

PHENOLICS AND FLAVONOIDS PROFILING IN *LEPIDIUM AFRICANUM* METHANOL EXTRACT AND EVALUATION OF ITS ANTIOXIDANT, ANTIDIABETIC, ANTI-INFLAMMATORY, AND ANTICANCER PROPERTIES

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Abstract

Lepidium africanum, a member of the Brassicaceae family, is a medicinal plant known for its diverse bioactive compounds. This study investigates the phytochemical composition and evaluates the antioxidant, anti-diabetic, anti-inflammatory, and cytotoxic activities of the methanol extract of *L. africanum*. Phytochemical analysis using HPLC revealed high concentrations of phenolic and flavonoid compounds, with gallic acid (48.46%) and catechin (77.42%) being the predominant constituents. The extract exhibited moderate antioxidant activity through ABTS radical scavenging (IC_{50} : 58.72 μ g/ml), suggesting its potential in mitigating oxidative stress-related conditions. Additionally, the extract demonstrated significant α -amylase inhibitory activity (IC_{50} : 57.46 μ g/ml), indicating its possible role in managing postprandial hyperglycemia. The anti-inflammatory potential was confirmed *via* COX-1 enzyme inhibition (IC_{50} : 33.16 μ g/ml), highlighting its ability to modulate inflammatory pathways. *In vitro* cytotoxicity assays on HepG-2, MCF-7, and CACO2 cell lines showed notable anticancer activity, with IC_{50} values ranging from 71.61 μ g/ml to 114.09 μ g/ml, demonstrating selective cytotoxic effects across different cancer cell lines. These findings suggest that *L. africanum* methanol extract holds promise as a natural source of bioactive compounds with therapeutic applications in oxidative stress-related diseases, diabetes, inflammation, and cancer..

Keywords: *Lepidium africanum*, Bioactive compounds, Antioxidant activity, Anticancer properties, Antidiabetic.



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1. INTRODUCTION

Medical plants are the main source of chemical compounds of treatment, and they have many pharmaceutical activities, while playing a decisive role in traditional medicine and contributing to the development and discovery of new drugs (Dar et al., 2017; Süntar, 2020). Several useful medications are found clinically to treat human diseases as a result of the search for natural resources, including plant excerpts, in the search for new active chemicals. In this context, the extraction, quantification, and identification of bioactive compounds from a wide variety of plants are carried out using diverse extraction and analytical techniques (Altemimi et al., 2017). Brassicaceae is a large and economically important family, and includes many types of cultivated food, oil production and other applications. It is characterized by its varied composition, which has been widely distributed through various climates, important environmental roles in many ecosystems (Long et al., 2011). *Lepidium* has received great attention in recent years due to its various biological activities and possible medical applications. *Lepidium*'s sex includes more than 200 species, many of which are traditionally used in folk medicine for its therapeutic properties (Shah et al., 2021). *Lepidium sativum*, in particular, was used in traditional african medicine to treat various diseases, including inflammation, diabetes, and microbes (Shah et al., 2021). *Lepidium africanum*, the subject of this study, is one such species predominantly found in Sub-Saharan Africa and South Africa, and is also native to the Al Baha region of Saudi Arabia (Tshikororo et al., 2023). While species like *Lepidium sativum* (garden cress) have been extensively researched for their rich profile of bioactive compounds such as glucosinolates, flavonoids, and phenolic acids, which contribute to noted anti-inflammatory, antidiabetic, and antioxidant activities (AL-SNAFI, 2019; Olotu et al., 2018; Shah et al., 2021; Vazifeh et al., 2022) scientific literature on *L. africanum* is remarkably scarce. No detailed phytochemical profiling or comprehensive evaluation of its biological potential has been reported to date. Vegetarian chemical studies have revealed that this plant is rich in biological active compounds such as glucose, Flavonoid, phenolic acids, and terpenoids (Olotu et al., 2018). These secondary interlocutors contribute greatly to the factory's biological activities, making them a promising candidate for the discovery of drugs and development. The ethnic importance of *Sativum* extends beyond its medical uses. The factory is also estimated for its nutritional content and cooking applications. Small leaves and buds are consumed as vegetables, as basic nutrients such as vitamins, minerals and dietary fiber provide (AL-SNAFI, 2019). Moreover, *Lepidium Sativum* seeds are used as a spice and flavor factor in different dishes, which adds a unique taste and smell to food preparations.

The phytochemical compositions of *Lepidium* species are diverse and complex, ranging from several classes of bioactive compounds. Among the constituents found in this plant, glucosinolates, a class of sulfur-containing compounds, are the most prominent. These compounds are known for their potent antioxidant and anticancer activity (Abu Ali et al., 2025). Another of the important classes of phytochemicals present in *Lepidium* species is flavonoids, which possess the activities of potent anti-inflammatory and antimicrobial agents (Ait-yahia et al., 2018; Amer et al., 2022; Ouattar et al., 2022; Vazifeh et al., 2022). The abundant phenolic acids are key contributors to the antioxidant ability of the plant (Ait-yahia et al., 2018; Amer et al., 2022; Vazifeh et al., 2022). *Lepidium* species have been the subject of extensive biological activity investigations and a therapeutic plant of multifaceted potential. One of its most noteworthy activities is anti-inflammatory. Inflammation is an elaborate physiological response that is central to the mechanism of several ailments, including arthritis, cardiovascular disorders, and cancers (Dzoyem et al., 2017). The anti-inflammatory activity of *Lepidium* species can be attributed to the flavonoids and phenolic acids readily present inside the plants while inhibiting the production of pro-inflammatory mediators, such as prostaglandins and cytokines (Elgorashi & McGaw, 2019). Another significant biological activity of *Lepidium sativum* is its antidiabetic effect. Diabetes mellitus is Chronic metabolic disorder characterized by hyperglycemia and severe complications in the absence of treatment (Olotu et al., 2018). It has been proven that extracts of *Lepidium sativum* reduce blood glucose levels and increase insulin sensitivity in diabetic animals. Hypoglycemic activity is attributed to increased glucose uptake in peripheral tissues and decreased intestinal absorption of glucose (Mu et al., 2023). Moreover, the antioxidant properties of *Lepidium sativum* are essential in protecting cells from oxidative stress and hence preventing the onset of numerous diseases (Al-Sheddi et al., 2016). The plant's antioxidant ability, being flavonoid and phenolic acid-rich, works through the scavenging of free radicals and inhibition of lipid peroxidation (Mu et al., 2023). This antioxidant capacity of *Lepidium sativum* makes it a potent candidate for the prophylaxis and management of disorders associated with oxidative stress, such as neurodegenerative disorders and cardiovascular diseases. The best possible understanding of the modes of action responsible for the biological activities of *Lepidium* species will lead to the use of this plant for therapeutic purposes. The anti-inflammatory actions of this plant are hypothesized to occur through several pathways. It is possible that flavonoids and phenolic acids found in *Lepidium* may exert their effect by depressing the activation of nuclear factor-kappa B (NF- κ B), a transcription factor known for regulating inflammatory genes (Oguntibeju, 2018). These compounds also inhibit

the production of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α) or interleukin-6 (IL-6) by modifying the activity of mitogen-activated protein kinases (MAPKs) (Kulkarni-Almeida et al., 2016). The action of *Lepidium sativum* as an antidiabetic is mediated through several mechanisms. The extracts of *Lepidium sativum* can enhance glucose uptake in peripheral tissues via the activation of glucose transporters such as GLUT4 (Sobhi et al., 2023). Other mechanisms include the inhibition of enzyme activity in carbohydrate metabolism, such as α -amylase and α -glucosidase, leading to the reduction of glucose absorption in the intestines (Naquvi et al., 2011). In addition, *Lepidium sativum* increases insulin sensitivity by stimulating insulin receptor translocation to the cell surface (S. S. et al., 2017). The antioxidant mechanism of *Lepidium sativum* acts through the scavenging of reactive oxygen species (ROS) and inhibition of lipid peroxidation. Flavonoids and phenolic acids present in the plant act as potent free radical scavengers, neutralizing ROS and preventing cellular damage (Selek et al., 2018). These compounds also upregulate the expression of antioxidant enzymes such as superoxide dismutase (SOD) and catalase, further enhancing the plant's antioxidant capacity.

Building on previous research, we were motivated to explore *Lepidium africanum*. It is crucial to reiterate that these potential biological activities are based on inferences from related species. Direct experimental validation is absolutely necessary to confirm these hypotheses and to fully characterize the biological activities of *L. africanum*. Therefore, drawing on the ethnobotanical and pharmacological evidence from its relatives, this study aims to address the critical knowledge gap surrounding *L. africanum*. We present the first comprehensive investigation into the phytochemical composition of its methanol extract, with a specific focus on phenolics and flavonoids profiling using HPLC. Furthermore, we systematically evaluate its antioxidant, antidiabetic, anti-inflammatory, and anticancer properties in vitro, thereby providing foundational data on the therapeutic potential of this underutilized plant.

