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Phytochemical Screening of *Echium angustifolium* and *Anchusa aegyptiaca* Extracts and their Cytotoxic, Antitoxic, and Antimicrobial Activities

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ABSTRACT

Medicinal plants have historically been a vital source of therapeutic agents and continue to play a significant role in the discovery of novel drugs due to their rich phytochemical diversity and biological activities. Despite their widespread use, systematic evaluation of their antimicrobial, antioxidant, and cytotoxic properties remains essential for validating their therapeutic potential. In this study, the antibacterial, antifungal, antioxidant, and cytotoxic activities of aqueous extracts from the medicinal plants *Anchusa aegyptiaca* L. and *Echium angustifolium* Mill. were evaluated at concentrations of 1%, 3%, and 5%. Antimicrobial efficacy was also assessed against five bacterial and five fungal strains using the agar well diffusion method. Cytotoxicity was determined via the *Vicia faba* root tip assay, with chromosomal aberrations serving as indicators of genotoxicity. *Echium angustifolium* exhibited the highest antioxidant activity, with an IC_{50} value of 0.0423 mg/mL, which may be attributed to its rich content of secondary metabolites, including phenols (1.04%), flavonoids (0.31%), and alkaloids (0.48%). The 5% aqueous extract of *E. angustifolium* was the most effective in inhibiting the growth of fungi such as *Aspergillus niger*, *A. flavus*, and *Alternaria alternata*, producing inhibition zones of 15 mm, 14.5 mm, and 14 mm, respectively. Additionally, the same 5% extract demonstrated potent antibacterial activity against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Ralstonia solanacearum*, and *Micrococcus luteus*, with inhibition zones measuring 21 mm, 31 mm, 34 mm, and 31 mm, respectively. Meanwhile, *Echium angustifolium* exhibited the highest concentrations of phytochemicals, antioxidant activity, and antimicrobial efficacy, while demonstrating relatively low levels of cytotoxicity and chromosomal aberrations. Notably, the 1% aqueous extract of *Anchusa aegyptiaca* reduced the incidence of chromosomal aberrations by 17.14% compared to the control group (which exhibited 20.81% aberrations). These findings suggest that these plant extracts possess cytoprotective properties that may mitigate genotoxic effects, supporting their safety at therapeutic doses. Consequently, the risk of adverse effects at clinically relevant concentrations appears to be minimal.

Keywords: *Anchusa aegyptiaca;* Antimicrobial activity; Antioxidant activity; Chromosomal abnormalities; *Echium angustifolium*.

INTRODUCTION

Medicinal plants are considered biochemical facilities that generate a wide range of chemical substances, including vitamins, minerals, fiber, essential oils, and phytonutrients, also known as phytochemicals (Sylaja *et al*., 2023). sources to address approximately 300 physiological and psychological ailments (Sbhatu and Abraha, 2020). According to the World Health Organization, a significant percentage (between 70% and 95%) of the population in underdeveloped countries depends on traditional medicines, primarily derived from plants, for their primary healthcare needs (Robinson and Zhang, 2011). Medicinal plants serve not only as a means of self-treatment but also as a significant source of revenue (IPBES, 2022). In addition, the utilization of medicinal herbs is increasing, particularly in the wake of the COVID-19 pandemic (Smith *et al*., 2021; Timoshyna *et al*., 2020; WHO, 2019). The presence of different phyto-constituents in many medicinal plants can result in outstanding pharmacological activities. This, in turn, has the potential to lead to the development of new classes of potentially safer drugs for disease treatment (Ganaie *et al*., 2018). Additionally, they serve as significant reservoirs of several bioactive substances that can be utilized in specific treatments because of their pain-relieving and anti-inflammatory characteristics (Boujbiha *et al*., 2023). Phytochemical and pharmacognostic studies are necessary to assess the safety and efficacy of the use of medicinal plants. Consequently, numerous herbs possess medicinal qualities and are readily accessible and user friendly (Boujbiha *et al*., 2023; Eissa *et al*., 2023).

The Boraginaceae family consists of approximately 115 genera and 2500 species. The distribution of these species is mostly concentrated in tropical, subtropical, and partly northern temperate latitudes. Many members

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of this family possess significant medical importance because of their notable pharmacological effects and biological activity (Panchenko *et al*., 2022). Several species of the Boraginaceae family synthesize secondary metabolites, including alkaloids, naphthaquinones, polyphenols, phytosterols, and terpenoids (Gottschling *et al*., 2001 and Iqbal *et al*., 2005). The family Boraginaceae produces polyphenols, such as flavonoids and phenolic acids, which exhibit many medicinal properties, including anti-inflammatory, antiviral, and antibacterial activities (Iqbal *et al*., 2005; Abdelaal *et al*., 2024). The Boraginaceae family is important because of its ability to produce beneficial secondary metabolites, such as antioxidants, that allow plants to combat oxidative stress caused by unfavorable environmental conditions (Panchenko *et al*., 2022).

Phenolics and polyphenols are a significant category of secondary metabolites that have demonstrated antibacterial action (Stefanović *et al*., 2012). Plant extracts are considered to be natural sources of antimicrobial agents that are both nutritionally safe and quickly degradable (Cowan, 1999; Duffy and Power, 2001; Berahou *et al*., 2007; Chika *et al*., 2007). Naturally occurring plant flavonoids exhibit antibacterial properties (Xu and Lee, 2001 and Cushnien Lamb, 2005). Multiple studies have verified the ability of plant extracts from the Boraginaceae family to combat cancer, act as antioxidants, and fight against microorganisms (Bošković *et al*., 2018; Khurm *et al*., 2016; Erdogan *et al*., 2020; Paun *et al*., 2020). Additionally, various studies have identified plant compounds with antimicrobial properties that are effective against a broad range of bacteria (both gram-positive and gram-negative), fungi, and viruses (Ali-Shtayeh *et al*., 1998) and antibacterial action (Stefanović *et al*., 2012). Plant extracts are considered to be natural sources of antimicrobial agents that are both nutritionally safe and quickly degradable (Cowan, 1999; Duffy and Power, 2001; Berahou *et al*., 2007; Chika *et al*., 2007). Naturally occurring plant flavonoids exhibit antibacterial properties (Xu and Lee, 2001 and Cushnien Lamb, 2005). Multiple studies have verified the ability of plant extracts from the Boraginaceae family to combat cancer, act as antioxidants, and fight against microorganisms (Bošković *et al*., 2018; Khurm *et al*., 2016; Erdogan *et al*., 2020; Paun *et al*., 2020). Additionally, various studies have identified plant compounds with antimicrobial properties that are effective against a broad range of bacteria (both gram-positive and gram-negative), fungi, and viruses (Ali-Shtayeh *et al*., 1998).

Boraginaceae includes the genus *Echium* L., which includes 67 officially recognized species (Jin *et al*., 2020). *Echium* L. has imported numerous species into North America and Australia for the purpose of ornamental and garden cultivation (Mack, 2003). *Echium* species have been utilized for centuries in the Mediterranean region as traditional remedies with purifying, sweat-inducing, urine-promoting, and mood-boosting qualities (Heidari and Azad, 2006; Shafaghi *et al*., 2010 and Abbasi and Jamei, 2019). *Echium* spp. have been scientifically proven to exhibit antioxidant, analgesic, anxiolytic, anti-inflammatory, antibacterial, and antiviral activities, as demonstrated by recent pharmacological research (Jin *et al*., 2020).

Echium angustifolium Mill., a perennial plant belonging to the Boraginaceae family, is found in the northwestern coastal region of Egypt. It is used for grazing, medical, and fuel reasons (Bidak *et al*., 2015). Although *Echium angustifolium* is utilized for grazing and medicinal purposes in Egypt, there is a lack of scientific information regarding the chemical and biological characteristics of this plant. Research has indicated that certain lignans isolated from plants exhibit potent cytotoxic activity (El-Tantawy *et al*., 2021). The utilization of this substance in the treatment of ciguatoxin poisoning and inflammation caused by snake venom has been documented by Sadawe *et al*. (2020). *Echium angustifolium*, a plant species, is rich in pyrrolizidine alkaloids, phenolic acid derivatives, flavonoids, and other compounds that have diverse biological effects (Sadawe *et al*., 2020).

