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*Original Research Article*

# *Fagonia longispina* Batt. extracts: Antioxidant, Antibacterial, and Antiproliferative effects on Cancer Cells

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ABSTRACT

*Fagonia longispina* (Zygophyllaceae family) has long been employed in traditional practices for combating cancer, with scientific research yielding insights into its biological impact. This study aimed to validate the potential of this plant by evaluating the antioxidant, antiproliferative, and antibacterial properties of the *n*-butanol, ethyl acetate, and chloroform extracts of *F. longispina* (BuE, EAE, and ChE, respectively). The most significant total phenolics (TPC) were recorded in EAE (377 ± 24.33 µg GAE/mg extract), whereas BuE showed the most important quantity of flavonoids (TFC) (87.14 ± 1.56 µg QE/mg extract). Outcomes indicated that EAE displayed the highest DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging effect ($IC_{50}$ = 37.82 ± 7.83 µg/mL) compared to BuE ($IC_{50}$ = 59.49 ± 6.16 µg/mL, $p$ = 0.0197) and ChE ($IC_{50}$ =111.89 ± 6.10 µg/mL, $p$ = 0.0002). Despite the increased activity of EAE, it remained lower than ascorbic acid ($IC_{50}$ = 5.00 ± 0.1 µg/mL, $p$ = 0.02). Moreover, EAE was the strongest at scavenging free radicals from $FeSO_4$ ($IC_{50}$ = 126.66 ± 5.76 µg/mL). All extracts except BuE showed antiproliferative efficacy against HeLa and HT-29 cells. Various bacterial strains underwent testing to demonstrate their antibacterial effectiveness, which was assessed by measuring inhibition zones. The results showed enjoyable antimicrobial activity against gram-positive bacteria. In conclusion, this study proves the application of *F. longispina* in traditional medicine. Furthermore, the results from the antioxidant tests confirmed that *F. longispina* had an antioxidant effect and may be used as a new natural antioxidant in therapy.

***Keywords***: *Fagonia longispina*, phenolic compounds, lipid peroxidation, antioxidant activity, antibacterial activity, antiproliferative activity.

## Introduction

As civilization and technology progress, individuals are increasingly encountering pollution of the environment, smoking, alcoholism, inadequate nutrition, stress, and unhygienic habits, all of which pose substantial threats to health. Oxidative stress is believed to underlie various illnesses such as cardiovascular diseases (CVDs), loss of muscle and strength, nervous system diseases, emphysema, and chronic bronchitis disease.[1,2] However, antioxidants have attracted increasing attention for their protective roles against pathological procedures induced by oxidative stress.[3]Two distinct categories of herbal medicine are traditional herbal medicine, which primarily addresses disease symptoms, and clinical herbal therapy, which adopts a holistic approach by considering the patient's overall health and environment alongside clinical assessment to determine treatment.[4]

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Traditional medicines constitute the essential knowledge, based on theories specific to various cultures, used in the treatment of physical and mental illnesses.[5] Hence, medicinal plants represent invaluable global resources that must be substituted with "phytosubstance" products, yet regrettably, they are vanishing rapidly.[6] Numerous natural antioxidants and phenolic compounds, including flavonoids and phenolic compounds, have attracted much attention. These phytochemicals, found in food, medicinal plants, flavonoids, and several other phenolic components, are said to be powerful antioxidants, anticancer, antibacterial, anti-inflammatory, protective of the skin against UV rays, and interesting candidates for pharmacological and therapeutic application.[7] Southern Algeria boasts abundant floral resources, rendering it an optimal site for exploring biological activities, often leading to the research and development of novel pharmaceutical products. *Fagonia longispina* (*F. longispina*) is a small, thorny shrub commonly found in southwest Algeria and southeast Morocco.[8] *Fagonia* is one of 27 genera in the Zygophyllaceae family. It comprises around 34 species restricted to warm and dry regions of the new and ancient worlds.[9] With 17 species, this genus is found in Algeria.[10] *F. longispina*, also referred to as "*Chouika*" or "*Atlihia*",[11] is a common plant used in herbal therapy as a protective syrup against cancer, urinary infection, rheumatoid arthritis, and insect repellent.[8] Furthermore, the upper part of the plant

is believed to be a treatment for cancer during its first stages, as well as a treatment for a variety of other digestive and blood circulatory system illnesses. In previous studies, plants' therapeutic benefits were acknowledged for their many active phytochemical ingredients.[12] In addition, it is important to point out that no phytochemical investigation has been published on this plant to date, except for the study carried out using GC/MS on the volatile compounds of this species.[9] To the best of our knowledge, a few studies evoke the biological activities of *F. longispina*. Hence, our study is to assess its antioxidant, anti-proliferative, and antibacterial activities using various *in vitro* analysis tests.