2. EXPERIMENTAL

2.1 Extraction

The aerial parts of *Lepidium africanum* were collected from the Albaha region, KSA, and taxonomically authenticated. The extraction protocol was designed to comprehensively isolate a broad spectrum of medium- and high-polarity bioactive compounds, with a specific focus on phenolic and flavonoid compounds. Methanol was selected as the solvent due to its well-established efficacy in extracting such polar metabolites. A sequential extraction strategy was employed, an initial maceration step was used to gently extract heat-labile compounds at room temperature, followed by a Soxhlet extraction to

ensure exhaustive recovery of intracellular metabolites using continuous solvent washing. A total of 500 g of the dried aerial plant material was soaked in 2,000 mL of methanol for 72 h to facilitate the extraction of methanol-soluble constituents. After soaking, the plant material was subjected to Soxhlet extraction using methanol as the extraction solvent. The combined methanolic extracts obtained from the soaking and Soxhlet processes were filtered and concentrated under reduced pressure using a rotary evaporator, and the resulting methanol extract was thoroughly dried under vacuum and preserved under refrigeration at 4 °C for further usage. The final dried extract weighed 3.37 g. Subsequently, the extract was subjected to phytochemical screening, evaluations of its antioxidant, antidiabetic, anti-inflammatory, and anticancer properties, and HPLC analysis.

2.2 Phytochemical analysis

2.2.1 Total Phenolic Content (TPC)

The TPC was determined using the Folin-Ciocalteu method (Sembiring et al., 2017). Briefly, 1 mg/mL of the extract solution was prepared. A 500 μ L aliquot of this solution was mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and allowed to stand for 5 minutes. Then, 2.5 mL of sodium carbonate solution (75 g/L) was added. The mixture was vortexed and incubated in the dark for 2 hours at room temperature. The absorbance of the resulting blue complex was measured at 765 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). A standard curve was prepared using gallic acid (0-100 μ g/mL), and the TPC was expressed as mg of Gallic Acid Equivalents (GAE) per gram of dry extract.

2.2.2 Total Flavonoid Content (TFC)

The TFC was determined using an aluminum chloride colorimetric method (Sembiring et al., 2017). Briefly, 200 μ L of the extract solution (1 mg/mL) was mixed with 75 μ L of a 5% sodium nitrite (NaNO_2) solution. After 5 minutes, 1.25 mL of a 7% aluminum chloride (AlCl_3) solution was added. After another 5 minutes, 0.5 mL of 1 M sodium hydroxide (NaOH) was added, and the total volume was made up to 2.5 mL with distilled water. The mixture was vortexed, and the absorbance was immediately measured at 510 nm. A standard curve was prepared using quercetin (0-100 μ g/mL), and the TFC was expressed as μ g of Quercetin Equivalents (QE) per gram of dry extract.

2.3 HPLC Analysis of Phenolics and Flavonoids

The phenolic and flavonoid profile of the extract was analyzed using an Agilent 1260 series HPLC system (Agilent Technologies, USA) equipped with a quaternary pump, an autosampler, and a diode array detector (DAD). Separation was achieved on a Zorbax Eclipse Plus C8 column (4.6 $\times$ 250 mm, 5 μ m). The mobile phase consisted of water with 0.1% formic acid

(A) and acetonitrile with 0.1% formic acid (B). A linear gradient elution program was used as follows: 0–5 min, 10% B; 5–25 min, 10–55% B; 25–30 min, 55–100% B; 30–35 min, 100% B; 35–36 min, 100–10% B; and 36–40 min, 10% B for column re-equilibration. The flow rate was 1.0 mL/min, the injection volume was 10 µL, and the column temperature was maintained at 30°C. Detection was carried out at 280 nm and 330 nm. Compounds were identified by comparing their retention times and UV spectra with those of authentic standards (Ahmed et al., 2024). Quantification was performed based on the peak areas of the external standards.

2.4 Antioxidant activity

The antioxidant activity of the extract was determined using the (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ABTS and Ferric reducing antioxidant power (FRAP) free radical scavenging assay in triplicate. Average values were considered (Ling et al., 2009; Samaniego Sánchez et al., 2007).

2.4.1 ABTS Radical Scavenging Assay

The antioxidant activity of the extract was determined using the (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) ABTS radical scavenging assay in triplicate, according to the method of (Ling et al., 2009). The ABTS radical cation (ABTS^{•+}) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12-16 hours before use. The ABTS^{•+} solution was then diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. For the assay, 1 mL of the diluted ABTS^{•+} solution was mixed with 10 µL of different concentrations of the extract or standard (Ascorbic acid). The reaction mixture was vortexed and incubated for 6 minutes in the dark, after which the absorbance was measured at 734 nm. The percentage inhibition was calculated using the formula:

% Inhibition = $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$, where A_{control} is the absorbance of the ABTS solution with methanol, and A_{sample} is the absorbance of the ABTS solution with the extract/standard. The IC_{50} value was determined from the dose-response curve.

2.4.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed as described by (Samaniego Sánchez et al., 2007). The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM FeCl₃ solution in a 10:1:1 ratio. The reagent was warmed to 37°C before use. An aliquot of 10 µL of the extract or standard (Ascorbic acid) was mixed with 1 mL of the FRAP reagent and incubated at 37°C for 4 minutes. The absorbance of the colored product (ferrous tripyridyltriazine complex) was measured at

593 nm. A standard curve was prepared using ferrous sulfate (FeSO₄·7H₂O), and the results were expressed as µM Fe(II) equivalents per gram of extract. The IC_{50} value was determined from the dose-response curve of the percentage activity.

2.5 Antidiabetic activity based in α-amylase inhibition assay

The assay performed for the inhibition of α-amylase followed the DNSA method (Miller, 1959). The extract was dissolved in 10% DMSO and diluted subsequently in phosphate buffer (0.02 M Na₂HPO₄/NaH₂PO₄, 0.006 M NaCl, pH 6.9) to prepare a set of concentrations that ranged from 10 to 1000 µg/mL. A 200 µL aliquot of the α-amylase solution (2 U/mL) was mixed with 200 µL of extract and incubated for 10 minutes at 30°C. After incubation, 200 µL of a 1% starch solution was added and further incubated for 3 minutes. The reaction was stopped by adding 200 µL of DNSA reagent followed by heating at 85–90°C for 10 minutes. Then, the mixture was cooled, diluted to 5 mL with distilled water, and absorbance was recorded at 540 nm. Acarbose was used as a positive control, and appropriate blanks were prepared. The percentage of α-amylase inhibition was calculated, and IC_{50} values were determined from the inhibition curve.

2.6 Anti-inflammatory activity based on Cyclooxygenase (COX-1) Inhibition Assay

The assay for inhibition of COX-1 was carried out according to an established method (Abdel-Aziz et al., 2016). The test sample concentrations were subjected to a pre-incubation with the COX-1 enzyme at (25 °C) for 5 minutes in the presence of hematin for test samples ranging from 7.8 to 1000 µg/mL. After this step, the premixed phenol (500 µM), 1-leuco-dichlorofluorescein (20 µM), arachidonic acid (50 µM) and hematin (1 µM) in 1 mL of 0.1 M Tris-buffer (pH 8) was added to the enzyme in order to start the reaction. For the first 15 seconds after the start of the reaction, the absorbance was measured at 502 nm using UV-visible spectrophotometry. A blank control was prepared without enzyme for recording non-enzymatic activity. It was used a standard inhibitor for celecoxib. The IC_{50} was determined from the concentration-response curve, defined as the concentration of inhibitor required to inhibit 50% of COX-1 activity (units: µg/mL).

2.7 Anticancer activity

The aforementioned extract was evaluated for cytotoxicity using the MTT assay (Nazreen et al., 2022) against the cell lines MCF-7, Caco-2, and HepG2. These were previously described and were acquired from the American Type Culture Collection (ATCC) situated in Rockville, Maryland. The human cancer cell lines HepG-2 (hepatocellular carcinoma), MCF-7 (breast adenocarcinoma), and Caco-2 (colorectal adenocarcinoma) were selected for this study. This selection was based on the high global prevalence and

clinical significance of liver, breast, and colon cancers. Using these three distinct cell lines allows for an initial assessment of the extract's potential broad-spectrum cytotoxic activity against cancers of different tissue origins. Cisplatin was used as the positive control in this assay. It was selected as a reference standard because it is a well-established, potent, and broad-spectrum chemotherapeutic agent. Its use provides a robust benchmark for maximal cytotoxicity, allowing for a clear and standardized comparison of the relative potency of the plant extract, which is a common practice in preliminary phytochemical cytotoxicity screening. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a humidified atmosphere of 5% CO₂. For the assay, cells were seeded in 96-well plates at a density of 1x10⁴ cells/well and allowed to adhere for 24 hours. The cells were then treated with various concentrations of the extract (ranging from 3.9 to 1000 µg/mL) or the positive control, cisplatin, for 72 hours. The final concentration of DMSO in the culture medium was kept below 0.5% to avoid solvent toxicity. After the treatment period, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 4 hours at 37°C. The resulting formazan crystals were dissolved in 100 µL of DMSO, and the absorbance was measured at 570 nm using a microplate reader (BioTek, USA). Cell viability was

calculated as a percentage relative to the untreated control cells, and the IC₅₀ values were determined from the dose-response curves. The percentage of cell viability was calculated using the following formula: Cell Viability (%) = [(Absorbance of Treated Well - Absorbance of Blank) / (Absorbance of Untreated Control - Absorbance of Blank)] × 100, the half-maximal inhibitory concentration (IC₅₀ value), defined as the concentration of the extract that caused a 50% reduction in cell viability, was determined from the dose-response curves using non-linear regression analysis.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis of *Lepidium africanum* Methanol Extract—Composition

L. africanum, a member of the Brassicaceae family, contains important classes of compounds like phenolics and flavonoids, as exhibited by the phytochemical analysis of its methanol extract. The phytochemical composition of its methanol extract has garnered significant interest due to the potential health benefits of its bioactive compounds, particularly phenolic and flavonoid derivatives, which are known for their antioxidant and pharmacological properties (Sembiring et al., 2017). This study provides a detailed analysis of the phytochemical composition of the methanol extract of *L. africanum*, integrating data from Table-1.

