5189±0.7598
6	10 µg/ml	74.2436±0.2110

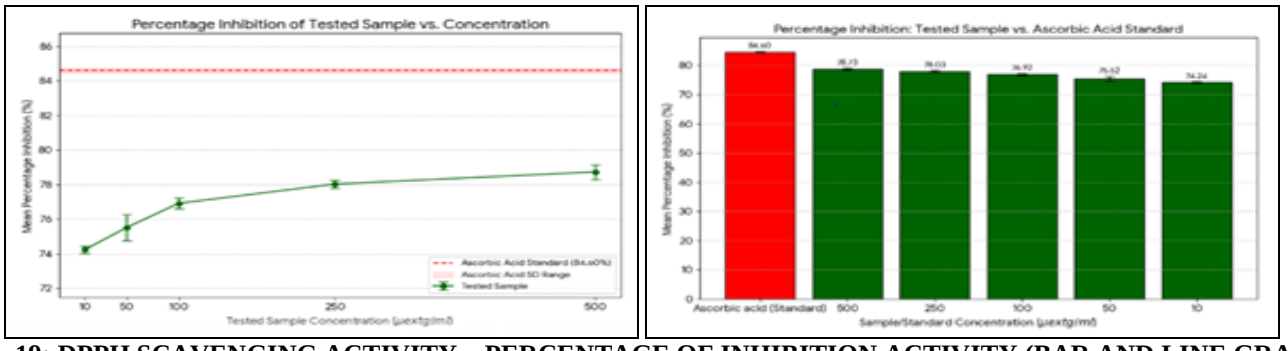


FIG. 19: DPPH SCAVENGING ACTIVITY – PERCENTAGE OF INHIBITION ACTIVITY (BAR AND LINE GRAPH PRESENTATION)

TABLE 7: IC50 VALUE OF TESTED SAMPLE: 83.06 MG/ML

Log (inhibitor) vs. normalized response -- Variable slope	
Best-fit values	
Log IC50	1.919
Hill Slope	-1.805
IC50	83.06

Std. Error	
LogIC50	0.03723
Hill Slope	0.2891
95% CI (asymptotic)	
LogIC50	1.839 to 2.000
Hill Slope	-2.430 to -1.181
IC50	69.02 to 99.96
Goodness of Fit	
Degrees of Freedom	13
R squared	0.9493
Sum of Squares	1058
Sy.x	9.022
Number of points	
# of X values	15
# Y values analysed	15

In-vitro Antidiabetic Activity:

α - Amylase Inhibition Assay:

TABLE 8: STANDARD (ACARBOSE) AND EXTRACT PERCENTAGE INHIBITION

S. no.	Concentration (µg/ml)	Percentage inhibition (%) Acarbose	Percentage inhibition (%) Extract
1	250	58.3100±0.0010	17.4209±0.0003
2	500	76.3716±0.0002	35.6428±0.0005
3	1000	89.7877±0.0003	55.5467±0.0004

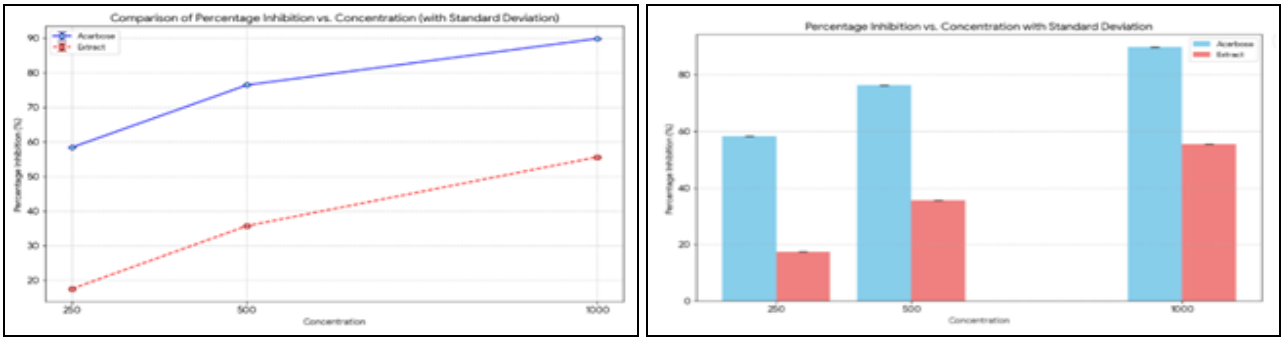


FIG. 20: PERCENTAGE INHIBITION OF ACARBOSE AND EXTRACT-(BAR AND LINE GRAPH PRESENTATION)

IC_{50} Value-Standard: 214.371µg/mL (Calculated using ED_{50} PLUS V 1.0 Software):

IC_{50} Value-Extract: 863.415 µg/mL (Calculated using ED_{50} PLUS V 1.0 Software):

Alpha Glucosidase Inhibitory Assay:

TABLE 9: STANDARD (ACARBOSE) AND EXTRACT PERCENTAGE INHIBITION

S. no.	Concentration (µg/ml)	Percentage inhibition (%) acarbose	Percentage inhibition (%) Extract
1.	250	53.230±0.0021	11.103±0.0030
2.	500	73.240±0.0038	29.340±0.0040
3.	1000	84.580±0.0031	49.753±0.0031

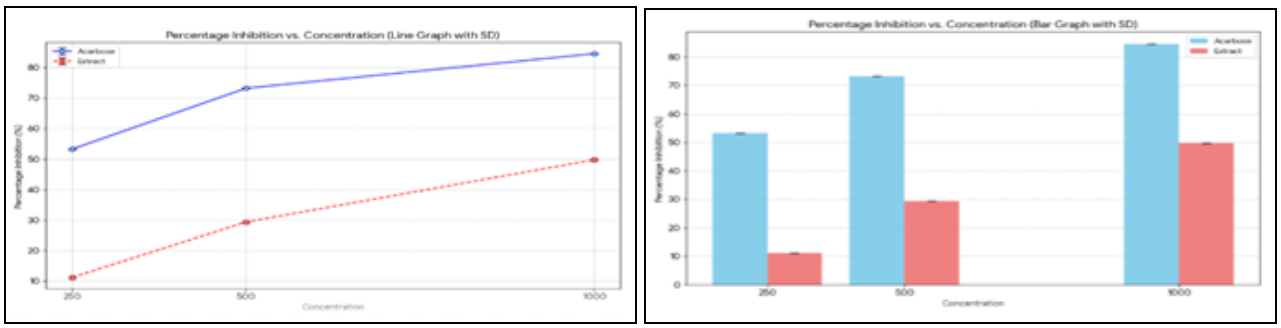


FIG. 21: PERCENTAGE INHIBITION OF ACARBOSE AND EXTRACT-(BAR AND LINE GRAPH PRESENTATION)

IC_{50} Value –STANDARD –228.758µg/mL (Calculated using ED_{50} PLUS V1.0 Software):

IC_{50} Value –EXTRACT –973.461µl/mL (Calculated using ED_{50} PLUS V1.0 Software):

***In-vitro* Parkinson's Activity:**

Acetyl Cholinesterase Assay:

OD Value at 410 nm:

TABLE 10: CONTROL MEAN OD VALUE: 1.048

S. no.	Concentration (µg/ml)	Tested Sample Percentage inhibition (%)	Physostigmine Percentage inhibition (%)
1	500	77.3855±0.0025	98.71±0.0030
2	250	74.4593±0.0130	96.82±0.0028
3	100	67.4618±0.0040	93.45±0.0043
4	50	46.5013±0.0033	86.19±0.0015
5	10	27.3219±0.0043	65.42±0.0021

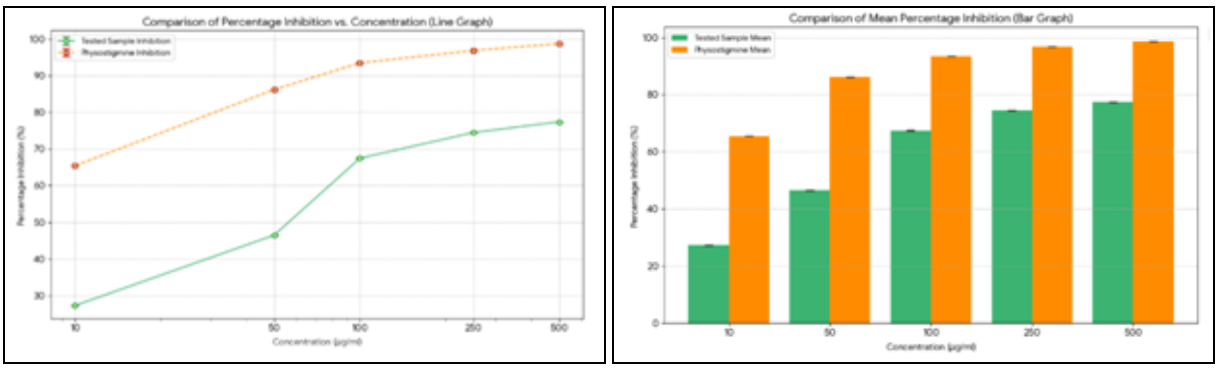


FIG. 22: PERCENTAGE INHIBITION OF SAMPLE AND PHYSOSTIGMINE (ACETYL CHOLINESTERASE ASSAY –INHIBITION ACTIVITY -LINE AND BAR GRAPH PRESENTATION)

TABLE 11: IC50 VALUE OF TESTED SAMPLE: 59.77 MG/ML

Log (inhibitor) vs. normalized response - Variable slope	
Best-fit values	
LogIC50	1.776
HillSlope	-2.598
IC50	59.77
Std. Error	
LogIC50	0.01257
HillSlope	0.2133
95% Confidence Intervals	
LogIC50	1.749 to 1.804
HillSlope	-3.058 to -2.137
IC50	56.14 to 63.62
Goodness of Fit	
Degrees of Freedom	13
R square	0.9917
Absolute Sum of Squares	180.6
Sy.x	3.727
Number of points	
Analyzed	15

