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TURGENIA LATIFOLIA (L.) HOFFM: CHEMICAL PROFILING AND ANTIOXIDANT, ANTI-INFLAMMATORY, AND ANTICANCER ACTIVITIES

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ABSTRACT. *Turgenia latifolia* is a medicinal plant that has not been studied previously. This study explores the first determination of its chemical profile and biological activities. LC-ESI-MS/MS identified *trans*-ferulic acid as the main compound in the Et_2O extract (10.012 mg/g) and chlorogenic acid in the EtOAc and BuOH extracts (1.944 mg/g and 3.539 mg/g, respectively). All extracts exhibited greater reducing power than the standards. The BuOH extract excelled in DPPH and reducing power (IC_{50} = 70.09 µg/mL, $A_{0.5}$ = 0.83 µg/mL), while the Et_2O extract excelled in *o*-phenanthroline ($A_{0.5}$ = 6.34 µg/mL) and showed the highest UV-B protection (SPF = 35.18). The EtOAc and BuOH extracts surpassed acarbose in α-amylase inhibition. The EtOAc extract demonstrated superior inhibition of xanthine oxidase and α-glucosidase (IC_{50} = 195.18 and 432.42 µg/mL, respectively), while the BuOH extract notably inhibited tyrosinase (IC_{50} = 360.00 µg/mL) and showed potent protein denaturation inhibition (76.16%). The Et_2O extract exhibited significant cytotoxicity against breast cancer cells (IC_{50} = 63.85 and 28.37 µg/mL for MCF-7 and MDA-MB-231, respectively). These findings highlight the pharmacological potential of *T. latifolia* as a promising source of bioactive compounds for therapeutic applications.

KEY WORDS: Biological activities, Breast cancer, Enzyme, *Turgenia latifolia*, LC-ESI-MS/MS

INTRODUCTION

During aerobic metabolism, byproducts are produced, known as reactive oxygen species (ROS). These species participate in essential signaling pathways [1]. In contrast, high amounts of ROS are unsafe and toxic, leading to oxidative harm to essential cellular components such as proteins, lipids, and nucleic acids. Numerous illnesses, including cancer, diabetes mellitus, inflammatory conditions, and neurological disorders, are believed to be caused or aggravated by oxidative stress [2].

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Cancer and diabetes are significant illnesses with considerable social and economic impacts on society [3]. The astounding number (almost 2.1 million) of cases recorded in 2018 proves that breast cancer substantially influences the death rate of women worldwide. Additionally, this illness resulted in around 630,000 death cases within the same period [4]. Moreover, among the most significant public health issues, diabetes mellitus is an epidemic that affects individuals worldwide. As per the World Health Organization, if the current alarming incidence rates persist, diabetes is projected to overtake heart disease as the seventh leading cause of death globally by 2030 [5].

Medicinal plants are essential for maintaining the health of the world's rural populations, particularly in developing countries. However, in several countries, people continue to use traditional treatments with plants [6]. Research into natural products extracted from medicinal plants has recently gained prominence due to the wealth of bioactive compounds found in various parts of plants [7-9]. Phenolics and flavonoids are secondary plant metabolites with an aromatic cycle containing at least one hydroxyl (OH) group. These phytochemical elements can be found in nutrients and herbal remedies. Both flavonoids and several other phenolic compounds are documented to have effective antioxidant, anti-cancer, anti-bacterial, cardioprotective, anti-inflammatory, immune-boosting, and UV-protective properties. They are promising candidates for pharmaceutical and medical applications [10].

Due to its geographical location, Algeria boasts a diverse floral landscape, encompassing 3,139 species distributed across approximately 150 botanical families. Notably, 653 species are endemic [11]. Many therapeutic plants in this rich flora are currently unstudied. *Turgenia latifolia* (L.) (Syn. *Caucalis latifolia* L.) is a species of the genus Turgenia of the family Apiaceae, found in central and southern Europe, Siberia, western Asia, and northern Africa [12]. The aerial parts of *T. latifolia* are traditionally used as an oral infusion to treat urinary tract problems [13]. To our knowledge, the phytochemical investigation and comprehensive biological activities of this species have not been previously reported, except for its antimicrobial and antimutagenic activities [14]. Therefore, the objective of this study is to determine, for the first time, the phenolic profile and assess the antioxidant properties, enzymatic inhibitory activity, anti-inflammatory effects, and cytotoxic effects on breast cancer cells of the phenolic extracts from the aerial parts of *T. latifolia*. This research aims to provide insights into its pharmacological properties and highlight its potential as a novel source of bioactive compounds for therapeutic applications.

EXPERIMENTAL

Apparatus and instrumentation

A rotary evaporator (BÜCHI R-300, Switzerland) with a rotation speed of 10–280 rpm and heating bath up to 220 °C (±1 °C), connected to a vacuum pump (5 mbar, V-300) was used for solvent evaporation. A UV-VIS spectrophotometer (Shimadzu UV-1601, Japan) with a wavelength range of 190–1100 nm (±0.5 nm) was used to assess antioxidant and anti-inflammatory activities. The Agilent Epoch-2 Microplate Reader (BioTek, USA) with a wavelength range of 200–999 nm (±2 nm) was used for enzyme inhibitory activity. A cell culture incubator (Thermo Electron Corporation Class 100, USA) maintained conditions of 37 °C, 95% humidity, and 5% CO_2 to ensure optimal cell growth. HPLC (Agilent 1260 Infinity II, USA) equipped with a Poroshell 120 EC-C18 column (100 mm × 4.6 mm) coupled to a 6460 Triple Quad mass spectrometer, with ESI source parameters: 4000 V capillary voltage, 11 L/min nebulizing gas (N_2), 15 psi nebulizer pressure, and 300 °C gas temperature was used for determining the chemical profile.