Table-1: Percentage Composition of Phenolic and Flavonoid Compounds in *Lepidium africanum* Methanol Extract

Compound	Retention Time (min)	Concentration (µg/mg)	Composition (%)
Phenolic Compounds			
Gallic acid	3.50	1.64	48.46
Chlorogenic acid	4.20	0.88	26.00
Methyl gallate	5.39	0.078	2.30
Syringic acid	6.31	0.398	11.76
Ellagic acid	7.21	0.0048	0.14
p-Coumaric acid	8.53	0.075	2.22
Vanillin	8.89	0.0137	0.41
Ferulic acid	9.58	0.0795	2.35
Rosmarinic acid	11.72	0.194	5.73
Total Phenolics		3.382	
Flavonoid Compounds			
Catechin	4.41	2.65	77.42
Naringenin	10.33	0.026	0.76
Daidzein	15.86	0.071	2.07
Quercetin	17.21	0.676	19.75
Total Flavonoids		3.423	

These compounds, identified through HPLC as shown in Figure-1, are critical secondary metabolites with established roles in antioxidant defense, enzyme inhibition, and disease prevention(Chatzitzika et al., 2020). The concentrations are converted into percentage compositions (w/w) to facilitate a comprehensive understanding and visual representation, correlating with the chromatographic peaks. The total concentration of phenolic compounds is 3.3821 $\mu\text{g}/\text{mg}$, with gallic acid emerging as the predominant component, accounting for nearly half of the phenolic content with 1.64 $\mu\text{g}/\text{mg}$ (48.46% of total phenolics). This dominance is reflected in the HPLC chromatogram (as shown in Figure S-1) by the prominent peak at ~3.50 minutes, indicating high abundance and purity. The retention times align with standard phenolic profiles, as gallic acid typically elutes early due to its polarity, followed by less polar compounds like rosmarinic acid. Methyl gallate (2.30%), syringic acid (11.76%), ellagic acid(0.14%), p-coumaric acid(2.22%), vanillin(0.44%), ferulic acid(2.53%), and rosmarinic acid are also present, albeit in lower concentrations. Notably, rosmarinic acid, with a concentration of 0.194 $\mu\text{g}/\text{mg}$, is recognized for its anti-inflammatory and neuroprotective effects.

The predominance of gallic acid and catechin in the methanol extract of *Lepidium africanum* further supports its potential as a rich source of antioxidants. Gallic acid, a polyphenol, exhibits strong radical-scavenging activity, chelating metal ions, and inhibiting lipid peroxidation, as supported by studies on Brassicaceae plants(Singh et al., 2019). Its high concentration (48.52%) and prominent HPLC peak (~3.50 min) suggest a robust capacity to mitigate oxidative stress, a key factor in chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders. Other phenolic compounds, such as chlorogenic acid (26.00%, ~4.20 min) and rosmarinic acid (5.73%, ~11.70 min), may contribute additional antioxidant and anti-inflammatory activities(Sforcin & Bankova, 2011). Chlorogenic acid, is known to inhibit glucose absorption and exhibit antimicrobial properties, while rosmarinic acid has been associated with anti-allergic and antiviral effects in plants like *Lepidium* species(Jaiswal & Kumar, 2022). Minor components e.g., ellagic acid(0.14%, ~7.20 min) may play synergistic roles, enhancing the overall bioactivity of the extract, as observed in recent phytochemical studies of methanol extracts from related medicinal plants(Pacifico et al., 2021).

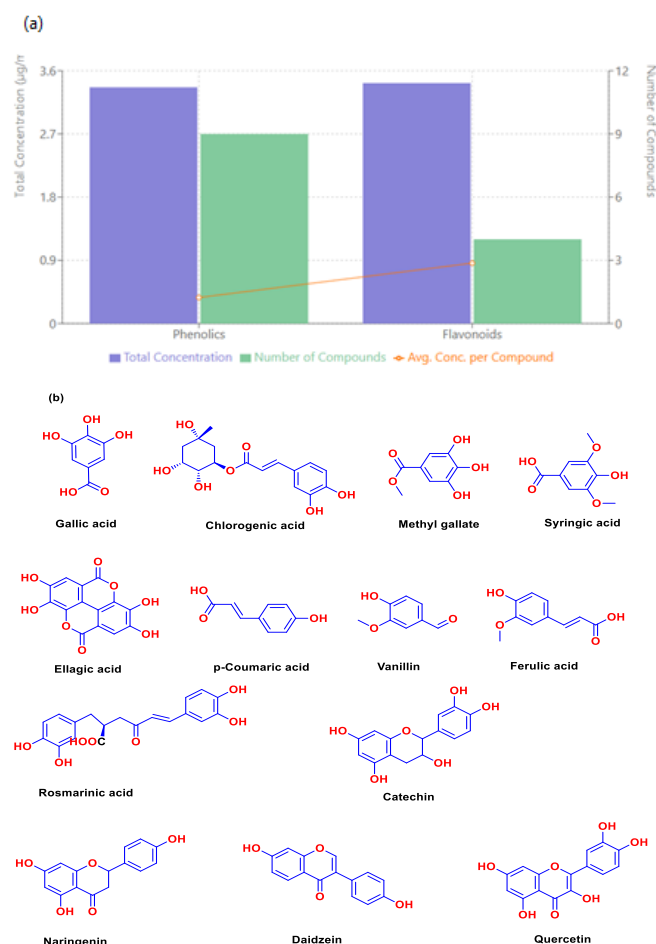


Figure-1: (a) A composite chart comparing the total concentration, number of compounds, and average concentration per compound between the two groups. (b) Chemical structure for all compounds.

The flavonoid compounds identified, along with their concentrations, retention times (as shown in Table-1), and percentage contributions, are as follows: Catechin: 2.65 $\mu\text{g}/\text{mg}$ (77.42% of total flavonoids, retention time ~ 4.41 min), Naringenin: 0.026 $\mu\text{g}/\text{mg}$ (0.76%, ~ 10.33 min), Daidzein: 0.071 $\mu\text{g}/\text{mg}$ (2.07%, ~ 15.85 min), Quercetin: 0.676 $\mu\text{g}/\text{mg}$ (19.75%, ~ 17.21 min). The total concentration of flavonoid compounds is 3.423 $\mu\text{g}/\text{mg}$, with catechin constituting the majority of the flavonoid fraction. The HPLC chromatogram (as shown in Figure S-1) shows a dominant peak at ~ 4.41 minutes for catechin, consistent with its high polarity and early elution, followed by less polar flavonoids like quercetin at ~ 17.21 minutes (Nwozo et al., 2023). Catechin, is a potent flavonoid with well-documented antioxidant, anti-inflammatory, and anticancer properties. Its hydroxyl-rich structure enables effective neutralisation of reactive oxygen species (ROS), as demonstrated in studies on related Brassicaceae plants, such as *L. sativum*, where flavonoids contribute to therapeutic efficacy (Raza et al., 2020). The presence of quercetin is another potent flavonoid, further enhances the extract's antioxidant profile, as quercetin is known for its ability to inhibit lipid peroxidation and modulate cellular signalling pathways involved in inflammation and cancer prevention (Nwozo et al., 2023). The phytochemical profile of *L. africanum* methanol extract, aligns with recent findings on Brassicaceae plants, which are rich in glucosinolates, phenolics, and flavonoids, contributing to their medicinal properties (Singh et al., 2019). Studies on *L. sativum* and *L. meyenii* have demonstrated that methanol extracts, due to their polarity, effectively extract polar compounds like phenolics and flavonoids, which are critical for antioxidant activity. For instance, study on

L. meyenii roots and leaves has identified high levels of saponins, phenols, and flavonoids, correlating with strong antioxidant capabilities, as measured by DPPH and FRAP assays (Raza et al., 2020). Similarly, the high phenolic and flavonoid content in *Lepidium africanum* suggests potential applications in antioxidant therapy, disease prevention, and pharmaceutical development. Generally (as shown in Figure-1), The dominance of gallic acid and catechin may also indicate anti-cancer and anti-inflammatory potential, as these compounds have been shown to modulate key signalling pathways (e.g., NF- κ B, MAPK) in cellular models (Nwozo et al., 2023). The presence of chlorogenic acid and quercetin could potentially contribute to antidiabetic and cardioprotective effects, as evidenced by studies on related Brassicaceae species (Jaiswal & Kumar, 2022). However, the low concentrations of minor compounds (e.g., ellagic acid, naringenin) suggest that their contributions may be supplementary, requiring further investigation to elucidate synergistic or additive effects.