The *Anchusa* genus has fifty species that are found in the Iran–Turanian and Mediterranean regions (Jaradat *et al*., 2020). *Anchusa* L. is a sizable genus belonging to the Boraginaceae family. It consists of over 170 species, both annual and perennial, found in the temperate and subtemperate regions of the Old World (Akcin *et al*., 2010). *Anchusa* species serve various purposes in different regions, including ornamental, medicinal, and edible uses. *Anchusa* species have been utilized in traditional medicine to heal open wounds, cuts, rheumatism, arthritis, gout, and stomach ailments and aid in weight loss. Additional species within the *Anchusa* genus, such as *Anchusa italica* and *Anchusa strigosa*, have many uses in traditional medicine. These species possess antibacterial, anticancer, antiviral, anti-inflammatory, antidiabetic, and numerous other properties (Al-Snafi, 2014 and Sahranavard *et al*., 2009). *Anchusa aegyptiaca* is widely recognized in the Eastern Mediterranean Basin, including the Saharo-Arabian, Eastern Mediterranean, and Western Irano-Turanian biogeographic regions. It is considered a native species in these areas. In more Western locations, such as Tunisia, it is considered native (Greuter *et al*., 1984).

These herbs are extensively employed in Arabian medicine as powerful antibacterial agents for treating various bacterial diseases of the respiratory, gastrointestinal, and urinary systems (Abu--Rabia, 2005; Alachkar *et al*., 2011; and Al-Khateeb *et al*., 2019). The objective of this investigation was to examine two species belonging to the Boraginaceae family, namely, *Echium angustifolium* and *Anchusa aegyptiaca*. This study involves analyzing the primary and secondary constituents of these plants, evaluating their antioxidant activity, testing the potential antimicrobial properties of their aqueous extracts against various fungal and bacterial species, and assessing the cytotoxic effects of the aqueous extracts of the selected taxa via a chromosomal aberration assay.

MATERIALS AND METHODS

Plant materials

Two species from the Boraginaceae family, *Echium angustifolium* Mill. and *Anchusa aegyptiaca* L., were collected from their natural habitats and taxonomically identified by Prof. Ibrahim A. Mashaly, Professor of Plant Flora and Ecology, Botany Department, Faculty of Science, Mansoura University (Fig. 1). These species were selected due to the well-documented phytochemical richness of the Boraginaceae family, which is known to contain diverse bioactive compounds such as alkaloids, flavonoids, saponins, and phenolic acids. These secondary metabolites are associated with a wide range of pharmacological activities, including antimicrobial, antioxidant, antiinflammatory, and anticancer effects. Furthermore, species within the Boraginaceae family have a long history of traditional medicinal use across various cultures, underscoring their potential therapeutic relevance.

Proximate constituents and phytochemical analysis

Proximate composition and phytochemical analysis were conducted on the shoots of selected wild medicinal plants. The shoots were harvested, dried under controlled conditions, and ground into a fine powder. Approximately 10 grams of each powdered sample were used for subsequent phytochemical analyses as described below:

Total Ash Determination

Approximately 3 grams of dried, powdered material from each medicinal plant species under investigation were placed in a pre-weighed silica crucible and initially incinerated over a low flame to avoid loss of sample. The partially charred residue was then transferred to a muffle furnace and subjected to ignition at a controlled temperature range of 500–600 °C for 6 hours, ensuring complete oxidation of the organic matter.

Following incineration, the crucibles were cooled in a desiccator and the ash content was accurately weighed using ash-free filter paper. The procedure was conducted in accordance with the official methods outlined by the Association of Official Analytical Chemists (AOAC, 1990).

Crude Fiber Determination

Crude fiber content was determined using the gravimetric method outlined in AOAC (1990; Method 962.09). Briefly, 1 g of each powdered plant sample was sequentially digested with 0.255 N sulfuric acid (H_2SO_4) and 0.313 N sodium hydroxide (NaOH) to hydrolyze soluble carbohydrates, proteins, and lipids. The remaining residue was washed, dried, and combusted in a muffle furnace at 550°C for 4 hours to remove organic matter. The final ash-free residue, representing acid- and alkali-insoluble fiber, was quantified gravimetrically.

Total lipid content

The extraction process involved the use of a Soxhlet apparatus and petroleum ether (boiling point 60-80°C) to extract 10 grams of powder from each sample for 16 hours. The extract was dehydrated by evaporating with anhydrous Na_2SO_4. The lipid content was assessed by subjecting the residue to a drying process at a temperature of 80°C for a duration of ten minutes, allowing it to cool; thereafter, its weight was measured (AOAC, 1990).

Figure (1): Morphological overview of medicinal plant species collected from sandy dune habitats to carry out the study.

Total protein content

The protein content was determined via the equation provided by AOAC (1990), which was generated from measuring the nitrogen content of each sample via the micro-Kjeldahl method as described by Chibnall *et al.* (1943).

Total carbohydrate content

The total carbohydrate content was estimated via the Hedge and Hofreiter (1962) method. Following a 3-h immersion in a vigorously boiling water bath, the powder, approximately 0.2 g of each sample, was subjected to hydrolysis using 5 cm^3 of HCl (2.5 N) and subsequently cooled to ambient temperature. Following the addition of Na_2CO_3 to the mixture and allowing ebullition to cease, the volume was then increased to 100 cm3. The mixture was then subjected to centrifugation to separate the supernatant. The liquid portion was heated in a water bath at boiling temperature together with 4 cm^3 of anthrone reagent for 8 minutes. The intensity of the color was assessed as ranging from green to dark green at a wavelength of 630 nm.

Total phenols

The Folin-Ciocalteu (FC) technique, adapted by Singleton and Rossi (1965), was employed to measure the total phenols in the crude peels. Sigma-Aldrich gallic acid (GA) at a concentration of 5% was used to construct the curve. The extracts were diluted to a volume of 10 microliters and transferred into test tubes.

Next, a 0.5 ml FC reagent was added, and the mixture was observed for 4 minutes. Following a 2-hour incubation period at room temperature in the absence of light, each sample was treated with 1 mL of Na_2CO_3 (7.5%, W/V). The absorbance of each sample was subsequently measured at a wavelength of 760 nm.

Total Flavonoid content

Total flavonoid content was determined using the aluminum chloride ($AlCl_3$) colorimetric method (Ahmad *et al.*, 2018). Briefly, 1 ml of each extract was mixed with 100 µl of 10% (w/v) $AlCl_3$ solution and incubated at room temperature for 5 minutes. Subsequently, 1 ml of 1 M sodium hydroxide (NaOH) was added, and the mixture was adjusted to a final volume of 5 ml with distilled water. After 15 minutes of equilibration, the solution was vortexed thoroughly, and absorbance was measured at 510 nm using a spectrophotometer (UV-VIS spectrometer (Jenway, Essex, UK). A blank sample, prepared identically but without the plant extract, served as the reference. Total flavonoid concentration was calculated using a catechin standard curve (0-100 µg/ml) and expressed as catechin equivalents (CE) per milliliter of crude extract (mg CE/ml). All chemicals, including $AlCl_3$ and catechin, were sourced from BDH Chemicals Ltd. (Poole, England).

Total Alkaloids

The mixture was subjected to 10% acetic acid in ethanol for four hours at room temperature, with a mass of 1 g and a volume of 50 mL. The substance was subsequently concentrated in a water bath following filtration. To induce separation of the extracted mixture, concentrated ammonium hydroxide was carefully added in small increments. Once the solution had been allowed to settle, the solid particles that formed, known as the precipitate, were collected. The precipitate was then rinsed with a solution of diluted ammonium hydroxide, filtered, and dried until its weight remained unchanged (Harborne, 1973).

Tannin content

The vanillin-hydrochloride test was employed to quantify the amounts of tannins, as described by Burlingame (2000) and Aberoumand (2009). The tannin content of the extracted plant samples was measured in grams of tannic acid equivalents per hundred grams of dry plant. The tannin concentration was quantified by analyzing the data via a standard curve derived from tannic acid ($y= 0.0009x$; $r^2 = 0.955$).