## Materials and methods

*Chemicals*

Folin-Ciocalteu (Merck, with a density of 1.24 g/cm³ at 20°C), $Na_2CO_3$ (Merck, meeting ISO standards), Aluminum nitrate (Sigma Aldrich, with a density of 880 g/cm³), Potassium Acetate (Merck, with a solubility of 2530 g/L), Methanol (Sigma Aldrich, with a purity of ≥99.8%), DPPH (Sigma Aldrich, with a purity of ≥90% as determined by elemental analysis), Trichloroacetic Acid (TCA) (Merck, with a purity of ≥99% as determined by titration), Thiobarbituric Acid (TBA) (Sigma Aldrich, slightly soluble with a solubility of 50 g/L), DMSO (Sigma Aldrich, with a purity of ≥99.0%), Ascorbic Acid (Merck, with a solubility of 330 g/L), and Quercetin (Merck, with a purity of ≥95% as determined by HPLC).

*Plant collection and Identification*

In April 2012, samples of *F. longispina* were collected close to Bechar in southwest Algeria (latitude N: 31° 37' 5.697", longitude W: 2° 12' 51.563") under the auspices of the National Natural Resources Conservation Agency. A voucher specimen (FL N° 38/2015) was archived in the Herbarium of VARENBIOMOL at Constantine 1 University.

*Extraction*

The upper parts of *F. longispina* (Zygophyllaceae), 2200 g, were cut into sections and subjected to extraction. This process involved three cycles of extraction at a moderate temperature; each process lasted 24 hours after using a mixture of methanol and water (MeOH-$H_2O$-7:3 v/v). Following the filtration process, the combined methanol extracts were evaporated under reduced pressure until reaching a temperature of 37°C. They were thus dissolved in 400 ml of distilled water. After filtering the resultant solution, EAE, ChE, and BuE were extracted one after the other. In the end, three extracts were obtained: BuE (25.27 g), EAE (3.5 g), and ChE (3.27 g).

*Quantification of the total phenolic content*

TPC was conducted following the method outlined by Laraba *et al.* (2022)[13] whereby 20 µL of various extracts from *F. longispina* (1 mg/mL) or gallic acid (a standard phenolic compound) were diluted with 1580 µL of distilled water. Subsequently, 100 µL of 2N Folin-Ciocalteu reagents were added. Afterward, 300 µL of a 20% $Na_2CO_3$ aqueous solution was added and thoroughly mixed. The absorbance of all samples was measured at 765 nm.

As for the determination of TFC, the adjusted method mentioned by Mechri *et al.* (2021)[14] was employed. In short, 10 µL of 10% aluminum nitrate, 1 M potassium acetate, and 130 µL of methanol were added to 50 µL of the extract (1 mg/mL). Absorbance was measured by spectrophotometer at 415nm using the Jenway™ 6305 UV/Visible spectrophotometer from France. TPC was expressed as grams of gallic acid equivalents per 1 milligram of extract, while TFC was measured as micrograms of standard quercetin equivalents (QE) per 1 milligram of extract using the formula provided below (1):

$$Absorbance = 0.34 \times Quercetin\ (\mu g) + 0.015;\ R^2 = 0.983 \ldots\ldots (1)$$

*In vitro antioxidant activity*

*DPPH scavenging assay*

The capacity of extracts to donate hydrogen atoms was evaluated by decolorizing a methanol solution of DPPH, initially displaying a purple coloration.[15] The stable DPPH free radical served as the reagent in the spectrophotometric test. Different concentrations (1, 2.5, 5, 10, 15, 20, 30, 50, 75 µg/mL) of the extracts were added to 3 mL of a 0.004% DPPH solution. Following a thirty-minute dark incubation period, the absorbance was measured against a blank at 517 nm.[16] The scavenging activity of the free radical was measured according to formula (2):

$$\%\ DPPH\ scavenging\ activity = [(Absc - Abss)/Absc]\ x\ 100 \ldots\ldots (2)$$

Where:

Absc: Absorbance of the control solution.

Abss: Absorbance of the sample (in the presence of the extract).

Ascorbic acid was utilized as a reference.

$IC_{50}$ is an antioxidant concentration necessary to scavenge 50% of the DPPH free radical.