Chemicals and reagents

All solvents and reagents used were HPLC grade and obtained from Sigma-Aldrich (St. Louis, MO, USA), including the phenolic standards, enzymes, substrates, and other chemicals for enzyme activity studies. The breast cancer cell lines MCF-7 and MDA-MB-231 were sourced from ATCC (Germany).

Plant material

In May 2019, during the flowering period, *Turgenia latifolia* was harvested in Aïn Azel, Sétif, North Algeria. Dr. Hocine Laouer, a botanist from the Department of Biology at Ferhat Abbas University in Sétif, identified the plant. The aerial parts were cleaned, dried, uniformly powdered, and stored in paper for future use.

Extraction of phenolic compounds

The extraction procedure followed the protocol established by Markham with slight modifications from Bruneton [15, 16]. The powder was macerated in methanol for 24 hours repeated three times. Following each filtration, the filtrates underwent evaporation using a rotary evaporator. The obtained residue was diluted with boiling distilled water to yield an aqueous solution and left to stand overnight to eliminate chlorophyll. After filtration, sequential liquid-liquid extractions were conducted on the aqueous phase using solvents of increasing polarity. The process commenced with petroleum ether, which dissolved lipophilic constituents and residual chlorophyll, followed by diethyl ether, ethyl acetate, and n-butanol. Each fraction was evaporated to obtain the extract, which was stored in darkness at 4 °C for future use.

Antioxidant activity

Antioxidant proprieties of *T. latifolia* extracts were conducted spectrophotometrically using five colorimetric methods. Extracts were dissolved in the appropriate solvent (DMSO) and then diluted sevenfold, where the solvent was used as a control. A graph was plotted, depicting the inhibition percentage (%) and absorbance versus the concentration of extracts, and the IC_{50}, $A_{0,5}$ values were determined using the slope equation derived from the graphs. BHA, BHT and ascorbic acid were used as reference compounds.

DPPH radical scavenging assay

The free radical scavenging assay was evaluated following to the method of Blois [17]. In this method, 400 µL of the investigated extracts were combined with 1600 µL of the DPPH solution (1 mM). The mixture was then incubated in darkness for 30 minutes. Subsequently, the absorbance was quantified at 517 nm. The inhibition percentage was computed via Eq. (1).

$$\%\, I \;=\; [\frac{A_c \;-\; A_s}{A_c}] \;\times\; 100 \qquad (1)$$

where, A_c denotes the control absorbance, and A_S denotes the absorbance after the sample addition.

Hydroxyl radical scavenging assay

The assessment of hydroxyl radical scavenging followed the modified technique developed by Smirnoff and Cumbes [18]. The protocol consists of mixing 400 µL of the studied extract at

several concentrations with 800 µL of salicylic acid (3 mM), 240 µL of $FeSO_4$ (8 mM), and 200 µL of H_2O_2 (20 mM) in succession. The mixing reaction was allowed to occur at 37 °C for half an hour. Then, 360 µL of distilled water was added, and the absorbance was quantified at 510 nm. The percentage of hydroxyl radical inhibition was computed via Eq. (1).

Superoxide radical scavenging assay

Based on the method of Kunchandy and Rao superoxide radical activity was determined [19]. In this experiment, 300 µL of NBT (1 mg/mL) and 400 µL of the examined samples at various dilutions were added to 1300 µL of alkaline DMSO (5 mM NaOH, 100 µL H_2O, 900 µL DMSO). The absorbance of the resulting mixture was read at 560 nm. The percentage of superoxide radical inhibition was computed via Eq. (1).

Reducing power assay

The reducing properties of *T. latifolia* extracts were measured using the procedure of Oyaizu [20]. Initially, 100 µL of the examined extracts were mixed with 400 µL of phosphate buffer solution (pH 6.6) and 500 µL of $K_3Fe(CN)_6$ (1%). Following incubation for 20 min at 50 °C, the mixture was supplemented with 500 µL of TCA (10%), 400 µL of distilled water, and 100 µL of $FeCl_3$ (0.1%). The absorbance of the combination was subsequently quantified at 700 nm.

o-Phenanthroline assay

The assessment of this test followed the protocol described by Szydłowska-Czerniak *et al.* [21]. The reaction combination comprised 100 µL of investigated samples, 500 µL of $FeCl_3$ (0.2%), 300 µL of *o*-phenanthroline (0.5%), and 1100 µL of MeOH. The absorbance was quantified at 510 nm following an oven incubation period of 20 min at 30 °C.

Photoprotective activity

The evaluation of photoprotective ability was conducted following the procedure described in the literature [22]. The sun protection factor (SPF) of a sunscreen measures how effectively photoprotective agents reduce short-term effects of UV-B radiation exposure. For this assessment, the extract was dissolved in MeOH at 2 mg/mL, and the absorbance was quantified at seven wavelengths ranging from 290 nm to 320 nm, in 5 nm intervals. The SPF value was computed via Eq. (2).

$$SPF = CF \times \sum EE\,(\lambda) \times I\,(\lambda) \times Abs\,(\lambda) \quad (2)$$

EE: erythemal effect spectrum, I: solar intensity spectrum, Abs: absorbance of sunscreen product, CF: correction factor (= 10). The values of EE (λ) × I (λ), which are constants provided by Sayre *et al.* [23].

Enzyme inhibitory activity

The analyzed samples were dissolved in the appropriate solvent, DMSO, and then diluted tenfold with the pure water. The inhibitory potential of these extracts on the enzymes was investigated across a concentration range of 1-1000 µg/mL in a 96-well microplate. All enzymes absorbance changes during the kinetic measurement were recorded using a microplate reader. The findings were quantified as IC_{50} values, indicating the concentration at which the enzyme activity was inhibited by 50%. Galantamine, allopurinol, acarbose, and kojic acid were used as reference compounds.