3.2 Biological study:

3.2.1 Antioxidant activity of *L. africanum* methanol extract using ABTS scavenging capacity method

The methanol extract of *L. africanum* has garnered significant interest due to its potential antioxidant capacity, attributed to its rich phytochemical profile, particularly phenolic and flavonoid compounds. This analysis evaluates the antioxidant activity of the methanol extract using the ABTS radical scavenging assay, (Table S-1).

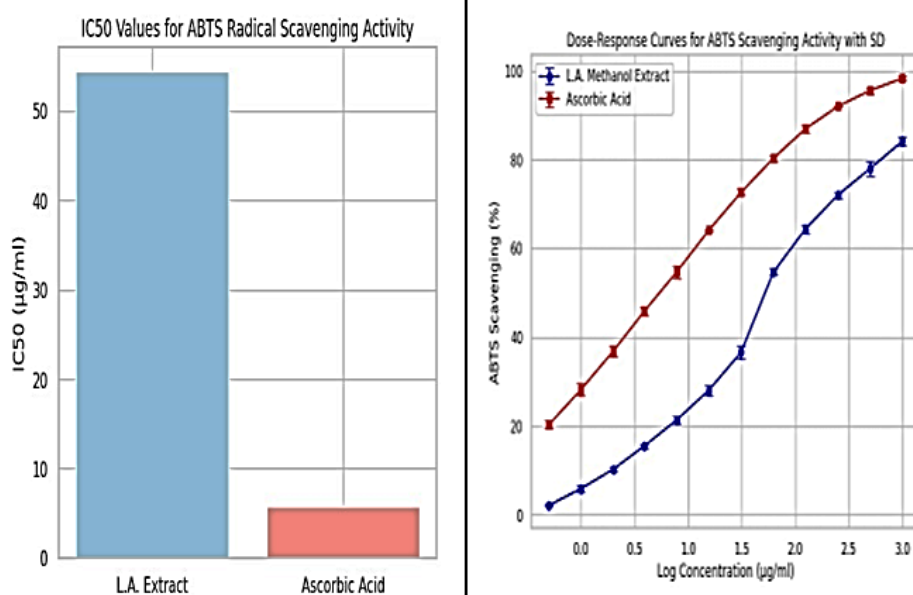


Figure-2. ABTS scavenging activity (from left to right pannel)for *L. africanum* and Ascorbic Acid, Bar chart comparing the IC50 values ($\mu\text{g}/\text{ml}$), Dose-Response Curves, ABTS Scavenging Percentage at Different Concentrations.

A potent polyphenol (Gallic acid ; 48.46% of phenolics) with strong radical-scavenging activity, capable of donating hydrogen atoms to neutralize ABTS radicals(Singh et al., 2019). Its high concentration (1.64 $\mu\text{g}/\text{mg}$) likely contributes significantly to the extract's antioxidant capacity (as shown in Figure-2). While Catechin (77.42%) with multiple hydroxyl groups, enabling effective electron donation to stabilize ABTS radicals. Its dominance (2.65 $\mu\text{g}/\text{mg}$) could be a major driver of the extract's antioxidant potential(Raza et al., 2020). Chlorogenic acid (26.00% of phenolics) and Quercetin (19.75% of flavonoids), these compounds further enhance antioxidant activity through their ability to scavenge free radicals and chelate metal ions(Jaiswal & Kumar, 2022). The synergistic interaction of these compounds, particularly gallic acid and catechin, likely amplifies the extract's ABTS scavenging capacity, as phenolic and flavonoid mixtures are known to exhibit additive or cooperative antioxidant effects(Pacifico et al., 2021). In conclusion, the methanol extract of *L. africanum* demonstrated moderate antioxidant activity in the ABTS assay, with an IC_{50} value of 58.72 $\mu\text{g}/\text{ml}$ (as shown in Figure-2). As expected, the standard antioxidant ascorbic acid was significantly more potent (IC_{50} : 6.45 $\mu\text{g}/\text{ml}$). The observed activity is may be attributed to the extract's rich phenolic and flavonoid content (Table-1), particularly the high concentrations of gallic acid and catechin, which are established potent radical scavengers(Chatzitzika et al., 2020; Nwozo et al., 2023). The dose-response curve shows a clear concentration-dependent increase in

activity, reaching >84% scavenging at the highest tested concentration (1000 $\mu\text{g}/\text{ml}$), confirming its potential to mitigate oxidative stress.

3.2.2 Evaluation of Antioxidant Activity using Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing power of a compound is a key indicator of its potential antioxidant activity. The FRAP assay was employed to evaluate the ability of the *L. africanum* methanol extract to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). As illustrated in Figure-3, both the extract and the ascorbic acid standard demonstrated a concentration-dependent increase in ferric reducing power. The extract exhibited considerable reducing activity, reaching a maximum of $89.54 \pm 0.98\%$ at the highest concentration tested (1000 $\mu\text{g}/\text{mL}$). However, as quantified by the IC_{50} value (Table S-2), the reducing potency of the extract ($\text{IC}_{50} = 71.73 \pm 9.62 \mu\text{g}/\text{mL}$) was significantly lower than that of the potent standard antioxidant, ascorbic acid ($\text{IC}_{50} = 12.72 \pm 0.36 \mu\text{g}/\text{mL}$).

This result is consistent with the findings from the ABTS and FRAP assays, confirming the antioxidant capacity of the *L. africanum* extract. The observed activity can be attributed to its high content of phenolic compounds (like gallic acid) and flavonoids (like catechin), which are known to act as electron donors, effectively reducing oxidized intermediates and thereby terminating radical chain reactions (Chatzitzika et al., 2020; Nwozo et al., 2023).

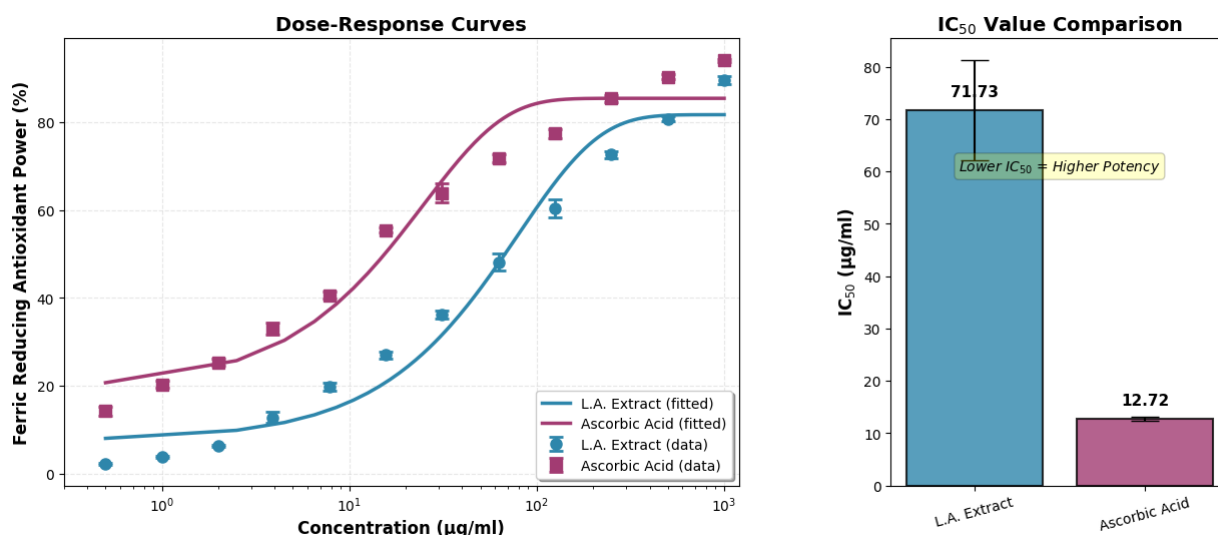


Figure-3. Evaluation of Antioxidant Activity using FRAP Assay for L.A

3.2.3 Anti-diabetic activity of *L. africanum* methanol extract using α -amylase enzyme inhibition

Diabetes mellitus, particularly type 2 diabetes, is characterized by chronic hyperglycemia resulting from impaired insulin action or secretion. One therapeutic strategy to manage postprandial hyperglycemia is to inhibit α -amylase, a key enzyme in the digestion of dietary starch. By reducing starch digestion, α -amylase inhibitors can lower the glucose absorption rate and thereby help manage blood glucose levels (Alasmari et al., 2025). The α -amylase enzyme inhibition assay is a

valuable method to assess the potential of plant extracts and compounds as anti-diabetic agents. To evaluate and compare the potency, we analyze the IC₅₀ values and dose-response relationships (Table S-3). The IC₅₀ for *L. africanum* methanol extract is 57.46 μ g/ml, while positive control acarbose is lower IC₅₀ of 4.65 μ g/ml.