*Lipid peroxidation test*

The lipid peroxidation test was estimated according to the modified protocol using egg vitellus as a source of lipid.[17] A 10% fresh vitellus homogenate was prepared in PBS (20 mM, pH 7.4). The mixture was subsequently centrifuged, and 1 mL aliquots of the supernatant were exposed to increasing concentrations of either the extract or ascorbic acid in the presence of 50 µL $FeSO_4$ (0.07 M) and then incubated at 37°C for 1 hour. 1 mL of TCA (20% w/v) was added, followed by 1.5 mL of TBA (1% w/v). After thorough mixing, samples were incubated for 15 minutes at 100°C (Memmert p2205-1462, France). Subsequently, the resulting thiobarbituric reacting substance (TBARS) in the supernatant was quantified at 532 nm following centrifugation (HET-MICRO120, France) at 4000 rpm for 20 minutes. Under similar conditions, the control group was performed without extract or ascorbic acid. The inhibition (I%) was calculated using formula (2).

$IC_{50}$ is the quantity of extract necessary to neutralize 50% of free radicals, and it is inversely proportional to the extract's activity.

*Antiproliferative activity*

The antiproliferative activity was assessed on HeLa (human cervix carcinoma) and HT-29 (human colorectal adenocarcinoma) cells using the xCELLigence RTCA system (Agilent xCELLigence Real-Time Cell Analysis (RTCA) MP 6 x 96-well E-Plates, Japan). The EAE, ChE, and BuE extracts of *F. longispina* were prepared in DMSO to achieve a concentration of 20 mg/mL. Subsequently, 25 µL of each extract was added to 475 µL of medium.

The medium was poured into each of the 96 E-Plate wells. Subsequently, the E-plate was placed in a sterile cabinet and then transferred to the incubator, where it remained for 30 minutes. Following that, a background measurement was done.

With the exception of the last three wells, which contained medium devoid of cells and unexposed cells served as a positive control, the cell suspension ($2.5x10^4$ cells/100L) was then added to each well. For half an hour, the plate was submerged in the hood. After that, the plate was placed inside the incubator unit and left for 80 minutes. Then, 250, 100, and 50 µg/mL of each fraction of the *F. longispina* solution were applied to the wells. Cell index (CI) assessment started after the sample was placed into the xCELLigence and continued for a duration of 70 hours.[18]

*Antibacterial activity*

The disc diffusion method was utilized to assess the antibacterial activity of *F. longispina*. [19] The agar diffusion method involves placing paper discs saturated with extracts onto agar previously inoculated by spreading them with the bacterium under investigation. Following incubation, an inhibition zone is formed around each disc. Measuring the diameter of this inhibition zone allows for the determination of whether the strain under study is sensitive, intermediate, or resistant. [20]

*Bacterial strains*

The extracts were tested against *Pseudomonas aeruginosa* (ATCC1117), *Escherichia coli* (ATCC25922), and *Staphylococcus aureus* (ATCC29213). Erythromycin, an antibiotic, was utilized as the positive control, and various solvents were used as the negative control.

The bacterial strains were cultured on nutrient agar plates and then incubated at 37°C for 24 hours to obtain young, isolated colonies.

*Preparation of inoculum*

Colonies sufficiently isolated from the respective bacterial species were extracted using a sterile platinum loop and homogenized in 5 mL of sterile physiological water (9%) until a bacterial suspension with turbidity matching the 0.5 McFarland standard (=1.5 x $10^8$ cfu/mL) was achieved. The tubes were thoroughly mixed, and the bacterial inoculum was utilized within 15 minutes of its preparation.

*Agar diffusion assay*

The bacterial suspension was inoculated using a Mueller Hinton (MH) agar swab. The process was carried out in triplicate, rotating the disc 60° each time, then running the swab around the agar's perimeter. Therefore, discs of sterile Whatman paper (No. 1, 6mm) were impregnated with ten µL at different concentrations (50, 100, and 200 mg/mL). Finally, discs were carefully placed on inoculated agar for a day at 37°C. The inhibition zone diameters were expressed in millimeters.

*Statistical analysis*

Results are presented as the mean ± standard deviation (SD). All tests were performed in triplicate. Statistical analysis was conducted using the Student t-test, with significance accepted at $p < 0.05$. Pearson correlation analysis examined the relationships between antioxidant activity and TPC and TFC levels and the relationships between total phenolic and flavonoid content. Statistical analyses were performed using GraphPad Prism version 8.4.3 software based in Portugal.