Inhibition of α-amylase

In evaluating α-amylase inhibition, the method involved utilizing 3,5-dinitrosalicylic acid (DNS) to quantify the reducing sugars released from starch, as outlined in previous studies [24]. Initially, the enzyme and starch were prepared in a solution containing sodium chloride (6.7 mM) and phosphate buffer (100 mM, pH 7.00). Following this, 100 µL of pure water was combined with 50 µL of starch solution (1%) and 50 µL of the enzyme. The mixture was kept at room temperature for 5 min. To stop the reaction, 100 µL of DNS was added. After incubating the combination in boiling water for ten minutes, the reaction system temperature was gradually decreased to 37 °C in a water bath. Then, 100 µL of the reaction mixture and 100 µL of distilled water were added to a microtiter plate, and the absorbance at 546 nm was determined. Eq. (3) was utilized to compute the inhibition percentage of α-amylase.

$$\% I = \left[\frac{A_0 - A_1}{A_0}\right] \times 100 \quad (3)$$

where, A_0 denotes the control absorbance, and A_1denotes the absorbance after the sample addition.

Inhibition of α-glucosidase

The technique described in the literature was used to study the inhibition of α-glucosidase by measuring the amount of *p*-nitrophenol produced from para-nitrophenyl-α-D-glucopyranoside (pNPG) fragments [24]. Briefly, 10 µL of enzyme, 100 µL of phosphate buffer solution (0.1 mM, pH 6.8), 90 µL of distilled water, and 40 µL of pNPG substrate (5 mM) were combined to create a reaction mixture in a 96-well microplate. The resultant solution absorbance was measured at 405 nm, and α-glucosidase inhibition percentage was computed using Eq. (3).

Inhibition of tyrosinase

The procedure described by Mason and Peterson was used to for the tyrosinase inhibitory assessment [25]. Briefly, in a 96-well microplate, 80 µL of phosphate buffer (0.1 M, pH: 6.8), 80 µL of distilled water, and 10 µL of tyrosinase enzyme prepared in buffer solution (pH: 8.0) were added to each well. The mixture was incubated at 37 °C. After 10 min, the reaction was initiated by adding 20 µL of L-dopa (2.5 mM, dissolved in buffer). Tyrosinase inhibition was estimated by measuring dopachrome production at 475 nm. The inhibition percentage was determined using Eq. (3).

Inhibition of xanthine oxidase (XO)

Xanthine oxidase inhibition was performed using a modified technique as previously outlined [26]. In a 96-well microplate, a solution containing 10 µL of enzyme, 50 µL of potassium phosphate buffer (500 mM, pH: 7.5), and 90 µL of distilled water was prepared. This mixture was incubated at 37 °C for 15 min. Subsequently, 60 µL of hypoxanthine substrate (3 mM) was added to each well, and the microplate was further incubated at 37 °C for 20 min. After 7 min, the change in absorbance at 295 nm was measured. XO inhibition percentage was determined via Eq. (3).

Anti-inflammatory activity (in-vitro)

The anti-protein denaturation potential of the samples was spectrophotometrically assessed with a slight modification of the method of Kandikattu *et al.* [27]. To elaborate, 1 mL of various dilutions of the tested extracts was combined with 1 mL of bovine serum albumin (0.2%). The reaction combination was incubated for 15 min at 37 °C, followed by another 5 min in a water

bath heated to 72 °C. After cooling, the turbidity absorbance was read at 660 nm. Diclofenac served as the reference compound, and the inhibition of protein denaturation was computed via Eq. (4).

$$Denaturation\ inhibition\ (\%) = [\frac{A_c - A_s}{A_c}] \times 100 \quad (4)$$

where, A_c denotes the control absorbance, and A_S denotes the absorbance after the sample addition.

Cytotoxicity on breast cancer cells (in-vitro)

The MCF-7 and MDA-MB-231 breast cancer cell lines were separately cultivated in 25 and 75 cm^3 flasks using DMEM medium (Biological Industries), which included 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin-streptomycin. The cells were maintained in a cell culture incubator at 37 °C, 95% humidity, and 5% CO_2, and they were passaged every 2-3 days. When the cells reached 80-90% confluency, they were detached from the flasks using trypsin to facilitate subsequent cell counting and seeding. The trypan blue dye exclusion test was employed under a light microscope to assess cell viability. This test allowed us to examine the number of viable cells by excluding cells that take up the dye, indicating cell membrane integrity. For the subsequent cytotoxicity assays, 1.5 x10^4 cells were seeded into individual wells for both cell lines. The cells adhered and stabilized for 24 hours, ensuring uniform cell distribution across the well surface. After 24 hours of seeding the cells, *T. latifolia* extracts were dissolved in the appropriate solvent DMSO and then diluted to the desired concentrations (200, 100, 50, 25, 12.5, and 5 µg/mL) for the cytotoxicity assay. Extracts in different concentrations were applied to the designated wells. No treatment wells were considered as controls. Following the application of the extracts, the 72-hour experiment was initiated at the xCELLigence system. Throughout the 72-hour experiment, the xCELLigence system continuously monitored and recorded cell proliferation, cell death, and cytotoxicity data. These data were subsequently analyzed to evaluate the cytotoxic effects of *T. latifolia* extracts on the MCF-7 and MDA-MB-231 cell lines.

Phenolic profile analysis

The phenolic profile analysis of *T. latifolia* extracts was conducted using multiple reaction monitoring (MRM) techniques, following the method described previously [28]. The analysis employed 45 standards using An HPLC coupled to a mass spectrometer, and ionization was performed with ESI. Chromatographic separation was achieved using a Poroshell 120 EC-C_{18} column with dimensions of 100 mm length and 4.6 mm inner diameter, packed with 2.7 µm particles. The injection volume consisted of 4 µL of extracts at a 2 mg/mL concentration in the full loop injection mode. A 0.4 mL/min flow rate was maintained using a gradient solvent system. The gradient system involved a flow starting with ultrapure water and methanol (containing 0.1% formic acid and 5 mM ammonium) for optimal compound separation. The gradient system flow was obtained from methanol at 15.12% for 1 min, followed by increments to 50% over 30 min, 90% over the next 30 min, and finally returning to 10% over 32 min. Validation studies for the quantitative analytical methods were assessed based on parameters such as linearity and limits of detection and quantification (LOD/LOQ), as described by Yilmaz [29].