DISCUSSION:

Macroscopic and Microscopic Characteristics: The physical properties of *Kalanchoe fedtschenkoi* leaves-including their appearance, texture, odor, taste, and tactile response-were thoroughly assessed to support taxonomic identification. The leaves exhibited a smooth texture with a distinctive odor and characteristic taste. Microscopic examination of the transverse section (T.S.) revealed a clearly defined upper and lower epidermis,

parenchymatous tissue, and well-developed vascular bundles containing both xylem and phloem. Stomata were predominantly confined to the lower epidermis, confirming the amphistomatic nature of the leaves with anisocytic stomatal arrangement. Xylem vessels showed spiral and pitted thickenings, while epidermal fragments displayed uniformity, supporting accurate species authentication.

Physicochemical Evaluation: The physicochemical parameter -including ash values, moisture content, and extractive yields-served as essential tools for determining the purity and quality of the plant material. These parameters contribute to the pharmacognostic standardization of *K. fedtschenkoi*, ensuring reliability and reproducibility in herbal formulations.

Phytochemical Screening: Preliminary phytochemical screening of the ethanolic leaf extract indicated the presence of major secondary metabolites such as alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, glycosides, and carbohydrates. These bioactive constituents are known to exhibit a wide range of therapeutic activities, validating the traditional medicinal use of the plant.

Chromatographic Profiling (HPTLC): High-Performance Thin Layer Chromatography (HPTLC) was utilized for chemical fingerprinting and quality control. Detection was carried out at 245 nm, 366 nm, and 575 nm, revealing distinct bands corresponding to various phytochemical classes, including flavonoids, alkaloids, and phenolic acids. The optimized mobile phase-ethyl acetate: toluene: formic acid (6:1:0.1)-produced well-resolved Rf values, establishing a reproducible chromatographic profile for the ethanolic extract.

GC–MS Analysis: Gas Chromatography–Mass Spectrometry (GC–MS) analysis identified more than 69 compounds in the ethanolic extract. The constituents included phenols, organic acids, esters, furans, alcohols, amines, heterocyclic compounds, and polycyclic aromatic hydrocarbons, reflecting the chemical diversity and complexity of *K. fedtschenkoi* leaves. This broad spectrum of metabolites suggests potential for multiple pharmacological applications.

Antioxidant Activity: The antioxidant potential of the ethanolic extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The extract demonstrated strong free radical scavenging capacity with an IC_{50} value of 83.06 µg/mL, comparable to that of the standard ascorbic acid. This indicates a significant hydrogen-donating ability and highlights the extract's potential as a natural antioxidant source.

Antidiabetic Activity: The ethanolic extract exhibited concentration-dependent inhibition of **α-**amylase and α-glucosidase enzymes.

α-Amylase Inhibition: 17.42% (250 µL/mL), 35.64% (500 µL/mL), and 55.55% (1000 µL/mL).

α-Glucosidase Inhibition: 13.21% (250 µL/mL), 30.89% (500 µL/mL), and 51.07% (1000 µL/mL).

These results indicate the extract's potential to modulate carbohydrate metabolism, suggesting a postprandial glucose-lowering effect. The activity is likely attributed to the synergistic action of phenolic and flavonoid compounds.

Anticholinesterase Activity: Acetylcholinesterase (AChE) inhibitory activity was determined using the modified Ellman's method. The ethanolic extract (KF) demonstrated a clear dose-dependent inhibition ranging from 27.32% at 10 µg/mL to 77.39% at 500 µg/mL, with an IC_{50} value of 59.77 µg/mL. A strong correlation ($R^2 = 0.9917$) between concentration and enzyme inhibition and a steep Hill slope (-2.598) indicated a high degree of enzyme–ligand affinity and potential cooperative binding. These findings suggest moderate to strong neuroprotective potential of the extract.

CONCLUSION: A detailed analysis of *Kalanchoe fedtschenkoi* leaves highlights their medicinal potential through morphological, microscopic, and phytochemical evaluation, ensuring authenticity and quality of the plant material. Phytochemical screening revealed bioactive compounds such as flavonoids, alkaloids, tannins, terpenoids, glycosides, carbohydrates, and phenolic substances, which are associated with diverse health benefits. The ethanolic extract exhibited significant antioxidant activity (IC_{50} = 83.06 µg/mL), suggesting its potential as a natural source of free radical scavengers.

It also showed moderate inhibitory effects on digestive enzymes α-amylase (IC_{50} = 863.42 µg/mL) and α-glucosidase (IC_{50} = 973.46 µg/mL) indicating possible antidiabetic properties through regulation of postprandial glucose levels. Furthermore, strong acetylcholinesterase inhibition (IC_{50} = 59.77 µg/mL, R^2 = 0.9917) points to potential neuroprotective effects, relevant to disorders like Parkinson's disease. These findings support further research into its therapeutic applications and development of antioxidant, antidiabetic, and neuroprotective formulations.

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CONFLICTS OF INTEREST: Nil

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