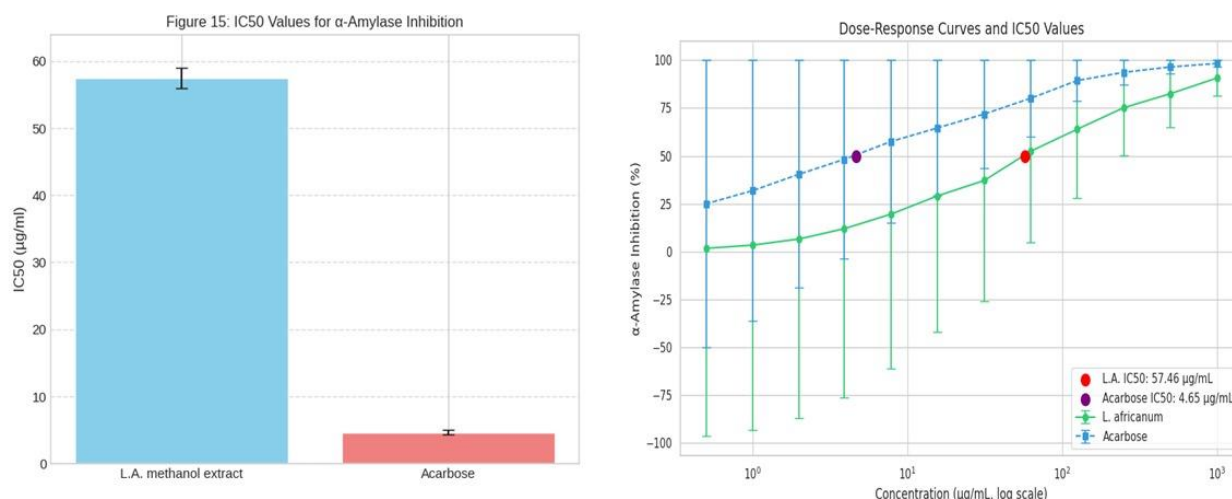


Figure-4. illustrates the dose-response curves for α -amylase enzyme inhibition for both *Lepidium africanum* methanol extract and acarbose. a dose-response curve graph showing % α -amylase inhibition vs. concentration for both extract and acarbose. Include error bars for SD, using data from Table S-3)

While acarbose, is more potent, the extract's inhibitory capacity is still noteworthy. At higher concentrations (e.g., 1000 μ g/ml), the extract achieves a substantial enzyme inhibition of approximately 91%, demonstrating its effectiveness at relevant concentrations (Figure-4). The observed α -amylase inhibitory activity of *L. africanum* methanol extract can be plausibly attributed to its complex phytochemical profile, especially flavonoids and phenolic acids, are well-documented for their ability to inhibit carbohydrate-hydrolyzing enzymes like α -amylase and α -glucosidase (Zainab et al., 2025). Chlorogenic acid, present in the *L. africanum* extract, is a known inhibitor of α -amylase and has been shown to reduce postprandial blood glucose levels in animal studies (Yan et al., 2022). Its mechanism involves competitive inhibition of the enzyme, thereby slowing down glucose release from starch digestion (Zheng et al., 2020). Catechin, the most abundant flavonoid in the extract, has also demonstrated α -amylase inhibitory effects (Xu et al., 2013). Catechins, and flavonoids in general, can interact with enzymes through hydrogen bonding and hydrophobic interactions, leading to conformational changes that reduce enzyme activity (Telagari & Hullatti, 2015). Quercetin is another flavonoid in the extract known for its anti-diabetic

properties, including α -amylase inhibition. Quercetin's inhibitory action may also involve similar enzyme-substrate interaction mechanisms, along with potential antioxidant protection of pancreatic β -cells (COSKUN et al., 2005). Other phenolic acids like gallic acid, syringic acid, and ferulic acid, also found in *L. africanum* extract, have been reported to exhibit α -amylase and α -glucosidase inhibitory activities, albeit to varying extents (Tan et al., 2017). The synergistic action of these and potentially other unidentified compounds in the extract may contribute to the overall anti-diabetic effect.

in conclusion, The *L. africanum* extract exhibited significant α -amylase inhibitory activity with an IC₅₀ value of 57.46 μ g/ml, suggesting its potential for managing postprandial hyperglycemia (as shown in Figure-4). Although the standard drug acarbose was more potent (IC₅₀: 4.65 μ g/ml), the extract's inhibitory capacity is notable, achieving ~91% inhibition at 1000 μ g/ml. This bioactivity can be plausibly linked to its flavonoid and phenolic acid content. Compounds like chlorogenic acid and catechin are well-documented for their ability to inhibit carbohydrate-hydrolyzing enzymes through interactions with the enzyme's active site (Telagari &

Hullatti, 2015; Xu et al., 2013; Zainab et al., 2025).

3.2.4 Anti-inflammatory activity of *L. africanum* methanol extract based on Cox-1 enzyme inhibition

Inflammation is a complex biological response to tissue injury, infection, or irritation. While acute inflammation is a protective mechanism, chronic inflammation underlies numerous diseases, including arthritis, cardiovascular diseases, and neurodegenerative disorders (Medzhitov, 2008). Cyclooxygenase-1 (COX-1) is a key enzyme involved in the biosynthesis of prostaglandins, which are crucial mediators of inflammation and pain (Simmons et al., 2004). Inhibition of COX-1 is a well-established therapeutic strategy for reducing inflammation, with non-steroidal anti-inflammatory drugs (NSAIDs) being primary examples (Vane & Botting, 1998). However, synthetic NSAIDs are often associated with adverse effects, prompting the search for safer, naturally derived

anti-inflammatory agents, particularly from plant sources (Bajpai et al., 2013). This analysis examines the anti-inflammatory potential of *L. africanum* methanol extract by evaluating its inhibitory activity against COX-1 enzyme and comparing it to celecoxib acid, a known COX inhibitor. The inhibitory potency of *L. africanum* methanol extract against COX-1 enzyme was evaluated using the COX-1 inhibition assay. Table S-4 presents the percentage inhibition of COX-1 enzyme activity at various concentrations of the extract and celecoxib acid. The IC₅₀ value, a key parameter for quantifying and comparing inhibitory potency. The IC₅₀ value for *L. africanum* methanol extract is $14.47 \pm 0.30 \mu\text{g/ml}$. Celecoxib acid, exhibited a significantly lower IC₅₀ of $7.19 \mu\text{g/ml} \pm 0.35 \mu\text{g/ml}$, indicating its greater potency as a COX-1 inhibitor in this assay. Figure 4 illustrates the dose-response curves for COX-1 enzyme inhibition by *Lepidium africanum* methanol extract and celecoxib acid.

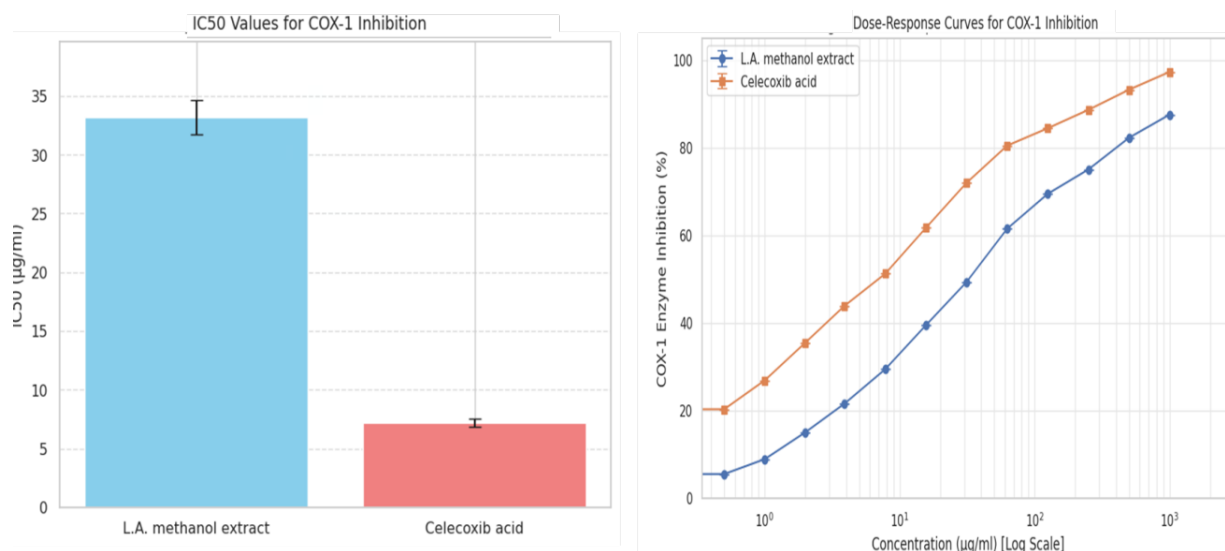


Figure-5: Dose-Response Curves for COX-1 Enzyme Inhibition for both extract and celecoxib acid. Include error bars for SD using data from Table S-4)

The dose-response curves in Figure 5 indicates that as the concentration of either the extract or celecoxib acid increases, the percentage of COX-1 enzyme inhibited also increases. While both exhibit this trend, celecoxib acid consistently shows a higher degree of inhibition at each concentration point compared to the *L. africanum* extract, which is consistent with its lower IC₅₀ value and greater inhibitory potency. At the highest concentration tested (1000 µg/ml), *L. africanum* methanol extract achieves approximately 87.5% COX-1 inhibition, demonstrating substantial, albeit not complete, enzyme inhibitory capacity. To visually compare the inhibitory strengths, Figure 5 presents a bar chart comparing the IC₅₀ values of *L. africanum* methanol extract and celecoxib acid for COX-1 inhibition. The observed COX-1 inhibitory activity

of *L. africanum* methanol extract indicates its potential to modulate inflammatory processes. While celecoxib acid, a selective COX-2 inhibitor with some COX-1 activity, is more potent, the extract's IC₅₀ of 33.16 µg/ml suggests a significant level of COX-1 inhibition that could contribute to anti-inflammatory effects. This activity can be attributed to the phytochemical constituents present in the extract, particularly the phenolic and flavonoid compounds identified in previous analyses (Table-1).