Statistical analysis

All tests were conducted in triplicate and the results were reported as mean ± standard deviation (SD) of three measurements. Comparisons of means were performed using one-way analysis of variance (ANOVA), followed by Turkey's test and differences were considered significant for $p < 0.05$.

RESULTS AND DISCUSSION

Characterization of T. latifolia extracts using LC-ESI-MS/MS

A comprehensive phytochemical analysis of *T. latifolia* aerial parts extracts was conducted using MRM-based LC-ESI-MS/MS technique. Compound identification and quantification were carried out utilizing a set of 45 standards. The chromatograms of the detected compounds of each extract are illustrated in Figure 1.

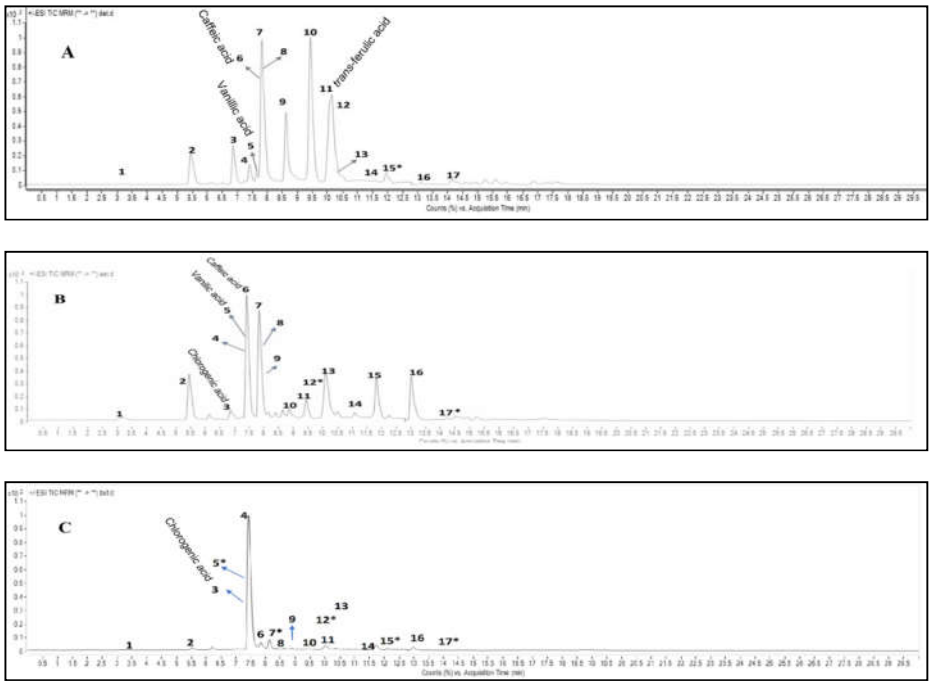

Figure 1. LC-ESI-MS/MS chromatograms of (A): Et_2O extract, (B) EtOAc extract and (C) BuOH extract.

Identification and quantification of phenolic compounds

Table 1 presents the compounds identified and quantified in each extract. The findings indicate that phenolic acids are the major compounds in all extracts, with minor or trace amounts of other compounds also present. The Et_2O extract has the highest concentration of phenolic compounds at 18.317 mg/g, followed by the EtOAc extract with 6.555 mg/g, and the BuOH extract with 3.806 mg/g. In the Et_2O extract, quantifiable amounts of protocatechuic acid (0.713 mg/g), vanillic acid (3.005 mg/g), caffeic acid (2.374 mg/g), *o*-coumaric acid (0.702 mg/g), chlorogenic acid (0.508 mg/g), and *trans*-ferulic acid (10.012 mg/g) were detected. Similarly, the EtOAc extract contained quantifiable amounts of chlorogenic acid (1.944 mg/g), vanillic acid (1.141 mg/g), caffeic acid (1.003 mg/g), syringic acid (0.628 mg/g), protocatechuic acid (0.585 mg/g), and *trans*-ferulic acid (0.913 mg/g). Finally, the BuOH extract exhibited a quantifiable amount of chlorogenic acid (3.539 mg/g).

Classification and biological effects of the identified compounds

The analysis revealed two categories of phenolic compounds in the plant extracts: phenolic acids and flavonoids. Phenolic acids encompassed hydroxybenzoic acids (such as gallic, protocatechuic, vanillic, and syringic acids) and hydroxycinnamic acids (including chlorogenic, caffeic, *o*-coumaric, and *trans*-ferulic acids), along with cinnamic acid (*trans*-cinnamic). Flavonoids identified were flavanones (hesperidin) and flavonols (isoquercitrin, rutin, and kaempferol-3-glucoside). Upon comparing the extracts and observing both similarities and differences in the compounds, it became evident that quantitative variations existed among them. Previous studies have highlighted the diverse biological activities of phenolic acids. For example, caffeic acid and chlorogenic acid, both hydroxycinnamic acids, demonstrate natural antioxidant properties and offer cardio-protective benefits. These acids can lower blood pressure and inhibit the activities of key enzymes involved in the development of hypertension in rats with cyclosporine-induced conditions [30].