Antioxidant activity

Free-radical scavenging activity (DPPH Assay)

The efficacy of the produced extracts in eliminating the "stable" free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was assessed via a modified version of the approach developed by Mekni *et al.* (2013). A solution of DPPH (0.3 mm) in ethanol was combined with 2.0 ml of the test materials. The reaction mixture was subsequently allowed to incubate for 30 minutes at ambient temperature in a light-restricted environment. The change in hue from deep violet to light yellow at a wavelength of 517 nm was measured via a spectrophotometer (Lambda 265, Perkin Elmer). The quantification of the capacity to eliminate free radicals was assessed by the decrease in absorbance, which was subsequently utilized to determine the percentage of antioxidant activity, as a percentage of DPPH radical scavenging activity using the formula:

$$Inhibition\ \% = \frac{(A_{control} - A_{sample})}{A_{control}} X\ 100$$

Where, $A_{control}$ is the absorbance of the DPPH solute-ion without extract, and A_{sample} is the absorbance of the DPPH solution with the extract. Ascorbic acid was used as a positive control.

The free-radical scavenging activity of the plant extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, following the method described by Brand-Williams *et al.* (1995) with slight modifications. Briefly, 1 ml of each extract at various concentrations was mixed with 1 ml of 0.1 mM DPPH solution prepared in methanol. The reaction mixture was incubated in the dark at room temperature for 30 minutes. The decrease in absorbance was measured at 517 nm using a UV-Vis spectrophotometer against a methanol blank. The percentage of DPPH radical scavenging activity was calculated using the formula:

Antimicrobial activity

The antimicrobial efficacy of *Echium angustifolium* and *Anchusa aegyptiaca* aqueous extracts was evaluated using the agar well diffusion assay. Aqueous solutions of the crude extracts (5% w/v) were prepared by dissolving 5 g of extract in 100 mL of sterile distilled water. The assay was performed against five fungal strains (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Alternaria alternata*, and *Rhizoctonia solani*) and five bacterial species (*Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Ralstonia solanacearum*, and *Micrococcus luteus*). Sterile filter paper discs (6 mm diameter) impregnated with 50 µL of extract were aseptically placed on inoculated agar plates and incubated at 37°C (bacteria) or 28°C (fungi) for 24-48 hours. Bacterial cultures were evenly spread on Mueller-Hinton agar (MHA) plates (Difco Laboratories, Detroit, USA), while fungal strains were cultured on potato dextrose agar (PDA) and incubated at 27°C for 7 days. Subsequently, 100 µL of each plant extract (5% w/v) was aseptically introduced into wells cut into the agar plates. Bacterial plates were incubated at 37°C for 24 hours, and fungal plates at 25°C for 48 hours. Antimicrobial activity was assessed by measuring the diameter of inhibition zones (mm) around the wells. Tetracycline served as the positive control antibiotic for bacteria, and fluconazole for fungi. All assays were performed in triplicate, and inhibition zones were compared to those produced by the standard antibiotics following CLSI guidelines (2009).

Chromosome aberration assay

The *Vicia faba* L. (Misr 1) seeds were donated by the National Gene Bank, Ministry of Agriculture and Land Reclamation. The seeds underwent germination in Petri dishes and were placed between two layers of

cotton after being immersed in distilled water at 26°C for 24 h, resulting in the growth of roots that were 1.5-2.0 cm in length. *Vicia faba* seeds were subjected to treatment with three different concentrations (1%, 3%, and 5%) of aqueous extracts derived from studied wild plants.

The root tips of *V. faba* were subsequently fixed in a solution of glacial acetic acid and ethanol at a ratio of 1:3 (known as Carney's solution) and stored in a refrigerator for a minimum of 48 hours. The process involves subjecting the substance to hydrolysis in a solution of 1 N hydrochloric acid at a temperature of 60°C for a duration of 6-8 minutes. This was then followed by rinsing the substance in distilled water for a period of 5 minutes. Next, a slide was made by immersing the root tips in water and applying aceto-orcein stain (Chattopadhyay, 1988) for duration of 2-4 hours. A single droplet of 45% acetic acid was applied onto a pristine slide, and the deeply colored root ends were compressed beneath a cover glass to disperse the cells. Both normal and diseased cells were observed at different stages of mitosis via electron microscopy (Olympus CX 40). Cytotoxicity throughout the cell cycle was evaluated via the mitotic index (MI), phase index (PI), and overall abnormality percentage. T tests were employed for statistical analysis to assess the disparity between various concentrations in each sample and the control.

Statistical analysis

All data are presented as means ± standard deviations (SD). Statistical analyses were performed using SPSS version 16.0 (IBM Corp., Armonk, NY, USA). Differences among treatments were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to evaluate the efficacy of different treatments. Statistical significance was set at $p \leq 0.05$. Additionally, the correlation coefficients between major constituents, phytochemical parameters, and antioxidant activities of the studied wild plants were calculated using PAST software (version 4.0; Paleontological Statistics Software Package, University of Oslo, Norway).

RESULTS

Phytochemical analysis

Primary constituents

Table (1) presents a comparative analysis of key nutritional components between *Atriplex aegyptiaca* and *Euphorbia angustifolium*. Significant differences were observed across most measured traits, suggesting species-specific variations in biochemical composition. For crude fiber (%), *E. angustifolium* exhibited a significantly ($p \leq 0.05$) higher crude fiber content (0.33%) compared to *A. aegyptiaca* (0.23%). This suggests that *E. angustifolium* may contribute more effectively to dietary fiber intake, which is beneficial for gastrointestinal health and metabolic regulation. However, for Total Lipids (%), *A. aegyptiaca* had a markedly higher lipid content (4.87%) than *E. angustifolium* (2.01%), which almost represent the double content (Table 1). This may reflect species-specific adaptations to arid environments, where lipids serve as energy reserves. In addition, total proteins (%), although both species contained high protein levels, *E. angustifolium* showed a slightly but significantly ($p \leq 0.05$) higher content (18.49% ± 1.56) than *A. aegyptiaca* (18.19% ± 1.34). For total Ash (%)**,** ash content, representing total mineral residue, was significantly ($p \leq 0.05$) higher in *A. aegyptiaca* (10.58 ± 0.98) than in *E. angustifolium* (6.71% ± 0.76). Furthermore, total carbohydrates (%) the content was also greater in *A. aegyptiaca* (4.21% ± 0.23) relative to *E. angustifolium* (2.07% ± 0.23). However, both species showed relatively low carbohydrate levels, which might make them suitable for low-carb dietary uses.

Secondary metabolites

The comparative analysis of secondary metabolites reveals clear interspecific differences in phytochemical content between *A. aegyptiaca* and *E. angustifolium*, which may reflect variations in their adaptive strategies, ecological roles, or pharmacological potential (Table 2). For total phenols (%), *E. angustifolium* exhibited a higher total phenolic content (1.04%) compared to *A. aegyptiaca* (0.41%). Phenolic compounds are key contributors to antioxidant activity and are often associated with anti-inflammatory and antimicrobial effects. The significantly ($p \leq 0.05$) elevated levels in *E. angustifolium* suggest a stronger potential for therapeutic applications. Total flavonoids (%) was also greater in *E. angustifolium* (0.31%) than in *A. aegyptiaca* (0.19%). The higher concentration in *E. angustifolium* reinforces its potential as a source of bioactive compounds for medicinal use.

Total Alkaloids, represented as percentage, in *E. angustifolium* (0.48%) was significantly (($p \leq 0.05$) higher than that in *A. aegyptiaca* (0.30%). Alkaloids

Table (1): Comparative analysis of primary composition of studied plants: *Anchusa aegyptiaca* and *Echium angustifolium.*

Measured Traits	Studied species *	
	A. aegyptiaca	***E. angustifolium***
Crude Fiber %	0.23±0.02 [b]	0.33±0.02 [a]
Total Lipids %	4.87±0.65 [a]	2.01±0.34 [b]
Total Proteins %	18.19±1.34 [b]	18.49±1.56 [a]
Total ash	10.58±0.98 [a]	6.71±0.76 [b]
Total Carbohydrates %	4.21±0.23 [a]	2.07±0.23 [b]

*Different letters within the same row indicate statistically significant differences between species at $p \leq 0.05$. Data are expressed as means of three replicates ± standard deviations (SD).

Table (2): The secondary metabolite constituents in *A. aegyptiaca* and *E. angustifolium*.