Compounds like gallic acid, chlorogenic acid, syringic acid, catechin, and quercetin, all identified in *L. africanum* methanol extract, have been reported to possess COX-1 inhibitory properties. Their mechanisms of action may involve direct binding to the enzyme

active site, competitive or non-competitive inhibition, or modulation of enzyme expression. The most abundant phenolic compound (Gallic acid) in the extract (1.64 $\mu\text{g}/\text{mg}$), has demonstrated COX-inhibitory and anti-inflammatory effects in several studies (Kaur et al., 2009). Similarly, chlorogenic acid (0.88 $\mu\text{g}/\text{mg}$) and quercetin (0.676 $\mu\text{g}/\text{mg}$) have shown inhibitory effects on COX enzymes and prostaglandin production, contributing to their anti-inflammatory properties (Bajpai et al., 2013). Catechin (2.65 $\mu\text{g}/\text{mg}$) and syringic acid (0.398 $\mu\text{g}/\text{mg}$) also contribute to the overall COX-1 inhibitory profile of the extract. The presence of these and other bioactive phytochemicals in *Lepidium africanum* methanol extract likely contributes synergistically to its observed COX-1 inhibitory activity and, consequently, its potential anti-inflammatory effects.

3.2.5 In Vitro Cytotoxicity Evaluation of *L. africanum* Against HepG-2, MCF-7, and CACO2 Cell Lines.

Table S-5, S-6 and S-7 present the cell viability percentages for HepG-2, MCF-7, and CACO2 cell lines treated with varying concentrations of *L. africanum* methanol extract and cisplatin, respectively. As evident from IC₅₀ values, cisplatin demonstrates significantly higher cytotoxicity across all three cell lines, with IC₅₀ values substantially lower than those of *L. africanum* methanol extract. However, the *Lepidium africanum* extract also exhibits notable cytotoxicity, with IC₅₀ values in the range of 71.61 $\mu\text{g}/\text{ml}$ to 114.09 $\mu\text{g}/\text{ml}$, depending on the cell line. HepG-2 cells appear to be the most sensitive to the extract, followed by CACO2 and then MCF-7 cells, based on their respective IC₅₀ values. To visually represent the cytotoxic effects and compare the dose-response relationships, Figure 6 presents dose-response curves for *L. africanum* methanol extract and cisplatin against HepG-2, MCF-7, and CACO2 cell lines.

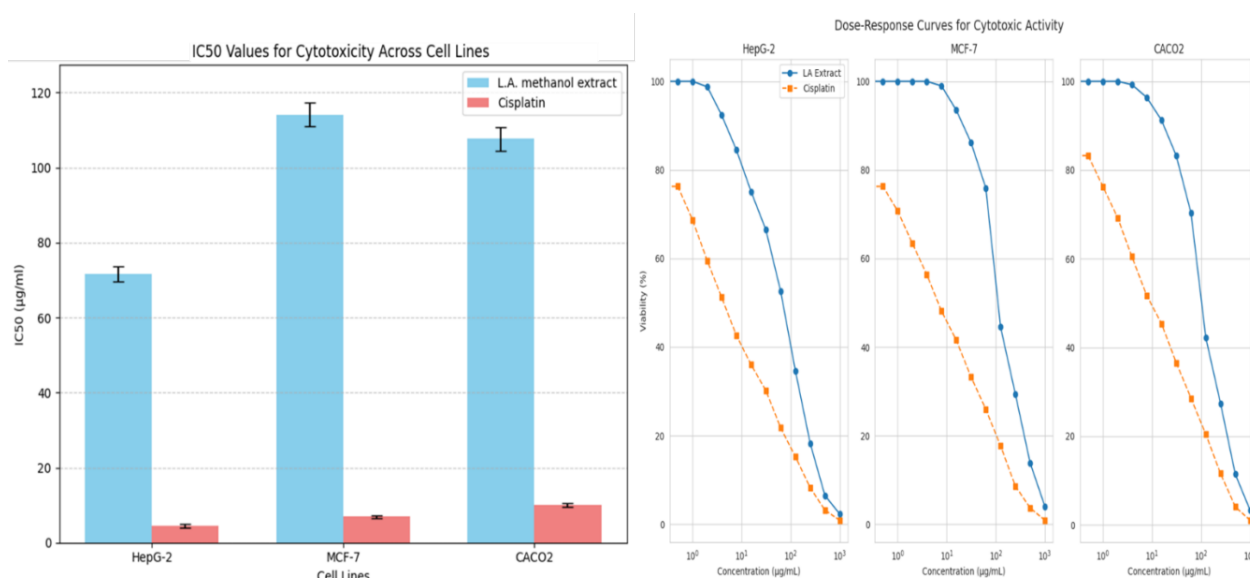


Figure-6: Dose-response curves and bar chart for *L. africanum* extract and Cisplatin against HepG-2, MCF-7, and CACO2 cell lines.

The MTT assay results demonstrate that *L. africanum* methanol extract exhibits promising anticancer activity against HepG-2, MCF-7, and CACO2 cell lines, with IC₅₀ values of 71.61 $\pm$ 1.96 $\mu\text{g}/\text{ml}$, 114.09 $\pm$ 3.13 $\mu\text{g}/\text{ml}$, and 107.63 $\pm$ 3.25 $\mu\text{g}/\text{ml}$, respectively, though significantly less potent than cisplatin (4.49 $\pm$ 0.44 $\mu\text{g}/\text{ml}$ for HepG-2, 6.94 $\pm$ 0.40 $\mu\text{g}/\text{ml}$ for MCF-7, 9.95 $\pm$ 0.53 $\mu\text{g}/\text{ml}$ for CACO2). However, the IC₅₀ values of *L. africanum* suggest moderate cytotoxic potential, comparable to other plant extracts rich in phenolics and flavonoids, such as those from *Lepidium meyenii*, reported to exhibit IC₅₀ values of 70–120 $\mu\text{g}/\text{ml}$ in MTT assays against similar cell lines (Raza et al., 2020). The extract's activity

is likely driven by gallic acid, catechin, chlorogenic acid, and quercetin, which induce apoptosis and may be inhibit cell proliferation through ROS generation and signaling pathway modulation. The dose-response curves (as shown in Figure-6) illustrate sigmoid relationships, with *L. africanum* showing more gradual increases in cytotoxicity, indicating a broader concentration range for effective cell inhibition compared to cisplatin's steeper curves. This suggests that the extract's complex phytochemical matrix may involve synergistic or competitive interactions, potentially reducing its potency relative to the pure cisplatin standard (Pacífico et al., 2021).

in conclusion, The MTT assay revealed that *L. africanum* extract possesses promising, dose-dependent anticancer activity against all three tested cell lines (Figure 6). The extract was most potent against the HepG-2 hepatocellular carcinoma cell line (IC₅₀: 71.61 µg/ml), followed by CACO2 colorectal cancer cells (IC₅₀: 107.63 µg/ml) and MCF-7 breast cancer cells (IC₅₀: 114.09 µg/ml). As anticipated, the chemotherapeutic drug cisplatin was markedly more cytotoxic (IC₅₀: 4.49 - 9.95 µg/ml). The selective cytotoxicity of the extract may be likely mediated by its prominent bioactive compounds, such as gallic acid and quercetin, which are known to induce apoptosis and inhibit proliferation in cancer cells through mechanisms involving ROS generation and modulation of key signaling pathways (Jaiswal & Kumar, 2022).

3.3 Overall Implications and Future Perspectives

The collective findings from this study firmly establish *L. africanum* as a promising medicinal plant with multifaceted biological activities. The strong correlations between its rich phytochemical profile and the observed bioactivities provide a scientific basis for its ethnobotanical use. However, this study has some limitations. The crude extract was used, and the contributions of individual compounds or potential synergistic effects remain to be determined. Future work should therefore focus on the bioassay-guided fractionation of the extract to isolate and identify the specific compounds responsible for each activity. Furthermore, the underlying mechanisms, such as apoptosis induction or NF-κB pathway modulation, should be investigated using molecular techniques. It is also noteworthy that Soxhlet extraction was used in this study; future work could employ greener and more efficient techniques like ultrasound-assisted extraction to minimize potential thermal degradation and compare the yield and bioactivity of the resulting extracts. Finally, in vivo studies are essential to confirm these promising in vitro results and assess the safety and efficacy of the extract.