## Results and discussion

As plants represent an essential source for human health, there is increasing interest in their studies in our time. Still, we cannot say that we will obtain an alternative medicinal revolution or parallel medicine, and therefore, we must keep abreast of developments. As scientific research shows, secondary metabolites have garnered attention for various purposes due to their antioxidant properties. These metabolites are classified into three main groups based on their biosynthetic pathway: phenolic compounds, terpenes and steroids, and nitrogen-containing compounds.[21]

Our findings indicate that EAE had the highest phenolic content with 377 ± 24.33 µg GAE/mg extract, followed by BuE and ChE with 253.33 ± 28.86 and 163 ± 20.66 µg GAE/mg extract, respectively (Figure 1). In addition, the results revealed that both EAE and BuE extracts contained a significant quantity of flavonoids (87.14 ± 1.56 and 86.98 ± 1.9 µg QE/mg extract, respectively). In contrast, the ChE extract exhibited a lower amount (21.17 ± 9.42 µg QE/mg extract) (Figure 1). Polyphenols stand out as vital constituents of plants, holding significant implications for human nutrition and health. Numerous studies have established a positive correlation between phenolic compounds and their antioxidant attributes, underscoring the relevance of phenolic compounds to antioxidant capacity. [22,23,24] In fact, due to their redox properties, polyphenols protect cells from oxidative damage caused by RONS, acting as reducing agents and hydrogen donors.[25] Current research estimates significant phenolic compounds for *F. longispina* extracts, notably EAE and BuE. *Fagonia* is a genus containing 34 species found in warm climates. It has been discovered that several phytochemical elements play a crucial role in disease prevention.[26]

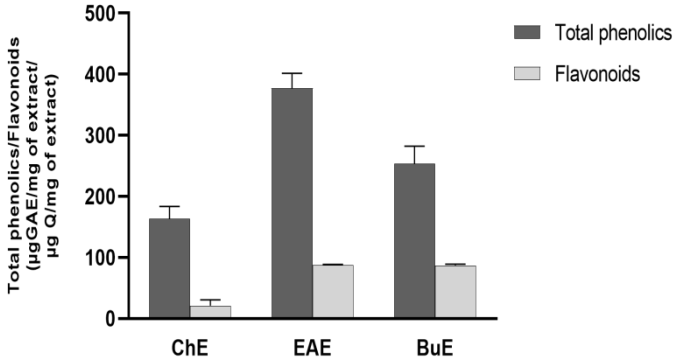


**Figure 1:** Total phenolic and flavonoid amount in various extracts of *F. longispina*. Values are means ± standard deviation (n = 3).

These outcomes indicate many other studies that describe that numerous *Fagonia* species, such as F. *cretica*, *F. olivieri*, and *F. bruguieri* DC, are rich in polyphenols.[26,27,28] As expected, there was a positive correlation between the total phenolic content of *F. longispina* extracts and the quantity of flavonoids present (r = 0.819; $p = 0.3861$). These findings suggest a positive correlation between the TFC and TPC, although it was not statistically significant, which aligns with existing literature data.[26]

Numerous *in vitro* experiments have been conducted to estimate the antioxidant potential of plants. Nevertheless, no universally accepted test has been devised to gauge or quantify this potential.[29] In the present investigation, we evaluated the antioxidant activity of *F. longispina* using *in vitro* spectrophotometric techniques, specifically through DPPH scavenging and lipid peroxidation inhibition assays. Figure 2 showed that *F. longispina* extracts exhibited DPPH scavenging activity in a concentration-dependent manner. It was discovered that EAE had potent activity ($IC_{50}$ was 37.82 ± 7.83 µg/mL) better than BuE ($IC_{50}$ = 59.49 ± 6.16 µg/mL) and ChE ($IC_{50}$ = 111.89 ± 6.10 µg/mL). However, EAE activity remained lower than ascorbic acid ($IC_{50}$ = 5 ± 0.1 µg/mL, $p = 0.02$) (Figure 3). The correlation analysis revealed a negative correlation between TPC and DPPH scavenging activity (r = -0.9477; $p = 0.2068$), which was not statistically significant. The same observation was observed between TFC and DPPH scavenging activity: (r = -0.9593; $p = 0.1823$).

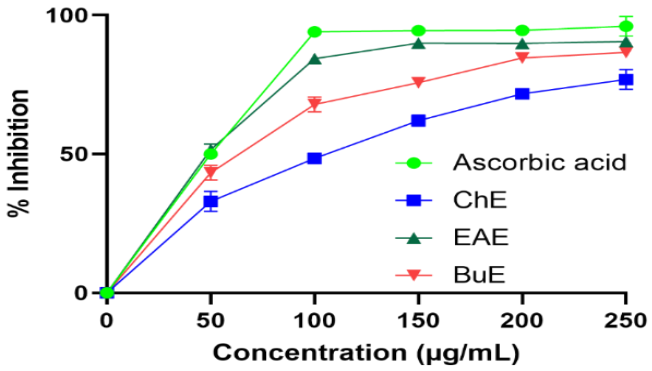


**Figure 2:** DPPH radical scavenging capacity of *F. longispina* extracts and ascorbic acid (mean ± SD, n=3).

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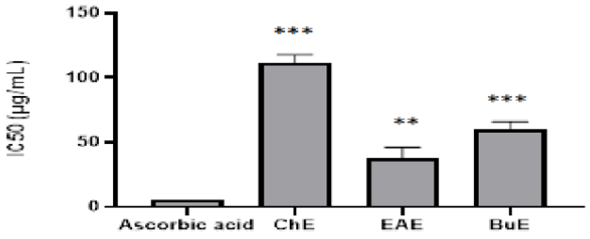