Measured secondary metabolites	Studied species*	
	A. aegyptiaca	***E. angustifolium***
Total Phenols %	0.41±0.001 [b]	1.04±0.10 [a]
Total Flavonoids %	0.19±0.001 [b]	0.31±0.01 [a]
Tannins %	0.01±0.001 [a]	0.01±0.001 [a]
Total Alkaloids %	0.30±0.010 [b]	0.48±0.001 [a]

*Means with different letters within the same row indicate statistically significant differences between species at $p \leq 0.05$. Data are expressed as means of three replicates ± standard deviations (SD).

are pharmacologically active compounds often associated with analgesic, antimalarial, and antibacterial properties. However, Tannins (%) recorded similar low level in both species with comparable tannin levels of 0.01%, with no significant difference observed.

Antioxidant activity using DPPH assay

For the Boraginaceae family, the highest IC_{50} value was 0.0423 mg/mL in *E. angustifolium*, and the lowest was 0.124 mg/mL in *A. aegyptiaca*, with ascorbic acid used as a standard (0.02 mg/mL), as shown in Figure (4). The figure illustrates the comparative antioxidant activity of *A. aegyptiaca*, *E. angustifolium*, and ascorbic acid. *A. aegyptiaca* exhibited the highest antioxidant activity (~0.125), indicating strong radical scavenging potential. This is distinguished given that *E. angustifolium* showed higher levels of total phenols, flavonoids, and alkaloids compounds typically associated with antioxidant properties. Despite its richer secondary metabolite profile, *E. angustifolium* demonstrated moderate antioxidant activity (~0.045), which is considerably lower than that of *A. aegyptiaca*. Interestingly, ascorbic acid, a well-established antioxidant, showed the lowest activity (~0.025) in this assay. This variation could be due to differences in concentration or specific assay conditions that favored the performance of the plant extracts, suggesting that *A. aegyptiaca* may contain compounds with higher efficacy under the tested conditions.

In general, *A. aegyptiaca* showed superior antioxidant performance, highlighting its potential as a promising natural source of antioxidants. Further phytochemical characterization and comprehensive bioactivity assays are recommended to identify the specific compounds responsible for its potent antioxidant effects.

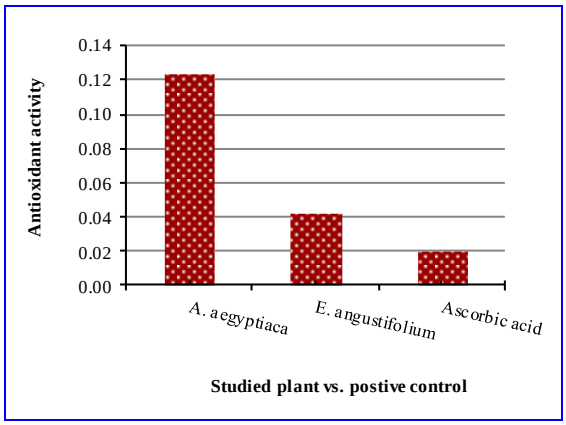


Figure (2): Antioxidant activity of studied plants: *A. aegyptiaca*, and *E. angustifolium* verses standard ascorbic acid antioxidant.

Correlation between measured parameters

The correlation matrix provides valuable insights into the interrelationships among the phytochemical and nutritional parameters measured in the studied plants (Figure 4). Antioxidant activity is strongly and positively correlated with total carbohydrates (r = 0.93), total phenols (r = 0.78), and tannins (r = 0.66), indicating that these components significantly contribute to the antioxidant potential. Conversely, a moderate negative correlation is observed between antioxidant activity and total flavonoids (r = -0.04), suggesting that flavonoids may play a less dominant role in antioxidant defense in this context.

Total lipids show a strong positive correlation with total ash (r = 0.95), suggesting a possible association between lipid content and mineral constituents. Crude fiber positively correlates with total flavonoids (r = 0.89), while total phenols exhibit strong positive associations with total alkaloids (r = 0.91) and flavonoids (r = 0.58), reinforcing their interlinked biosynthetic pathways. Generally, the data suggest that antioxidant activity is primarily influenced by phenolic compounds and carbohydrates, whereas other parameters like lipids and proteins show more diverse and less direct relationships with antioxidant capacity. This multivariate correlation analysis underscores the complex biochemical network underlying plant-based nutritional and functional properties.

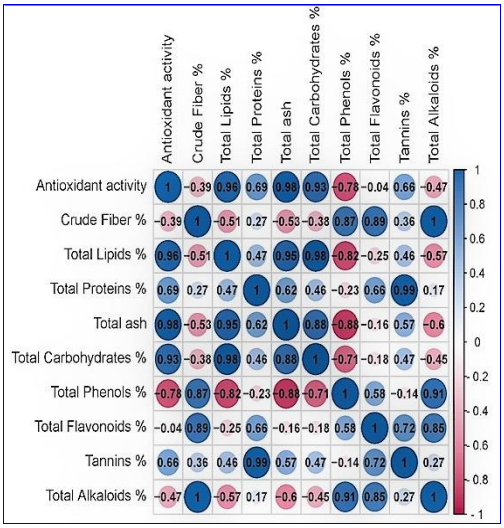


Figure (3): Interrelationships among antioxidant activity and bioactive compounds in studied plant species.

Antimicrobial activity

The antimicrobial activities of the aqueous extracts of the studied taxa against different species of fungi and bacteria, five bacterial strains (*B. subtilis*, *B. cereus*, *P. aeruginosa*, *R. solanacearum* and *M. leteus*) and five fungal species (*A. niger*, *A. flavus*, *A. terrus*, *A. alternata* and *R. solani*) determined using the agar well diffusion method are shown in Table (3).

Antibacterial activity

Figure (4) and table (3) illustrate the antimicrobial activity of aqueous extracts from *Aerva aegyptiaca* and *Echium angustifolium* against five pathogenic bacterial strains: *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Ralstonia solanacearum*, and *Micrococcus luteus*. Treatments T1, T2, and T3 represent different extract concentrations or fractions, while C denotes the negative control and S represents the standard antibiotic (positive control). For *A. aegyptiaca* (5%) against *B. subtilis* and *P. aeruginosa*, with inhibition zones of 33.0 and 31.0 mm, respectively, followed by the antibacterial activity of *E. angust-ifolium* (5%)

against *B. cereus*, *R. Solana-cearum* and *M. leteus*, with inhibition zones of 21.0, 34.0 and 31.0 mm, respectively (Figure 4). Both plant species demonstrated distinguished antimicrobial effects, as indicated by clear zones of inhibition surrounding the extract wells (T1–T3), particularly in *P. aeruginosa* and *M. luteus*, suggesting higher sens-itivity of these strains. Among the tested concent-ration, T3 generally produced the largest inhibition zones, implying a dose-dependent response or a more potent fraction. The antimicrobial performance of *E. angustifolium* appears slightly stronger overall, partic-ularly in comparison to *B. cereus* and *R. solanacearum*. In conclusion, the observed data support the potential of both plant species, especially *E. angustifolium*, as sources of bioactive compounds with promising antimicrobial activity against both Gram-positive and Gram-negative human pathogens.

Antifungal activity

For the Boraginaceae family, the greatest antifungal activity was recorded by *A. aegyptiaca* (5%). The antifungal activity of the tested treatments (Figure 5) varied significantly across the different fungal species (*Aspergillus niger*, *A. flavus*, *A. terreus*, *Alternaria*