Antioxidant activity of T. latifolia extracts

Results of antioxidant activity assays

The antioxidant effects of *T. latifolia* were assessed using five colorimetric methods, as shown in Table 2. The results demonstrate the efficacy of all extracts in the DPPH, reducing power, and *o*-phenanthroline assays. However, variations were observed among the extracts in the superoxide and hydroxyl radicals scavenging assays. The BuOH extract exhibited the most potent activity in scavenging DPPH radicals (IC_{50} = 70.09 µg/mL), followed by the Et_2O extract (IC_{50} = 95.40 µg/mL). In contrast, BHT and BHA showed significantly lower IC_{50} values (12.99 and 6.14 µg/mL, respectively). The EtOAc extract was the only extract that exhibited scavenging activity against hydroxyl radical (IC_{50} = 615.71 µg/mL), while ascorbic acid had an IC_{50} of 32.33 µg/mL. Both the Et_2O and EtOAc extracts demonstrated superoxide radical scavenging activity (IC_{50} = 642.66 and 626.55 µg/mL, respectively), whereas BHT exhibited a much lower IC_{50} value of 23.73 µg/mL. All extracts showed higher reducing power than the standards, as evidenced by their reduction potential. Notably, the BuOH extract ($A_{0.5}$ = 0.83 µg/mL) outperformed ascorbic acid ($A_{0.5}$ = 9.01 µg/mL) and BHA ($A_{0.5}$ = 8.41 µg/mL).

Mechanisms of antioxidant activity (HAT and SET)

In food systems and the human body, antioxidants play a crucial role by reducing oxidative processes and adverse impact of reactive oxygen species (ROS) [31]. Antioxidant reactions to free radicals involve two mechanisms of action: the transfer of hydrogen atoms (HAT) and the transfer of electrons (SET). In hydrogen atom transfer, antioxidant eliminates free radical by providing a hydrogen atom. In the transfer of electrons, the antioxidant donates an electron to decrease the reactivity of substances such as metals, carbonyls, and radicals [32]. Due to the diverse mechanisms of action of antioxidants, more than one method is required to assess their antioxidant potential. Therefore, the antioxidant properties of *T. latifolia* extracts was evaluated by five complementary methods. The results obtained indicate that all extracts exhibit antioxidant activity through both mechanisms, confirming their antioxidant properties. These findings suggest that *T. latifolia* extracts are more effective in exerting their antioxidant effects through SET compared to the HAT mechanism. Phenolic acids and flavonoids, due to their double bonds and hydroxyl groups, possess significant antioxidant properties, enabling them to chelate metal ions and neutralize free radicals [33]. Moreover, many identified compounds in *T. latifolia* are known for their substantial antioxidant potential; for instance, hydroxycinnamic acids such as chlorogenic acid the main compound in the EtOAc and BuOH extracts have demonstrated robust antioxidant activity against DPPH [33, 34].

Table 1. Identified compounds in different extracts of *T. latifolia*.

No	Compounds	RT (min)	Et_2O (mg/g)	EtOAc (mg/g)	BuOH (mg/g)	Precursor ions (m/z) MS	Product ions (m/z) MS/MS	Ion mode	LOD (µg/L)	LOQ (µg/L)	Linearity range (µg/L)	R^2
1	Gallic acid	3.221	0.022	0.039	0.011	169.0	125.1	Neg	7.1674	18.5862	31.25-500	0.9986
2	Protocatechuic acid	5.451	0.713	0.585	0.042	153.0	109.0	Neg	3.1564	13.1729	15.625-250	0.9969
3	Chlorogenic acid	7.396	0.508	1.944	3.539	353.0	191.0	Neg	11.589	25.9023	31.25-500	0.9981
4	4-Hydroxybenzaldehyde	7.642	0.074	0.006	0.003	121.0	92.0	Neg	4.9742	12.8651	15.625-250	0.9993
5	Vanillic acid	7.766	3.005	1.141	ND	167.0	151.8	Neg	219.0421	1424.2132	1250-20000	0.9958
6	Caffeic acid	7.820	2.374	1.003	0.067	178.9	135.1	Neg	6.9205	24.162	31.25-500	0.9994
7	Syringic acid	8.359	0.434	0.628	ND	197.1	181.8	Neg	358.5	857.3388	1250-20000	0.9996
8	Caffein	8.357	0.003	0.010	0.002	195.0	137.9	Pos	6.8099	15.4959	18.75-300	0.9986
9	Vanillin	8.584	0.351	0.022	0.006	153.0	125.0	Pos	14.5885	40.5411	62,5-1000	0.9949
10	*o*-Coumaric acid	9.362	0.702	0.053	0.005	163.0	119.1	Neg	4.0164	7.9973	15.625-500	0.9996
11	*trans*-Ferulic acid	10.041	10.012	0.913	0.047	193.1	133.9	Neg	6.1184	11.5276	31.25-1000	0.9953
12	Coumarin	11.611	0.011	ND	ND	147.1	91.3	Pos	15.63	23.04	62.5–2000	0.9999
13	Hesperidin	11.789	0.004	0.029	0.012	611.0	302.9	Pos	4.139	17.675	31.25–500	0.9957
14	Isoquercitrin	11.781	0.008	0.110	0.008	464.9	302.8	Pos	9.9382	11.268	18.75-300	0.9982
15	Rutin	12.242	ND	0.072	0.064	611.0	302.8	Pos	59.5597	240.672	125-2000	0.9987
16	Kaempferol-3-glucoside	13.139	0.006	0.080	0.010	448.8	286.9	Pos	4.5238	1.1609	7.8125-125	0.9997
17	*trans*-Cinnamic acid	14.230	0.096	ND	ND	149.0	131.1	Pos	11.1853	22.0279	31.25-500	0.9999
	Total quantification		18.317	6.555	3.806							

RT: retention time. ND: not detected. LOD: limit of detection. LOQ: limit of quantification.

Table 2. Antioxidant results of *T. latifolia* extracts.