**Figure 3:** Different $IC_{50}$ values of BuE, EAE and ChE of *F. longispina* and ascorbic acid. **: $p < 0.001$, ***: $p < 0.0001$

The DPPH scavenging assay provides essential insights into the antiradical capability of an extract, and its outcomes can suggest that plant extracts are rich in phenolic compounds.[30,31,32] Based on the results depicted in Figure 2, which involved measuring the $IC_{50}$ values of each extract alongside those of ascorbic acid, it is evident that various extracts of *F. longispina* demonstrated significant antioxidant effects. Among these, EAE displayed the highest potency, with an $IC_{50}$ value of 37.82 ± 7.83 µg/mL. Nevertheless, it stayed lower than ascorbic acid ($IC_{50}$ = 5.00 ± 0.1 µg/mL, $p$ = 0.02). These findings were further corroborated by previous studies.[8,11]

In many cases, the antioxidant activity of all extracts is typically linked to their primary components. However, there is no significant correlation between DPPH scavenging activity, TPC, and TFC. Prior studies have corroborated our findings.[26]

Lipid peroxidation inhibition is utilized to assess the capacity of extracts to prevent lipid peroxidation (LPO) in biological systems. It is worth noting that this oxidation creates many degrading and cytotoxic chemicals, such as MDA. It has been reported that free radicals damage fatty acids, which results in various cellular disorders such as carcinogenesis.[26,33,34] Figure 4 showed that all *F. longispina* extracts and ascorbic acid inhibited the LPO induced in egg vitellose homogenate by the $FeSO_4$ system in a concentration-dependent manner. Among all extracts, EAE had the highest effect in scavenging free radicals from $FeSO_4$ ($IC_{50}$ = 126.66 ± 2.35 µg/mL), followed by BuE ($IC_{50}$ = 206.89 ± 6.09 µg/mL), and then ChE ($IC_{50}$ = 301.71 ± 3.31 µg/mL) (Figure 5). This effect seems low compared to ascorbic acid ($IC_{50}$ = 20 ± 1.06 µg/mL), but it stays intriguing. In comparison, at 0.3 mg/mL, EAE demonstrated a 60.93% inhibition of lipid peroxidation. In contrast, the percentage of ascorbic acid at the same concentration was 97.50%. In our study, correlation analysis revealed a negative correlation, although not statistically significant, between both TPC and TFC and the $IC_{50}$ values of the inhibitory effect (r = -0.8875, $p$ = 0.3048, and r = -0.9913, $p$ = 0.0843, consecutively).

In a previous study, extracts of the aerial parts, fruit, and roots of *F. cretica* demonstrated a promising ability to inhibit lipid peroxidation.[26] Moreover, an *in vivo* study revealed that the methanol extract of *F. olivieri* efficiently decreased elevated levels of thiobarbituric acid reactive substances (TBARS) in rats treated with acetaminophen, and this effect was dose-dependent.[27] These observed effects could be attributed to bioactive components such as phenolic acids and flavonoids.[34, 35]

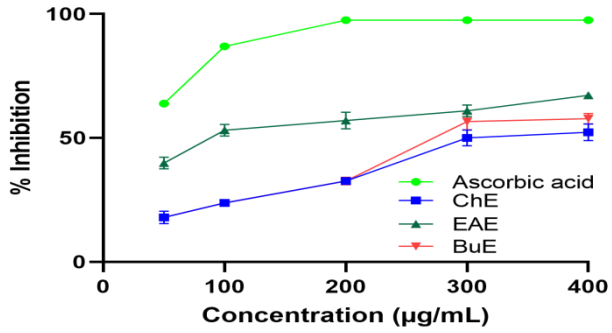


**Figure 4:** Inhibition of lipid peroxidation by various extracts of *F. longispina* and ascorbic acid in different concentrations (mean ± SD, n=3).