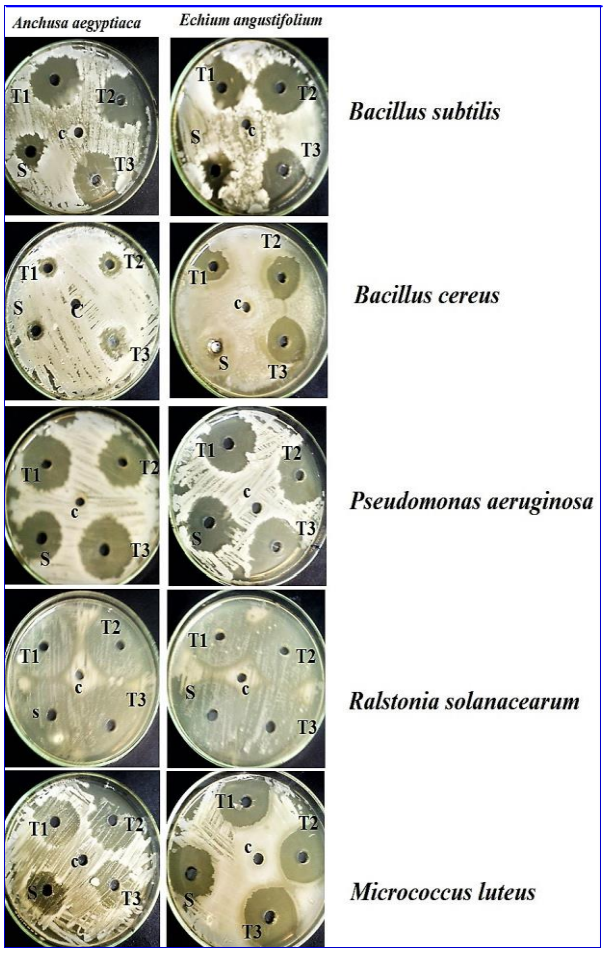


Figure (4): Comparative antibacterial activity of studies plant species: *Anchusa aegyptiaca* and *Echium angustifolium*-aqueous extracts against Gram-Positive and Gram-Negative human pathogens. Different concentrations of aqueous plant-extract were used. T1, 1%; T2, 2%; T3, 5%. C, represented negative control; S, standard, 10 µg/30 µg (0.5% DMSO) of gentamicin per paper disc.

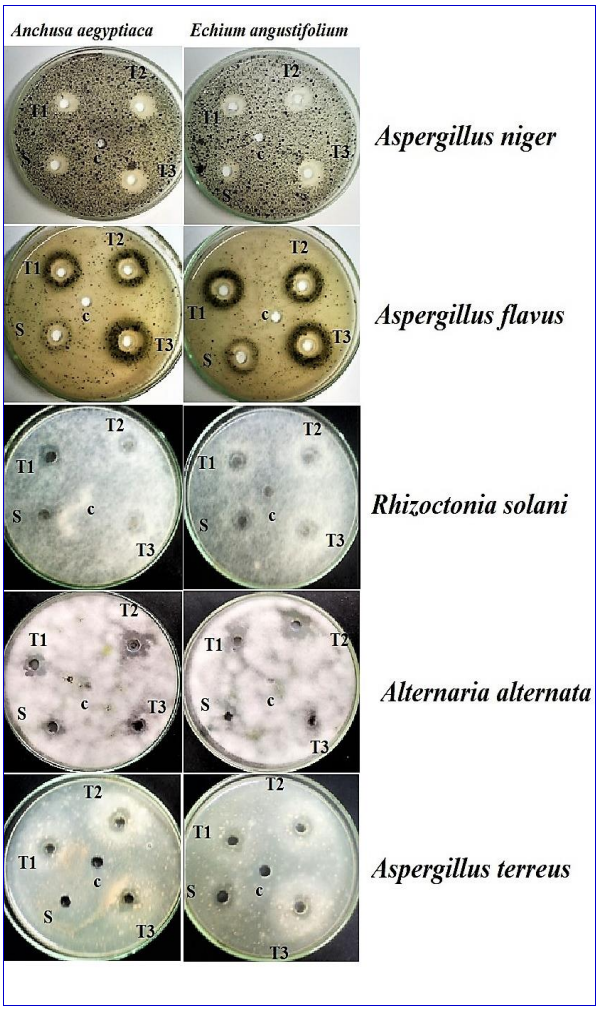


Figure (5): Comparative antibacterial activity of studies plant species: *Anchusa aegyptiaca* and *Echium angustifolium*-aqueous extracts against fungus pathogens. Different concentrations of aqueous plant-extract were used. T1, 1%; T2, 2%; T3, 5%. C, represented negative control; S, standard, 10 µg/30 µg (0.5% DMSO) of gentamicin per paper disc.

alternata, and *Rhizoctonia solani*). The inhibition zones indicate a treatment-dependent response (Table 3). Both plant extracts demonstrated moderate to strong antifungal activity, especially at higher concentrations. The highest antifungal activity was recorded for *E. angustifolium* at 5%, with inhibition zones of 15 ± 1.23 mm against *A. niger* and 14.5 ± 1.43 mm against *A. flavus*. Meanwhile, the lowest antifungal activity was observed for *A. aegyptiaca* at 1%, particularly against *A. terreus* (6 ± 0.76 mm) and *R. solani* (6.5 ± 0.45 mm), reflecting comparatively weak inhibition. These values may suggest lower susceptibility of these fungi to the applied treatment or differences in extract penetration or fungal resistance mechanisms. In general, the data demonstrate concentration- and species-dependent antifungal efficacy, with *A. niger* being the most susceptible and *A. terreus* the most resistant among the tested pathogens.

Cytotoxicity assay

The cytotoxic effects of three different aqueous extract concentrations (1%, 3%, and 5%) of the two

studied medicinal plants are presented in Table (4) and illustrated in photomicrographs (1) and (2). Table (4) summarizes the mitotic index (MI%), phase index (PI%), and total abnormality percentage (Tab%). Compared to the control, the highest increase in MI was observed in *A. aegyptiaca* at a concentration of 3%, reaching 13.38%. In contrast, the most pronounced decrease in MI was recorded in *E. angustifolium* at 5%, with a value of 8.05%.

The highest significant phase index for prophase was found in *E. angustifolium* at a concentration of 1%, with a value of 32.97%, and for metaphase, it was 37.64% for *E. angustifolium* at a concentration of 1%. The highest phase index for anaphase (24.33%) was recorded for *E. angustifolium* at a concentration of 3%, whereas the highest phase index for telophase was recorded for *A. aegyptiaca* at a concentration of 1%, with a value of 32.43. The greatest percentage of abnormalities in prophase was 0.61% in *A. aegyptiaca* at a concentration of 3%, whereas the highest percentage of abnormalities in metaphase was 9.47% in *A. aegyptiaca* at a concentration of 5%, whereas the highest percentage of abnormalities in anaphase was 7.27% in *A. aegyptiaca* at a concentration of 3%, and the highest percentage of abnormalities in telophase was 9.86% in *A. aegyptiaca* at a concentration of 5% compared with the control.

The greatest increase in the percentage of total chromosomal abnormalities (26.32%) was recorded in *A. aegyptiaca* at a concentration of 5%. In contrast, the lowest percentage of total abnormalities (12.52%) was detected for *E. angustifolium* at a concentration of 1%. Chromosome abnormalities are presented in Plates 1 and 2 for the three concentrations of *A. aegyptiaca and E. angustifolium*.

The photomicrograph (1 and 2) shows different chromosome aberrations for the three concentrations of *A. aegyptiaca* and *E. angustifolium.* Microscopic examination of *Vicia faba* root tip meristematic cells treated with *A. aegyptiaca* aqueous extract (photomicrograph 1) revealed a variety of mitotic abnormalities, indicating the extract's cytotoxic and genotoxic potential. Compared to normal mitotic divisions, treated cells exhibited pronounced chromosomal disturbances, including C-metaphase configurations, which suggest interference with spindle fiber formation. Chromosomal bridges and lagging chromosomes were also frequently observed, particularly during anaphase and telophase, implying chromosomal breakage and segregation defects. Sticky chromosomes were noted, reflecting possible chemical interactions with chromatin leading to impaired chromosomal condensation and separation. Additionally, the presence of micronuclei in interphase cells signifies genomic instability and residual DNA fragments resulting from improper chromosomal segregation or damage. Multipolar mitotic figures and disturbed metaphases were evident, further supporting the notion of disrupted spindle apparatus function. These abnormalities collectively suggest that the phytochemicals present in *A. aegyptiaca*, such as alkaloids and phenolics, may exert clastogenic or aneugenic effects on dividing cells. The results underscore the need for further investigation into the specific bioactive compounds responsible for these cytogenetic effects, as well as their potential applications or toxicological implications.