Extract	DPPH radical scavenging IC_{50} (µg/mL)	Reducing power $A_{0.5}$ (µg/mL)	*o*-Phenanthroline $A_{0.5}$ (µg/mL)	Superoxide DMSO alcalin IC_{50} (µg/mL)	Hydroxyl radical scavenging IC_{50} (µg/mL)
Et_2O	95.40 ± 1.18^{b}	1.11 ± 0.02^{c}	6.34 ± 0.03^{c}	642.66 ± 3.2^{b}	>800
EtOAc	111.90 ± 1.77^{a}	1.89 ± 0.02^{c}	13.31 ± 0.26^{b}	626.55 ± 3.96^{b}	615.71 ± 0.62^{b}
BuOH	70.09 ± 0.52^{c}	0.83 ± 0.01^{d}	23.33 ± 0.72^{a}	>800	>800
BHT *	12.99 ± 0.41^{d}	>50	$2.24\pm0.17_{d}$	23.73 ± 1.11^{c}	NT
BHA *	6.14 ± 0.41^{e}	8.41 ± 0.67^{b}	0.93 ± 0.07^{c}	NT	NT
Ascorbic acid *	NT	9.01 ± 1.46^{b}	NT	NT	32.33 ± 1.17^{c}
$LSDp_{0.05}$	3.26	0.72	0.51	23.55	28.10

IC_{50} and $A_{0.5}$ were calculated by linear regression analysis and expressed as the mean ± SD (n = 3). (LSDp = 0.05) indicated the least significant difference. Different letters (^{a}, ^{b}, ^{c}, ^{d}, ^{e}) indicate significantly different values ($p < 0.05$), while the same letter indicates no significant difference. NT: Not tested. *Reference compounds.

Photoprotective effects of T. latifolia extracts

UV-B protection and SPF of extracts

Solar radiation's harmful impact is mainly in the ultraviolet part of the spectrum. UV-B (290-320 nm) is particularly dangerous to human skin, leading to allergic reactions, skin tanning, and an

increased risk of cancer [35]. SPF is a quantitative measure of a sunscreen product's effectiveness. According to the SPF results, all the tested extracts were found to offer skin protection against UV-B radiation, which fall within the range of [30→50], thus classified as possessing high sun protection action [36]. The Et_2O extract exhibited the highest photoprotective efficacy, comparable to that of commercial sunscreens and cosmetics (Table 3).

Table 3. Photoprotective efficacy of *T. latifolia* extracts.

Extracts	Nivea*	Vichy*	Et_2O	EtOAc	BuOH
SPF	50.11 ± 0.53^{a}	44.22 ± 0.35^{b}	35.18 ± 0.24^{c}	35.12 ± 0.26^{c}	34.01 ± 0.11^{c}
$LSDp_{0.05}$	2.01				

SPF values are expressed as the mean ± SD (n = 3). (LSDp = 0.05) indicated the least significant difference. Different letters ($^{a, b, c}$) indicate significantly different values ($p < 0.05$). The same letter indicates no significant difference. *Reference products.

Mechanism of extracts in UV-B protection

The effectiveness of the extracts is attributed to the presence of phenolic compounds, which are recognized as a promising source of UV protection [37, 38]. Previous studies have suggested a connection between phenolic content and the photoprotective properties of plant extracts [36]. Due to their chemical structures, consisting of cyclic and aromatic hydrocarbons, these compounds can absorb ultraviolet light within the wavelengths of 240-285 nm and 300-550 nm [39].

Enzyme inhibitory activity of T. latifolia extracts

Results of enzymes inhibitory activity assays

The inhibitory effects of the extracts on xanthine oxidase, tyrosinase, α-amylase, and α-glucosidase were evaluated and are shown in Figure 2. The results reveal that all extracts had an impact on xanthine oxidase, α-glucosidase, and tyrosinase, with the exception of the Et_2O extract, which exhibited no activity against α-amylase. In xanthine oxidase inhibition, the EtOAc extract demonstrated the highest efficacy among the extracts (IC_{50} = 195.18 µg/mL), followed by the BuOH extract (IC_{50} = 477.11 µg/mL), although both were less potent than allopurinol (IC_{50} = 13.78 µg/mL). Regarding tyrosinase inhibition, the BuOH extract showed the most significant inhibitory effect among the extracts (IC_{50} = 360.00 µg/mL), followed by the EtOAc extract (IC_{50} = 595.38 µg/mL), though both were lower in potency than kojic acid (IC_{50} = 25.23 µg/mL). Both the EtOAc and BuOH extracts exhibited inhibitory effects against α-amylase (IC_{50} = 675.65 and 691.34 µg/mL, respectively), showing greater potency than acarbose (IC_{50} = 3650.93 µg/mL). For α-glucosidase inhibition, the EtOAc extract displayed the most significant activity (IC_{50} = 432.42 µg/mL), followed by the BuOH extract (IC_{50} = 527.34 µg/mL), while acarbose exhibited an IC_{50} of 275.43 µg/mL.

Enzyme inhibition effects in disease management

Enzymes are critical in the development and progression of many diseases, making the inhibition of key enzymes a fundamental strategy in various treatments. These enzymes are frequently used to evaluate the bioactivity of natural substances [33]. Among these, the xanthine oxidase is a key factor in joint inflammation associated with hyperuricemia, leading to uric acid accumulation in the joints. Despite its potential, there remains unexplored territory in developing natural products to prevent or manage XO-related diseases [40]. Tyrosinase, a vital enzyme governing melanin metabolism, regulates skin and hair colour in animals. Overactive tyrosinase leads to excessive epidermal pigmentation, contributing to dermatological issues like melasma, age spots, and

actinic damage [41]. In diabetes mellitus therapy, α-glucosidase and α-amylase inhibitors curb the rapid surge in blood glucose levels following meals by impeding carbohydrate digestion in the small intestine [42, 43]. In this context, *T. latifolia* extracts were examined for their ability to inactivate key enzymes. The findings highlight the potential of *T. latifolia* extracts as enzymes inhibitors, particularly as anti-diabetic compounds. Several previous investigations have demonstrated the ability of phenolic compounds to inhibit enzymes. Notably, caffeic and chlorogenic acids, the major compounds in *T. latifolia* extracts, have demonstrated significant inhibition of α-amylase, α-glucosidase, and XO [44-46].