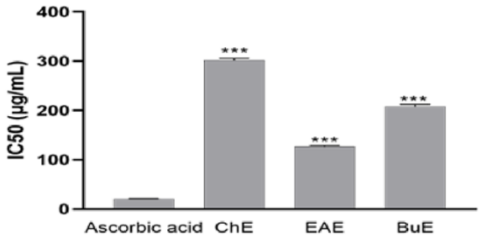


**Figure 5:** Different values of $IC_{50}$ of *F. longispina* extracts and ascorbic acid. ***: $p < 0.0001$.

The Zygophyllaceae family has been used as a medicinal plant, and their polyphenols have been studied for their chemopreventive properties against cancer.[36] This research examined the *in vitro* cancer cell growth inhibitory effects of BuE, EAE, and ChE extracts from *F. longispina* on two human cell lines, HeLa and H-T29. During the initial 80 minutes, unexposed cells in the control group (red line) exhibited regular multiplication. Nevertheless, no improvement was observed in the medium wells (represented by the green line). All extracts exhibited different profiles at different concentrations and time points, but the most exciting effect was between 10 and 50 h. EAE and ChE fractions consecutively demonstrated an intriguing impact against HeLa and HT-29 cells (Figure 6 and Figure 7) and (Figure 8 and Figure 9). The most effective activity observed compared to the control is that with a 250 µg/mL concentration, cell proliferation and viability are greatly affected after 48 h post-treatment. At the same time, both concentrations (50 and 100 µg/mL) presented low action compared to the high concentration during the period.

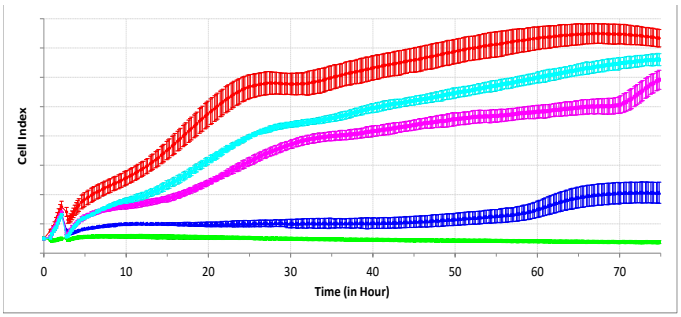


**Figure 6:** Antiproliferative activity of EAE extract of *F.longispina* (250 100 50 Control Medium (µg/mL)) against HeLa (2.5x104cell/well) cell line.

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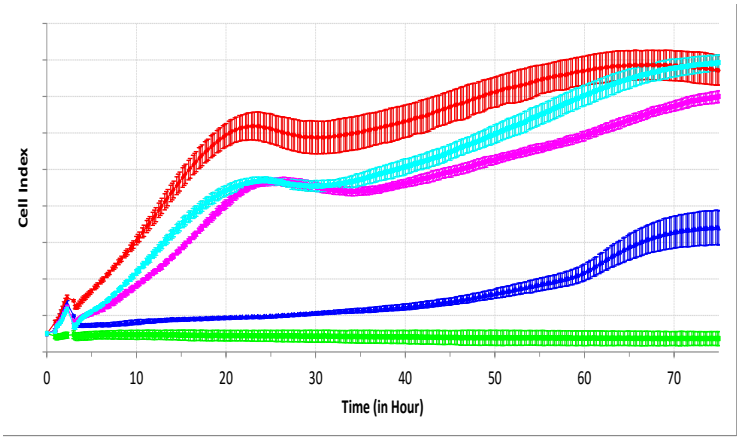


**Figure 7:** Antiproliferative activity of EAE extract of *F. longispina* (— 250 — 100 — 50 — Control — Medium (µg/mL)) against HT-29 ($2.5x10^4$cell/well) cell line.

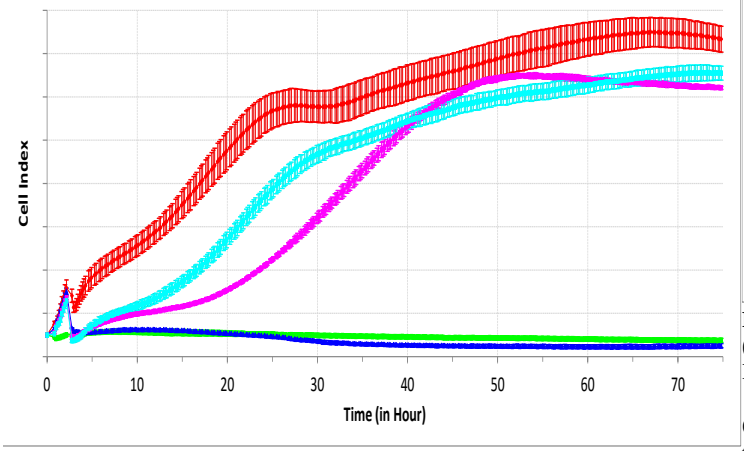


**Figure 8:** Antiproliferative activity of ChE extract of *F. longispina* (— 250 — 100 — 50 — Control — Medium (µg/mL)) against HeLa ($2.5x10^4$cell/well) cell line.