4. Conclusion

The present study comprehensively investigates the phytochemical composition and biological activities of *Lepidium africanum*, a plant predominantly found in Sub-Saharan Africa and Saudi Arabia. Our analysis reveals that *L. africanum* is a rich source of bioactive compounds, particularly phenolic acids and flavonoids, with significant antioxidant, antidiabetic, anti-inflammatory, and anticancer potentials. The HPLC analysis identified various phenolic and flavonoid compounds in the methanol extract of *L. africanum*, with gallic acid and catechin being the predominant components. The extract demonstrated significant multifunctional bioactivity, exhibiting antioxidant (ABTS IC₅₀: 58.72 µg/ml), antidiabetic (α-amylase IC₅₀: 57.46 µg/ml), anti-inflammatory (COX-1 IC₅₀: 14.47 µg/ml), and anticancer

effects (IC₅₀: 71.61-114.09 µg/ml across HepG-2, MCF-7, and Caco-2 cell lines). These findings validate the plant's traditional use and highlight its potential as a source of therapeutic agents. Future work should focus on compound isolation, mechanism elucidation, and exploring alternative extraction methods like ultrasound-assisted extraction.

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M
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M (Sequence Method)
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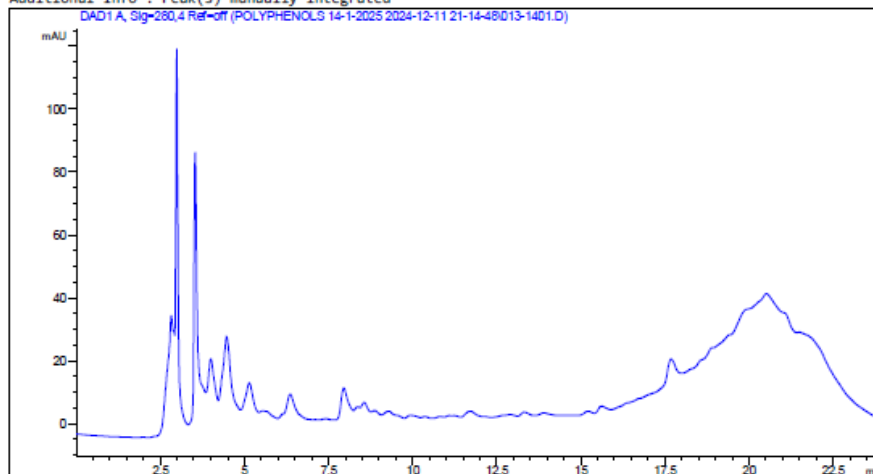


Figure S-1: HPLC Chromatogram of *L. africanum* methanol extract, showing the total phenolic and flavonoid content (µg/mg).

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                                           Inj Volume: 5.000 µl
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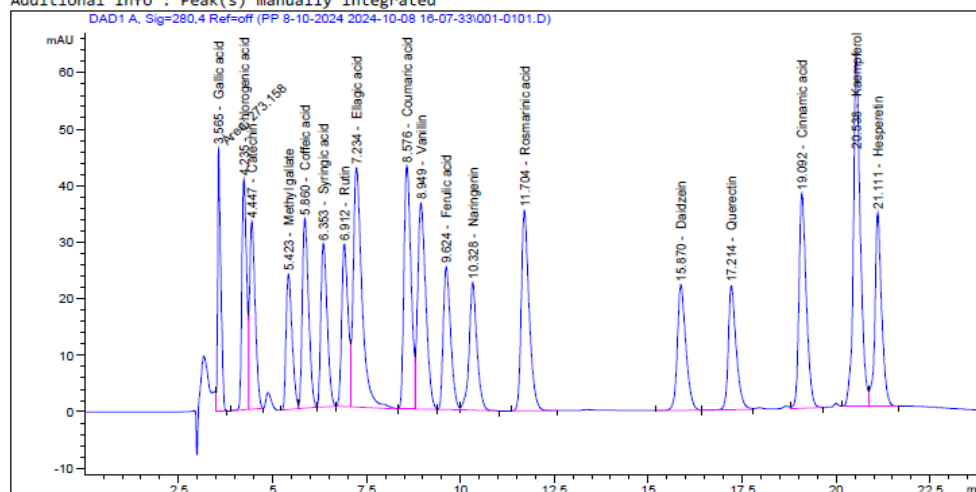


Figure S-2: HPLC Chromatogram of the polyphenols standards .

Table S-1: Evaluation of antioxidant of L.A. activity using ABTS. radical scavenging

Sample conc. (µg/ml)	ABTS scavenging %					ABTS scavenging %				
	3 Readings			Mean	S.D. (±)	3 Readings			Mean	S.D. (±)
	<i>L.A. methanol extract</i>					<i>Ascorbic acid</i>				
1000	83.16	85.09	84.26	84.17	0.97	98.13	98.78	98.06	98.32	0.40
500	76.34	79.53	78.14	78.00	1.60	95.02	95.42	96.31	95.58	0.66
250	71.96	72.88	71.32	72.05	0.78	91.86	91.97	92.15	91.99	0.15
125	63.8	65.49	64.07	64.45	0.91	86.31	86.74	87.89	86.98	0.82
62.5	54.91	55.23	53.85	54.66	0.72	80.42	79.53	80.94	80.30	0.71
31.25	36.78	38.02	35.14	36.65	1.44	72.96	71.85	73.12	72.64	0.69
15.6	27.19	29.41	27.68	28.09	1.17	63.88	64.19	64.19	64.09	0.18
7.8	21.37	22.13	20.64	21.38	0.75	54.09	53.67	56.28	54.68	1.40
3.9	15.28	16.04	15.28	15.53	0.44	46.21	44.82	46.57	45.87	0.92
2	10.45	10.67	9.85	10.32	0.42	37.07	35.49	38.12	36.89	1.32
1	6.71	5.94	5.23	5.96	0.74	28.42	26.84	29.47	28.24	1.32
0.5	1.89	2.16	2.43	2.16	0.27	19.86	19.65	21.38	20.30	0.94
0	0	0	0	0	0	0	0	0	0	0
	IC50					IC50				
	54.04	53.00	56.07	54.37	1.56	5.78	6.18	5.28	5.75	0.45

Table S-2: Evaluation of antioxidant of L.A. activity using FRAP.

Sample conc. (µg/ml)	ABTS scavenging %					ABTS scavenging %				
	3 Readings			Mean	S.D. (±)	3 Readings			Mean	S.D. (±)
	<i>L.A. methanol extract</i>					<i>Ascorbic acid</i>				
1000	89.43	90.57	88.62	89.54	0.98	93.47	94.05	94.31	93.94	0.43
500	80.57	81.32	80.19	80.69	0.58	89.62	90.53	90.53	90.23	0.53
250	72.38	73.45	71.86	72.56	0.81	84.59	85.23	86.14	85.32	0.78
125	60.51	62.38	58.27	60.39	2.06	76.14	77.89	78.06	77.36	1.06
62.5	49.69	48.73	46.08	48.17	1.87	70.87	71.94	72.51	71.77	0.83
31.25	37.08	36.41	35.29	36.26	0.90	61.34	65.29	64.85	63.83	2.16
15.6	26.45	27.98	26.74	27.06	0.81	54.93	56.04	55.42	55.46	0.56
7.8	20.78	19.83	19.05	19.89	0.87	39.75	41.53	40.68	40.65	0.89
3.9	13.92	13.25	11.43	12.87	1.29	31.42	34.21	33.56	33.06	1.46
2	6.71	6.48	6.25	6.48	0.23	24.59	26.08	25.37	25.35	0.75
1	4.08	3.92	3.64	3.88	0.22	19.56	20.91	20.68	20.38	0.72
0.5	2.45	2.31	1.96	2.24	0.25	13.48	15.32	14.05	14.28	0.94
0	0	0	0	0	0	0	0	0	0	0
	IC50					IC50				
	64.29	68.32	82.60	71.73	9.62	13.07	12.35	12.73	12.72	0.36

Table S-3: Evaluation of Antidiabetic of L.A. activity using α - Amylase Enzyme Inhibition (%)