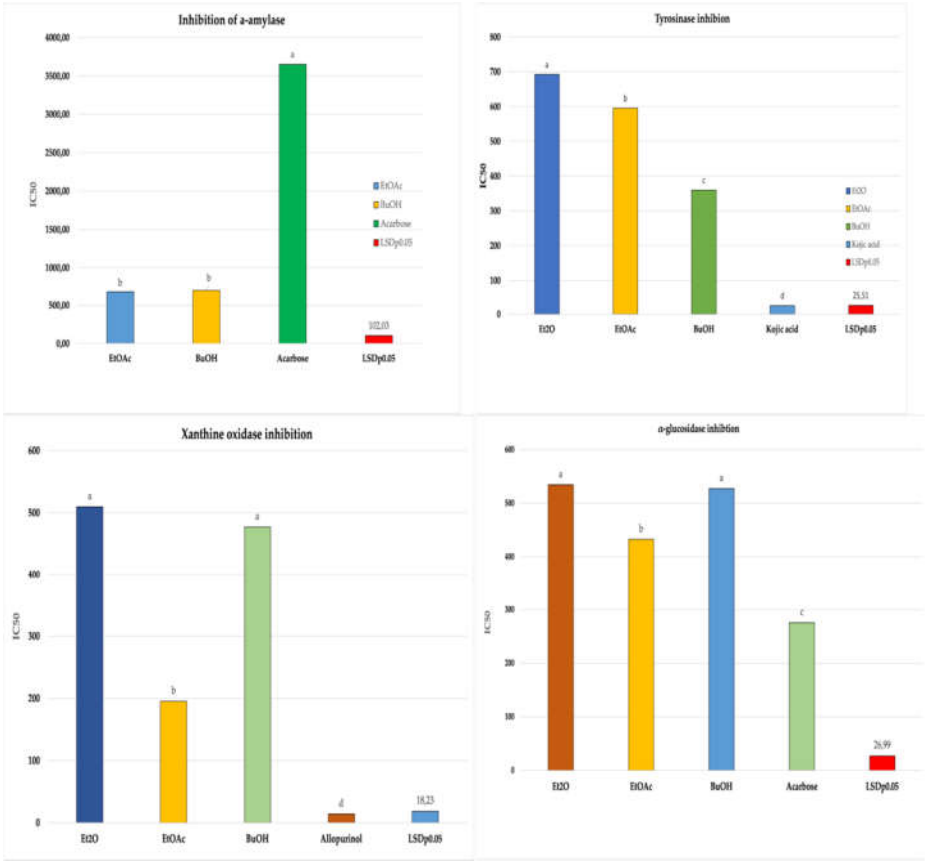

Figure 2. Enzyme inhibition results of *T. latifolia* extracts. IC_{50} values were calculated by linear regression analysis and expressed as the mean ± SD (n = 3). (LSDp = 0.05) indicated the least significant difference. Different letters (a,b,c,d) above the bars indicate significantly different values ($p < 0.05$), while the same letter indicates no significant difference.

Anti-inflammatory effects of T. latifolia extracts

Protein denaturation inhibition

The anti-inflammatory capacity of *T. latifolia* extracts was assessed using a protein denaturation method, specifically through a BSA denaturation assay, which is commonly associated with

inflammation. Protein denaturation, a potential mode of action for this effect, involves the modification of electrostatic, hydrogen, hydrophobic, and disulfide bonds that stabilize the three-dimensional arrangement of proteins [11]. Following denaturation, the majority of proteins forfeit their biological functions, resulting in the generation of autoantigens capable of triggering diverse autoimmune dysfunctions, such as rheumatic and inflammatory diseases. Consequently, compounds that impede protein denaturation are regarded as potent treatments for arthritis and inflammatory conditions [47].

Anti-inflammatory results of extracts

The impact of *T. latifolia* extracts on the protein denaturation revealed that all extracts exhibited inhibition of protein denaturation ranging between 76.16% and 16.22% (Figure 4). The BuOH extract exhibited the most significant inhibition (76.16%) closely comparable to diclofenac's (83.02%), which suggest its potential to act as anti-inflammatory agent. Previous research has shown that phenolic compounds possess powerful anti-inflammatory properties and interact with crucial molecules in inflammation signalling pathways [48].