Microscopic analysis of *Vicia faba* root meristematic cells exposed to *Euphorbia angustifolium* aqueous extract revealed a range of mitotic abnormalities, indicating the extract's cytotoxic and genotoxic potential (photomicrograph 2). The observed anomalies included C-metaphases, indicative of spindle apparatus disruption, which can interfere with proper chromosomal alignment and segregation. Chromosomal bridges and laggards during anaphase and telophase stages were also frequently noted, suggesting chromosome mis-segregation or breakage. Sticky chromosomes were present, likely resulting from the extract's interference with chromatin condensation, leading to improper separation during mitosis. Multipolar divisions and disoriented spindles were visible, highlighting the destabilization of spindle microtubules. Furthermore, the presence of micronuclei during interphase points to genetic damage and incomplete chromosomal segregation. These aberrations collectively demonstrate that the phytoconstituents in *E. angustifolium*, such as phenols and alkaloids, may possess clastogenic or aneugenic effects. Despite its moderate antioxidant activity, the extract appears to exert significant mitodepressive and mutagenic effects under the tested conditions.

DISCUSSION

In recent years, the popularity of traditional herbal medicines has increased, as they are being utilized as substitutes for mainstream pharmaceuticals. This is mostly because these products are natural and have few or no adverse effects (Malgaonkar *et al.,* 2020). Medicinal plants (MPs) are valuable reservoirs of biologically active molecules that can be utilized in the creation of novel chemical compounds for pharmaceutical purposes (Basudan, 2023; Mahdi *et al*., 2023). Natural products and their associated medications possess a wide range of pharmacological properties and are employed in the treatment and/or prevention of numerous prevalent human disorders (Newman *et al*., 2003; Ramzan *et al.,* 2024). Phytochemicals in these classes are recognized for their diverse range of biological functions, which include antibacterial, antioxidant, anti-inflammatory, antiplasmodial, and anticancer actions (Ruch *et al*., 1989; Rice-Evans *et al*., 1995).

The Boraginaceae family consists of a collection of plants that are significant in the fields of pharmacology and cosmetology. The therapeutic impact of these plants is attributed to the presence of several physiologically active chemicals, such as flavonoids, terpenoids, and phenols (Dresler *et al.*, 2017). Extracts of *E. angustifolium* and other species of *Echium* plant

Table (3): Antimicrobial efficacy of aqueous extracts from the wild medicinal plants *Anchusa aegyptiaca* L and *Echium angustifolium* Mill. (Family: Boraginaceae) against selected bacterial and fungal pathogens.

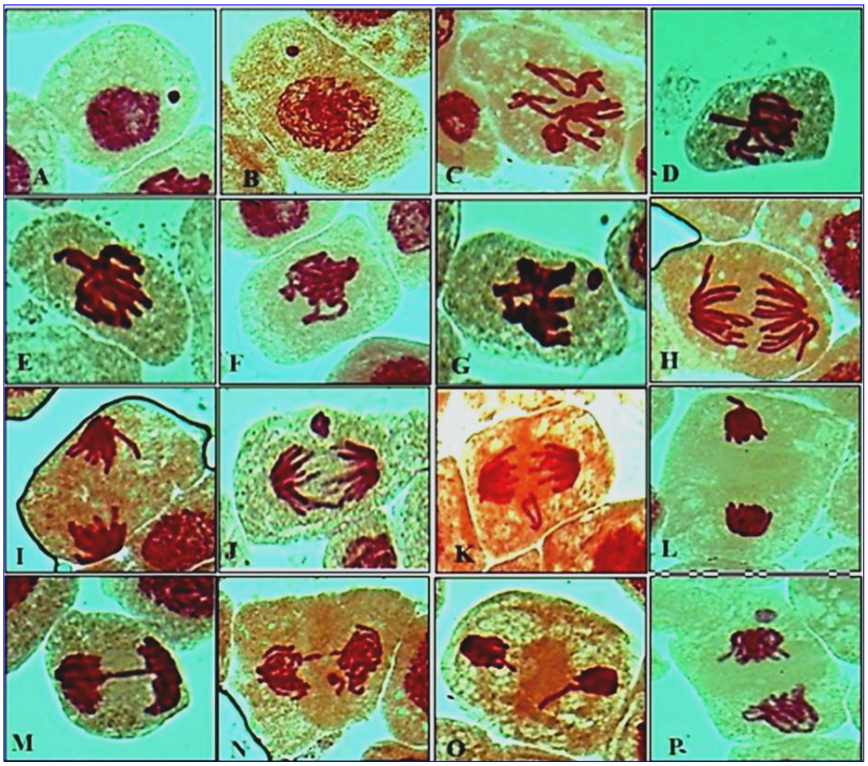
Plant species	Aqueous-extract conc.	Diameter of inhibition zone (mm)									
		Bacterial pathogens					Fungal pathogens				
		B. subtilis	*B. cereus*	*P. aeruginosa*	*R. solanacearum*	*M. leteus*	*A. niger*	*A. flavus*	*A. terrus*	*A. alternata*	*R. solani*
A. aegyptiaca	1%	19±1.87^{e}	8±0.86^{d}	22±1.65^{d}	30±2.11^{d}	21±1.11^{d}	8±0.76^{d}	12±1.12^{c}	6±0.76^{c}	10±0.98^{c}	6.5±0.45^{e}
	3%	24±2.11^{c}	10±0.98^{c}	25±0.87^{c}	31±2.12^{c}	25±0.78^{c}	11±0.98^{c}	12.5±1.32^{c}	12±1.12^{b}	12±1.12^{b}	12.5±1.12^{b}
	5%	33±2.34^{a}	14±2.10^{b}	31±0.98^{a}	33±2.13^{b}	30±2.12^{a}	13.5±1.21^{b}	13.5±1.34^{b}	13±1.34^{a}	13.5±1.54^{a}	13.5±1.21^{a}
E. angustifolium	1%	21±0.87^{d}	6±0.56^{e}	27±0.78^{b}	30±2.11^{d}	24±1.45^{c}	10.5±0.98^{c}	12±1.1^{c}	12±1.21^{b}	8.5±0.87^{d}	10.5±0.98^{d}
	3%	25±1.89^{c}	15±0.12^{b}	30±2.11^{a}	31±2.09^{c}	29±1.98^{b}	11.5±1.03^{c}	13±1.23^{b}	12.5±1.24^{a}	12±1.32^{b}	11±1.09^{c}
	5%	31±2.13^{b}	21±1.65^{a}	31±2.12^{a}	34±2.31^{a}	31±2.15^{a}	15±1.23^{a}	14.5±1.43^{a}	13±1.34^{a}	14±1.54^{a}	12.5±1.32^{b}

*Means with different letters, per column, indicate statistically significant differences between species at $p \leq 0.05$. Data are expressed as means of three replicates ± standard deviations (SD).

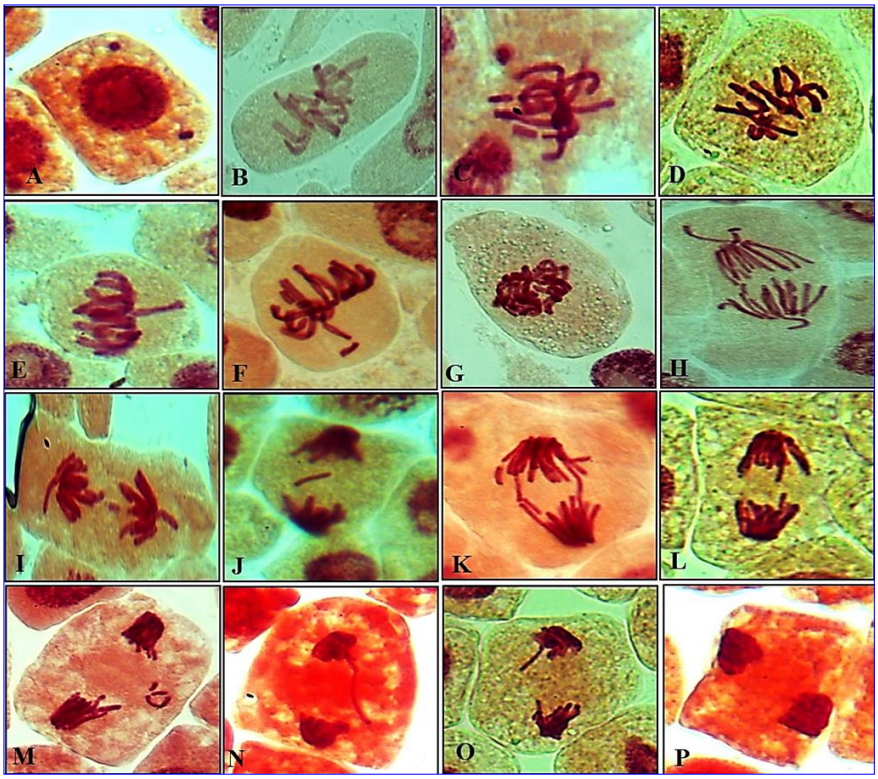
Table (4): Cytotoxicity of aqueous extracts of wild medicinal plant extracts, *A. aegyptiaca* and *E. angustifolium,* from the family Boraginaceae. Mitotic indices (MI), normal and abnormal phase indices, and total abnormalities in nondividing and dividing cells after treating *Vicia faba* root tips with different concentrations of aqueous extracts of wild medicinal plant extracts.