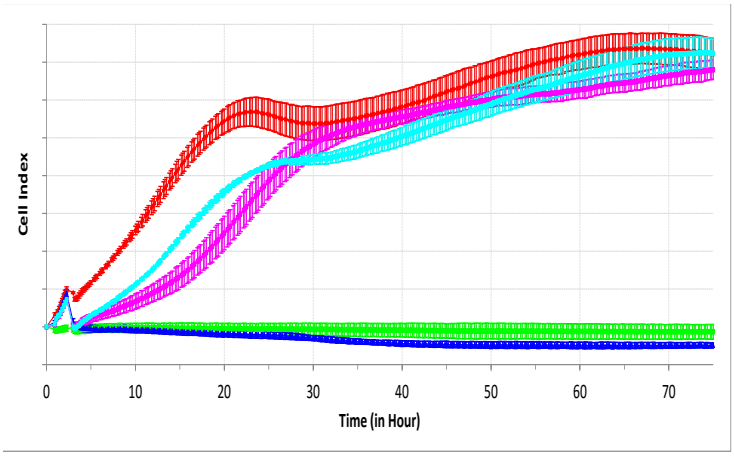


**Figure 9:** Antiproliferative activity of ChE extract of *F. longispina* (— 250 — 100 — 50 — Control — Medium (µg/mL)) against HT-29 ($2.5x10^4$cell/well) cell line.

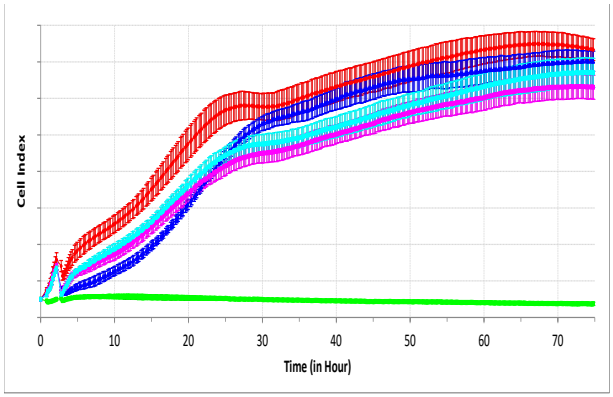


**Figure 10:** Antiproliferative activity of BuE extract of *F. longispina* (— 250 — 100 — 50 — Control — Medium (µg/mL)) against HeLa ($2.5x10^4$cell/well) cell line.

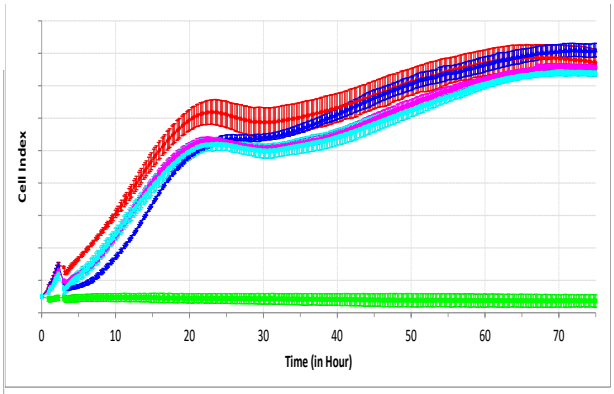


**Figure 11:** Antiproliferative activity of BuE extract of *F. longispina* (— 250 — 100 — 50 — Control — Medium (µg/mL)) against HT-29 ($2.5x10^4$cell/well) cell line.

On the other hand, after 24 hours, the EAE extract inhibited 17% of the HT-29 cell line at 50 and 100 µg/mL. However, at 250 µg/mL, 92.8% of HT-29 cells were inhibited. After 48 hours, the extract's effectiveness seemed to diminish, as 250 µg/mL of the extract only inhibited 77.17% of HT-29 cells, suggesting that the quantity of the extract may have been insufficient. The same effect was observed with HeLa cell lines after 24 and 48 hours. Antiproliferative activity decreased gradually after 60 h, and CI increased slowly until the end of the experiment at 250 µg/mL. The 100 µg/mL concentration (represented by the pink line) exhibited a similar effect to the 250 µg/mL concentration, and it was more effective than a lower dose (50 µg/mL). However, as depicted in Figures 10 and 11, the BuE fraction exhibited no activity compared to the control across all three doses against HeLa and HT-29 cells. It can be hypothesized that the antiproliferative properties of EAE and ChE of *F. longispina* may be attributed to the existence of flavonoids such as cirsimaritine, hispiduline, quercetin, kaempferol, and astragaline, as reported in the previous study,[9] which indicate that *F. longispina* can be a promising source of potential antitumor agents.