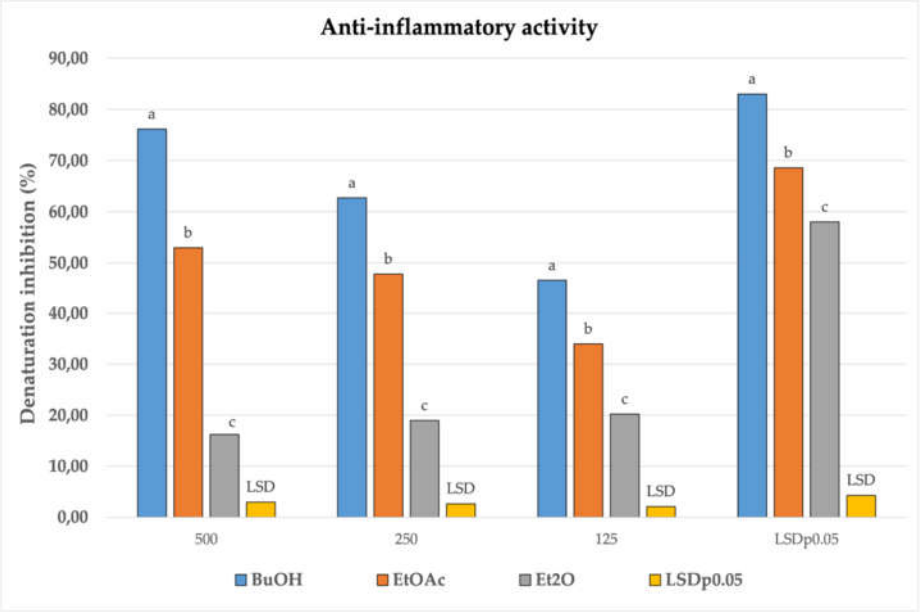

Figure 3. Inhibition of protein denaturation effect of *T. latifolia* extracts. Denaturation inhibition (%) are expressed as the mean ± SD (n = 3). (LSDp = 0.05) indicated the least significant difference. Different letters above the bars (a, b, c) indicate significantly different values ($p < 0.05$). The same letter indicates no significant difference.

Cytotoxicity on breast cancer cells effects

As mentioned previously, the escalating breast cancer statistics increase the mortality risk among women. Therefore, many recent studies focus on breast cancer and evaluate the anticancer capacity of plant extracts [49]. For both cell lines, the cytotoxicity results show that the BuOH and EtOAc extracts did not show statistical significance. However, the Et_2O extract demonstrated significant cytotoxic effects in MCF-7 and MDA-MB-231 breast cancer cell lines, as calculated

from the graphs in Figures 4 and 5 (IC_{50} = 63.85 and 28.37 µg/mL, respectively). These results may be attributed to the phenolic compounds in the Et_2O extract, which, according to its chemical profile analysis, has the highest concentration. Additionally, previous studies have demonstrated the potential efficacy of *trans*-ferulic, caffeic, and vanillic acids the main compounds in the Et_2O extract against breast cancer cells [50].

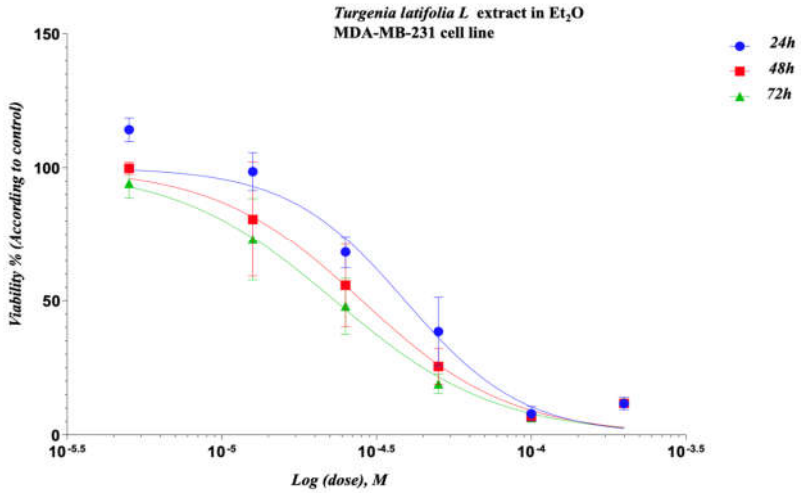

Figure 4. IC_{50} determination for Et_2O extract on MDA-MB-231 cell line at 48 h. The plot represents the percentage of cell viability as a function of extract concentration. IC_{50} value was calculated using nonlinear regression. Data are expressed as mean ± standard deviation (SD) of three independent experiments.

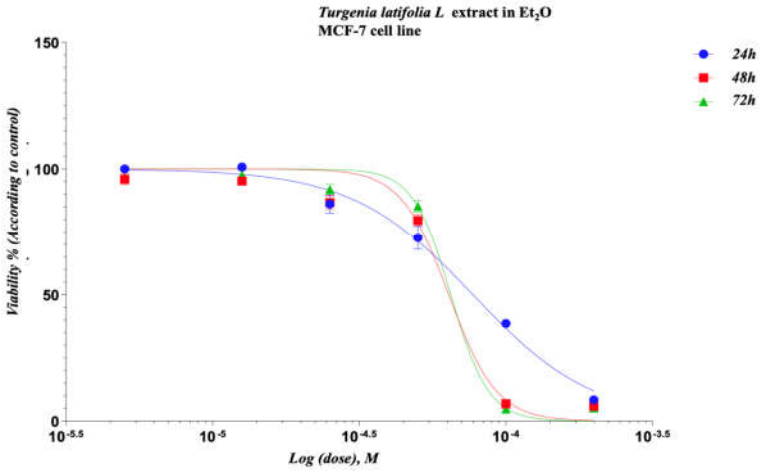

Figure 5. IC_{50} determination for the Et_2O extract on MCF-7 cell line at 48 h. The plot represents the percentage of cell viability as a function of extract concentration. IC_{50} value was calculated using nonlinear regression. Data are expressed as mean ± standard deviation (SD) of three independent experiments.

The explanation for the difference in activity of *T. latifolia* extracts observed in each test can be attributed to its complex mixture. Extracts may react according the majority of compounds or act synergistically.

CONCLUSION

Our study identifies the chemical profile and evaluates the biological activities of *Turgenia latifolia* extracts. The chemical profile indicates that phenolic acids as the main compounds. The antioxidant and UV-B protective properties suggest their potential as natural additives in the food industry and natural-based sunscreens. Enzyme inhibitory activity offer therapeutic prospects for the treatment of dermatological disorders, hyperuricemia, and diabetes. The high denaturation inhibition points to anti-inflammatory potential, while the cytotoxicity against breast cancer cells highlights the species' anticancer properties. The findings encourage more detailed *in vivo* studies to ensure the safety of the plant's therapeutic applications before it is exploited for pharmaceutical and industrial purposes.

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