Studied plant species	Aqueous-extract conc. (%)	MI %	Phase index % (PI)								Total abnormal % (Tab)	
			% Prophase		% Metaphase		% Anaphase		% Telophase		Interphase	Mitosis
			Mitotic (normal)	Abn.	Mitotic (normal)	Abn.	Mitotic (normal)	Abn.	Mitotic (normal)	Abn.		
A. aegyptiaca	Control	10.70± 0.45	22.76	0.0	69.88	16.77	0.51	2.47	6.75	1.56	0.03±0.02	20.81±2.09
	1%	11.34±0.87*	22.56	0.59	29.98	4.88	16.64	5.08	32.43	6.58	0.85±0.04*	17.14±0.97^{ns}
	3%	13.38±0.96*	25.37	0.61	33.04	9.23	18.76	7.27	22.57	7.09	0.56±0.03*	24.21±1.04*
	5%	9.55±0.54^{ns}	23.24	0.0	28.18	9.47	18.76	6.99	26.66	9.86	0.32±0.02*	26.32±1.23*
E. angustifolium	1%	10.43±0.67*	32.97	0.0	37.64	3.78	10.28	2.52	19.11	6.22	0.05±0.001*	12.52±0.87^{ns}
	3%	12.52±0.76*	19.35	0.0	34.45	8.24	24.33	3.89	23.87	5.75	0.18±0.01*	17.88±0.99^{ns}
	5%	8.05±0.45^{ns}	27.38	0.0	28.55	5.24	16.4	4.39	20.43	13.81	0.25±0.02*	23.43±1.03*

*, significant at level $p \leq 0.05$; ns: non-significant.



Photomicrograph (1): Different types of chromosome aberration for three concentrations (1%, 3% & 5%) of *A. aegyptiaca* eggus extract. (**A**) Micronucleus at the interphase stage (1%), (**B**) micronucleus at prophase (3%), (**C**) two groups at metaphase (1%), (**D, E**) disturbed at metaphase (3, 5%), (**F**) Stickiness at metaphase (1%), (**G**) Micronucleus at metaphase (5%), (H) Disturbed at anaphase (1%), (**I**) Diaggonal at anaphase (1%), (**J**) Micronucleus at anaphase (5%), (**K**) Laggerus at anaphase (3%), (**L**) Disturbed at telophase (1%), (**M, N**) Bridge at telophase (3%),(**O**) Late separation at telophase (3%) and (P) micronucleus at telophase (1%) (X =1000).



Photomicrograph (2): Types of chromosomal aberrations for three concentrations (1%, 3% and5%) of *E. angustifolium* aqueous extract. (**A**) Micronucleus at the interphase stage (1%), (**B**) Oblique at metaphase (1%), (**C**) Micronucleus at metaphase (1%), (**D**) Two groups at metaphase (1%), (**E, F**) Disturbed at metaphase (1, 5%), (**G**) Stickiness at metaphase (1%), (**H, I**) Disturbed at anaphase (1, 5%), (**J**) Laggard at anaphase (1%),(**K**) Bridge at anaphase (5%), (**L**) late separation at telophase (3%), (**M**) ring at telophase (5%), (**O**) late separation at telophase (1%) and (P) diagonal at telophase (1%), (X =1000).

have been extensively used in traditional medicine for various therapeutic purposes, primarily for the treatment of external wounds and ulcers (Jaradat, 2005 and Eruygur *et al*., 2012). Earlier research on *Echium* species identified flavonoids, phenolic acids, pyrrolizidine alkaloids, and fatty acids as the primary chemical components (Sarg *et al*., 1992; Albreht *et al*., 2009; Sakineh *et al*., 2011; Chaouche *et al*., 2012).

This study revealed that *E. angustifolium* presented the highest concentrations of total phenols, total flavonoids, and total alkaloids, with values of 1.04%, 0.31%, and 0.48%, respectively. This finding aligns with a previous study by Al-Rimawi *et al.* (2020), which reported that the polar fractions (methanol and aqueous) of E. angustifolium are abundant in phenols and flavonoids. These two classes of natural compounds are known for their strong antioxidant activity. El- Rokh *et al*. (2018) previously reported the anticancer efficacy of *E. angustifolium* in biological experiments. Antioxidants are present in numerous natural components and can be highly concentrated in various herbal extracts (Al-Rimawi *et al*., 2020). The antioxidant activity of plant extracts is a crucial characteristic that has received significant attention in recent decades. Researchers have extensively investigated the antioxidant capabilities of plants and essential oils (Boskovic *et al*., 2021). Phenolic compounds exhibit strong hydrogen-donating properties, which render them highly efficient antioxidants (Amić *et al*., 2003). Boskovic *et al*. (2021) demonstrated that plant extracts derived from the Boraginaceae family possess cytotoxic properties and that these plants exhibit the highest antioxidant activity. The antioxidant activity of *E. angustifolium* extracts has been reported in several studies (Eruygur *et al*., 201 and El-Tantawy *et al*., 2021).

The study revealed that *E. angustifolium* had the highest IC_{50} value of 0.0423 mg/ml, whereas *A. aegyptiaca* had the lowest value of 0.124 mg/ml. These findings align with the research conducted by Al-Rimawi *et al*. (2020) on the antioxidant activity of *E. angustifolium*. They used the DPPH method, a common approach to assess the ability of plant extracts to scavenge free radicals. Their study confirmed that this plant species exhibits promising antioxidant activity. In their study, Gharib and Godarzee (2016) and Baeissa *et al*., (2024) demonstrated a direct relationship between the antioxidant activity and the overall phenolic and flavonoid contents of the extracts. These data are consistent with previous studies on the diversity of species within the Boraginaceae family.

Antimicrobial resistance has been recognized by the World Health Organization as one of the foremost worldwide public health risks confronting humanity (World Health Organization, 2021). Antibiotic resistance poses a significant risk to world health, and the resistance of microorganisms to antibiotics is consistently increasing (Van Elsas and Bailey, 2002). Antimicrobial resistance poses a significant worldwide problem and is a grave danger to humans. This resistance is mostly generated by the widespread and improper utilization of antibiotics (Chebaro *et al.*, 2023). The significance of antimicrobial activity in medicinal plants lies in their ability to uncover novel and efficacious molecules for the management of infectious disorders (Yetgin, 2024; Serag *et al*., 2023).

The aqueous extracts of the studied taxa exhibited antimicrobial properties. The highest antibacterial activity was observed in *A. aegyptiaca* (5%) against *B. subtilis* and *P. aeruginosa*, with inhibition zones measuring 33 and 31 mm, respectively. *Echium angustifolium* (5%) also showed antibacterial activity against *B. cereus*, *R. solanacearum*, and *M. leteus*, with inhibition zones measuring 21, 34, and 31 mm, respectively. *Anchusa aegyptiaca* (5%) displayed the strongest antifungal activity, inhibiting *A. niger*, *A. terrus*, and *R. solani*, with inhibition zones measuring 13.5, 13, and 13.5 mm, respectively. In addition, E. angustifolium exhibited the highest level of antifungal activity against *A. flavus*, *A. terrus*, and *A. alternata*, resulting in inhibition zones measuring 14.5, 13, and 14 mm, respectively. The plants belonging to the Anchusa genus are extensively used in Arabian medicine, as documented in studies conducted in Palestine (Shtayeh *et al*, 1998) and Iran (Miri *et al*, 2013). *Anchusa* is regarded as a powerful antibacterial substance and is utilized to treat various bacterial illnesses in the respiratory, gastrointestinal, and urinary systems (Abu--Rabia, 2005; Alachkar *et al*., 2011; Al-Khateeb *et al.*, 2019). Boskovic *et al*. (2018) reported that *Anchusa officinalis* contains a significant amount of phenolic components in its various extracts. They evaluated the antimicrobial, antioxidant, and cytotoxic potential of these extracts and reported that they are rich in pharmacologically active compounds.