It's noteworthy that no previous studies have been conducted on the antiproliferative properties of *F. longispina* until now, unlike some other species of *Fagonia*, such as *F. schimperi* and *F. indica*. Multiple studies have been conducted on these species, revealing a potent antiproliferative effect against breast cancer MCF-7 cells *in vitro*.[36, 37]

The pathogenic bacteria used in this study, *E. coli*, can cause skin Antibacterial activity was assessed on *S. aureus*, *P. aeruginosa*, and *E. coli* using agar diffusion. The results obtained from this investigation are presented in terms of the diameters of the inhibition zones. The study found that, at three different doses (50, 100, and 200 mg/mL), none of the extracts exhibited antibacterial activity against the Gram-negative bacteria tested (*E. coli* and *P. aeruginosa*), as compared to the positive control (erythromycin). However, all *F. longispina*

extracts, except BuE, demonstrated antibacterial activity against gram-positive *S. aureus* strains at a concentration of 200 mg/mL (Table 1). The inhibition zones for *S. aureus* at 200 mg/mL with EAE and ChE extracts of *F. longispina* were 8 ± 1 and 7 ± 1 mm, respectively. However, no effect was mentioned at 50 mg/mL and 100 mg/mL (Table 2). This plant's flavone monoterpene, para-hydroxybenzoic acid, quercetin, and sterol content account for the ChE and EAE fractions' antibacterial activity.[9] These results agreed with other studies, which reported that *F. longispina* essential oils had moderate activity against *S. aureus*.[11] infections, gallbladder problems and urinary tract infections. Likewise, food poisoning, rashes, ulcers, and lung infections have all been linked to *S. aureus*.[38] Regarding *P. aeruginosa*, it has been linked to fatalities in certain populations after a frequent opportunistic infection.

**Table 1:** Antibacterial activity of *F. Longispina* extracts.

| **Extracts** | **EAE** | | | **ChE** | | | **BuE** | | | **Erythromycin** |
|---|---|---|---|---|---|---|---|---|---|---|
| Concentrations | C1 | C2 | C3 | C1 | C2 | C3 | C1 | C2 | C3 | |
| *S.aureus* | - | - | + | - | - | + | - | - | - | + |
| *E. Coli* | - | - | - | - | - | - | - | - | - | + |
| *P. aeruginosa* | - | - | - | - | - | - | - | - | - | + |

C1=50 mg/mL, C2=100 mg/mL, C3=200 mg/mL; (-) = No activity; (+) = Positive activity.

**Table 2:** Antibacterial activity of different extracts of *F.longispina* against *S.aureus* strain.

| **Extracts** | **EAE** | | | **ChE** | | | **BuE** | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentrations | C1 | C2 | C3 | C1 | C2 | C3 | C1 | C2 | C3 |
| Inhibition zone diameter (mm) | 0 | 0 | 8±1 | 0 | 0 | 7±1 | 0 | 0 | 0 |

C1=50 mg/mL, C2=100 mg/mL, C3=200 mg/mL.

Typically, it is found abundantly in everyday food items, particularly vegetables and drinking water, albeit in trace amounts.[39] Natural alternative treatments for fungal and bacterial infections may lead to the discovery of new antimicrobial agents.[40] The resistance of *E. coli* and *P.aeruginosa* strains to all concentrations of extracts used may be attributed to their membrane structures. The outer layer, composed of lipopolysaccharides and proteins, forms a barrier that prevents the entry of substances and inhibits the growth of gram-positive bacteria. The antimicrobial activity of different extracts of *F. indica* against several fungal and bacterial pathogens has been studied, and the results demonstrated that all extracts possess antifungal and antibacterial properties. [41] In another study, the extract from the same species of *F. indica* showed an antibacterial effect against *P. aeruginosa*.[37] Furthermore, the assessment of the antimicrobial activity of various extracts (methanolic root, methanolic leaves, ethanolic root, and ethanolic leaves) of *F. cretica* revealed that this species, traditionally used in traditional therapy, could serve as a novel origin of antimicrobial agents. These findings provide a scientific foundation for integrating the plant into modern medicine.[40] However, EAE and ChE of the same species presented a strong antibacterial effect against *S. aureus*.[42]

## Conclusion

Our results indicates that *F. longispina* extracts have a significant concentration of flavonoids among their many other phenolic components. Additionally, the findings suggest that *F. longispina* shows potential as both an antioxidant and an antibacterial agent against the *S. aureus* strain. Notably, this study marks the first exploration of *F. longispina's* anti-proliferative properties in scientific research. The results indicate that *F. longispina* extract holds promise as a source of anti-proliferative agents for HeLa and HT-29 cell lines, highlighting its potential as a candidate for further investigation in cancer treatment. Future research will expand upon these findings through experimentation on animal models, aiming to provide additional validation of *F. longispina's* antiproliferative and antioxidant effects.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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