Sample conc. ($\mu\text{g/ml}$)	α - Amylase Enzyme Inhibition (%)					α - Amylase Enzyme Inhibition (%)				
	(3 Replicates)			Mean	S.D. ($\pm$)	(3 Replicates)			Mean	S.D. ($\pm$)
	<i>L.A. methanol extract</i>					<i>Acarbose acid</i>				
	1 st	2 nd	3 rd			1 st	2 nd	3 rd		
1000	91.54	90.73	89.86	90.71	9.29	98.27	98.64	98.01	98.31	1.69
500	82.76	83.24	81.63	82.54	17.46	96.58	97.06	95.89	96.51	3.49
250	75.82	74.91	74.91	75.21	24.79	93.12	94.28	93.67	93.69	6.31
125	63.59	63.24	65.06	63.96	36.04	88.21	90.63	89.42	89.42	10.58
62.5	52.34	51.86	53.18	52.46	47.54	80.68	79.41	80.23	80.11	19.89
31.25	37.16	36.03	38.2	37.13	62.87	72.94	71.28	71.39	71.87	28.13
15.6	29.08	28.46	29.72	29.09	70.91	65.36	64.59	63.84	64.60	35.40
7.8	18.73	19.51	20.35	19.53	80.47	58.13	57.42	56.92	57.49	42.51
3.9	11.45	11.27	13.14	11.95	88.05	49.05	48.36	47.18	48.20	51.80
2	6.29	5.98	7.52	6.60	93.40	40.62	41.29	39.57	40.49	59.51
1	3.41	3.06	3.69	3.39	96.61	32.51	30.65	32.43	31.86	68.14
0.5	1.76	1.54	1.82	1.71	98.29	24.97	23.19	26.82	24.99	75.01
0	0	0	0	0		0	0	0	0	
	IC50					IC50				
	57.68	58.83	55.87	57.46	1.49	4.31	4.61	5.03	4.65	0.36

Table S-4: Evaluation of Anti-inflammatory of L.A. activity using Cox-1 enzyme inhibition (%)

Sample conc. ($\mu\text{g/ml}$)	COX-1 Enzyme Inhibition (%)					COX-1 Enzyme Inhibition (%)				
	(3 Replicates)			Mean	S.D. ($\pm$)	(3 Replicates)			Mean	S.D. ($\pm$)
	<i>L.A. methanol extract</i>					<i>Celecoxib drug</i>				
	1 st	2 nd	3 rd			1 st	2 nd	3 rd		
1000	92.03	92.46	91.89	92.13	7.87	97.64	97.23	96.87	97.25	2.75
500	87.64	88.31	86.47	87.47	12.53	93.56	93.14	92.85	93.18	6.82
250	82.75	84.69	81.23	82.89	17.11	89.12	88.71	87.92	88.58	11.42
125	75.86	77.28	74.19	75.78	24.22	85.03	84.95	83.16	84.38	15.62
62.5	69.42	70.54	67.85	69.27	30.73	81.36	80.92	78.93	80.40	19.60
31.25	60.54	63.18	59.43	61.05	38.95	73.59	71.87	70.26	71.91	28.09
15.6	51.98	52.37	51.54	51.96	48.04	61.34	62.16	61.34	61.61	38.39
7.8	38.17	39.42	37.18	38.26	61.74	50.63	51.95	50.87	51.15	48.85
3.9	29.46	31.63	28.88	29.99	70.01	43.28	44.39	43.85	43.84	56.16
2	21.08	22.19	20.67	21.31	78.69	35.16	36.24	34.92	35.44	64.56
1	14.92	15.87	14.53	15.11	84.89	26.93	27.8	25.74	26.82	73.18
0.5	8.75	9.58	8.49	8.94	91.06	19.42	21.83	19.42	20.22	79.78
0	0	0	0	0		0	0	0	0	
	IC50					IC50				
	14.48	14.17	14.76	14.47	0.30	7.47	6.79	7.32	7.19	0.35

Table S-5: Cytotoxicity Evaluation of L.A Against HepG-2

Sample conc. (µg/ml)	Viability (%)						Viability (%)					
	(3 Replicates)			Mean	Inhibitory %	S.D. (±)	(3 Replicates)			Mean	Inhibitory %	S.D. (±)
	<i>L.A. methanol extract</i>						Cisplatin Standard					
	1 st	2 nd	3 rd				1 st	2 nd	3 rd			
1000	2.64	1.87	2.39	2.30	97.70	0.39	0.81	0.94	0.87	0.87	99.13	0.07
500	6.93	5.64	6.85	6.47	93.53	0.72	3.17	3.42	2.96	3.18	96.82	0.23
250	18.76	17.59	18.47	18.27	81.73	0.61	8.29	8.57	7.83	8.23	91.77	0.37
125	34.68	35.72	33.51	34.64	65.36	1.11	15.32	16.08	14.27	15.22	84.78	0.91
62.5	51.97	53.06	52.84	52.62	47.38	0.58	21.79	22.93	20.54	21.75	78.25	1.20
31.25	65.42	66.89	67.13	66.48	33.52	0.93	30.45	31.79	28.32	30.19	69.81	1.75
15.6	73.81	75.43	76.09	75.11	24.89	1.17	37.18	36.26	34.59	36.01	63.99	1.31
7.8	84.19	84.19	85.32	84.57	15.43	0.65	43.21	44.05	40.71	42.66	57.34	1.74
3.9	91.73	92.36	93.17	92.42	7.58	0.72	52.37	51.13	50.34	51.28	48.72	1.02
2	98.67	98.59	99.06	98.77	1.23	0.25	60.78	59.32	58.29	59.46	40.54	1.25
1							69.83	68.14	68.14	68.70	31.30	0.98
0.5							76.13	75.06	74.98	75.39	24.61	0.64
0	100	100	100	100	0		100	100	100	100	0	
	IC50						IC50					
	69.62	73.53	71.68	71.61		1.96	4.91	4.52	4.04	4.49		0.44

Table S-6: Cytotoxicity Evaluation of L.A Against MCF-7

Sample conc. (µg/ml)	Viability (%)						Viability (%)					
	(3 Replicates)			Mean	Inhibitory %	S.D. (±)	(3 Replicates)			Mean	Inhibitory %	S.D. (±)
	<i>L.A. methanol extract</i>						Cisplatin Standard					
	1 st	2 nd	3 rd				1 st	2 nd	3 rd			
1000	4.07	3.85	4.21	4.04	95.96	0.18	0.83	0.96	0.88	0.89	99.11	0.07
500	13.74	13.12	14.68	13.85	86.15	0.79	3.46	3.71	3.92	3.70	96.30	0.23
250	29.03	28.46	30.71	29.40	70.60	1.17	8.03	8.69	9.14	8.62	91.38	0.56
125	44.96	42.78	45.83	44.52	55.48	1.57	16.37	18.24	18.53	17.71	82.29	1.17
62.5	76.02	74.23	77.49	75.91	24.09	1.63	24.52	26.03	27.31	25.95	74.05	1.40
31.25	85.94	84.71	87.85	86.17	13.83	1.58	32.19	33.46	34.06	33.24	66.76	0.95
15.6	93.28	92.83	94.39	93.50	6.50	0.80	40.56	41.82	42.39	41.59	58.41	0.94
7.8	99.15	98.76	99.02	98.98	1.02	0.20	47.08	48.23	49.12	48.14	51.86	1.02
3.9	100	100	100	100.00	0	0	56.27	55.92	57.03	56.41	43.59	0.57
2	100	100	100	100.00	0	0	61.88	63.15	65.03	63.35	36.65	1.58
1							69.84	70.49	71.81	70.71	29.29	0.99
0.5							77.16	75.82	77.16	76.71	23.29	0.77
0	100	100	100	100	0		100	100	100	100	0	
	IC50						IC50					
	114.86	110.65	116.77	114.09		3.13	6.56	6.90	7.37	6.94		0.40

Table S-7: Cytotoxicity Evaluation of L.A Against CACO2

Sample conc. (µg/ml)	Viability (%)						Viability (%)					
	(3 Replicates)			Mean	Inhibitory %	S.D. (±)	(3 Replicates)			Mean	Inhibitory %	S.D. (±)
	<i>L.A. methanol extract</i>						Cisplatin Standard					
	1 st	2 nd	3 rd				1 st	2 nd	3 rd			
1000	3.92	3.05	2.98	3.32	96.68	0.52	1.03	0.98	1.07	1.03	98.97	0.05
500	11.89	11.64	10.67	11.40	88.60	0.64	4.18	3.75	4.29	4.07	95.93	0.29
250	27.35	27.96	26.83	27.38	72.62	0.57	11.43	10.68	12.57	11.56	88.44	0.95
125	42.79	43.18	40.65	42.21	57.79	1.36	21.37	19.52	20.43	20.44	79.56	0.93
62.5	70.63	71.84	68.42	70.30	29.70	1.73	29.56	28.17	27.91	28.55	71.45	0.89
31.25	82.79	85.06	81.91	83.25	16.75	1.63	36.45	37.01	36.24	36.57	63.43	0.40
15.6	91.48	91.76	90.58	91.27	8.73	0.62	45.26	46.12	44.83	45.40	54.6	0.66
7.8	97.23	95.49	96.42	96.38	3.62	0.87	51.24	52.03	51.96	51.74	48.26	0.44
3.9	99.85	99.12	98.79	99.25	0.75	0.54	60.37	61.56	59.42	60.45	39.55	1.07
2	100	100	100	100.00	0	0	69.51	70.28	67.86	69.22	30.78	1.24
1							76.24	77.46	75.13	76.28	23.72	1.17
0.5							83.92	85.05	82.19	83.72	16.28	1.44
0	100	100	100	100	0		100	100	100	100	0	
	IC50						IC50					
	108.81	110.13	103.96	107.63		3.25	9.42	10.48	9.94	9.95		0.53