Although medicinal plants have therapeutic benefits, the general public and professional groups of traditional medicines do not acknowledge their potential toxicity (Soetan and Aiyelaagbe, 2009). Importantly, many plant species that are commonly used for medicinal purposes may contain substances that can be potentially harmful (Rodrigues *et al.*, 2011). Thus, it is crucial to assess the toxicological impacts of any herbal extract intended for human consumption (Ribeiro *et al.*, 2016). Cytotoxicity studies serve as a valuable first step in assessing the potential toxicity of a test substance, such as plant extracts or physiologically active chemicals derived from plants (McGaw *et al.*, 2014). The screening of plant cytotoxicity is a preliminary method used to detect the active chemicals present in plants (Hashemi *et al.*, 2011). The root tip meristems of *Vicia faba* have been utilized as primary cytogenetic material in numerous studies to identify genotoxicity. These investigations include the works of Ma *et al.* (2005) and Dong and Zhang (2010). The study conducted by Leme and Marin-Morales in 2009, as well as the research by Firbas and Amon (2014), demonstrated the rapidity, convenience, and sensitivity of the method.

The *Vicia faba* plant system is a dependable, uncomplicated, responsive, and effective biological system for genotoxicity studies and other relevant

investigations (Prabhu *et al*., 2017). Genetic mutation of chromosomes is a valuable technique for assessing the detrimental impacts of various substances, such as fungicides, pesticides, plant extracts, and medications (Pandey and Pandey, 2014). Chromosomal aberrations (CAs) refer to alterations in the structure of chromosomes caused by the breaking or swapping of chromosomal material (Aşkin Çelik and Aslantürk, 2010). The existence of chromosomal abnormalities in the investigated cells may indicate the presence of genotoxicity (Grant, 1978). Chromosome fragments are evidence of cytotoxic effect of plant extracts.

Chromosome breaks can occur due to anaphase/telophase bridges (Sharma and Sen, 2002 and Singh, 2003; Soliman *et al.*, 2023). The current investigation demonstrated that three concentrations (1%, 3%, and 5%) of the chosen medicinal plants resulted in a substantial and noteworthy increase in the overall percentage of abnormalities. The highest recorded percentage of abnormalities, amounting to 26.32%, was observed in *A. aegyptiaca* at a concentration of 5%. Conversely, *E. angustifolium* 1% had the lowest proportion of overall anomalies, accounting for only 12.52% of all anomalies. Ateşşahin *et al*. (2023) reported that extracts of *Echium vulgare* did not have a notable cytotoxic effect on the quantities that were examined.

The present investigation revealed many chromosome abnormalities, including micronuclei at interphase, disrupted chromosomes, noncongression and stickiness during metaphase, laggard chromosomes, late separations, and diagonal chromosomes at anaphase and telophase where there is a structural or numerical change in one or more chromosomes. It is crucial to identify these chromosomal errors accurately for the purpose of implementing prevention strategies, providing genetic counseling, and administering appropriate treatment (Milani and Tadi, 2023). Disruption of metaphase may indicate partial disruption of the spindle apparatus or suppression of spindle formation, as proposed by El-Ghamery *et al.* (2003).

The stickiness also observed, is a result of the interchromosomal connection between the strands of chromatids, which is caused by excessive mutation in the nucleoprotein (Soliman *et al*., 2014). Chromosome breaks result in mismatches and mutations, such as noncongression (Ma, 1982, Soliman *et al.*, 2023, Khedr *et al.*, 2024). Plants with chromosomal abnormalities experience not only enlargement effects but also increased phytochemical compounds. From an ecological perspective, chromosomal abnormalities in plants have been identified. Hromosomal abnormalities also alter secondary metabolites, especially phytochemical compounds, in several plant species (Gantait and Mukherjee, 2021). For example, natural components observed in tetrasomic tetraploid opium poppy (*Papaver somniferum* L.) increased the morphine content by 25-50% by altering the expression of several genes regulating the alkaloid biosynthesis pathway (Mishra *et al*., 2010).

CONCLUSION

Echium angustifolium contains high concentrations of flavonoids, alkaloids, and phenols, which are well known for their anti-inflammatory, anticancer, and neuroprotective activities. Furthermore, this plant has low prevalence of chromosomal abnormalities, which provides support for the utilization of synthesized drugs. The antioxidant activity of E. angustifolium may help decrease oxidative stress. The occurrence of disease and the extent of free radical damage may diminish. *Echium angustifolium* possesses antibacterial and antifungal properties that have medical benefits. This chemical has the potential to be utilized in the development of new antimicrobial drugs to combat resistance by eradicating these infections. *Echium angustifolium* exhibits minimal chromosomal abnormalities, indicating its potential for therapeutic use. The endurance of a plant makes it a reliable and secure source of natural medication.

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دراسة التركيب الكيميائى لمستخلصات نباتات الحمحم المصرى وزهرة الافعى والسمية الخلوية والجينية ونشاطات مضادت الميكروبات

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الملخـــــص العـربـي

النباتات الطبية منذ القدم مصدرًا للاستخدامات الطبية والعلاجية ولا تزال تلعب دورًا مهمًا في اكتشاف عقاقير جديدة نظرًا لاحتوائها على الكثير من المواد الكيميائية المتنوعة بها وأنشطتها البيولوجية. وعلى الرغم من انتشار استخدامها على نطاق واسع، لا يزال من المهم دراسة التقييم لخصائصها المضادة للميكروبات ومضادات الأكسدة والسمية للخلايا ضروريًا للتحقق من صحة إمكاناتها العلاجية. في هذه الدراسة، تم تقييم الأنشطة المضادة للبكتريا والفطريات ومضادات الأكسدة والسمية الخلوية للمستخلصات المائية من النباتات الطبية (حمحم مصرى) *Anchusa aegyptiaca* L. و(زهرة الأفعى) *Echium angustifolium* Mill. كما تم تقييم الفعالية المضادة للميكروبات ضد خمس سلالات بكتيرية وخمس سلالات فطرية. تم تحديد السمية الخلوية باستخدام بذور نبات الفول الاختبارى، باستخدام التشوهات الكروموسومية كمؤشرات على السمية الجينية. أظهر نبات *Echium angustifolium* أعلى نشاط مضاد للأكسدة، حيث بلغت قيمة IC_{5000} 0.0423 مللجرام/مللى، بما في ذلك الفينولات (1.04%) والفلافونويدات (0.31%) والقلويدات (0.48%). أما بالنسبة لللمستخلص المائي لنبات *E. angustifolium* بتركيز 5% هو الأكثر فعالية في تثبيط نمو الفطريات مثل *Aspergillus niger*, *A. flavus* و *A. flavus* و *Alternaria alternata*، حيث بلغت أقطار مناطق التثبيط الى 15 و14.5 و14 مللى ميتر على التوالي. بالإضافة إلى ذلك، أظهر تركيز 5% من نفس النبات نشاطًا مضادًا قويًا للبكتيريا ضد *Bacillus cereus*, *Pseudomonas aeruginosa*, *Ralstonia solanacearum*, و*Micrococcus luteus*, حيث بلغت أقطارمناطق التثبيط 21 و31 و34 و31 و31 مللى ميتر على التوالي. وفي الوقت نفسه، أظهر نبات *Echium angustifolium* أعلى تركيزات من المواد الكيميائية النباتية والنشاط المضاد للأكسدة والفعالية المضادة للميكروبات، بينما أظهر مستويات منخفضة نسبيًا من السمية الخلوية والتشوهات الكروموسومية. ويرجع ذلك الى أن المستخلص المائي لنبات *Anchusa aegyptiaca* بتركيز 1%أظهر أقل نسبة للتشوهات الكروموسومية بنسبة 17.14%مقارنة بالماء (التي أظهرت انحرافات بنسبة 20.81%). تُشير هذه النتائج إلى أن هذه المستخلصات النباتية تمتلك خصائص وقائية خلوية قد تخفف من التأثيرات السمية الجينية، مما يدعم سلامتها عند استخدامها فى الاغراض الطبية والعلاجية.